Immunology of the genital tract
A review

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I. Introduction

A. General introduction

The mucosal immune system in the female reproductive tract has evolved to meet the unique requirements of dealing with sexually transmitted bacterial and viral pathogens, allogeneic spermatozoa, and the immunologically distinct fetus.
Despite years of immunologic investigation, the mechanisms that regulate this mucosal immune system in the human reproductive tract have received only little attention. This is due to the complexities of the immune and endocrine system and also to the difficulties of conducting experiments in this field.
During the last years there has also been an increasing awareness of the need for further investigation of this area due to the continuously rising prevalence of sexually transmitted diseases (STDs), among them the pandemics caused by the human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS). Also in another field of gynecology and obstetrics, the reproductive medicine, the knowledge of immunological mechanisms and disturbances has gained growing attention.
However, recent progress in the field of genital tract immunology has shed light on some of the mechanistically most important functions of this immune system. Most importantly, we are beginning to understand how immune responses should best be stimulated at the genital tract mucosal level. On the basis of this information, the attempt to construct new mucosal vaccines specifically targeted to the genital tract is one of the ambitious goals of research in this field.

B. Methods and goals of this study

The objective of this work was to systematically review and discuss recent studies and articles dealing with the subject of the immunology of female genital tract tissue.
To this purpose, a computerized search of PubMed databases was first performed on general terms such as “immunology of genital tract”, “mucosal immunology”, “mucosal immunity”, or “immunology in gynecology”. On the basis of references of these newer articles, other studies or older literature could be found as well.

To further explore studies on this topic, the comprehensive database of medical magazines (DocumentWeb) of the Ludwig-Maximilians-Universität München was used. Archives of relevant magazines dealing with immunology and/or gynecology were searched with the help of these general terms again.

Particular attention was then given on a more specialized computerized search concerning the chapters on immunology of genital tract infections and immunology of reproductive medicine by using more defined keywords.

Besides research via internet databases, I have used public institutions and libraries such as the Bayerische Staatsbibliothek München, Medizinische Universitätsbibliothek München Großhadern and the Deutsche Zentralbibliothek für Medizin Köln.

After giving a brief introduction on the basic principles of human innate and specific immunity, and further of mucosal immunology, the overall goal of the first chapter is to define the mucosal immune system in the female reproductive tract. The focus is thereby to identify what is known about humoral and cellular factors of this particular mucosal immune system and to define the regulatory influences by sex hormones and cytokines. The unique immunologic characteristics of the female genital tract are then considered in respect to the design of mucosal vaccines for the protection against microbial disease.

The second chapter deals with the most important infections of the female genital tract and describes the latest results on innate and adaptive immune responses and vaccine development for each infection.

These are the following:

Viral infections

Herpes simplex virus (HSV)

Human immunodeficiency virus (HIV)

Human papillomavirus (HPV)

Bacterial infections

Neisseria gonorrheae

Chlamydia trachomatis

Bacterial Vaginosis (BV)

Mycoses

Candida albicans

Parasites

Trichomonas vaginalis

The third chapter then reports on different important fields of immunology in reproductive medicine. After describing immunologic principles at the fetomaternal interface during normal pregnancy and labor, another emphasis lies on different disturbances in maternal-fetal interactions and their immunologic background.
These are:

Preeclampsia

Preterm labor/preterm birth

Fetal growth retardation

Early pregnancy loss

Furthermore, the topic of infertility is further elucidated from an immunological point of view. The remarks on latest developments on immunocontraception conclude this chapter.

II. Immunology of the genital tract

A. Fundamentals of immunology

1. Concepts of innate and specific immunity

The term immunity derives from the Latin word *immunitas* which stands for the privilege of the Roman senators to be protected from legal punishment or exempted from certain public duties (1). At all times immunity has been described as the protection against illness, especially infectious illness. In modern times immunity is more exactly defined as reaction of the body against unknown substances and the capability to distinguish infectious nonself and non-infectious self.

The immune system has evolved to provide appropriate defense systems at various levels of innate or unspecific and acquired or specific immune responses (578). Innate immunity is the ancient part of the host defense mechanisms and lies behind most inflammatory responses. Acquired immunity can provide specific recognition of foreign antigens, an immunological memory of infection and pathogen-specific adaptor proteins. However, the adaptive immune response is also responsible for allergy, autoimmunity and the rejection of tissue grafts.

In most cases, components of both systems interact to create an appropriate immune response to an infectious agent. But it is not only the protection from penetration by foreign or modified cells but also the elimination of old and deficient cells which characterizes a functioning immune system.

a) The innate immune response to infectious agents

Recognized as the first line of defense, innate immunity consists of different mechanisms which are already available before exposition with pathogens or unknown molecules and do not need prior activation or induction. Innate immune responses do not change in type or magnitude if there is more than one encounter with the same antigen and differences between unknown substances cannot be distinguished (302). In the following, a short overview of the different factors of innate immunity is given (Table 1).

Mechanical and chemical barriers
Skin, mucosal surfaces
Enzymes (lysozyme), peptides (defensins), fatty acids, acidic pH, etc.
Cellular factors
Mononuclear phagocytes (blood monocytes/tissue macrophages)
Granulocytes
Dendritic cells
Mast cells
Natural killer cells
Humoral factors
Complement
Acute-phase proteins
Interferons

Table 1: Factors of the innate immune system

Preventing microorganisms from gaining access to the body is achieved by mechanical barriers such as skin and surface epithelia (1019). These are also equipped with additional chemical features including fatty acids in the skin, low pH in the stomach or antibacterial enzymes in saliva, for example. Once the pathogen has crossed the epithelial barrier, cellular effector mechanisms involving granulocytes and mononuclear phagocytes are activated to eliminate the intruder by phagocytosis (1213).

To recognize foreign structures that are not normally found in the host, the innate immune system relies on conserved germline-encoded receptors that recognize conserved pathogen-associated molecular patterns (PAMPs) found in groups of microorganisms (615). These are conserved products of microbial metabolism produced by microbial pathogens but not by the host, such as lipopolysaccharide (LPS) in the outer membrane of gram-negative bacteria. Recognition of these molecular structures allows the immune system to distinguish infectious nonself from non-infectious self (615, 909).

Among receptors for PAMPs which are expressed on cells of the innate immune system, Toll-like receptors (TLRs) are of particular importance. Signaling through

TLRs in response to PAMPs leads to recruitment of other immune cells and production of antimicrobial factors that kill invading microbes as well as link innate and acquired immunity (16). Studies have found ten different subtypes of mammalian TLRs spread on the surface of different immune cells (534).
Among the cells that bear innate immune or germline-encoded recognition are mononuclear phagocytes, dendritic cells (DCs), mast cells, granulocytes, and natural killer (NK) cells (615).

(1) Cellular factors

Phagocytic cells are located at strategically important spots in the organism (Table 2) and are all derived from pluripotent stem cells of the bone marrow.

Localisation	**Phagocytic cells**
Blood	Monocytes Neutrophils (Eosinophils, Basophils)
Lung	Alveolar macrophages
Liver	Kupffer cells
Brain	Microglia
Bone	Osteoclasts
Kidney	Mesangial cells in glomeruli
Joint	Synovia-A-cells

Table 2: Localisation of phagocytic cells

Monocytes circulating in blood vessels and macrophages in other tissues like lung, liver and lymph nodes as well as granulocytes are capable of killing microorganisms. After activation by cytokines, especially interferon-γ (IFN-γ), macrophages produce toxic effector molecules as reactive oxygen intermediates (ROI) and reactive nitrogen intermediates (RNI) which are interactively able to kill the pathogen. RNI have proved to be the most effective defense mechanism in murine macrophages but also an increasing amount of studies supports RNI production of human macrophages (667). Lysosomal enzymes within the phagosome or depletion of intraphagosomal iron function as other mechanisms of phagocytic cells to kill intracellular pathogens (412, 817).

The group of granulocytes consists of neutrophils, eosinophils and basophils but the uptake and intracellular killing of microorganisms is in the first place the task

of neutrophils. By expriming Fc receptors for immunoglobulin (Ig) G (CD16) and receptors for activated complement factors (C3b), neutrophils can phagocyte pathogens coated by antibodies or complement factors (1266). Neutrophils are able to release azurophil granule with myeloperoxidase and lysozyme, specific granule with lactoferrin or alkaline phosphatase as well as superoxid radicals, which leads to an inflammatory reaction of tissue (1266).
Extracellular killing of large parasites such as helminths is performed by eosinophils. They also provide surface receptors for complement factor C3b and release their granule with major basic protein (MBP), cationic protein and anti-inflammatory enzymes (1213).
Basophils play an important role in allergic reactions and in immune responses against parasites where they release mediators such as histamine or heparine and chemotactic factors and therefore cause an anaphylactic reaction. They have surface receptors for both IgE and activated complement factors C3a and C5a (1266).

Other cellular components of the innate immunity are NK cells which are large granular lymphocytes making out 5-10% of the peripheral lymphocytes in the blood (1266). They exprime surface receptors for IgG (CD16) as well as T cell markers such as CD8 and are able to mediate antibody-dependent cellular cytotoxicity (ADCC). Thereby, they are able to destroy target cells such as cancer or virus-infected cells by binding these antibody-loaded cells to the Fc receptor of NK cells via the Fc region of the antibody (1266).

(2) Humoral factors

The complement system is a multicomponent triggered enzyme cascade (Figure 1) and is used to attract phagocytic cells to the pathogens.

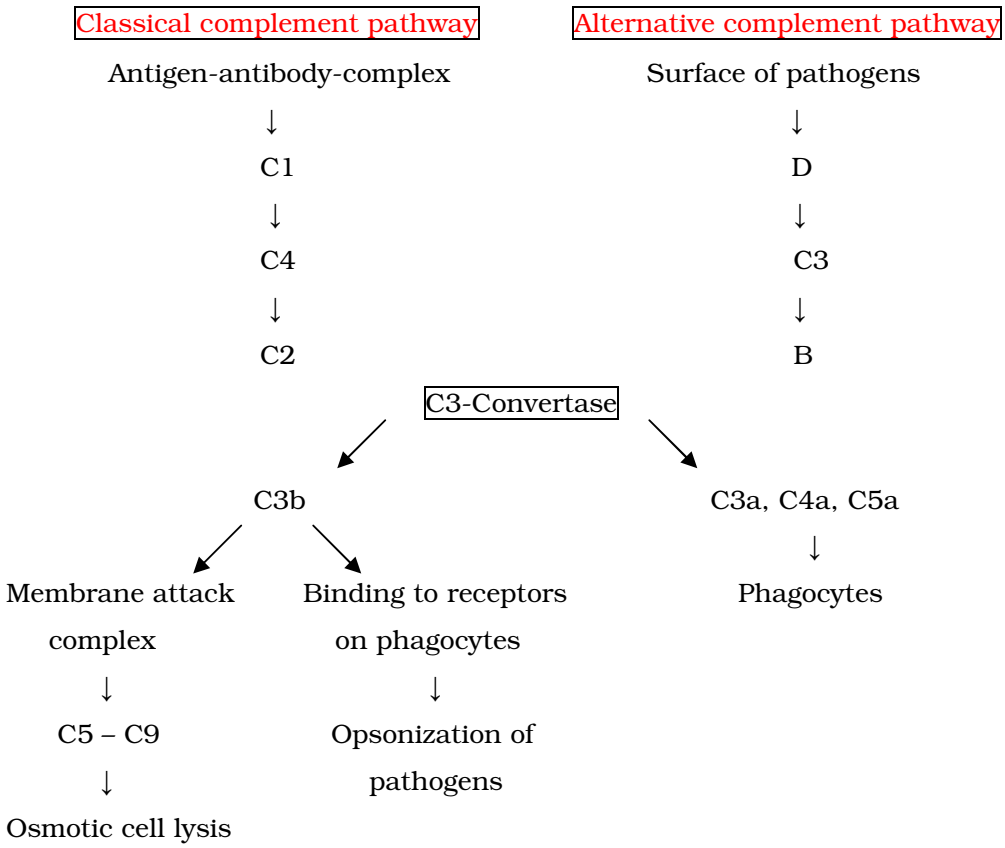


Figure 1: Components and effector mechanisms of the complement system (616)

Complement activation can start via the classical pathway with Igs in complex with antigens binding to complement protein C1. As it requires the presence of specific antibodies this way is delayed upon microbial infection (177). The alternative pathway beginning with the binding of complement factor C3b on different activated bacterial surfaces is initiated in the absence of antibodies and therefore is the more important one among the humoral mechanisms of the innate immune system (667). Both ways lead to the splitting and activation of factor C3 whose activated fragments enable the opsonization of antigens or bacteria surfaces. The terminal components C5-C9 form a membrane attack complex (1213) which enables the osmotic lysis of the pathogen.

Other humoral factors of the innate immune system involve IFNs and acute-phase proteins (1266). IFN-α produced by leukocytes and IFN-β synthesized by fibroblasts and other cell types block viral replication in virus-infected cells. C-reactive protein as the most common representative of acute-phase proteins promotes the binding of complement to bacteria and facilitates their phagocytosis.

b) Specific acquired immunity

If the intruding microorganism cannot be eliminated by the effector mechanisms of the innate immune system mentioned above, the components of the specific or adaptive immune system are activated. Specific immunity is characterized by an adaptation to the first reaction to an antigen (1213). The following responses to the same antigen are specific and quantitatively and qualitatively different from the primary response. This specificity is achieved through usage of clonally distributed antigen receptors, i.e. surface Ig on antibody-producing B lymphocytes and T cell receptors (TCR) on the surface of T lymphocytes. Another feature of the adaptive immune system is that it develops memory which allows a faster response of specific effector cells when encountering the relevant antigen a second time (667).

Responding to antigens is either realized by producing specific antibodies (humoral immunity) or direct specific lymphocyte contact with host cells expressing foreign antigenic peptides (cell-mediated immunity). The cell-mediated response needs the cooperation of different subclasses of T cells, macrophages and perhaps NK cells. Humoral responses involve the interaction of B cells, T cells and antigen-presenting cells (APCs) (302).

(1) The major histocompatibility complex (MHC)

T and B lymphocytes need a system to be able to distinguish between “self” and “nonself”, which is done by the MHC (1482). This group of genes encodes for several proteins, also called human leukocyte antigens (HLA) (Table 3). MHC class I molecules, which include HLA-A, HLA-B and HLA-C, are being expressed by all nucleated cells whereas MHC class II molecules including HLA-DR, HLA-DP and HLA-DQ are being expressed by APCs, B and T cells (1266).

MHC class Ia	HLA-A, HLA-B, HLA-C
MHC class Ib	HLA-E, HLA-F, HLA-G
MHC class II	HLA-DP, HLA-DQ, HLA-DR
MHC class III	Complement components, TNF-α, TNF-β

Table 3: MHC classes with examples for encoding proteins

(2) B lymphocytes

After the process of B cell differentiation in bone marrow each mature B cell bears a surface receptor, an Ig, which is different in its antigen specificity from all other B cells. These mature but naïve B cells circulate in the blood, lymph and secondary lymphoid organs waiting to encounter antigen. By interaction with antigen, B cells produce and secrete large amounts of antigen-specific memory B cells and effector plasma cells which generate large amounts of soluble but otherwise identical versions of the membrane-bound Ig (1).

This clonal selection hypothesis (1387) explains why following responses to the same antigen are more effective and longlasting as in the initial response (302). In the first encounter with antigen, a primary antibody response is generated; later, a re-encounter with the same antigen causes a more rapid secondary response, producing high levels of antibodies with a high binding affinity for the target antigen. This process is also exploited in prophylactic vaccination.

Characteristic for the primary immune response are primarily IgM class antibodies whereas the secondary and all subsequent responses to the same antigen may be of the IgG, IgE or IgA subclasses, depending on the location (302). Proliferation and differentiation of B cells as well as the Ig isotype class switching are driven by cytokines, especially by interleukins (IL)-4 and -5 (1266). The differences between the Ig subclasses shows Table 4.
In addition to their unique role as antibody-producing plasma cells, B cells have the capacity to present antigen to T lymphocytes. Upon binding of antigen to membrane-bound Ig, antigen-antibody complexes are internalized and degraded. Antigen-derived peptides are then introduced to MHC-II-dependent pathways and can be presented to peptide-specific CD4+ T cells (1482)

	IgG	**IgA**	**IgM**	**IgD**	**IgE**
Form	Monomer	Monomer Dimer	Pentamer Hexamer	Monomer	Monomer
Subclasses	G1,G2,G3,G4	A1,A2	—	—	—
Percent of Ig	75-85	7-15	5-10	0,3	0,019
Binds to	Macrophages NK cells Neutrophils	Lymphocytes	Lymphocytes	—	Mast cells Basophils B cells
Complement fixation	Classical	Alternative	Classical	—	—
Cross Placenta	Yes	—	—	—	—

Table 4: The different classes of immunoglobulins

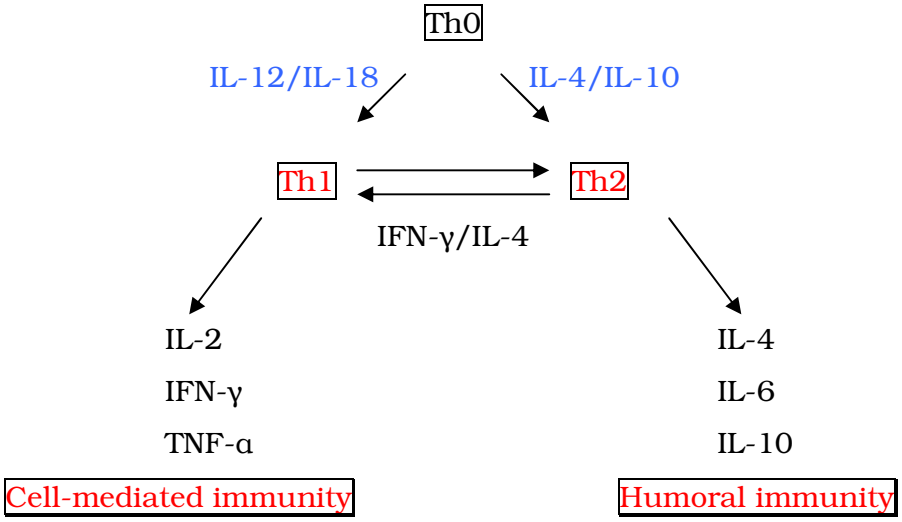
(3) T lymphocytes

In contrast, T cells only recognize antigen when it is presented by appropriate MHC molecules on APC such as DCs or macrophages (667).
During their differentiation in thymus, T lymphocytes learn to recognize MHC molecules and develop the cluster of differentiation (CD) 4 and 8 surface receptors which mark them as CD4+ T helper cells or CD8+ cytotoxic T cells (CTL).
Antigenic peptides presented to T cells by MHC class I molecules stimulate the CD8+ T cells whose primary function is to destroy intracellular pathogens. Peptides presented by MHC class II molecules stimulate the CD4+ T cells which are able to eliminate both intracellular and extracellular pathogens. They produce various cytokines required for the activation of leukocytes and are therefore also termed T helper (Th) cells (667).

Activation of T cells requires signaling mediated through both the TCR and costimulatory receptor-ligand interactions which involve the costimulatory molecules CD80 (B7.1) and CD86 (B7.2) on APCs (667). These can bind to T cell surface molecule CD28 and CD125 (CTLA-4).

As CD4+ Th cells produce different cytokines upon antigenic stimulation to activate B cells and macrophages, they can be divided into the two subpopulations of Th1 and Th2 cells (977). Th1 cells are characterized by secretion of INF-γ and IL-2,

whereas Th2 cells produce IL-4, IL-5 and IL-10 (977). Th2 cells are therefore important for the induction of humoral immune responses by controlling B cell activation while Th1 cells initiate cell-mediated immune responses by activating macrophages by IFN-γ and CD8+ T cells by IL-2 (667, Table 5). The differentiation of these subsets from Th0 precursor cells is driven by cytokines, especially by IL-12/IL-18 and IL-4, respectively (667).



▪ Activation of cytotoxic T cells → Protection against viruses ▪ Macrophage activation → Protection against intracellular microbes ▪ Ig class switch to IgG (complement activation/opsonization) → Protection against extracellular microbes ▪ Th1 activation → Protection against all microbes and viruses	▪ B cell maturation → Protection against extracellular microbes, virions and helminths ▪ Ig class switch to IgE (mast cell, basophil, eosinophil) → Protection against helminths ▪ Ig class switch to IgG (neutralization) → Protection against virions, toxins ▪ Ig class switch to IgA (mucosa) → Protection against many pathogens ▪ Eosinophil activation → Protection against helminths

Table 5: The role of Th1 and Th2 cells in immunity, adapted from Kaufmann et al. (667)

A third category of T cells, regulatory T cells (Tregs) with the phenotype CD4+CD25+, usually secretes IL-10 and tumor growth factor-β (TGF-β). Cells with this phenotype are thought to recognize self-antigens and function to prevent

autoimmunity, but they also regulate responses to exogenous antigens, and have been implicated in chronic and immunopathologic viral infections (1220).

CTLs are able to eliminate virus-infected cells or tumor cells by lysis or apoptosis (1266). Cytotoxicity is mediated by pore-forming proteins (perforins) and enzymes (granzymes) that perforate the target cell. CTLs can also trigger apoptosis, i.e. programmed cell death, in target cells through receptor-ligand interaction (667). Upon activation, CTLs are induced to express Fas-ligand (FasL) which interacts with the corresponding receptor Fas expressed on virus-infected target cells.

The division of the immune system into innate and specific immunity (Table 6) does not mean the strict separation of both when encountering pathogens. Instead, it is required that both systems closely cooperate and that components of both activate each other. Over the recent years, it has become increasingly clear that the two systems cannot be seen separately and that the innate immune system is even instrumental for the development of the adaptive immune response.

	Innate immune system	Adaptive immune system
General characteristics	First line of defense No specificity and no adaptation following antigen exposure	Specific recognition of foreign antigens Immunological memory Responsible for allergy, autoimmunity, rejection of tissue grafts
Physical and chemical barriers	Skin Mucosal membranes	Immune systems of skin and mucosal membranes Antibodies in secretions
Circulating molecules	Complement	Antibodies
Cellular factors	Macrophages/Monocytes Granulocytes Natural killer cells	Lymphocytes
Soluble mediators effective on other cells	Cytokines like α- and β-interferons, tumor necrosis factor (derived from macrophages)	Cytokines like γ-interferons (derived from lymphocytes)
Receptors	Genes encoded in germline DNA, no gene rearrangement	Encoded in gene segments Rearrangement necessary
Recognition	Conserved molecular patterns (PAMPs)	Details of molecular structure (proteins, peptides)
Self-Nonself discrimination	Perfect (selected over evolutionary time)	Not perfect (selected in individual somatic cells)
Response	Immediate activation of effectors	Delayed activation of effectors

Table 6: The concepts of innate and specific acquired immunity, adapted from Janeway and Travers (616)

2. Mucosal immunology

The immune system can be divided into two compartments that display considerable functional independence; on the one hand, the systemic compartment represented by the bone marrow, spleen, and lymph nodes, and on the other hand, the mucosal compartment, represented by lymphoid tissues in mucosae and external secretory glands. Numbers and types of cells involved in immune responses and their soluble products, primarily antibodies, are remarkably different in the mucosal and systemic compartments of the immune system, which should be further elucidated here.

A particular immune system of mucosal surfaces in humans was first presumed during the times of Paul Ehrlich in the 19th century (1266). Further anatomical and biological studies of a common mucosa-associated immune system go back to the seventies where **Tomasi** started to describe the function of secretory (S)-IgA and cellular immune mechanisms of a common mucosal immune system as components of mucosa-associated immune system (1423).

Several hundred square meters comprise the surface areas of mucosal membranes where antigens from ingested food or inhaled air and resident pathogens represent the most important exogenous stimulants (762). Due to the high antigen load of mucosal surfaces the mucosal immune system exhibits immunologic hyporesponsiveness or unresponsiveness to most antigens, on the other hand, it must also be capable of inducing effective cell-mediated and antibody-mediated immune responses towards selected antigens. To meet this task, mucosal surfaces possess a unique immune system that tightly controls the balance between responsiveness and non-responsiveness (tolerance). Besides mechanical barriers, humoral factors such as lysozyme, peroxidase and specific antibodies as well as cellular mechanisms contribute to the protection of mucosal surfaces (Table 7).

Mechanical barriers and peristalsis
Desquamation of epithelial cells with attached microorganisms
Humoral factors: Mucin, acids, lysozyme, lactoferrin, peroxidase system antimicrobial proteins, interferon-α, complement Specific antibodies: IgA>>IgG>IgM
Cellular factors: Phagocytic cells, T cells, NK cells

Table 7: Protection of mucosal surfaces, adapted from Kutteh and Mestecky (762)

Immune responses generated by organized lymphoid structures in the mucosa-associated lymphoreticular tissue (MALT) result in the development of B cells capable of producing antigen-specific Igs that can reach the draining lymph nodes and other mucosal tissues where they differentiate into plasma cells (1018). A second major outcome of the entry of antigen and antigen presentation by DCs is the activation and differentiation of T cells that can subsequently migrate out of the MALT and reach mucosal as well as peripheral non-mucosal tissues.

The mucosal immune system is structurally and functionally divided into sites for antigen uptake and processing at inductive sites on the one hand, and effector sites

engaging lymphocytes, granulocytes and mast cells on the other hand (1018). Besides the nasal-asssociated lymphoreticular tissue (NALT), the gut-associated lymphoreticular tissue (GALT) is the prototype of MALT and posesses APCs, T lymphocytes and IgA-committed B cells.

a) S-IgA as the major Ig subclass in the mucosal immune system

Igs of all isotypes have been detected in various human external secretions. The predominant Ig isotype in normal human serum is IgG, followed by IgA and IgM. In contrast to serum, the major isotype in human excretions such as saliva, tears, bile, urine and milk is IgA (762).
IgA is the most important subclass of Igs that can actively and efficiently be secreted through epithelia (1). Due to the size of mucosal surfaces the total amount of daily IgA production was quantified by 66mg/kg body weight, which is more than twice the rate of IgG (247). Approximately 1500mg/day IgA are produced systemically in bone marrow, lymph nodes or spleen but twice as much IgA is released in the mucosal immune system.

Most of the serum-IgA is found in a monomeric form with two heavy and two light chains whereas S-IgA is mainly polymeric with presence of a J chain and the so-called secretory component (SC). IgA is produced by plasma cells in the lamina propria and combined with the SC, a glycoprotein expressed on the surface of mucosal epithelial cells (ECs). This protein then handles the active transport of polymeric IgA into the intestinal lumen where IgA is released by proteolytic cleavage again (1). Investigations concerning the origin of S-IgA have demonstrated that it is produced locally at the mucosal sites and is not derived to a significant degree from the circulation (764).

The two different molecular forms also show different effector functions, which is illustrated in Table 8.

	Secretory-IgA	Serum IgA
Molecular form	polymeric	monomeric
Subclass	IgA1 ≥ IgA2	IgA1 >>> IgA2
SC-mediated transport into secretions	Yes	No
J-chain expression	Yes	Mostly no
Origin of precursor cells	Bone marrow, no circulation	Peyer`s patches, IgA cells in circulation
Neutralization of antigens	Yes	Yes
Inhibition of bacterial adherence	Yes	?
Loss of bacterial plasmid	Yes	?
Inhibition from antigen uptake from mucosa	Yes	No
Enhancement of innate factors	Yes	Yes (?)
Suppression of inflammatory effects (phagocytosis, lysis, NK cell activity etc)	Yes	Yes

Table 8: Effector functions of secretory and serum IgA (762)

IgA can also be divided into two subclasses IgA1 and IgA2 which differ in primary structure, carbohydrate composition and their sensitiveness to bacterial proteases. *Neisseria gonorrhoeae*, for instance, is evidently a producer of IgA protease which constitutes its most important virulent factor.
In serum, the monomeric IgA1 predominates over IgA2 whereas in external secrections almost exclusively polymeric forms of approximately equal proportions of IgA1 and IgA2 are found (925). Furthermore, specific antibodies to viral antigens, including HIV, are often found in the IgA1 subclass, whereas IgA2 antibodies in external secretions are associated with specificity for common structural microbial antigens as LPS and lipoteichoic acid (922).

b) Inductive sites of the mucosal immune system, in the example of the GALT

The primary inductive sites for mucosal immune responses are organized lymphoid aggregates such as Peyer`s patches placed in the wall of the intestine or tonsils in the upper respiratory tract. The Peyer`s patches of the GALT consist of a follicle-associated epithelium with specialized ECs known as membranous epithelial (M)

cells, a subepithelial dome overlying B cell follicles, and interfollicular regions enriched in T cells (1528, Figure 2).

Following ingestion, antigens and microorganisms are transported from the gut lumen to the dome region through specialized M cells (237) where they encounter APCs such as DCs leading to cognate interactions between APCs and T cells. DCs can also migrate to the interfollicular regions which are enriched with T cells and containing high endothelial venules (HEV) and efferent lymphatics to initiate immune responses upon antigen uptake. DCs as the APCs bind bacterial products with their TLRs, process antigen as relative immature cells and then migrate to the T cell region and present antigen to naïve T cells. There they have the properties of mature and immunogenic DCs with high surface expression of costimulatory molecules such as CD40, CD80, and CD86 and adhesion molecules such as CD44 (843).

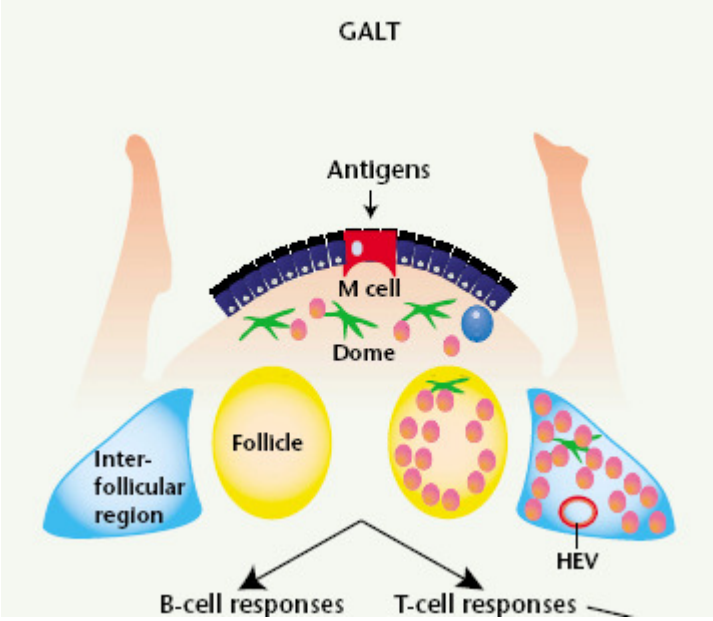


Figure 2: Inductive site of the GALT, adapted from Neurath et al. (1018)

After antigen uptake via M cells B cells are induced to switch into IgA-secreting cells. Following IgA switch and affinity maturation, B cells migrate from the Peyer`s patches to the mesenteric lymph node via efferent lymphatic vessels and finally to the lamina propria where they undergo terminal differentiation to plasma cells.

Peyer`s patches are an enriched source of IgA precursor cells capable of lodging in the recipient`s gut as well as in other glands and mucosal tissue (762). Depending on the type of antigen and the duration of stimulation, ingestions of pathogens induce local and systemic immune responses with parallel appearance of specific S-IgA in saliva, milk and tears, for example. The production of IgA is therefore

induced in lymphoid follicles such as the Peyer`s patches from where the cells recirculate through lymph and blood to diffusely populate other mucosal tissues and exocrine glands where terminal differentiation into IgA plasma cells under the influence of locally produced cytokines occurs.

This provides the mucosal immune system with the ability to induce responses at sites that are distant from the immediate inductive environment, or even in different mucosal tissues. This has led to the concept of generalised functioning of mucosal tissues with some cross talk between them (857). Substantial dissemination of primed immune cells from GALT to exocrine effector sites beyond the gut is also the rationale for many desired oral vaccines.

c) Effector sites of the mucosal immune system, in the example of the GALT

Following induction in the MALT, mature lymphocytes leave the inductive sites and migrate to the effector sites such as the lamina propria where they can induce proinflammatory as well as suppressive immune responses. Effector mechanisms that protect mucosal surfaces include CTLs and effector CD4+ T cells for cytokine production and IgA response (1528).
Lamina propria T cells are mainly CD4+ Th cells (60-70%), the majority of which also express the TCR, just as in peripheral blood. However, lamina propria T cells are in a more activated state than blood lymphocytes and have a mature or memory phenotype, indicated by the surface markers $CD44^{high}$, $CD62^{low}$, CD45RO+ (1264).

Cytotoxic CD8+ T cells account for about 30-40% of T cells in the lamina propria. They control the level of viral infection and have a cellular memory (1266). A more restricted T cell population, the intraepithelial lymphocytes (IEL), mainly CD8+ T cells, may play a role in maintenance of epithelial integrity and in class switching to IgA (219, 1528). Zytologically, they are T lymphocytes but their function is equivalent to NK cells of the innate immunity.

Besides an inflammatory phenotype T cells can adopt immunosuppressive function. These cells have been termed Th3 and Treg cells, and it is currently not clear if these cells are identical cell types or different immunoregulatory cells (537). Treg cells can actively inhibit activation or differentiation of other T cells and also

express the CD25 marker besides their CD4 marker (1266). Treg cells have been shown to produce large amounts of IL-10 and/or TGF-β and their immune-suppressive properties are most likely explained by the ability of these cytokines to inhibit APC function and to mediate direct antiproliferative effects on T cells (866).

Another task which is presumably done by T cells is the constant distinguishing of harmless antigens in food and on commensal bacteria from pathogenic microbes. Oral tolerance is defined as the induction of a state of systemic immune non-responsiveness to orally administered antigen upon subsequent antigen challenge (1528). This mechanism seems to prevent the development of an immune reaction or allergy against intestinal intraluminal antigens. T cells appear to be the major target of tolerance and the reduction in antibody responses after antigen exposition are due to the reduction in T helper activity rather than to a tolerization of B cell directly.
In addition to active suppression by Treg cells, tolerance is also maintained by deletion and anergy of T cells specific for luminal antigens (537, 1528). Deletion of specific T cells occurs by apoptosis whereas anergy of mucosal T cells is believed to be induced when cells are stimulated without proper costimulatory molecules.

d) Cytokine regulation of the gut mucosal immune response

The differentiation into different Th cells and Treg cells in the mucosa results in secretion of proinflammatory cytokines such as IFN-γ and TNF-α by Th1 cells; Th2 cells secrete IL-4, IL-5, IL-6, IL-10, IL-13 and promote IgA expression, Th3 cells secrete TGF-β and Treg produce predominantly IL-10 (1266, Table 9).
Several reports suggest that the level and type of costimulation a naïve T cell receives influences whether Th1 or Th2 cells develop. Further, different types of APCs may selectively trigger either Th1 or Th2 responses (824); however, the same APC cell can function equally well for inducing a Th1 or Th2 response.

Th subset	**Cytokine production**	**Effect on IgA response**
Th1	IL-2 IFN-γ Lymphotoxin β	Synergizes with IL-5/TGF-β → IgA synthesis Ig switch to IgG2a Development of Peyer`s patches
Th2	IL-4, IL-5 IL-6 IL-10	Differentiation to plasma cells IgA synthesis IgA synthesis
Th3	TGF-β	IgA isotype switching
T_R1	IL-10, TGF-β	Suppression of immune responses Downregulation of Th1

Table 9: Cytokine help for the regulation of mucosal immunoglobulin response (1528)

B. General immunology of the genital tract

1. Distinct features of the genital mucosal tissue

Mucosal surfaces have evolved to handle potential pathogens against a background of selective physiological functions. The mucosal immune system of the female genital tract has developed to meet the unique requirements of dealing with the presence of a resident population of bacteria in the vagina with periodic exposure of antigens in the uterus and fallopian tubes as well as with the presence of sexually transmitted pathogens. It also has to balance allogenic spermatozoa and the longterm exposure of an immunologically distinct fetus which should also be part of this review later on.

The lower genital tract in women is comprised of four discrete anatomical regions (1146):

- The introitus, which is covered by a keratinized stratified squamous epithelium resembling skin
- The vaginal mucosa, which is covered by an aglandular non-keratinized stratified squamous epithelium
- The ectocervix, which is covered by a mucosal layer histological similar to that of the vagina
- The endocervix, which consists of a simple columnar epithelium with numerous glands

The transformation zone represents an abrupt transition between ectocervix and endocervix. The susceptibility of these regions to infectious organisms differs. The transformation zone is the main target of HPV infection whereas *Candida albicans* and *Trichomonas vaginalis* colonize the vagina, and the cervix is susceptible to infection by *Chlamydia trachomatis* and *Neisseria gonorrhoea* (1146).
Among the tissues of the female reproductive tract, the upper genital tract consisting of the endometrium, the myometrium, the fallopian tube and the ovary needs to be investigated as well. These separate compartments have evolved to meet the different challenges and are precisely regulated by the endocrine system.

The reproductive tract has various systems of defenses against the risk of infection, which appear complementary and synergistic. These defenses comprise non-

immune strategies, namely passive factors such as pH, mucus, epithelial barrier on the one hand as well as active factors such as inflammatory reaction and secretion of humoral soluble factors on the other hand. Pre-immune humoral and cellular defense strategies are also possibly involved in rapid protection before antigenic stimulation. When these initial lines have been failed, a third strategy which is acquired and antigen-specific, occurs and associates humoral response with S-IgA/IgM and locally produced IgG as well as cellular immune responses. The latest knowledge of these lines of defense should be reviewed here.

Although the genital tract is considered to be a component of the mucosal immune system, it displays several distinct features not shared by other mucosal tissues or the systemic compartment. Differences include the endogenous flora, the predominance of IgG, hormonal fluctuations that may modify mucosal immunity, and the need to be tolerant to sperm and to a developing fetus but also the ability to respond to sexually transmitted bacterial and viral pathogens.

Despite of the fact that genital and intestinal tract share a common embryologic origin and remain in anatomical proximity, these two compartments display distinct immunologic features that reflect their very different physiologic functions (Table 10).

	Genital tract	Intestinal tract
Dominant Ig isotype	IgG ≥ IgA	IgA >>> IgG
Hormonal regulation	+++	-
Contribution of Ig from the circulation	++ (50%)	- (1%)
Inductive site for local and generalized humoral responses	- to +	++
Effector site	+	++
Expression of homing receptors on lymphocytes and ligands on ECs	LFA-1, ICAM-1, VCAM-1, α4β1	CCR9, CCR10, CCL25, CCL28, MAdCAM-1, α4β7, αEβ7
Response after intranasal immunization	++	+
Dominant function	Resident commensal flora in vagina vs exposure to sexually transmitted pathogens Acceptance of histoincompatible sperm/allogenic fetus	Induction of effective cell-mediated and antibody-mediated immune responses towards selected antigens Oral tolerance

Table 10: Comparative characteristic features of humoral compartments of the human genital and intestinal tract, adapted from Mestecky et al. (924, 779)

A major difference between the genital tract and the intestinal tract is that part of the genital mucosa is sterile, lacking the presence of a microbial flora. The female reproductive tract can thus be divided into two compartments, the vagina and ectocervix which host a commensal flora with predominantly lactobacilli which may play an important part in host defense, and endocervix, the uterus and fallopian tubes which are sterile (630).

Sterility in the endocervix depends on the hormonal phases of the menstrual cycle, which should be discussed in further detail later (1151). The epithelium of the vagina and ectocervix therefore is required to provide a strong barrier whereas the epithelium of uterus and endocervix is less afflicted by microorganisms. A sophisticated barrier function is also given by the cervical mucus that filters bacteria but allows sperm to ascend to the uterus (630). Whether related to the

microbial flora or not, the vagina seems to be in a hypo-responsive state. This may be a protective measure as a result of the massive antigenic load in the lower genital tract. By contrast, the endocervix, uterus and fallopian tubes respond to bacteria with distinct patterns of cytokines and chemokines (1151).

Several basic characteristics of mucosal immunity in the intestine and respiratory tract are absent in the genital tract. Mucosal lymphoid nodules and M cells for antigen uptake overlaying organized lymphoid tissue like in the intestinal tract are missing in the reproductive tract (630). There are lymphoid aggregates in the basal layers of the uterus but their presence and distribution strongly varies under the hormonal influence. Besides lymphoid aggregates, the genital tract mucosa contains APCs such as macrophages and DCs as well as genital ECs. Due to the specialized functions of the genital tract immune system it seems reasonable to assume that this system hosts unique regulatory cells. Both pregnancy and infection are associated with increased numbers or alterations of function of T cells which is discussed later on more specifically.

The mucosal immune system of the female genital tract is under strong hormonal control that regulates the transport of Igs, the levels of cytokines, the distribution of various cell populations, and antigen presentation in the genital tissues during the reproductive cycle (1512). In addition to protecting against infectious agents, it must adapt to a spectrum of physiological events that includes fertilization, implantation, pregnancy, and parturition. A balance is maintained by sex hormones throughout the menstrual cycle to respond to the challenges of bacteria, yeast, and viruses without interfering with events that surround conception.
The hormonal influence on the ability to respond to antigen is considerable, because vaginal immunization only in the follicular, but not in the luteal, phase gave strong local IgA antibody responses (745). Also the ability to present antigen is a property that is under strong hormonal control.

Although sometimes disputed, several recent studies have revealed that human urine, seminal plasma, and cervicovaginal washings collected at various stages of the menstrual cycle contain IgG rather than IgA as the dominant isotype (764). In contrast to the predominance of IgA-producing cells in most mucosal tissues, the endocervix was found to contain a high proportion of IgG-secreting cells whose product, IgG, reaches the cervical fluid by a currently unknown mechanism (273).

2. Functions and regulations of innate immune responses in the human reproductive tract

While much attention has been paid to innate immune function in other mucosal tissues like lung and intestine, very few studies have investigated presence and function of the innate immune system in the female reproductive tract. What is becoming clearer is that the innate immune system is present throughout the reproductive tract and functions in synchrony with the adaptive immune system to provide protection in a way that enhances the chances for fetal survival, while protecting against potential pathogens.

The focus of the next chapter is on the different cells of the innate immune system defining the role of ECs, macrophages, DCs, neutrophils, and NK cells throughout the female reproductive tract, their functions and regulation during the menstrual cycle, and the ways in which these cells communicate with the adaptive immune system.

a) Epithelial cells in mucosal immunity

The epithelium of endocervix, uterus and fallopian tubes is composed of polarized ECs connected by tight junctions. This single cell layer was initially thought to reside in a sterile environment that was only infrequently exposed to bacteria constituitively present in the ectocervix and vagina (1514). However, studies demonstrate that polarized ECs of the reproductive tract are exposed to bacteria at a frequency not previously appreciated (1093). Furthermore, routine histological analysis has determined that uterus and fallopian tubes have a relatively low incidence of chronic infections (1514).

Once thought to function only by providing a physical barrier between the lumen and internal tissue, mucosal ECs are now known to be a part of the mucosal immune system, protecting against the organisms present throughout the reproductive tract. They function as sentinels that recognize antigen and also respond in ways that lead to the production of antimicrobial molecules that either leads to killing or inactivation of the pathogen (1514). Their ability to signal to underlying immune cells when pathogenic challenge exceeds their protective capacity is also investigated. Estradiol and progesterone regulate their proliferation,

apoptosis, secretions and effects on pathogenic microbes. Finally, uterine ECs are involved, through their cytokine and chemokine secretions, in normal physiological processes such as menstruation and receptivity (1513).

(1) The role of epithelial cells as mechanical barrier

Throughout the reproductive tract ECs form an uninterrupted physical barrier between the lumen and underlying cell layers. This aims at preventing opportunistic and pathogenic microbes from infiltrating the body. However, they also permit the transport of sperm or ovum to the site of fertilization and implantation or support the conceptus through gestation (1512, 1514).

Each site of the female reproductive tract has a unique morphological form of ECs. Whereas the vagina and the lower part of the cervix are lined with stratified squamous ECs, uterus and fallopian tubes have a columnar epithelium as well as the upper part of the cervix (1146). For the integrity of these cell formations the presence and maintenance of tight junctions is essential. Paracellular permeability is regulated by these most apical epithelial intercellular junctions which form a regulated, semipermeable barrier and act as a fence that segregates protein components of the apical and basolateral plasma membrane domains (1034). Built of several different proteins, tight junctions seal off the lumen from the basolateral compartment. However, this barrier is continuously regulated by calcium, cytokines, leukocytes and, above all, hormones (1034).

When being established in culture, ECs from the female genital tract form polarized monolayers with distinct luminal and basolateral surfaces. They also establish an electrochemical gradient and a transepithelial resistance (TER) which illustrates the tightness of the epithelial barrier (372). **Grant-Tschudy et al**. demonstrated lately that estradiol significantly decreased TER within 24 hours when incubated with polarized uterine ECs from mice (474). ICI 182,780, an estradiol receptor (ER) antagonist, neutralized this effect whereas incubation with progesterone, cortisol, aldosterone and dihydrotestosterone had no effect on uterine epithelial TER. This demonstrated that epithelial monolayer integrity is directly influenced by estradiol via ER.

(2) Stromal cell regulation of epithelial cell function

Stromal cells and ECs in the genital tract act as a single unit with each cell type producing factors to regulate each other (1514). The stroma is critically important for endometrial function by influencing epithelial development and differentiation. Conversely, ECs influence stroma cell function through soluble factors and cell-cell-contact. Stromal cells from neonatal tissues are able to change the phenotype of adult epithelium (277). Regional differentiation in the reproductive tract epithelium is therefore directed by the inductive capability of the stroma.
Uterine stromal cells also communicate with ECs via soluble factors to maintain epithelial barrier function, i.e. TER, and secretory activity. Previous studies showed that human uterine stromal cells modulate the barrier function of ECs by decreasing TER (372).

Also the influence of stromal cells on the release of the cytokines TNF-α and TGF-β by ECs was object of studies. Mouse uterine ECs, which reach confluence as indicated by high TER, set free TGF-β into the basolateral compartment and TNF-α into the apical lumen. When brought together with mouse uterine stromal cells in culture, release of TNF-α of ECs went down whereas the amount of released TGF-β was not affected (473). This indicated that uterine stromal cells communicate with ECs via soluble factors to maintain uterine barrier function and epithelial secretory activity. They produce soluble factors that regulate EC` TER and release of TNF-α without effecting TGF-β release (473).

The proliferation of ECs due to estrogen is also dependent on underlying stromal cells and their release of mediators. Several studies suggested that estrogen regulation of EC proliferation is mediated indirectly by uterine stroma (250, 595, 1121). Also mediated by uterine stroma is the effect of estradiol on EC TNF-α release (476). While estradiol treatment on ECs alone led to a significant decrease in TER, the amount of released TNF-α did not change. But when ECs were cocultured together with stromal cells and treated with estradiol, apical TNF-α release was significantly decreased (476). However, the amount of released TGF-β was not altered by estradiol. One can conclude that estradiol directly influences epithelial electrical integrity whereas its effect on TNF-α release is dependent on the presence of uterine stromal cells.
Further aim is to identify the mechanisms of stromal cell effects on uterine epithelium. Estradiol acts through the ER to regulate uterine growth and functional

differentiation. Studies detected that epithelial ER is neither necessary nor sufficient for estradiol-induced uterine epithelial proliferation. Instead, estradiol induction of epithelial proliferation appears to be a paracrine event mediated by ER-positive stroma (250, 278).

Stromal fibroblasts release soluble factors such as hepatocyte growth factor (HGF), insulin-like growth factor (IGF) and keratinocyte growth factor (KGF) as mediators of estrogen-induced proliferation of uterine ECs.
HGF, a mesenchymal growth factor, is expressed by uterine stromal cells and mediates EC proliferation via HGF-receptor. HGF was shown to increase TER so that it was concluded that stromal cells act through HGF receptors on ECs to increase TER (475). At the same time, HGF was shown to decrease apical TNF-α release. Both effects could be blocked by incubating epithelial and/or stromal cells with anti-HGF or anti-HGF receptor antibody. HGF receptor, which is located at the basolateral surface of ECs, seems to mediate the effect of HGF on TER and TNF-α release. Moreover, as neutralization of stromal media failed to affect TNF-α secretion, these results suggest that other growth factors, in addition to HGF, affect EC cytokine production (475).

(3) Production of antimicrobial molecules

To encounter microbial pathogens, the genital tract ECs produce soluble factors of the innate immune system with microbicidal effects. Among these secretions of ECs that inhibit the growth of pathogens are the enzymes lysozyme and lactoferrin, defensins, secretory leukocyte protease inhibitor (SLPI) and the tracheal antimicrobial peptide (TAP) among other peptides (1512).

Defensins

Defensins, also called “natural antibiotics”, are small cationic peptides with proven effectiveness against bacteria, fungi and some viruses and contribute to mucosal immune responses at epithelial sites (436). Defensins are abundant in microbicidal granules of polymorphonuclear leukocytes and epithelia, including human intestinal Paneth cells or bovine tracheal epithelium (1436). Human defensins can be divided into two classes, the α- and β-defensins, which are both expressed in ECs and granulocytic white cells (325). The crucial step in defensin-mediated antimicrobial activity and cytotoxicity is the permeabilisation of target membranes; they seem to kill pathogens via creating pores in their membranes (436, 1271).

ECs at mucosal surfaces, and especially in the genital tract, are known to produce human β-defensins (HBD)-1 and -2.
HBD-1 is an essential part of epithelial secretions in the genitourinary tract and was detected in the epithelial layers of vagina, cervix, uterus and fallopian tubes as well as in vaginal secretions (1436). The highest concentrations of HBD-1 were noted in the urine of pregnant women, intermediate concentrations in nonpregnant women, and the lowest concentration in men (1436). Exposure to microbial products, microtrauma and hormonal influences may regulate HBD-1 synthesis.
HBD-2 was originally isolated from psoriatic skin lesions and is inducibly expressed in inflamed skin lesions and lung tissues upon treatment with bacterial LPS and cytokines (1023). It was shown that its expression is increased at infection or inflammation sites, for example the endometrium, and that HBD-2 is a potent chemoattractant of human neutrophils.

The expression of these defensins may be regulated by cycle-associated changes in sex hormones. **Fleming et al**. demonstrated that HBD-1 expression was highest during the secretory phase while HBD-2 expression peaks during menstruation (415). The use of the oral contraceptive pill downregulates expression of both which may among other factors contribute to altered susceptibility to infection.
Concerning HBD-3 and HBD-4, it was shown that HBD-3 messenger ribonucleid acid (mRNA) expression in the human endometrium is highest during the secretory stage of the menstrual cycle, HBD-4 mRNA expression peaks in the proliferative phase (707). Expression is altered due to hormonal contraceptive use which may contribute to differential infection rates in oral contraception users relative to non-users. HBD-3 is also upregulated during infection allowing an increased immune response at that time.

Concerning the α-defensins, leukocytes express human neutrophil peptides (HNP) 1-4 whereas human defensin (HD)-5 and -6 are expressed mainly by intestinal cells. Lately, HD-5 was detected in female genital tract epithelia with variable expression in upper genital tract, but also in the lower genital tract (1152). It was also found in cervicovaginal lavages (CVL), with its highest concentration during the secretory phase of the menstrual cycle.
In addition to their bactericidal activity, defensins have also been shown to have multiple functions in innate immunity. For example, β-defensins have been shown chemotactic for immature DCs and memory T cells by binding to the chemokine receptor CCR6 (1554). Uterine ECs also produce CCL20/macrophage inflammatory

protein (MIP) 3α, a chemokine ligand of CCR6, which has significant homology to defensins and has been shown to have significant antimicrobial activity (564).

SLPI

SLPI is a neutrophil elastase inhibitor which also has antibacterial and antiinflammatory properties (704). It is not only produced by macrophages but also by uterine and cervical ECs and is active against a variety of pathogens including gram-positive and gram-negative bacteria and HIV-1 (557).

King showed that the primary site of its synthesis in the endometrium is the glandular epithelium and that secretory peaks are higher in the progesterone-dominated late secretory phase than in the proliferative phase of the menstrual cycle (704). Expression of endometrial SLPI has been shown to be upregulated by estrogen in the rat (214) and by progesterone in the human (709). Studies also proved that uterine SLPI productions of premenopausal women were significantly higher than those of postmenopausal women (376). These results confirm the suggestions that expression of SLPI varies in cervical mucus during the different stages of the menstrual cycle and menstrual status.

Furthermore, it increases in amniotic fluid during gestation and labor (323). This may be to modulate proinflammatory paracrine interactions for the maintenance of pregnancy and limit those occurring at parturition within the uterus. There is also a positive correlation between SLPI expression and implantation as well as early pregnancy which could be a benefit due to its antibiotic action and anti-inflammatory effects of inhibiting elastase and nuclear factor- κB (NF-κB) (1512). NF-κB is responsible for the production of inflammatory cytokines in response to pathogens.

Surfactant protein A and D

Previous studies have demonstrated the essential role of Surfactant protein A (SP-A), a member of the collectin family of proteins, in protecting the respiratory system from infections (811). **MacNeill et al**. have now identified SP-A in two layers of vaginal epithelium which makes SP-A an essential component of the host defense system in the genital tract as well (855). SP-A can facilitate phagocytosis by opsonizing bacteria, fungi and viruses. It can also modulate proinflammatory cytokine production by phagocytic cells and provides a link between innate and adaptive immunity by promoting differentiation and chemotaxis of DCs.

SP-D, originally detected in alveolar cells type II, was also recently demonstrated in cells lining the epithelium and secretory glands in the vagina, cervix, uterus, fallopian tubes and ovaries (809). Endometrial presence of SP-D varied according to stage of menstrual cycle with highest concentrations in the secretory phase. SP-D may play a role in preventing intrauterine infection at the time of implantation and during pregnancy.

(4) Cytokine and chemokine production

In the reproductive tract, growth factors and cytokines are synthesized in abundance at almost every level as partly mentioned above. There is evidence that they are not only regulators of immune function but also local modulators of steroid hormone action. Cytokines are small secreted proteins regulating immunity, inflammation and hematopoiesis by either acting on the cells that secrete them (autocrine action), on nearby cells (paracrine action) or distant cells (endocrine action). Although still considered a subclass of cytokines, chemokines are developing their own identity (674). Their hallmark is their ability to induce chemotaxis, but they are also involved in cellular proliferation and differentiation, angiogenesis and inflammation.
The known chemokines form a large subsets of cytokines and basically consist of four families, CC, C, CXC and CXXXC, their receptors (-R) and ligands (-L) corresponding (674). The CC chemokines are functionally and structurally different from the CXC chemokines, stimulating multiple cell types such as monocytes, lymphocytes, basophils and eosinophils. In contrast, most of the CXC chemokines are specifically chemotactic for neutrophils with only minor effects on other cells (1249).

Cytokines are the mediators of communication between ECs as first line of defense and other immune cells and therefore guarantee a successful interactive immune response. They are produced by ECs, macrophages and T lymphocytes and act through different receptors in the reproductive tract to regulate differentiation, maturation and recruitment of lymphocytes (1514). Cytokine and adhesion molecule expression are also decisive for endometrial growth in preparation for fertilization, implantation and successful pregnancy but also for the renewal of the uterus during each menstrual cycle.

Several studies described the production of numerous cytokines including granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), TNF-α, IL-1, IL-6, leukaemia inhibitory factor (LIF), TGF-β and of chemokines such as MIP-1β, monocyte chemoattractant protein-1 (MCP-1), RANTES (regulation upon activation, normal T cell expressed and secreted) and IL-8 by endometrium ECs (374, 672, 673, 674).

They are constitutively produced by the polarized ECs, preferentially secreted apically and contribute to the resident and temporary populations of immune cells in the subepithelial layers of the endometrium, for example by recruiting neutrophils, monocytes and T cells (374).

Besides HBD-2 (1023), another chemokine of uterine ECs, MIP3α/CCL20, attracts B cells, memory T cells and immature bone-marrow derived DCs. Its levels significantly increased together with levels of TNF-α by the presence of *Escherichia coli* and PAMPs (265, 264). Chemokines could also account for the influx of leukocytes and lymphocytes that form lymphoid aggregates observed during the secretory phase of endometrium (1560).

Kayisli et al. stated that IL-8 and MCP-1 among others produced by endometrial, myometrial and trophoblast cells also display specific roles in endometrial angiogenesis, apoptosis, proliferation and differentiation (674). IL-8 takes part in cervical ripening and parturition and is also found at high levels in the peritoneal fluid of women with endometriosis. Besides mesothelial cells that form the majority of the peritoneal cells, macrophages and endometrial cells are potential sources of this chemokine. It was found that there are menstrual cycle-dependent changes in IL-8 mRNA in the endometrium (48). IL-8 mRNA levels in the late secretory and early to midproliferative phase samples are higher than the level observed in the middle of the cycle. It can be speculated that IL-8 may modulate the timely recruitment of neutrophils and lymphocytes into the endometrium.

Endometrial stromal and ECs produce IL-6 in response to hormones and other activators. Besides its role in inflammation and cell differentiation of immunocompetent cells, it also regulates ovarian steroid production, folliculogenesis, and embryo implantation. Endometrial stromal cell IL-6 protein is induced by IL-1β or IL-1α, TNF, platelet-derived growth factor (PDGF) and INF-γ. IL-6 is said to inhibit the proliferation of human endometrial stromal cells, dependent on cell density, which suggests that IL-6 may play a role in epithelial-stromal interaction in the normal uterus (1574).

IL-6 fluctuations during the menstrual cycle reflect an inverse relationship to estrogen action; IL-6 levels are high during the secretory phase and low during the proliferative phase (1382). **Yoshioka et al.** observed that IL-6 has no effect on the growth of endometrial stromal cells from the proliferative phase, but it inhibits proliferation of endometrial stromal cells from the secretory phase (1564).

Concentrations of cytokines and chemokines vary in the endometrium during physiological processes as well as pathological conditions. Many cytokines such as GM-CSF, TNF-α, TGF-β and LIF show temporal release patterns and are regulated by sex hormones.
Four different patterns of cytokine expression related to the menstrual cycle were identified in CVL obtained from healthy ovulating women (880):

- LIF, RANTES, and MIP-1α are detectable at menses only
- IL-8, IL-6, TGF-β, and IL-1β are detected throughout the cycle but at highest levels at menses
- M-CSF and epidermal growth factor (EGF) are found throughout the cycle but peak during the late proliferative phase
- IFN-γ and TNF-α are detectable in a subpopulation of women during non-menses stages of the cycle and may be associated with inflammatory events

For example, progesterone withdrawal results in upregulation of MCP-1 and IL-8 leading to chemotaxis and activation of monocytes and neutrophils (268). A correlation between high levels of IL-6, IL-8 and MCP-1 and amniotic microbial infections has been found in cervicovaginal fluids and amniotic fluids from women in preterm labor (610, 1512). However, low cervicovaginal concentrations of IL-6 and IL-8 were found in patients with chorioamnionitis in early pregnancy (1307). The conclusion was that low concentrations of multiple cytokines indicated a broad immune hypo-responsiveness that could create a permissive environment for infection whereas high levels correlated with a dangerous infection.

Among other cytokines, GM-CSF, which regulates granulocyte and macrophage proliferation, is released by ECs of the pregnant and non-pregnant uterus, which **Robertson et al**. have shown in mice (1205). They also demonstrated that GM-CSF synthesis and release by uterine EC cultures is stimulated by estrogen and moderately inhibited by progesterone (1204). GM-CSF can synergize the effect of IL-8 of attracting neutrophils (1292).

TNF-α plays a dominant role in inflammatory processes and had been demonstrated in both normal pregnancies and conditions of pregnancy loss (470). It is also promoted by estrogen in uterine mast cells whereas studies in macrophages suggested that TNF-α expression is unaffected by estrogen but inhibited by progesterone (580).
TGF-β seems to regulate cellular proliferation, migration, differentiation and protein expression. Diethylstilbestrol, a synthetic estrogen, was proved to increase TGF-β mRNA expression and protein for TGF-β1, -2 and -3 in the immature mouse uterus (1386).

(5) Regulation of Ig secretions into the lumen

At genital tract mucosal surfaces, the polymeric Ig receptor (pIgR), a transmembrane glycoprotein created by ECs, is responsible for transporting polymeric IgA across ECs (1514). It was shown that SC as the external part of the pIgR was synthezised by ECs and tended to accumulate in the apical compartment, especially in endocervix and ectocervix. It binds polymeric IgA at the basolateral surface of ECs and builds a receptor-ligand-complex which is internalized and transported to the apical surface. There the ectoplasmic portion of the receptor is cleaved from the transmembrane and cytoplasmic part.

The female sex hormones estradiol and progesterone influence the local production and Ig transport in ECs in the reproductive tract (1519). During the estrous cycle and after the administration of estradiol in rats, IgA, SC and IgG accumulation are stimulated in the uterine lumen and are inhibited in cervicovaginal secretions (CVS) (1519, 1520).
In the uterus, estradiol increases vascular permeability, which results in serum transudation of IgA and IgG into the uterine tissues. In contrast, movement of IgA and IgG in CVS is inhibited by estradiol and progesterone. Estrogen increases expression of pIgR and therefore IgA transport into the lumen when ECs were cultured with IL-4 and IFN-γ (918). IgG, however, moves down a concentration gradient from blood to uterine lumen under the influence of estrogen.

Other data demonstrated that Ig levels in cervical mucus corresponded to hormonal fluctuations during the human menstrual cycle (423). Peak concentrations of IgA and IgG occurred approximately one day before the estradiol peak around ovulation and decreased from the time of ovulation in cervical mucus secretions of normal

ovulating women (423, 764). The decrease of Ig levels could represent an effect of dilution secondary to the increased volume of cervical mucus and estradiol produced at the time of ovulation. When compared with women on birth control pills, the mean peak of IgA detected was about one third less in normal ovulating women. This supports the general agreement that reproductive hormones enhance immunity.

(6) Antigen presentation

As mentioned in the last chapters, the role of APCs throughout the body as well as at mucosal surfaces, is central to the generation of immune protection. An effective immune response requires that exogenous antigen has to be internalized, processed and returned to the cell surface of APCs in association with MHC class II for recognition by CD4+ T cells. It can also stimulate MHC class I-restricted T cell activation after uptake by APCs via a phagocytic pathway. After antigen presentation, lymphocyte effector functions including cytokine production, cytotoxicity and antibody synthesis are activated.

Previous experiments investigated mixed cell suspensions from throughout the genital tract and found cells capable of presenting foreign antigen to autologous T cells, independent from menstrual status (373). Isolated uterine ECs expressed MHC class II antigen and were able to process and present tetanus toxoid to T cells, as well as cells from basolateral subepithelial stroma (1470).
Lately, this was supported by the finding that preparations of endometrial ECs without being contaminated with professional APCs such as DCs or macrophages presented tetanus toxoid to autologous T cells (375). ECs also had the ability to express CD1d and CD40 which shows that these cells interact with CD8+ T cells. In addition to MHC class II molecules, CD40 and CD1d proteins are another family of antigen-presenting molecules that bind bacterial and lipid antigens for presentation to T cells.

Antigen presentation in the female reproductive tract is supposedly regulated by sexual hormones and soluble factors of stromal cells. Isolated uterine and vaginal cells from ovariectomized rats treated with estrogen were incubated with sensitized T cells. ECs from animals treated with estrogen presented more antigen than ECs from saline controls did. In contrast, uterine stromal and vaginal cells from estrogen-treated animals presented fewer antigens than the saline control group.

Estradiol enhances antigen presentation by ECs of rat uterine at a time when uterine and vaginal stromal antigen presentation is inhibited (1515, 1516).
More recent studies demonstrated that stromal antigen presentation is regulated by cytokine production by ECs. In response to estrogen, uterine ECs produce TGF-β which suppresses underlying APCs in the stroma (1517).

It can be concluded that uterine ECs as well as APCs in the uterine stroma and vagina are capable of presenting antigen which initiates an immune response in the female reproductive tract. Moreover, sex hormones play a principal role in regulating antigen presentation in the genital tract.

(7) TLRs on epithelial cells

As mentioned above, TLR are a newly discovered family of integral membrane receptors which can stimulate cytokine and chemokine production after ligand recognition. First, a *Drosophila* Toll receptor made up of a type 1 transmembrane protein with a cytoplasmatic protein was described (520). Then, **Medzhitov et al**. described a human homologue of this receptor which was similar to the IL-1 receptor (910). The *Drosophila* Toll and human TLR both act through the NF-κB pathway.
The eleven known TLRs in mammals comprise a family of structurally related receptors that recognize specific products of pathogens referred to as PAMPs as well as endogenous ligands associated with cell damage. Just as TLRs are conserved from one species to another, PAMP ligands are repeated in a high variety of pathogenic microbes. An overview of TLRs and their ligands gives Table 11.

Receptor	Ligand	Origin of ligand
TLR1	Lipopeptides Soluble factors	Bacteria/mycobacteria *Neisseria meningitides*
TLR2	Lipopeptides Lipoteichoic acid Peptidoglycan Zymosan Lipoarabinomannan	Various pathogens Gram-positive bacteria Gram-positive bacteria Fungi Mycobacteria
TLR3	Double-stranded RNA	Viruses
TLR4	Lipopolysaccharide Taxol Fusion protein	Gram-negative bacteria Plants RS-Virus
TLR5	Flagellin	Bacteria
TLR6	Diacyl lipopeptides Lipoteichoic acid Zymosan	Mycoplasma Gram-positive bacteria Fungi
TLR7	Single-stranded RNA	Viruses
TLR8	Single-stranded RNA	Viruses
TLR9	CpG-containing DNA	Bacteria and viruses
TLR10	?	?
TLR11	?	Uropathogenic bacteria

Table 11: Toll-like receptors and examples for their ligands (15)

Stimulation of different TLRs induces distinct patterns of gene expression which not only leads to the activation of innate immunity but also instructs the development of antigen-specific acquired immunity. TLRs are expressed on immune cells such as lymphocytes or APCs as well as on ECs (1514).

Human uterine EC lines express TLR that are capable of recognising specific structural components of bacterial, fungal and viral pathogens.

Young et al. first examined the expression of TLRs in the endometrium and detected TLR1-6 and TLR9 mRNA in both whole endometrium and separated endometrial ECs (1567). Both whole endometrial samples and purified epithelium lacked detectable TLR 7, TLR 8 and TLR 10. Since B cells are the predominant cell type for expressing TLR 10, the absence of TLR 10 is consistent with data suggesting that B cells are rare in the endometrium.

Constitutive expression of TLR1-6 was observed throughout the female genital tract by **Pioli et al**. (1126). They also described the differential expression of TLR2 and

TLR4 in the human reproductive tract tissue. TLR2 mRNA levels were highest in fallopian tube and cervical tissues, followed by endometrium and ectocervix. In contrast, TLR4 expression declined along the tract with highest levels in fallopian tubes and endometrium. TLR4 is expressed in primary endometrial ECs but not in cervicovaginal epithelium. This is maybe because gram-negative bacteria in the upper genital tract are likely to be associated with infection whereas bacteria are usually found as commensal organisms in the lower genital tract.
Another study showed that TLR1-9 were expressed by the uterine EC line EEC1 and that PAMP agonists of TLR2, TLR4 and TLR9 stimulated expression of IL-6, IL-8 and MCP-1 (1261). However, it is not known if changes in sex hormones affect TLR expression (91).

More recently, studies were undertaken to examine the expression of TLRs on human primary uterine ECs and to determine if exposure to the TLR agonist poly(I:C) would induce an antiviral response. The ligand for TLR3, dsRNA, is produced by virtually all viruses at some point in their life. When investigating exposure of poly(I:C), a synthetic dsRNA which binds TLR3 to uterine ECs, the expression of the proinflammatory cytokines IL-6, G-CSF, GM-CSF and TNF-α, of the chemokines IL-8, MCP-1 and MIP-1β as well as of MIF and HBD1/2 was induced (1262, 1263). Also the uterine ECs initiated an antiviral response when inducing IFN-β and IFN-β-stimulated antiviral genes myxovirus resistance gene 1 and 2`,5`-oligoadenlyate synthetase mRNA.
This also suggests that ECs in the female reproductive tract are sensitive to viral infection and possess the capability to respond to RNA viruses.

To conclude, the following figure shows the multiple functions carried out by uterine ECs as sentinels of immune protection in the female reproductive tract. Acting as the first line of defense, ECs provide host protection in a number of ways that includes providing a mechanical barrier, secreting antimicrobial molecules, transporting IgA, processing and presenting antigen and communicating with underlying immune cells by secreting cytokines and chemokines. Sex hormone regulation of EC function is both direct via ER in ECs and indirect via ER located in the underlying stromal cells (Figure 3).

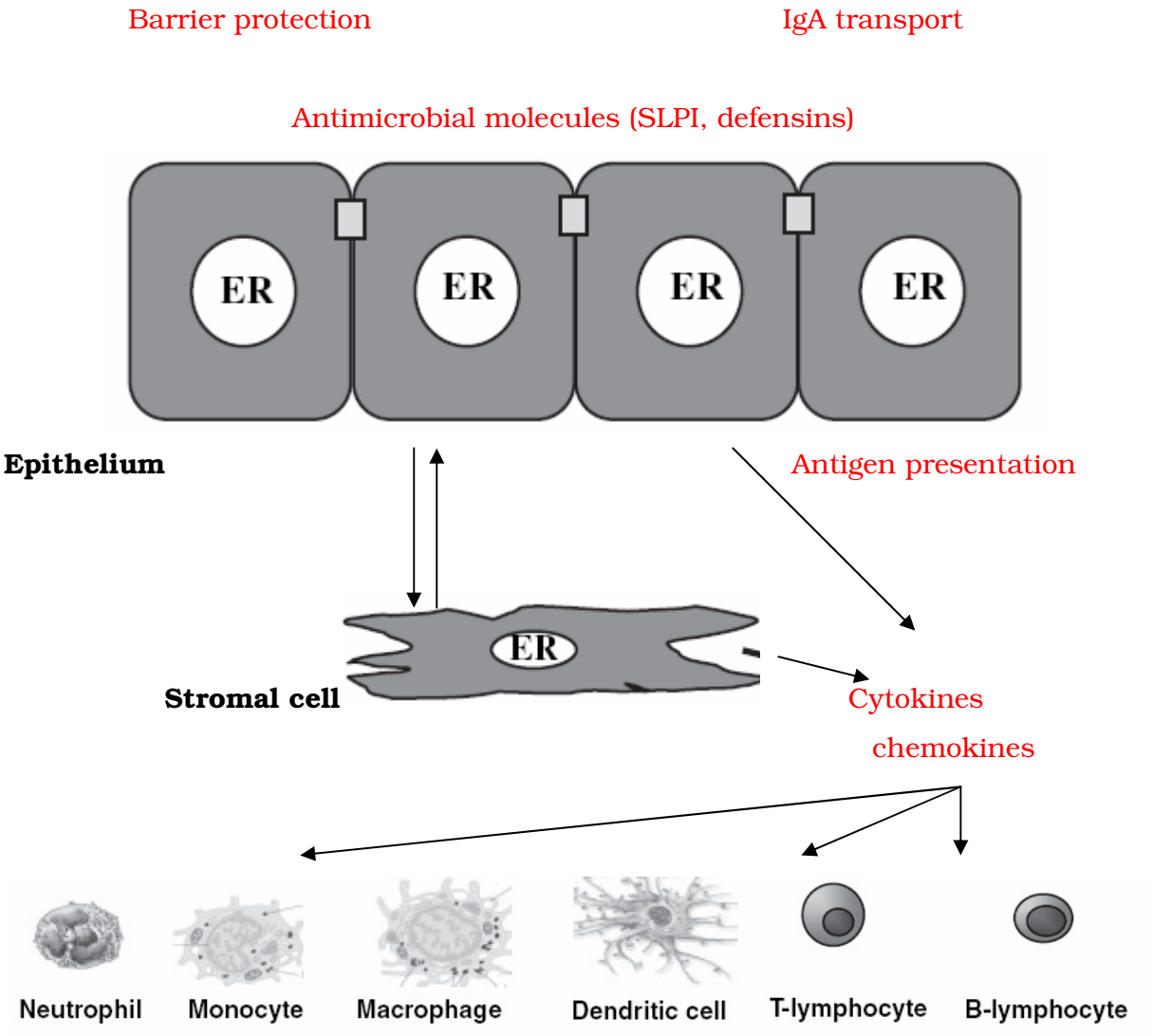


Figure 3: Different immunoregulatory functions of ECs in the female reproductive tract, adapted from Wira et al. (1514)

b) The role of macrophages in the female genital tract

As mentioned above, monocytes and tissue macrophages are important effector cells of innate immunity. The phenotypic characteristics of tissue macrophages reflect the unique tissue environment including cytokines, extracellular matrix and cellular components. They are identified in tissues by their expression of cell surface receptors and can execute diverse functional activities, including phagocytosis of foreign antigens, matrix dissolution and tissue remodelling, and production of cytokines, chemokines and growth factors.

(1) Distribution and function

Concerning distribution in the female reproductive tract, macrophages can be found throughout all tissues and represent about 10% of the total number of leukocytes (459). Macrophages in mouse, rat and human uteri are distributed throughout the endometrial stroma and myometrial connective tissue with myometrial macrophages usually larger in size than endometrial macrophages (584).

A large accumulation of endometrial leukocytes occurs in the periimplantation phase and during early pregnancy as well as a dramatic influx in the premenstrual phase and during menses. Pregnancy-associated leukocytes are predominantly a subpopulation of macrophages and uterine-specific NK cells whereas in the perimenstrual period there is a dramatic influx of inflammatory-type leukocytes: neutrophils, mast cells and macrophages (638). Immediately after implantation a redistribution of uterine macrophages takes place. Macrophages are reported to flee from the implantation site and are entirely absent from the primary decidua in mice and rats. They continued to be excluded from the decidua as pregnancy progresses and are only present in small numbers in maternal blood near the placenta (584).

Besides their role in the regulation of inflammation, macrophages have also been identified as important effector cells of ovarian function. The distribution of ovarian macrophages differs during the various stages of the menstrual cycle. The highest level of macrophages is in the vascular connective tissue and the theca-lutein areas of the corpus luteum during the periovulatory period (1538).
Their specific localization and variations in distribution in the ovary during different stages of the cycle as well as their presence in periovulatory human follicular fluid suggest that they play diverse roles in the folliculogenesis, tissue restructuring at ovulation and corpus luteum formation and regression. Ovarian macrophages secrete several cytokines such as TNF-α, IFN-γ, IL-1, IL-6, IL-10, IL-12 to regulate inflammation, as for example the state of ovulation (920).

(2) Regulation by sex hormones

Multiple studies have been examining the influence of estrogen on infectious diseases where results showed that physiologic levels of estrogen influence macrophage proliferation and function as well as cytokine production (1512). It

appears that the ability of estrogen to induce either pro- or antiinflammatory mediator production is concentration- and cell-context dependent.
Human peripheral blood monocytes and human macrophages have similarly shown expression of both receptor isoforms, ERα and ERβ (1114, 964). Although macrophages have been showing an effect to progesterone, nuclear progesterone receptor (PR) was rarely detected on macrophages with the work of **Vegeto et al**. as only exception (1451). This paradox between macrophage progesterone responsiveness and lack of PR expression is explained by cross binding of progesterone to the glucocorticoid receptor (1495).

There is high evidence that steroid hormones regulate the recruitment of uterine macrophages throughout the menstrual cycle due to regulation of cytokine and chemokine expression. The total uterine leukocyte population increased significantly on treatment with estrogen. This rise in leukocytes was due to a significant increase in both uterine macrophage populations and NK cells (318).
Human studies have demonstrated that macrophages selectively aggregate into premenstrual endometrial stroma, concurrent with depression of estrogen and progesterone levels as the result of luteolysis (639, 656).

One cytokine that controls macrophage migration and activation is MCP-1, which has also been shown on human uterine ECs (926). Highest levels of MCP-1 in the endometrium were detected perimenstrually at low estrogen levels and lowest levels around the time of ovulation when estrogen levels are high. This was coincident with macrophage accumulation and depletion. **Arici et al**. have demonstrated that treatment of endometrial stromal cells with estradiol significantly inhibits expression of MCP-1, which correlates with suppression of macrophage migration (48).

Estrogen also plays an important role in the modulation of MIF expression by macrophages. MIF is produced by monocytes and macrophages as a consequence of bacterial LPS stimulation and mediates lymphocyte activation and nitric oxide synthesis as proinflammatory functions (168). As a counterpart of glucocorticoid-regulated antiinflammatory effects, it stimulates monocyte production of IL-6, IL-8 and IL-1β (1211). Estrogen was demonstrated to downregulate MIF expression by LPS-activated human monocytes, directly mediated by ER (54).

c) Dendritic cells in the female genital tract

The APCs of the vaginal mucosa include the intraepithelial CD1a+, MHC class II+, fascinbundling protein (p55)+, CD11c+, CD123-, DCSIGN-, CD4+ Langerhans cells (LC), and CD1a- MHC II+ p55+, DC-SIGN+, and CD4+ DCs in the lamina propria of the mucosa. LCs are abundant in the epithelial layer of the vaginal and ectocervical mucosa of women (119), whereas DCs are predominant in the submucosal layers. These immune cells detect pathogenic invasion and/or damage of epithelial surfaces. Migration of LCs from epithelial surfaces is triggered by inflammatory cytokines induced either by pathogen invasion of mucosal surfaces, and/or by epithelial damage.

DCs as bone marrow-derived professional APCs are initiators and modulators of the immune response via stimulation of B and T lymphocytes (73). Immature DCs reside in peripheral lymphoid tissues, acquire antigen by phagocytosis, macropinocytosis or adsorptive pinocytosis and mature on pathogen stimulation, for example through LPS. This leads to the upregulation of MHC class II and the costimulatory molecules CD80 and CD86. After migration to T cell areas of secondary lymphoid tissue, they undergo terminal maturation through ligaturing of CD40 with CD154 (CD40L) on antigen-specific T lymphocytes. Terminally mature DCs are characterized by production of IL-12 which facilitates production of IFN-γ-producing Th1 cells (196).
DCs express mRNA for both ER isoforms at all stages in their differentiation. Data suggest that nonsteroidal anti-estrogens such as tamoxifen inhibit the differentiation of immature DCs and therefore the development of inflammatory Th1 responses (737). Anti-estrogens can act as estrogen agonists or antagonists depending on the target cells and are therefore called selective estrogen receptor modulators. Human monocytes incubated with GM-CSF and IL-4 in the presence of anti-estrogen fail to differentiate into immature DCs; however, this was not mediated by ER (737).
Others illustrated that estradiol promoted the differentiation of functional DCs from murine bone marrow precursor cells via ER (1060). *Ex vivo* DC differentiation was inhibited in steroid hormone-deficent medium and was restored by addition of physiological amounts of estradiol, but not dihydrotestosterone.

Estrogen has also been demonstrated to modulate the expression of cytokines and chemokines in human monocyte-derived DCs. Their treatment with estradiol

increased production of IL-6, IL-8 and MCP-1 (106). Moreover, mature DCs treated with estradiol had an increased ability to stimulate naïve CD4+ T cells.

d) Natural killer cells in the female genital tract

NK cells are large granular lymphocytes that utilize receptors encoded in the germline DNA to become specifically activated or inhibited and are thus part of innate immunity. NK cell function can be classified in three categories (Table 12):

Cytotoxicity
NK cells can kill virally infected cells and tumor target cells regardless of their MHC expression via their cytolytic granules containing perforin
Cytokine and chemokine secretion
Besides production of IFN-γ, NK cells also secrete TNF-α, GM-CSF, IL-5, IL-13, MIP-1 and RANTES. Killing and cytokine secretion seem to be mediated by different subsets of NK cells characterized by the intensity of expression of the CD56 marker on their surface
Contact-dependent cell costimulation
Serving as a bride between innate and adaptive immunity, NK cells express several costimulatory ligands including CD40L which allow them to provide a costimulatory signal to T cells or B cells

Table 12: Different functions of NK cells (1051)

Human NK cells are found in the blood, lymphoid organs, liver and various mucosal tissues including lung, intestine and uterus. They comprise about 15% of all lymphocytes and are defined phenotypically by their expression of CD56 and lack of expression of CD3 (252).

Human blood NK cells can be divided into two major subsets based on the density of CD56 expression (252, 1051, 1512). $CD56^{dim}$ cells comprise the majority of peripheral blood NK cells (about 95%) and express high levels of the Fc-γ receptor CD16 and killer cell Ig-like receptors (KIRs) as well as perforin. Thus, they have a high spontaneous lytic activity. $CD56^{bright}$ NK cells express low levels of CD16, KIRs and perforin but high levels of cytokines and are thought to be an important inflammatory subset. This is the primary NK cell subset found in lymph nodes.

(1) Distribution and characteristics

Distribution

In the female reproductive tract, NK cells can be found in all tissues. Their numbers as a percentage of leukocytes vary in the different regions from 10 to 30% in nonpregnant women (459). NK cells account for a substantial presence in the uterus, and altered NK cell numbers and activity have been associated with a variety of clinical conditions involving reproductive organs and reproductive failure. Reduced NK cell activity is associated with an increase in the incidence of ovarian and endometrial malignancies (848) while higher NK cell activity has been associated with recurrent pregnancy loss (338).

In the human endometrium, NK cells localize in large numbers especially following ovulation and account for almost 70% of leukocytes prior to menstruation (459, 584) which again suggests the involvement of sex hormones in regulation of this NK cell migration.

Phenotype

Closer characterization of NK cells in reproductive tract tissues besides the endometrium has been performed rarely. **McKenzie** found CD3+, CD8+, CD16+, CD56-positive NK cells in the ectocervical epithelium which increase in number in relation to cervical intraepithelial neoplasia (CIN) (902). In fallopian tubes, cervix and ectocervix there seem to be up to 20% of all leukocytes of the CD45+, CD56+, CD3- cell type (1512).

Endometrial NK cells also have a unique cell-surface phenotype compared with peripheral blood NK cells. They have been previously described as endometrial granulocytes, endometrial stromal cells or decidual NK cells (1512). Uterine NK cells express CD56 and CD94, few express CD16 and none express CD8 or CD57 (370). A large amount express KIRs on their surface but unlike blood NK cells, uterine NK cells express also CD9 and CD69 on their surface (370).

Decidual NK cells were investigated more closely and it was revealed that they differ in 278 genes from blood NK cells (740). It was indicated that decidual NK cells were more similar in their gene expression profile to $CD56^{bright}$ N cells than to $CD56^{dim}$ NK cells.

Chemokine receptors

Blood NK cells express a variety of chemokine receptors such as CXCR3, CXCR4, CCR5 and CCR7 and specific migration of NK cells has been induced by chemokines *in vitro* (598, 858). Uterine NK cells demonstrated expression of CXCR3, CCR5 and CCR7 (1275). **Kitaya et al**. investigated the expression of chemokines in the human endometrium throughout the menstrual cycle (715). The expression of CCL4, CXCL9 and CXCL10 increases during the menstrual cycle which correlates with the increasing number of NK cells in the endometrium.

Sentman et al. showed that estrogen and progesterone were able to induce expression of the CXC chemokine ligands 10 and 11 (CXCL10 and CXCL11), which suggests that sex hormones induce specific chemokines in nonpregnant human endometrium that can activate NK cell migration (1275).

Several potential chemokine receptor-ligand pairs have been described in decidua that could be involved in the leukocyte trafficking during pregnancy. In the maternal decidua, CD16- NK cells are found in direct contact with the fetal extravillous trophoblasts. It is yet unknown which factors contribute to the specific homing of this unique NK subset to the decidua. **Hanna and colleagues** reported that CXCL12, a ligand for CXCR4, which preferentially recruits $CD56^{bright}$ NK cells, was shown to be expressed in the trophoblast (504). $CD56^{bright}$CD16- NK cells, which are the predominant type in decidual leukocyte population, have been reported to express chemokine receptor CCR5 and that its ligand CCL4 acts as a strong chemoattractant for these cells (1180).

(2) Functions and hormonal regulation

Functions

Large amounts of NK cells are populating the human decidua, often close to trophoblasts. These uterine NK cells have several functions in pregnancy (1512):

- Help shield trophoblasts bearing paternal antigens from the maternal immune system
- Protect the mother from trophoblast invasion and limit their expansion
- Be involved in regulation and restructuring of maternal spiral arteries
- Be a part of the innate immune system, protecting against infection in the uterus

NK cells are capable of amplifying an inflammatory response and promoting macrophage activation and generation of cytotoxic T cells by producing cytokines and chemokines. Uterine NK cells have been proved to secrete IFN-γ, CSF-1, GM-CSF, and TNF-α, IL-8, IL-10, and TGF-β1 (370, 634). Human NK cells cultured in the presence of IL-12 or IL-4 differentiate into cell populations with distinct patterns of cytokine secretion similar to Th1 and Th2 cells. NK cells grown in IL-12 produce IL-10 and IFN-γ while NK cells grown in IL-4 produce IL-5 and IL-13 (1107).
These cytokines may have significant effects on decidualization and trophoblast invasion. Uterine NK cells also produce other cytokines than blood NK cells such as angiogenic growth factors and LIF which constitutes the hypothesis that uterine NK cells may play a role in endometrial angiogenesis (815).

In the first trimester of pregnancy uterine NK cells accumulate as a dense infiltrate around the trophoblast cells and spiral arteries (156). With further progression of pregnancy these cells disappear from the decidua and are absent at term which suggests that specific signals are involved in the recruitment and localization of NK cells within the uterus. **Shao et al**. found that trophoblast cells induce a subset of regulatory CD8+ T cells during the first trimester which suggests that these cells probably regulate T cells as well as NK cell function at the maternal fetal interface (1288).
Also the general effects of human NK cell deficiency were investigated (1052). These deficiencies were correlated with an increase in infections, especially viral herpes infections. But the role of NK cell function alone is difficult to determine as many of the NK cell deficiencies involve other immune function as well. NK cells have also been shown to actively recognize fungal infections and to play a role in the immune response against *Cryptococcus*.

Regulation through cytokines

NK cells can be activated by several cytokines including IL-2, IFN-α/β as well as by prolaktin and IL-15 or IL-18 in combination with IL-12 (252, 387, 716). IL-15 and prolaktin are both produced by endometrium and influence NK cell differentiation. Uterine NK cell were shown to express prolaktin receptors (1371).
IL-15 is present during the whole menstrual cycle with increasing levels during the mid-secretory phase and early pregnancy and is produced by endometrial and decidual stromal cells (209, 348). The role of IL-15 seems to be determined for survival, proliferation and attachment of uterine NK cells. Perhaps expression of IL-

15 by decidual endothelium is important for specific localization of NK cells close to spiral arteries (30).
Another immunoregulatory molecule which has been observed to influence NK cell is TGF-β. Inhibition of uterine NK cell cytokine production by locally produced TGF-β is a likely mechanism to regulate NK cell function in the human endometrium (370).

Regulation by sex hormones

There is strong evidence that uterine NK cell numbers and migration are regulated by sexual hormones. NK cells are found widely within the nonpregnant endometrium and are associated with other leukocytes in small aggregates (1560). NK cell numbers are low in the early proliferative phase and increase as the menstrual cycle progresses (459, 702).
In the late secretory phase prior to menstruation NK cells account for up to 70% of the leukocytes in the endometrium and are often seen as loose aggregates in the *Stratum functionalis* lying next to the upper endometrial glands and the luminal epithelium. The cyclic nature of uterine NK cell appearance and the role of sex hormones in modifying changes in endometrium suggest hormonal regulation of NK recruitment and expansion in the endometrium.

(3) NK cell recognition and interactions

NK cells can distinguish between healthy cells and abnormal cells by using a sophisticated repertoire of cell surface receptors that control their activation, proliferation and effector functions (783). There is the hypothesis that NK cells co-evolved with T cells given that they share common killing mechanism using perforin and granzymes and a similar pattern of cytokine production. Both of these lymphocytes are focused on recognition of MHC molecules and in this regard, NK cells distinguish themselves from phagocytes which rely on conserved pattern-recognition receptors such as TLRs.

NK cell recognition includes the initial binding to potential target cells, interactions between activating and inhibiting receptors with ligands available on the target, and the integration of signals transmitted by these receptors, which determines whether the NK cell responds or detaches again (783).

Three families of cell surface receptors, KIR, Ly49 and CD94/NKG2, are expressed on NK cells which all recognize MHC I ligands and contain both activating and inhibitory isoforms (1177). They all share a common immunoreceptor tyrosine-based inhibitory motif (ITIM) in their cytoplasmatic domains and provide protection for all cells that express normal amounts of MHC I on the cell surface. The MHC-I-binding inhibitory receptors recruit tyrosine phosphatases which are believed to counteract activating receptor-stimulated tyrosine kinases. KIRs recognize epitopes on HLA-A, HLA-B and HLA-C classes while the CD94/NKG2 receptor recognizes human HLA-E (1177). Many of these receptors or related proteins are also found on NKT cells, memory T cells and other immune cells (905).

Downregulation of MHC I due to viral infection or transformation of cells would alleviate inhibition of NK cell positive signaling and may result in initiation of cytotoxicity and cytokine production. It appears that there is not a single antigen receptor responsible for NK cell activation but an activity of several receptors triggering effector function. Activating receptors on NK cells include molecules with immunoreceptor tyrosine-based activation motifs (ITAM) such as NKG2D that recognizes MHC-like proteins MICA and MICB as well as 2B4 which binds to CD48 (784). MICA and MICB are expressed by epithelial tumor cells and stressed cells; CD48 is significantly upregulated in B cell infected with Epstein-Barr-Virus (EBV), for example (483, 784). NKG2D triggering can induce NK cell lysis of tumor cells and cytokine secretion.

The invading trophoblasts express HLA-G and can interact with the KIR receptors on uterine NK cells. Membrane-bound HLA-G has been shown to stimulate uterine NK cells and IFN-γ production and to suppress mononuclear cell effector functions (1438). NK cell-derived IFN-γ seems to be necessary for vascular remodelling of spiral arteries and placental formation so that the recognition of HLA-G on trophoblasts by uterine NK cells may be important for placental development. Uterine NK cell-deficient mice demonstrated failure to sustain decidual integrity and loss of spiral artery modifications (481).

NK cell interactions with DCs are seen as an important part of forming an initial immune response. NK cells have been demonstrated to kill immature DCs (313). DC-derived IL-2 is essential for induction of NK cell production of IFN-γ during bacterial infection. Moreover, IFN-α helps activating NK cell cytotoxicity, and DCs are a good source of IFN-α after infection. NK cells can interact with endothelial

cells through adhesion molecules and chemokine receptors. NK cells are able to recognize and kill endometrial endothelial-derived cell lines, and ECs can produce cytokines upon exposure to pathogens that can activate NK cells. However, the interactions of NK cells with these cells are poorly understood so far.

(4) TLRs

Like other innate immune cells, NK cells also express various TLRs. Human blood NK cells were positively tested for the expression of mRNA of TLR1 to TLR10 (568). Agonists of TLR2, TLR3 and TLR9 are able to trigger IFN-γ production of NK cells while CpG DNA as the agonist of TLR9 is not certain to activate NK cells directly or via cytokines such as IL-12, IL-8, IL-15 or TNF-α (749).
Recent data suggest that uterine NK cells express several TLRs such as TLR2, TLR3 and TLR4 whose agonists can trigger these cells to produce IFN-γ (1512). The hypothesis is therefore that microorganisms may initially activate ECs which produce cytokines and these cytokines in combination with PAMPs will activate NK cell cytokine production, resulting in further activation of innate immune responses.

e) Neutrophils in the female genital tract

Neutrophils comprise 40 to 70% of circulating white blood cells and have always been considered as a first line of defense against pathogens (1266). They have a relatively short lifespan of about 6 hours circulating in blood before being removed by macrophages of the reticuloendothelial system (RES). When pathogenic microorganisms infect the host, neutrophils rapidly migrate to the site of infection during the first 1 to 4 hours.
Produced in bone marrow, they enter the blood stream by inflammatory signals originating from the infection sites, adhere to the vessel wall at the sites of tissue damage and transmigrate through endothelial cells to the infection site (600). When moving with the blood stream they roll along the vessel wall and continuously interact with the capillary walls to search for inflammation signs, what is called margination (812).

Tissue cell injury and infection result in increased expression of the adhesion molecules E- and P-selectins by endothelial cells as well as expression of

sialomucins which interact with L-selectins on neutrophils. When rolling across the endothelial surface the integrin molecules on the neutrophils` surface are activated, which leads to firmer adhesion. IL-8 is secreted by endothelial cells which induces shedding of integrins and L-selectin and therefore starts transmigration of neutrophils (576). By crossing the endothelial barrier neutrophils unpack their granules with tissue-degrading enzymes and microbicides which facilitate this passage (382).
Neutrophils follow both endogenous and bacterial chemoattractant signals to arrive at a site of infection (536). The presence of pathogens are detected by germline-encoded receptors that recognize PAMPs; human neutrophils express TLR 2 to 10 except TLR3 (525). The final elimination of pathogens is completed either by phagocytosis or production of toxic oxidate compounds by the cytoplasmatic and membrane-bound nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system. Other possible mechanisms are the release of microbicides such as defensins and serine proteases from intracellular granules (1512). The neutrophils themselves produce several cytokines, for example IL-1β, IL-8, TNF-α, TGF-β1 (182), that attract more neutrophils, macrophages and other immune cells helping to initiate the adaptive immune response.

(1) Distribution

Neutrophils can be found in all tissues of the female reproductive tract. Given the fact that the vagina is colonized by a mixture of commensal microorganisms one would expect the highest level of neutrophils in these lower regions of the female genital tract where the most pathogens can be found. However, the highest amount of neutrophils is recovered from fallopian tube with consistently decreasing numbers down through the lower genital tract (459).
Patton and colleagues examined vaginal tissue of ovulating and non-ovulating women during three phases of the menstrual cycle for the number of cell layers and epithelial immune cells (1098). The number of EC layers underwent a small reduction from days of menses to the postovulatory phase and non-ovulating women had a thinner vaginal epithelium than ovulating women which suggests a hormonal influence on vaginal EC growth. However, the numbers of neutrophils as well as these of T cells and macrophages remains constant throughout the cycle.

In mice, a large number of neutrophils infiltrate into the vaginal epithelium accompanied by an increased number of neutrophils in vaginal lavage fluid after

ovulation (1327). Correspondingly, concentrations of a functional IL-8 homologue, murine MIP-2 were significantly increased. This indicates the regulation of neutrophil migration into the vagina by MIP-2 in a sexual cycle-dependent manner.

IL-8 is a potent chemotactic and activating factor for neutrophils and high levels of IL-8 correlate with high levels of neutrophils in the female vagina (192). This corresponds with the findings that neutrophils cannot cross the epithelial barrier unless they are under the influence of a chemokine gradient of IL-8, for example (692). The same study showed no difference between the number of neutrophils or concentration of IL-8 in healthy women compared to women with bacterial vaginosis (BV). When women were experimentally infected with *Candida albicans*, either a commensal or a pathogen, there was no neutrophil infiltration in the vagina when the infection was asymptomatic or did not take place (400). In case of vaginal infection there was neutrophil infiltration and inflammation. Production of chemokines that attract neutrophils and neutrophil infiltration seem to occur only after infection of the vaginal epithelial layer. Vaginal lavage fluid from women with symptomatic infection, but not those asymptomatically colonized, promoted the chemotaxis of neutrophils (400).
In animals, a rapid subepithelial influx of neutrophils in the vagina occurs not only due to insemination but also to sterile liquid solution brought into the vagina (888). Similar results were produced in women after insemination, although more in association with the cervix (1074).

Cervix

Compared with other leukocytes, neutrophils represent the highest population with 83% of all leukocytes in human cervical secretions (1414). Both insemination with increased local production of IL-8 and infection of the cervix increase the number of neutrophils in the cervix significantly (125). In case of infection, cervical cells secrete IL-8 to promote neutrophil invasion in response to pathogen-associated substances such as LPS (1481) but human cervical tissue is generally capable of producing IL-8 in vitro with decreasing activity postmenopausal (125).

Uterus

The complex cycle of the endometrium is regulated by hormonally controlled interactions between cytokines, chemokines, endometrial cells and leukocytes. During most phases of the menstrual cycle the numbers of neutrophils are low. However, prior to menses a decrease in progesterone levels is responsible for a rise

in IL-8 production in perivascular cells, epithelia and glands (47, 267). At this time, there is also a significant increase in endometrial neutrophils which comprise up to 15% of all tissue cells (1249), which serves to guard against infection at this time of lower epithelial defenses.

Neutrophils may aid in endometrial breakdown as neutrophil granules release elastase and membrane-type matrix metalloproteinase (MT1-MMP) by chemotaxis which both are able to activate MMP 2 and 3 (1579). The degranulation of neutrophils also releases antimicrobial contents such as defensins, SLPI or the neutrophil protease inhibitor and microbicide related to SLPI, elafin, which also peaks at menses (706).

Endometrial neutrophils are also capable of producing IFN-γ both *in vitro* and *in vivo* (1559), especially in the endometrial stromal layer. This production of IFN-γ also shows no variation during the menstrual cycle. By altering the local cytokine environment, neutrophils seem to be able to biase immune responses in the endometrium.

Fallopian tube

The fallopian tubes represent the tissues with the highest number of neutrophils in the female reproductive tract (459) with most of them distributed in the lamina propria and a few in the epithelium (1529). The expression of IL-8 is highest in the late proliferative phase around ovulation (1067). This may suggest a greater influx of neutrophils at this time but there seem to be no studies on neutrophil number variation in the fallopian tubes during menstrual cycle (1512). IL-8 was also present in greater amounts in the distal compared with the proximal tube. The degradative enzyme aminopeptidase N is found in tubal stromal tissue at the epithelial stromal border and may limit the effect of epithelial IL-8 in recruiting neutrophils. However, the exact role of neutrophils in the fallopian tubes as part of the local immune system remains quite unclear.

Ovary

Also in the ovary neutrophils are present throughout the menstrual cycle. It has been show that low numbers of neutrophils infiltrate ovarian stroma but high numbers are present in the wall of the developing ovarian follicle (144). At ovulation, there was a marked increase in the density of these cells in the follicle wall, especially in the thecal layer, where they accumulate particularly around the point of imminent rupture. This suggests an active role for neutrophils in tissue remodelling during the ovulatory process.

Following rupture of the follicle and release of the ovum, the empty follicle becomes the corpus luteum. In the corpus luteum there is a higher density of neutrophils in the theca lutein area compared with the granulosa lutein area (144). There are no significant changes in the density during early and late luteal phase.
IL-8 concentrations in follicular fluid vary over the menstrual cycle and have their peak around ovulation which is consistent with the peak of neutrophil influx (1225). Exposure to follicle-stimulating hormone (FSH) and luteinizing hormone (LH) but not estradiol or progesterone increased the IL-8 secretion from granulosa cells.

(2) Characteristics and functions

The different numbers and distribution of neutrophils in the female reproductive tract explains the need for investigating the distribution of neutrophil chemoattractants in the different regions of the genital tract (1512).
It was found that cervical tissue expressed the highest amount of RNA for Gro-γ (CXCL3), ENA-78 (CXCL5), GCP-2 (CXCL6) and IL-8 (CXCL8). Fallopian tube expressed the second highest amount of IL-8, but ectocervix was the second to highest in expression of ENA-78 and NAP-2. Endometrium expressed the least amounts of RNA for Gro-γ, GCP-2, NAP-2 and IL-8. These findings do not correlate with neutrophil recovery from tissues by enzyme digestion (1512). The cervix should contain the greatest number of neutrophils if CXC chemokine expression was proportional to recruitment of neutrophils. Moreover, one would expect a higher amount of microorganisms at the cervix than in higher regions of the reproductive tract, resulting in greater need for innate immune protection by neutrophils.

In studies with EC cultures of the reproductive tract the high neutrophil chemoattractant activity of these cells was the result of synergistic action between IL-8 and GM-CSF secreted by ECs (1292).
This suggests that relatively low concentrations of GM-CSF can potentiate the activity of CXC chemokines in induction of neutrophil chemotaxis. Moreover, one of the many actions of GM-CSF on neutrophils could also be to downregulate responses when they are no longer necessary (1512). If neutrophils have entered the lumen of the genital tract after encountering high GM-CSF levels in the epithelium, a response to IL-8 which might attract the cells back into the tissue would be counterproductive.

Concerning neutrophil crossing of the epithelial barrier, it is known that they prefer to cross monolayers of cultured ECs from the basal to the apical side provided that an IL-8 gradient exists from apical to basal (692). **Wira and colleagues** presumed that unstimulated ECs in the female genital tract secrete chemokines basally to attract neutrophils to the epithelium. In addition, they produce a higher amount of chemoattractant on the luminal side that might serve to induce neutrophils to cross the epithelium and enter the lumen (1512).

Shen and colleagues have concentrated lately on the investigation of neutrophils of the fallopian tubes as this tissue has the highest numbers of neutrophils per gram (1512). Fallopian tube neutrophils compared with blood neutrophils expressed higher levels of the adhesion molecule CD31 (PECAM-1) and CD15.

CD31 is expressed on leukocytes and endothelial cells and is increased on transmigrated neutrophils (844), which suggests that fallopian neutrophils have crossed the endothelial barrier and are not only part of the marginated pool associated with the luminal surface of blood vessels. CD15 is a carbohydrate antigen involved in the binding of neutrophils to the endothelial lectins, E-selectin and P-selectin. When stimulated with chemotactic peptides during neutrophil migration, for example, CD15 is brought to the surface from intracellular pools. Another point is that CD15 ligation is reported to induce a release of granule content; the high CD15 expression may be important for innate immune responses in the fallopian tube.

Fallopian neutrophils have also shown lower amounts of specific granule-associated molecules which suggests that they have undergone some kind of degranulation. Furthermore, they also demonstrated high levels of intracellular VEGF and IFN-γ (1512). As VGEF plays an important role in vasodilatation and vascular permeability (86) it would make a leukocyte infiltration of tissues possible.

In summary, neutrophils in the reproductive tract seem to be regulated by the hormonal cycle as well as chemokines and cytokines.

3. Functions and regulations of adaptive humoral and cell-mediated immunity in the female genital tract

a) Immunoglobulins in the female genital tract

(1) Distribution of immunoglobulin classes and subtypes

Highly variable information has been reported about the presence of different Ig classes in human female genital secretions. Such discrepancies seem to reflect both the differences in the applied sampling method and individual variability such as age and stage of menstrual cycle (1090). The dominance of IgA in the majority of external secretions, such as intestinal and nasal fluids, saliva, tears and milk, has for decades been considered a cardinal characteristic of the humoral arm of the mucosal immune compartment.

Waldman et al. found in 1971 already that S-IgA was the predominant Ig class (45%) in normal CVS; the remainder consisting of monomeric IgA (20%) and IgG (30%) (1467). In the same study, IgA levels were found to decrease with age in contrast to IgG which increases.

In more recent studies results partly differ from those findings. Latest studies have revealed that humane urine, seminal plasma and cervical or vaginal washings collected at various stages of the menstrual cycle contain a higher proportion of IgG than IgA (608). Some studies showed a predominance of IgG in cervical secretions but a higher proportion of specific IgA antibodies in secretions from women with colonized group B streptococci in their cervix or rectum (566). **Hocini et al**. came to the result that IgG is also predominant in normal vaginal fluid (556) which fits to the findings of **Quesnel et al**. with IgG dominating in vaginal fluid and IgA in mucus mostly from endocervix (1155). However, **Kutteh et al**. showed in 1996 with the method of cervical washing the predominance of IgG also in cervical mucus with a peak before the time of ovulation together with smaller preovulatory peaks of IgA and IgM (764). **Parr and Parr** reported in their article that in several reports IgG appears to be the predominant Ig in CVS, but the ratios of IgG to IgA vary from 2:1 to 10:1 (1090). Measurements of Ig in humane uterine fluid also indicated the predominance of IgG; nevertheless, the uterus may be the primary source of IgA in

vaginal fluid since the concentration of IgA in vaginal fluid from hysterectomized women was only about 10% of normal (612).

IgG occurring in genital secretions was deemed to be mainly serum-derived, but a significant enrichment of the IgG1 subclass suggested also some local influence (556). However, the mechanisms involved in the appearance of IgG in cervical secretions remain unclear. There are strong suggestions that specific activity of IgG antibodies in genital tract secretions often correlates with that in serum which shows that part of IgG originates from circulation. Others discuss that part of IgG is secreted by plasma cells residing in genital tissues (1227). How IgG reaches the lumen whether by active transport or passive diffusion remains unknown.
Concerning IgM antibodies, very little is known so far. A short-lived IgM response has been found during herpes simplex infection.

Concerning the IgA subclasses, female genital tract secretions resemble secretions of the lower intestinal tract rather than the upper intestinal or respiratory tract where IgA antibodies mostly belong to the IgA1 subclass (922). Both IgA1 and IgA2 are found in equal proportions in the female genital tract as well as in the rectum and the large intestine (Figure 4).

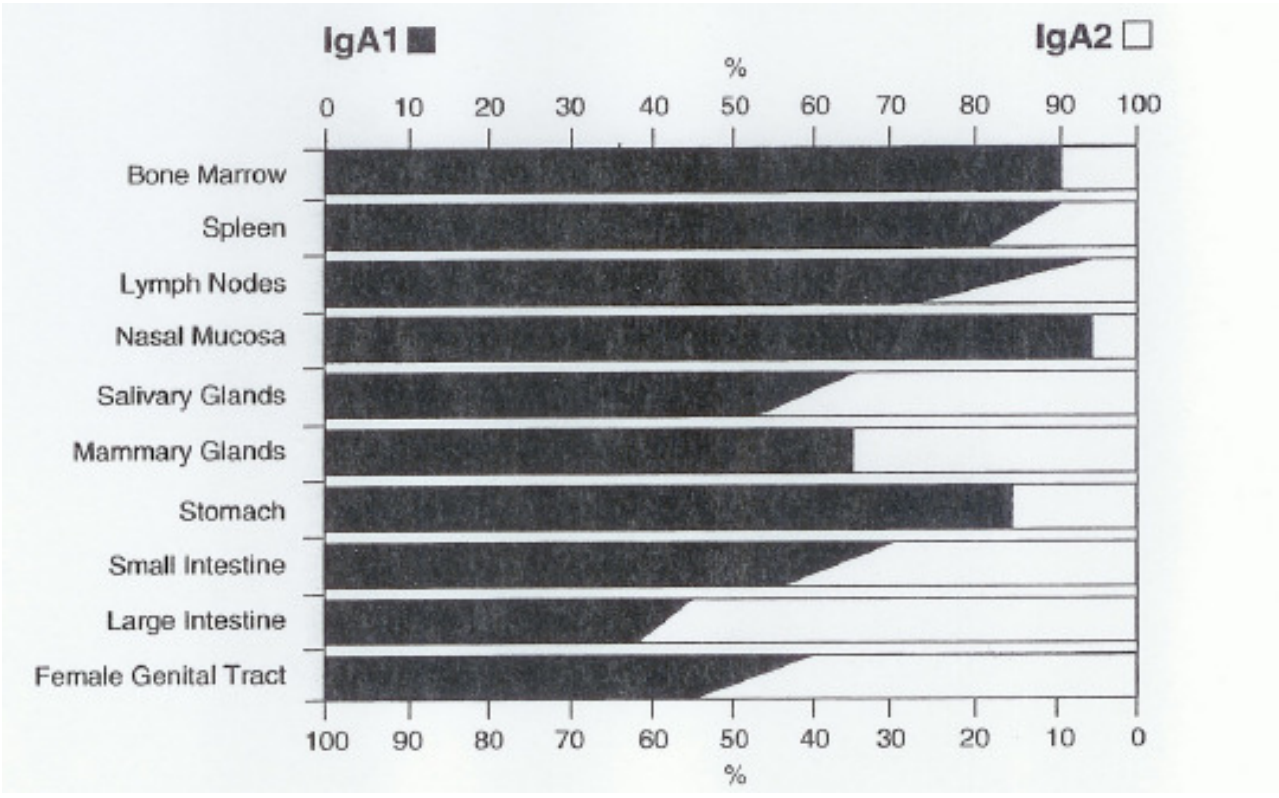


Figure 4: Distribution of IgA1- and IgA2 producing cellls in systemic and mucosal tissues in humans (925)

With respect to the molecular forms of IgA, the female genital tract secretions are again unique. In contrast to plasma in which monomeric IgA constitutes 90-99% of total IgA or to saliva and milk in which 95% of IgA is polymeric, cervical mucus contains about 70% polymeric IgA and vaginal secretions contain almost equal proportions of polymeric and monomeric IgA.
The ratios of IgA1 and IgA2 and the predominance of polymeric IgA in cervical secretions indicate that much of the IgA originates from local production, not from plasma (764). However, the high representation of the serum monomeric form of IgA in the vagina clearly demonstrates that, contrary to other mucosal surfaces, the genital tract relies heavily on antibodies derived from serum and systemic immunity. Because of the intrinsic resistance of IgA2 to IgA1 proteases of many pathogenic bacteria, the increased proportions of IgA2 may provide functional advantage to certain specific antibodies (763).

To conclude, antigen-specific IgA, IgG and IgM antibodies and SCs can be detected in the external secretions of the human genital tracts. IgG levels in the lower genital tract secretions seem to equal or even exceed the levels of S-IgA whereas the dominant isotype of other mucosal secretions is S-IgA (763). IgG and IgA are under hormonal influence and are derived from the systemic compartment, as well as being locally produced.

(2) Production of immunoglobulins in female genital tract tissue

Cervix, fallopian tubes and vagina
IgA and IgG antibodies in genital tracts can be transported from peripheral blood and can also be produced by resident Ig-secreting cells. Studies showed that the presence of antibody-containing and antibody-secreting cells is higher in the uterine endocervix than in the ectocervix, fallopian tubes and the vagina (273, 759). IgG and IgA-secreting plasma cells are abundant in the lamina propria of the endocervix and almost all IgA-producing cells contain J chain, a marker of synthesis of polymeric IgA. The immunocyte class proportions were similar to those reported for small intestine mucosa, with a striking predominance of IgA-producing cells.
Immunohistochemical examinations by **Mestecky and Kutteh** (759) found out that the number of Ig-producing cells of IgA, IgG and IgM is different at various spots in the female genital tract (Table 13). The endocervix and the ectocervix displayed the highest accumulation of Ig-forming cells and that such cells predominantly produce

antibodies of the IgA subtype, among that almost equal proportions of IgA1 and IgA2 subtypes.

Tissue	IgA	IgA1	IgA2	IgM	IgG
Fallopian tube	67	55	45	22	11
Endocervix	73	57	43	12	15
Ectocervix	79	59	41	10	11
Vagina	79	45	55	14	7

Table 13: Distribution of immunoglobulin-producing in different tissues of the female genital tract in percentage of total Ig-containing cells, adapted from Kutteh et al. (759)

However, in a study by **Crowley-Norwick et al**. on cervical cells with ELISPOT, at least four times more IgG- than IgA-producing cells were detected (89% versus 17%) (273). **Brandtzaeg** proposed that immunohistochemical studies have underestimated the IgG class because of interstitial staining (141). Moreover, the quantification of Ig-producing cells is difficult to perform due to their uneven distribution. The actual quantities of Ig-producing cells were measured as density by number of cells/10 low-power fields without any accurate definition of the evaluated tissue compartment. The endocervix was found to have the largest number followed by ectocervix, fallopian tubes and vagina (761).
While the expression of both CD19 and CD20 is lost in terminal differentiation of B cells, CD38 is strongly expressed by plasma cells. **Johansson et al**. could not detect any CD19- or CD20-expressing cells in cervix and vagina which agrees with the results of **Crowley-Norwick et al**. (626). CD38 plasma cells were abundant and scattered in subepithelial stroma reinforcing that the genital tract contains antibody-producing cells.

Mestecky et al. also found out that the ECs of fallopian tubes, uterus, endocervix and ectocervix express the pIgR, the extracellular part of which is called the SC (923). Thus, all components for an active transepithelial transport of polymeric IgA are present which takes place in the same manner like in other mucosal tracts.
As the pIgR is not distributed equally throughout the genital tract, secretion of S-IgA takes place primarily in the cervix and to a lesser extent in the fallopian tubes and uterus. Uterine expression of polymeric IgA is under the control of hormones, in a way that estradiol elevates whereas progesterone partly reverses this effect.

Moreover, in case of infection or inflammation its expression is also increased, possibly by cytokines such as IFN-γ, TNF-α and IL-4 (1227).

Table 14 shows an overview of the presence and frequency of IgA, IgG, J chain and SC in different female genital tract mucosa. Data suggest that the endocervix is likely to be a focal point for mucosal immunity in the genital tract.

	IgA	J chain	SC/sIgA	IgG
Myometrium	—	—	—	—
Fallopian tube	+	+	+	+
Ovary	—	—	—	—
Endometrium	+/—	+/—	+	+
Endocervix	+++	+++	++	+/+++
Ectocervix	++	+	+/—	+
Vagina	+	+	+/—	++

Table 14: Summary of results form different studies concerning relative distribution of IgA, IgG, SC, and J chain in the tissues of the female reproductive tract (conflicting results exist for IgG production in cervical mucosa), adapted from Brandtzaeg (141)

Endometrium

Studies have reported that only quite rare and scattered Ig-producing cells are present in the normal human endometrium (759). Others reported the predominance of IgG-containing cells in the human endometrium regardless of the stage in menstrual cycle (141), followed by IgM and IgA-containing cells.

When evaluated without the prefixation washing procedure, the endometrium`s stroma contained scattered IgG and less IgA but there was an accumulation of IgA within the glandular lumina and apically in the epithelium without the presence of IgA immunocytes (140). This strongly suggested selective external transport of serum-derived polymeric IgA, which was supported by staining for J chain. Monomeric IgA without co-localization of J chain was detected in vessel lumina, which supports this hypothesis.

Also the preferential epithelial localization of IgM compared with IgG supported the idea that polymeric Igs derived from serum can actively be transported externally through endometrial glands. This was underlined by the general appearance of SC throughout the endometrial epithelium, particularly in the glands (141). Glandular uptake of antibody molecules from the interstitial fluid therefore seems to be dependent on SC acting as pIgR with additional paracellular diffusion of IgG (141).

SC-expression and IgA uptake in the endometrium proved to be dependent on the phase of the menstrual cycle, showing a rise from the proliferative to the mid- and late secretory phase (118). The same study showed that the endometrial glands contained significantly more of all components of the secretory immune system in the mid- and late luteal phase than in the early half of the menstrual cycle (Figure 5).

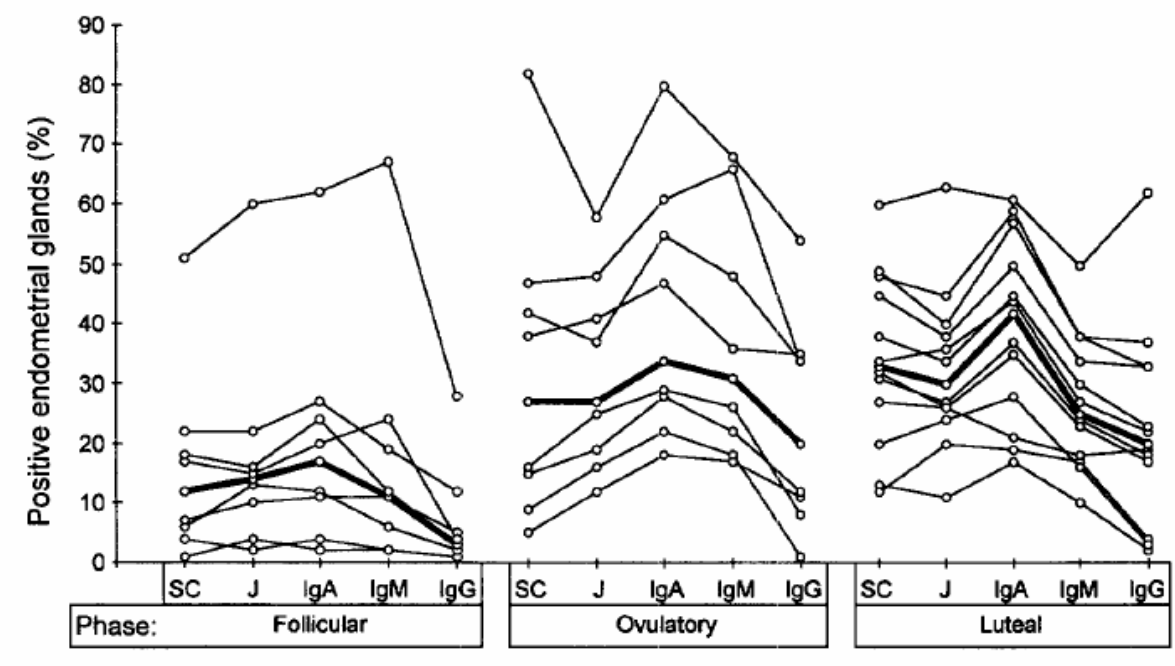


Figure 5: Scatter diagram displaying the percentage distribution of human endometrial glands containing different humoral immune components throughout the menstrual cycle; each thin line representing one specimen, thick lines connecting medians, adapted from Bjerke and Brandtzaeg (118)

(3) Regulation of Ig levels by sex hormones

Sex steroid hormones may be important in regulating both the systemic and secretory immune system. Estrogens, progesterone, and androgens directly and indirectly modify numerous immunological functions (1408).

Estrogens drive differentiation of CD4+ helper T cells toward Th2 regulation, increase production of Igs, and downregulate cell-mediated immunological processes. In contrast, androgens, such as dehydroepiandrosterone (DHEA), drive differentiation of CD4+ helper cells toward Th1-regulated functions and antagonize certain estrogen-mediated immunological effects. Progesterone increases the activity of suppressor T cells.

There is clear sexual dimorphism in the immune responses of males and females which is shown by higher Ig levels in females as compared with males. Together with more vigorous antibody responses to exogenous antigens of females this indicates a higher humoral immunity in females than in males (929). In addition, females seem to have a reduced incidence of tumors, a better resistance against viral and parasitic infections and seem to reject allograft more rapidly as compared with males which also suggests a higher cellular immunity in females (135).

From an endocrine point of view, each organ in the female genital tract acts like a discrete reproductive organ whose contribution to reproductive success is controlled by the ovarian release of estradiol and progesterone. They also enhance or suppress immunocompetency of the genital tract.
IgA and IgG levels in uterine secretions in rats were shown to change markedly during the reproductive cycle with highest levels at ovulation (1518). When ovariectomized rats were treated with estradiol, IgA and IgG levels in uterine secretions were elevated compared to saline controls (1519). In CVS, however, levels of IgG, IgA and SC were lowered in response to hormones (1520). This suggests that changes in hormone levels may have different effects on immune responses in different regions of the female reproductive tract.
Estradiol also upregulates the expression of pIgR on uterine ECs and thereby increase the transcytosis of polymeric IgA into the uterine lumen of intact and ovariectomized rats (1518, 1519).
Kutteh et al. demonstrated that Ig levels of IgA, IgG and IgM in cervical mucus are dependent on the stage of menstrual cycle reaching a peak before the time of ovulation (764). Previous experiments on IgA concentrations in cervical mucus and cervicovaginal secretions also show a decrease at ovulation from preovulatory peaks (1467). This relationship between estrous cycle and specific antibody levels likely reflects the changes that occur in the female reproductive tract during the course of the estrous cycle. Preovulatory until the time of ovulation, or at the time of mating, the female genital tract is subjected to numerous pathogens (1144). In fact, sperm has been shown to be a vector for bacteria, whereby the bacteria attach to the tails of sperm as they move up the reproductive tract.

Estrogen has been shown to increase total IgG and IgM production by human peripheral blood mononuclear cells (PBMCs) from normal individuals (1058, 661). In the former study, the Ig-enhancing effects of estradiol were due to inhibition of CD8+ suppressor cells (1058), while in the latter study the effect of estradiol was

neutralised by anti-IL-10 antibody (661) and the authors suggested that the increased IgG production was mediated by secretion of IL-10 by monocytes in the PBMC cultures.

b) Prevalence of T lymphocytes

Givan et al. estimated that leukocytes represent 6 to 20% of the total number of cells in fallopian tubes, endometrium, cervix and vaginal mucosa with greater cell numbers in the upper tract (459). B cells are present in low but measurable numbers but T cells accounted for about 50% of all leukocytes, with CD8+ T cells predominating over CD4+ T cells.

This was confirmed some years later in a study by **Johansson and colleagues** who also showed a characteristical distribution of immune cells (626). They found that both CD4+ and CD8+ T cells were concentrated in a band beneath the epithelium and dispersed in epithelium and lamina propria. They also found several lymphoid aggregates consisting of T cells and B cells in one cervical sample.

Also another study detected aggregates consisting of an inner core of B cells surrounded by mainly CD8+ T cells and an outer halo of macrophages in the human endometrium (1560). Size was found to vary with the stage of menstrual cycle with larger size during secretory stage than those at proliferative stage. The absence of these aggregates in uteri of postmenopausal women provided further evidence that these aggregates are under hormonal control (1560). These aggregates had multiple features of inductive lymphoid tissue, for example aggregated lymphocytes in follicle-like structures, lymphocyte invasion of overlying epithelium and the presence of HEV. This suggests that these structures represent an organization of immune cells that are not dependent on the presence of infection or malignancy.

White et al. used hysterectomy specimens cocultured with IL-2 that contained CD3+CD8+ T cells to demonstrate cytolytic activity in the genital tract (1497). Cytolysis by CD3+ CD8+ T cells was found throughout the reproductive tract and appeared to be hormonally regulated, since in the uterine endometrium, the capacity for CD3+ T cell cytolytic activity was present during the proliferative phase of the menstrual cycle and absent during the subsequent secretory phase. In contrast, in postmenopausal women the entire reproductive tract, including the uterus, retains the capacity for strong CD3+ T cell cytolytic activity (1497).

A study by **Pudney et al**. showed that in women without inflammation, T cells and APCs were most prevalent in the cervical transformation zone and surrounding tissue (1146). Intraepithelial lymphocytes were predominantly CD8+ T cells; most CD8+ cells in the transformation zone and endocervix, and a proportion of cells in the ectocervix, expressed T-cell internal antigen-1, a marker of cytotoxic potential.
In contrast, the normal vaginal mucosa contained few T cells and APCs. Cervicitis and vaginitis cases had increased numbers of intraepithelial CD8+ and CD4+lymphocytes and APCs. The menstrual cycle and menopause had no apparent effect on cellular localization or abundance in any of the lower genital tract tissues. These data indicated that the cervix, especially the transformation zone, is the major inductive and effector site for cell-mediated immunity in the lower female genital tract (1146).

Other data demonstrated that changes in circulating levels of estrogen can regulate the recruitment of bone marrow-derived cells to the uterine endometrium. In a study by **DeLoia et al**., the total uterine leukocyte population increased significantly when the women received oral estrogen, which resulted in higher serum estrogen levels (318). This rise in leukocytes was due to a significant increase in both the uterine NK cells and the macrophage populations whereas T-cell numbers did not change relative to circulating estrogen levels.
While further studies will be required to determine the effects of sex hormones on T cell function, receptors for estradiol have been demonstrated on both CD8+ and CD4+ T cell populations whereas the presence of progesterone receptors on T lymphocytes remains controversial (91).

To conclude, the human systemic and mucosal compartments of the immune system, especially the female genital tract, display a remarkable degree of independence. In contrast to other mucosal systems, the genital tract mucosal system is characterized by a significant contribution of the systemic compartment with respect to Ig isotype distribution, unique distribution and phenotypes of B and T cells, strong hormonal dependency and lack of typical lymphoepithelial inductive sites (Table 15).

	Humoral immunity			Cellular immunity					
	Ig-producing cells	J chain	pIgR	T cells CD4 - CD8		NK cells	MA	DC	N
Myometrium	–	–	–	–	–	–	–	–	–
Fallopian tube	+	+	+	++	++	+	+	+	+++
Ovary	–	–	–	+	+	–	+/–	+/–	+
Endometrium	+/–	+/–	++	++	++	++	+	–	++
Endocervix	+++	++	+++	++	++	+	+	++	++
Ectocervix	++	+	+	+++	+++	+	+	++	++
Vagina	+	+	–	+++	+++	+	+	++	++

Table 15: Components of humoral and cellular immunity at different sites of the female reproductive tract; MA=macrophages, N=neutrophils, adapted from Kutteh et al. (763)

4. Immunization studies

Based on the concept of a common mucosal immune system through which a fraction of lymphocytes activated at one mucosal site can disseminate immunity not only at this specific site, but also to other mucosal tissues, there has been much interest in the possibility of developing vaccines against mucosal infections in the genital tract (287).

Although the concept of the common mucosal immune system has been the dominant paradigm in mucosal immunology for more than two decades, it has become increasingly clear that there is a substantial degree of subcompartmentalization with the human reproductive tracts representing a component of this system with unique features, as discussed in the last chapters in detail. Indirect evidence suggests that female genital tract tissues may be preferentially supplied by cells from inductive sites located in intestine, rectum and nasal cavity.

It has been well established that the dissemination of cells from the inductive to the effector site is regulated by characteristic homing receptor-ligand interactions operational in mucosal tissue. In order to recruit leukocytes to mucosal tissue, the endothelium of the vessels expresses adhesion molecules, or addressins, that serve as ligands for homing receptors on the cells. **Johansson et al**. described the expression of intercellular adhesion molecule-1 (ICAM-1), VCAM-1, vascular

adhesion protein-1 (VAP-1) and P-/E-selectins in cervical and vaginal tissue (626). However, none of the samples expressed mucosal addressin cell adhesion molecule-1 (MAdCAM-1). MAdCAM-1 has been shown in the gut-associated lymphoid tissue and is the only known vascular adhesion molecule that specifically directs lymphocytes to mucosal tissue via binding to the lymphocyte integrin α4β7 (1364).
Immunization that produces secretory immunity in the female reproductive tract against STDs could have important practical applications. Investigators have attempted to elicit secretory immunity in the genital tract by using different routes of immunization. These local, remote and parenteral routes should be briefly outlined here.

a) Vaginal immunization

Earlier studies of immunization in the human uterine lumen with sperm, for example, failed to detect immune responses in the reproductive tract (966). Comparative studies have demonstrated that antibody titers in luminal fluids of the mouse female tract after intravaginal immunization are low in comparison with titers produced by systemic immunization (1410). Adjuvants increased local immune responses to intravaginal immunization in mice as they are likely to damage the uterine epithelium thus allowing antigen to reach lymphoid cells and vessels in stroma (1409).
However, in 1973, **Ogra and Ogra** observed that immunization with inactivated poliovirus placed in the uterine lumen resulted in IgG antibodies in uterine secretions but not in serum (1041).
More recent studies with the potent mucosal immunogen cholera toxin (CT) B yielded analogous results. In one study by **Kozlowski et al**. women were immunized three times either orally, rectally, or vaginally with a cholera vaccine containing the recombinant CTB subunit with or without killed *Vibrio cholerae* cells (743, 744). All three immunization routes increased levels of specific IgG in serum and specific IgA in saliva to similar extents. Only vaginal immunization significantly increased both specific IgA and specific IgG in both the cervix and the vagina but did not generate antibodies in the rectum (743, 744). As the quality of humoral immune response is significantly influenced by the time of immunization during the menstrual cycle, it may be necessary to administer vaccine during the follicular phase of the menstrual cycle in case of the vaginal route (745).

b) Rectal immunization

Follicular structures analogous to Peyer`s patches are also found in the appendix and the large intestine, with especially pronounced accumulations in the rectum. The fact that the distribution of plasma cells produces almost equal amounts of IgA1 and IgA2 subclasses in the large intestine and female genital tract suggests that rectal lymphoid tissue may be an important source of IgA precursor cells destined for the genital tract.
The potential importance of rectal lymphoid tissues as an inductive site for stimulation of humoral immune responses in the human female genital tract has been subject of several studies (272, 743, 744, 745, 760).

In the studies by **Kozlowski et al**., rectal immunization was superior to other routes for inducing high levels of specific IgA and IgG in rectal secretions but was least effective for generating antibodies in female genital tract secretions (743, 744). In the trial of **Crowley-Norwick et al**. with influenza virus, rectal immunization induced significant increases in the concentration of flu-specific IgA but not of IgG found in cervical secretions within 28 days after vaccination (272). Rectal administration did not induce significant IgA responses, and only small flu-specific IgG increases in serum. Six months after administration, IgA and IgG flu-specific antibody concentrations were significantly higher than baseline levels in vaginal/cervical secretions.

c) Nasal immunization

Animal studies also emphasized the importance of the inductive sites in the nasal cavity for the generation of mucosal, including genital, and systemic immune responses that may exceed in magnitude those induced by oral immunization (1227).
Limited studies on nasal immunization performed on women suggested that intranasal immunization with CTB or CTB-*Vibrio cholerae* vaccines induced respectable titers of anti-CTB IgA and IgG antibodies in vaginal washings and cervical fluid (111, 625, 1222).
The results of another study comparing nasal and vaginal immunization with CTB showed that both resulted in significant IgA and IgG anti-CTB responses in serum (627). Only vaginal vaccination given on days 10 and 24 in the cycle induced strong

specific IgG and IgA antibody responses in the cervix whereas modest responses were seen after nasal vaccination. Nasal vaccination was superior in inducing a specific IgA response in vaginal secretions, giving a 35-fold increase, while vaginal vaccination only induced a 5-fold IgA increase. It was concluded that a combination of nasal and vaginal vaccination might be the best vaccination strategy for inducing protective antibody responses in both cervical and vaginal secretions, provided that the vaginal vaccination is given on optimal time points in the cycle (627).

When mice were immunized intravaginally with a bacterial protein antigen coupled to CTB subunit plus CT as adjuvant, weak specific antibody responses in both IgA and IgG isotypes were detected in vaginal wash fluids 7 days after the last of three immunizations (1537). No antibodies were detected in saliva and only low levels of IgG antibodies were found in the serum. In contrast, when the same immunogen was administered without adjuvant intranasally, mice developed substantially greater levels of IgA and IgG antibodies in vaginal fluids, and also IgA antibodies in saliva as well as IgG and IgA antibodies in serum. Analysis of the molecular forms of IgA antibodies in murine vaginal washes indicated that these were predominantly polymeric and similar to those found in saliva, consistent with S-IgA, although smaller amounts of possibly monomeric IgA were also present (1537).

d) Systemic immunization

Systemic immunization is of unique relevance to the induction of the humoral immunity in the female genital tract. The significant contribution of IgG from the circulation to the pool of antibodies in the genital tract secretions clearly indicates that parenteral immunization may be of considerable value (1227). Indeed, systemic vaccination with the inactivated influenza virus TT elicited specific IgG antibodies in vaginal and cervical secretions (272). **Thapar et al**. immunized female mice at two parenteral sites in the pelvis which generated significant IgG and IgA titers in vaginal fluid (1410).

5. Immunology of menstruation – menstruation as an inflammatory process

In order to be prepared for implantation, the endometrium undergoes predictable, sequential phases of proliferation and secretory changes. Menstruation results from partial breakdown of the superficial or functionalis layer of the endometrium and ends in almost complete loss of the functionalis due to a fall in estrogen and progesterone. Re-epithelialization occurs simultaneously with the tissue destruction and is followed by regeneration of the stromal components (1248).
This also occurs by withdrawal of exogenous hormones as in the case of contraceptive use or by administration of progesterone receptor antagonists such as mifepristone at the appropriate stage of cycle (424). However, although levels of these hormones fall significantly with corpus luteum degeneration in all mammals with oestrous cycle, only women and some primates menstruate. The molecular mechanisms by which steroids induce these changes involve interactions between the endocrine and immune system.

a) Classic concepts of menstruation

Markee examined the morphological changes in autologous endometrium transplanted to the eye of the rhesus monkey in the year 1940 (873). Prior to menstruation there was vasoconstriction of the spiral arterioles followed by vasodilatation. His thesis was that the tissue destruction occurred by necrosis coming from anoxia.
Already 1961 it was observed that menstruation still occurred in primate atrophic endometrium without such coiled arteries (553). Overall endometrial blood flow is not reduced just prior to menstruation and the thesis of the presence of anoxia has not been demonstrated in human endometrium.

Later on it has been demonstrated that the onset of bleeding is clearly not the first significant event in the process. Prior to bleeding as the first outward sign of menstruation, one can see degeneration in the basal lamina supporting the decidualized endometrial cells and the endothelium of blood vessels in the late luteal phase of the cycle (1201). Small lesions in the luminal epithelium have been observed on day 28 of the cycle followed by degeneration of the functionalis layer

(838). The first event in menstruation seems to be the degradation of extracellular matrix which leads to a loss of blood vessel integrity resulting in bleeding. The disruption of luminal epithelium then allows the escape of blood into the uterine lumen.

Since the 1980s the concept of menstruation as the result of an inflammatory event has been coming up (413).
Several features during the late secretory phase involving the presence of tissue oedema and decidual cells and the influx of migratory cells have been observed as well as leukocyte invasion and subsequent production of inflammatory mediators. The more recent identification of a variety of types of inflammatory cells using specific markers and also of chemokines and MMPs in premenstrual and menstrual tissue strongly supports this hypothesis.
Current views of the mechanism of menstruation fall in two different categories, that supporting a key role for vasoactive substances and that proposing a central role for tissue destruction. Both theories fit the concept that menstruation is initiated at the stage when cells bearing proinflammatory molecules dramatically increase in number in the tissue (1249). During the immediate premenstrual phase the progesterone withdrawal induces expression of uterine cytokines that attract leukocytes into the uterine environment and the expression of MMPs by both endometrial and leukocytic cells. However, the knowledge of the exact local mechanisms involved is still incomplete.

b) Distinct features of endometrium during menstruation

During the secretory phase one of the characteristic features is a progressive rise in the number of apoptotic cells within the endometrial glands (1381). Withdrawal of factors such as ILs as well as of steroid hormones leads to apoptosis in steroid-sensitive tissues. Estrogen regulates proliferation and apoptosis in endometrium as studies on animals showed; its withdrawal inducing apoptosis (1384).
Secretory epithelium loses its integrity during the menstrual phase. The loss of key proteins in cell-cell adhesion may be responsible for the loss of integrity of the epithelial lining in endometrium during menstruation.
Menstruation is also associated with a compromise in vascular integrity. (1384). Progesterone withdrawal also involves a severe constriction of the spiral arteries with consequent hypoxia in the regions closest to uterine lumen (873). There have

been proposed other endometrial vasoconstrictors besides prostaglandins such as endothelins and angiotensin II whose activity was increased in perivascular stromal cells around spiral arterioles in the secretory phase (12).

Kelly has proposed two different phases of menstruation (686). Vasoconstriction and cytokine changes at the onset of menstruation are initiated by progesterone withdrawal and probably reversible. Subsequent activation of lytic mechanisms due to hypoxia is then inevitable. This latter phase seems to be progesterone independent and involves cells that may not express progesterone receptor such as uterine leukocytes.

Hypoxia stimulates local mediators such as the angiogenic vascular endothelial growth factor (VEGF) in endometrial stromal cells. VEGF, a heparin-binding glycoprotein, is a very potent mitogen for endothelial cells, and induces vascular permeability and acts as a chemoattractant for monocytes. It is also one of the most potent angiogenic factors and is produced by monocytes, macrophages and smooth muscle cells. It has been shown that VEGF protein is localized predominantly in endometrial glands (1299). Estradiol increases the expression of VEGF gene in normal human endometrium. Hypoxia, IL-1, PDGF and TGF-β, EGF, and prostaglandin E2 are other factors known to up-regulate VEGF expression. VEGF also induces expression of certain MMPs in the endometrium (268).

Endometrium repair starts as early as 36 hours after the beginning of menstrual bleeding while tissue breakdown is still in progress. Macrophages are partly responsible for the removal of detritus (1248). Cytokines and growth factors produced by inflammatory cells induce the process of wound healing. Macrophages, for example, express TGF-α, TGF-β, fibroblast growth factors, PDGF, VEGF and activin β_B (877).

(1) Non-resident cellular components of human endometrium

Both the extracellular matrix composition and the cellular components of the human endometrium change parallel with the hormonal changes during the normal menstrual cycle (1248).

Recently, the contribution of infiltrating cells of lymphomyeloid origin has been recognized. The population of various lymphomyeloid cells varies with the different phases of the cycle and has their highest levels during the premenstrual and menstrual phases. The distribution of inflammatory cells in the endometrium at three stages of the normal menstrual cycle shows Table 16.

	Proliferative phase (days 10-12)	Secretory phase (days 22-23)	Menses (days 26-28)
Macrophages	+	++	+++ (6-15%)
Eosinophils	-	-	++ (3-5%)
Neutrophils	-	-	+++ (6-15%)
Mast cells	++	++	++ (3-5%)
T lymphocytes	+	+	+ (1-2%)
B lymphocytes	+/-	+/-	+
NK cells	-	+/++	+++ (5-6%)

Table 16: Relative distribution of specific inflammatory cells in the functional endometrium at three stages of the normal menstrual cycle, with their percentage of total endometrial cells at menses (1249)

Evidence has emerged that at any time subsets of these cell types, represented by different phenotypes, are present in the endometrium and that apparently similar cells in the endometrium can be functionally different from those in the blood of the same individual (1497). Thus at least some of the cells which traffic into the endometrium must change in phenotype in response to the new environment.
However, there is no doubt that during the perimenstrual and menstrual phase there is a significant influx of inflammatory cells into the endometrium comprising up to 40% of total cell number within the functional endometrium.

Leukocyte population in the endometrium is dynamic. Fluctuations in the number of cells are probably a consequence of migration from the blood, cellular proliferation within the tissue, apoptosis and cell loss during the shedding of the functionalis. Eosinophils and neutrophils become apparent in the endometrium during the premenstrual phase only whereas increased numbers of macrophages and NK cells can be found earlier in the cycle (Table 16, 1250).

Neutrophils

Neutrophils, the most abundant leukocytes in the human, are closely related to tissue damage in inflammatory disorders.
During most of the cycle, neutrophils are barely detectable in normal endometrium but the amount raises perimenstrually, comprising about 6-15% of the total cell number at this time (1249). Densities compared to those seen during menses are also reached in areas of endometrial breakdown in patients treated with progestins (1326).

Neutrophils may aid in endometrial breakdown as neutrophil granules release elastase and membrane-bound MT1-MMP by chemotaxis which both are able to activate MMPs 2 and 3 (1579). The degranulation of neutrophils also releases antimicrobial contents such as defensins, SLPI or the neutrophil protease inhibitor and microbicide related to SLPI, elafin which also peaks at menses (706). However, not all neutrophils have been observed to be immunopositive for MMP-9, activin β_A which is responsible for cellular differentiation and MT11-MMP (1248) which suggests that there is more than one phenotype.
Endometrial neutrophils are also capable of producing IFN-γ both *in vitro* and *in vivo* (1559), especially in the endometrial stromal layer. This production of IFN-γ also shows no variation during the menstrual cycle.

Eosinophils

Eosinophils have been detected in the human endometrium by immunolocalization of eosinophil cationic proteins (ECP) 1 and 2. They are also absent from normal endometrium during most of the cycle but immediately prior to menstruation increase dramatically in their numbers. Most of them are found in aggregates and the extracellular localization of the ECP suggests activation of the cells (623). Also some eosinophils are positive for MMP-9 and also the eosinophil chemoattractant, the chemokine eotaxin, and its receptor CCR3 were localized in the endometrial cells (1580).

Macrophages

Macrophages (CD68+) are present throughout the cycle with an increase in the early secretory phase and a further increase in the late secretory phase (1250). On days 27 to 28 their numbers are similar to those of neutrophils comprising approximately 6-15% of all cells in the functionalis at this time (1249). These cells are distributed throughout the tissue with some aggregates and some concentration around endometrial glands. Increased numbers of macrophages are found in progestin-exposed endometrium, particularly in association with abnormal uterine bleeding (236). Macrophages also show phenotypic differences concerning the expression of MMP-9, activin β_B and MT1-MMP (1248).

Mast cells

Mast cells secrete many vasoactive and proinflammatory molecules and participate in inflammation through vasodilatation and enhancing leukocyte infiltration as well as causing direct tissue damage through their proteases.

Mast cells are to be found in endometrial tissue throughout the cycle without any changes in their number or distribution. However, by detecting the mast cell-specific proteases tryptase and chymase one can tell that there are defined phases of mast cell activation. Activated mast cells are seen in the mid-proliferative (days 10-12) and mid-secretory phase (days 20-23) phase and immediately prior to and during menstruation coinciding with the phases of tissue oedema (623). The mid-secretory activation occurs at a time when implantation would be initiated in a fertile cycle.

Two different phenotypes have been detected in the endometrium; those in the functionalis are positive for tryptase and negative for chymase while those in the basalis contain both mast cell-specific proteinases.

Thus, tryptase, more than chymase, seems to have an important function in the process of shedding of the functionalis (1249). It can activate proMMP-3, a key enzyme in the activation cascade of MMPs, and could be an important factor in degradation of the interstitial matrix through its actions on collagen type VI. Endometrial interstitial stroma contains a matrix composed of collagen type I, III, V and VI and fibronectin whereas basement membrane structures are composed of laminin and collagen type IV. As compositional changes during menstruation includes the depletion of collagen VI during periimplantation period and at times of oedema (623), tryptase seems to be of immediate relevance to the stromal disruption and oedematous changes that apparently coincide with endometrial mast cell degranulation.

Mast cell products histamine and heparine mediate changes in endometrial vasopermeability, bleeding and angiogenesis. Mast cell activation also has the potential to stimulate the production of matrix-degrading metalloproteinases through their expression of ILs and TNF-α.

NK cells

NK cells are among the most numerous haematopoietic cells in perimenstrual endometrium. Only few can be detected during the proliferative phase but numbers increase during the secretory phase and their numbers rise up to 15% of the total number of cells in the stroma perimenstrually (1249). They are found distributed throughout the stroma and in intraepithelial locations. They are present during the decidual changes and the very large number of these cells found in the decidua of pregnancy suggests an important role in the decidualization process (703). In contrast, the death of uterine NK cells could be an early event in the onset of endometrial breakdown at menstruation. In premenstrual endometrium, NK cells

undergo morphological changes with their nuclei becoming pyknotic and fragmented. Probably they influence the critical decision that the mid- to late secretory endometrium has to make either to decidualize or to undergo menstruation.

Due to their expression of $CD56^{bright}$, they seem to be relatives of the $CD56^{dim}$ CD16+ NK cells in peripheral blood (697). Compared to peripheral NK cells, EGLs have cytotoxic activity from the late proliferative phase on (637). At this phase the proportion of ELGs expressing the activation antigens CD69 and HLA-DR is highest and decreased during the menstrual phase (727) suggesting anti-infectious abilities. EGLs contain perforin, granzyme A, T cell intracytoplasmatic antigen-1 granules and MT1-MMP (1248) which all can contribute to cell and tissue degradation.

Lymphocytes

CD3+ T lymphocytes are present in the endometrium throughout the menstrual cycle and their numbers increase prior to menstruation. Their total numbers are much less than those of other leukocytes with only 1-2% of the total number of cells (1249). Concerning their distribution they are found as basal lymphoid aggregates, in intraepithelial sites and scattered throughout the stroma.

The CD4+ to CD8+ ratio in endometrium is inverted when compared with peripheral blood T cells (66% of CD8+ to 33% CD4+ in endometrium) (1248). The cells are cytolytically active during the proliferative phase but not in the same manner in the secretory phase which could mean that progesterone may downregulate its activity (1497). T cell activity may be related also to local secretion of cytokines such as IFN-γ, which is apparent in lymphoid aggregates in the stratum basalis but less consistently in functionalis throughout the cycle. Other T cell activation markers as CD69 and DR are similar in both proliferative and secretory phase and suggest that T cells are in a state of persistent activation in the activation (213). At the time when cytolytic activity is low in these cells in the endometrium it is high in such cells within the blood, indicating that endometrial CD3+ cells are functionally different. Subsets of endometrial T cells also express MMP-9 (1248).

CD45RA+ B lymphocytes can also be detected in low numbers during the entire cycle and are present in perimenstrual tissue in clusters among stromal cells (1249). So far Ig synthesis or its specificity at this site remains unknown.

(2) Regulation of leukocytes in endometrium

Steroid hormones

Although there is also the possibility of leukocyte regulation by estrogen, the greatest chances in leukocyte numbers and activation occurs during the changes in progesterone level. Leukocyte numbers are negatively regulated by progesterone in an ovine endometrium (471) while the influx is coincident with the fall in progesterone.

It seems to be the PR-expressing cells such as stromal, epithelial and endothelial cells that regulate the influx and activation of leukocytes. These act as effector cells that mediate the destruction and remodelling of the endometrial tissue together with factors produced by resident endometrial cells (1248).

The question arises how inflammatory cells differentiate to different phenotypic subsets in the endometrium and how are they activated to release their mediators at appropriate time and place for menstruation. Besides entering the endometrium from the blood, some leukocytes, especially granulocytes, have been observed to proliferate in the endometrium, mostly during the secretory phase (1383). CD45+ T cells, macrophages and CD56+ NK cells express the proliferation markers Ki67 and BrdU within endometrium throughout the menstrual cycle with a marked increase in the secretory phase.

Endometrial receptors of both hormones are upregulated during the follicular phase by ovarian estrogen and subsequently downregulated in the luteal phase by progesterone (268). The predominant form of the ER in all cell types in the uterus is ERα with only weak expression of ERβ (883). ERα expression in the functionalis increases in both glandular and stromal cells in the proliferative phase and declines in the secretory phase due to progesterone suppression (266). ERβ, however, has also been detected in vascular endothelial cells, which suggests a direct influence of estrogen on endometrial blood vessels. Such direct effects may be involved in endometrial angiogenesis and vascular permeability changes during the cycle.

The PR isoform PRA is present in human glandular and stromal cells during the proliferative phase but is present only in stromal cells by the end of the secretory phase (1474). The other isoform PRB is also detected in both cellular compartments in the proliferative phase and absent in the late secretory phase.

In rodents, however, steroid hormone receptors have been identified on several immune cell types with low binding affinities but numerous steroidal effects which

would support direct actions (940). Studies in sheep suggest that endometrial leukocytes are negatively regulated by progesterone, either directly or indirectly (471).

The finding of PR on leukocytes would confirm this hypothesis but most studies show a lack of evidence for PR or ER expression on endometrial leukocytes which suggests that effects of steroid hormones on human leukocytes may only be indirect (708, 1356). Except the study of **Paldi and colleagues** (1065) there was also no evidence for PR-encoding mRNA in peripheral blood lymphocytes. However, there are PR-independent effects of progesterone which still have to be investigated in the case of endometrial leukocytes.

Cytokines and adhesion molecules

Leukocyte migration into the endometrium is likely to be mediated by chemokines, which bind a large family of G-protein-coupled receptors on their target cells. In addition to their role in cell migration, they also stimulate degranulation and promote angiogenesis.

Most of these intercellular mediators are multifunctional and synergistic as well as antagonistic properties exist for specific cytokine combinations. Different chemokine receptors expressed on the same cell can induce specific signals, giving weight to the theory that the receptors couple to distinct pathways (1412). Steroid hormones could modulate the influx of inflammatory cells via actions on chemokines.

Most chemokines are stimulated by IL-1 and inhibited by glucocorticoids (105). Glucocorticoid receptors are similar to PRs; both belong to a superfamily of homologous transcription factors. Therefore it is highly likely that progesterone could act in progesterone-dependent tissues in a similar manner than glucocorticoids in other tissues.

Each of the non-resident cells in the endometrium described above is able to synthesize and release a variety of cytokines and growth factors. The distribution pattern of chemokines in the normal endometrium (Table 17) supports their role in the influx of inflammatory cells prior to menstruation although there are partly inconsistent data.

IL-8 was detected in the surface epithelium and glands but not in stromal cells (47) whereas others demonstrated the presence of IL-8 in perivascular cells of blood vessels increasing in the late secretory phase (639).

MCP-1 was also detected in perivascular cells of blood vessels and was increased in both late secretory and proliferative phase (639) while other studies localized MCP-1 in ECs (635). **Kelly and colleagues** demonstrated that progesterone inhibited the synthesis of MCP-1 from cell lines (684) and synthesis of IL-8 from endometrial explants (685). Progesterone withdrawal thus can stimulate the premenstrual rise in these chemokines.

RANTES production from endometrial stromal or ECs was stimulated by IL-1 and TNF-α in vitro, but not by sexual hormones (567).

Eotaxin, a chemoattractant for eosinophils which is only detectable in the late secretory phase, was found in eosinophils, perivascular and decidualized stromal cells at this stage (1580). The highest concentrations were seen in luminal and glandular ECs throughout the proliferative and secretory phases of the cycle. Its CCR3 receptor was expressed not only by eosinophils but also by endometrial ECs. Therefore the role of eotaxin and its receptor is not limited to recruitment of eosinophils premenstrually but they may also have additional functions due to their overall expression by ECs.

These results may give the conclusion that chemokines modulate the recruitment of leukocytes into the endometrium and their expression is stimulated by the withdrawal of progesterone at the end of cycle.

Adhesion molecules are necessary for the attachment of leukocytes to endothelium and for their extravasation and trafficking through tissues.

ICAM-1 has been demonstrated in the stroma of the functionalis during the menstrual phase of the cycle (1413). This molecule was also expressed by CD3+ cells in the lymphoid aggregates in the basalis layer and was present on vascular endothelium throughout the cycle with an overall peak in expression at menstruation. ICAM-2 expression was just detected in vascular endothelium without changes during the menstrual cycle. Platelet endothelial cell adhesion molecule (PECAM) was also stained in stroma during menstruation and in endothelial cells of all vessel types (1397). VCAM-1 and E-selectin appeared in stromal cells in the upper functionalis in the secretory phase (1380). It seems likely that unique cell and site-specific expression of adhesion molecules in the endometrium may account partly for the distinct distribution of leukocytes.

	Localization in endometrium	Cyclic expression
Chemokines		
IL-8 (47, 639)	Surface epithelium, glands, perivascular	Increase in late secretory phase
MCP-1 (501)	Epithelial>stromal>vascular	Proliferative and secretory phase
MCP-2 (501)	Epithelial	Proliferative and secretory phase
RANTES (567)	Stromal	
Eotaxin (1580)	Epithelial>decidualized stromal cells/perivascular/eosinophils	Proliferative and secretory phase
MIP-1α (17)	Epithelial	Proliferative and secretory phase
Adhesion molecules		
ICAM-1 (1413)	Epithelial, vascular, stromal	Entire cycle, peak at menses
ICAM-2 (1413)	Vascular endothelium	Entire cycle
PECAM (1397)	Vascular endothelium, stromal	Peak at menses
VCAM/ E-selectin (1380)	Glands Vascular endometrium, Stromal	Entire cycle Secretory phase Secretory phase

Table 17: Distribution of different chemokines and adhesion molecules in endometrium during the menstrual cycle

(3) Matrix metalloproteinases

The integrity of endometrial tissues is maintained by cell-cell and cell-matrix interactions and binding as well as an intact fibrovascular meshwork. Shedding of the endometrium seems to require participation of factors that display the ability to cause the breakdown of these cell-cell and cell-matrix adhesions and compromise the integrity of the fibrovascular stroma.

MMPs are a family of highly homologous endopeptidases that degrade components of both interstitial and basement membrane extracellular matrix (117). The MMPs can be divided into major subfamilies; collagenases, gelatinases, stromelysins and MT-MMPs. Most of them are secreted as zymogens that can be activated *in vitro* by a number of natural proteases and can be inhibited by specific inhibitors of metalloproteinases (TIMPs) by the formation of 1:1 complexes (117).

The sources of various MMPs and TIMPs in the human endometrium gives Table 18.

Most of the MMPs such as MMP-1, -2 and -3 are produced by endometrial stromal/decidual cells except MMP-7 which is an EC product and MMP-9 which is mainly produced by leukocytes including eosinophils, neutrophils and macrophages (1209). MMP-2 and MT1-MMP are distributed more widely and are found in almost all cells in endometrium (1579).

MMPs are regulated at the transcriptional level by a variety of cytokines, growth factors and steroid hormones by tissue and cell-specific mechanisms. The patterns of expression of MMPs during the menstrual cycles are different in normal human endometrium. MMP1, MMP-3, MMP-7, MMP-9 and MT1-MMP mRNA and protein are all substantially increased immediately prior to and during menstruation (1209, 1579) which let regard them as the mediators of tissue breakdown at menstruation. TIMPs have been demonstrated to be present throughout the cycle and can be localized in most cell types (1582).

MMP	Localization in human endometrium	Cyclic expression
Collagenases		
MMP-1 (1209)	Stroma, connective tissue cells	Increase prior and during menses
MMP-8	Neutrophils	
Gelatinases		
MMP-2 (1209)	Stroma, most cells	Entire cycle
MMP-7 (1209)	Glandular epithelium	Increase prior and during menses
MMP-9 (1209)	Neutrophils, macrophages	Increase prior and during menses
Stromelysins		
MMP-3 (1209)	Stroma	Increase prior and during menses
MMP-10 (1209)	Stroma	
MMP-11 (1209)	Stroma, epithelium	
Others		
MT1-MMP (1579)	Epithelium, most cells	Increase prior and during menses
MT2-MMP (1579)	Most cells	Increase prior and during menses
TIMPs		
TIMP-1 (1582)	Epithelium, stroma, vascular smooth muscle	Entire cycle
TIMP-2 (1582)	Epithelium, stroma, vascular smooth muscle	Entire cycle
TIMP-3 (1582)	Epithelium, stroma, vascular smooth muscle	Entire cycle

Table 18: Distribution of MMPs and TIMPs mRNA or protein in human endometrium during menstrual cycle

Leukocytes can produce MMPs and other enzymes with the potential to degrade components of the extracellular matrix (Table 19). These factors produced by leukocytes have the potential to stimulate the production of latent MMP produced by adjacent cells or participate in MMP activation.

Leukocyte	Protease	Potential substrate
Mast cell	Tryptase	proMMP-3
	Chymase	proMMP-1, pro-MMP-3
	Chymotrypsin	broad spectrum
	Plasminogen activator	plasminogen
Neutrophil	Elastase	Elastin, proteoglycans, collagens
	MMP-8	Collagens
	MMP-9	Collagens, elastin
	MT1-MMP	ProMMP-2, proMMP13
	Heparanase	Proteoglycans
	Cathepsin G	Elastin, proteoglycans, collagens
Eosinophil	MMP-1	Collagens
	MMP-9	Collagens
	β glucoronidase	Proteoglycans
	aryl sulphatase	Proteoglycans
Macrophage	MMP-9	Proteoglycans
	Metalloelastase	Elastase, collagens
	MT1-MMP	Elastase, collagens
	Plasminogen activator	Elastase, collagens
T lymphocyte	MMP-2	Elastase, collagens
	MMP-9	Elastase, collagens
NK cells	MT1-MMP	Elastase, collagens

Table 19: Leukocyte production of proteases relevant to menstruation (1248)

For example, when MMP-3 is present, a cascade of MMP activation can be initiated (1248). Besides production of plasminogen activator and chymotrypsin, mast cells produce tryptase which can activate proMMP-3 and chymase which can activate proMMP-1 and proMMP-3. Neutrophil elastase acts directly on substrates elastin, proteoglycan and collagen but they also produce MMP-8, MMP-9 and MT1-MMP themselves. Eosinophils can produce MMP-1 and MMP-9 while macrophages can generate MMP-9 and MT1-MMP. Thus activation of leukocytes at any site can result in substrate-degrading enzyme or activators of such enzymes being released into the surrounding tissue.

Studies have examined the regulation of MMP production and activation by endometrial cells *in vitro*. Endometrial stromal cells were put in culture with the human mast cell line (HMC)-1, or with peripheral blood neutrophils (1581). HMC-1 produces the mast cell products tryptase, IL-1 and TNFα. The HMC-1 cells stimulated stromal cell proMMP-1 and proMMP-3 and to a lesser extent also

proMMP-2, with an increasing stimulation as mast cell numbers increased. When cultured with peripheral blood neutrophils, proMMP-2, proMMP-3 and proMMP-9 were activated while TIMP-1 and TIMP-2 produced by stromal cells were degraded.

Withdrawal of progesterone may contribute to the recruitment and activation of leukocytes by inducing the production of cytokines and chemokines by endometrial and ECs of the endometrium. Furthermore, withdrawal of progesterone may directly promote MMP production by resident endometrial cells. Several *in vitro* studies demonstrate that progesterone is a potentially important player in regulation of MMPs in the endometrium.

Physiological concentrations of progesterone co-cultured with endometrial explants almost completely inhibited the release of both latent and active MMPs (868). **Salamonsen** demonstrated the upregulation of MMP-1, -2 and -3 productions by withdrawal of progesterone in a cell culture model (1247). There are several arguments for indirect actions of progesterone withdrawal accounting for the upregulation of MMPs at menstruation (1249). Menstruation exclusively happens in women and some primates while all mammals undergo demise of the corpus luteum in a nonfertile cycle. Furthermore, withdrawal of progesterone is an endocrine mechanism and would result in systemic actions whereas MMP production happens very focal.

There are also many local mediators in endometrial cells that modulate MMP production which are maximally produced during the late secretory and menstrual phase. These include TGF-β, TNF-α, IL-1, LIF, GM-CSF and prostaglandins (1249). *In vitro* studies support their potential for MMP regulation in the endometrium. For example, TNF-α and IL-1 are both products of epithelial and stromal cells and increase MMP-1 and MMP-3 production by stromal cells (1178).

Prostaglandins are present in high concentrations in endometrium where synthesis and metabolism of prostaglandin is regulated via estrogen and progesterone. Increased production of prostaglandin F2α induces myometrial contractions and vasoconstriction whereas other prostaglandins are vasodilatators that work together with bradykinin to increase pain and oedema (268). Cyclooxygenase 2 (COX2) is the inducible form of prostaglandin synthetase and is present in human endometrium, particularly in the menstrual phase (639).

Finally, a summary of the latest hypotheses of menstruation gives Figure 6.

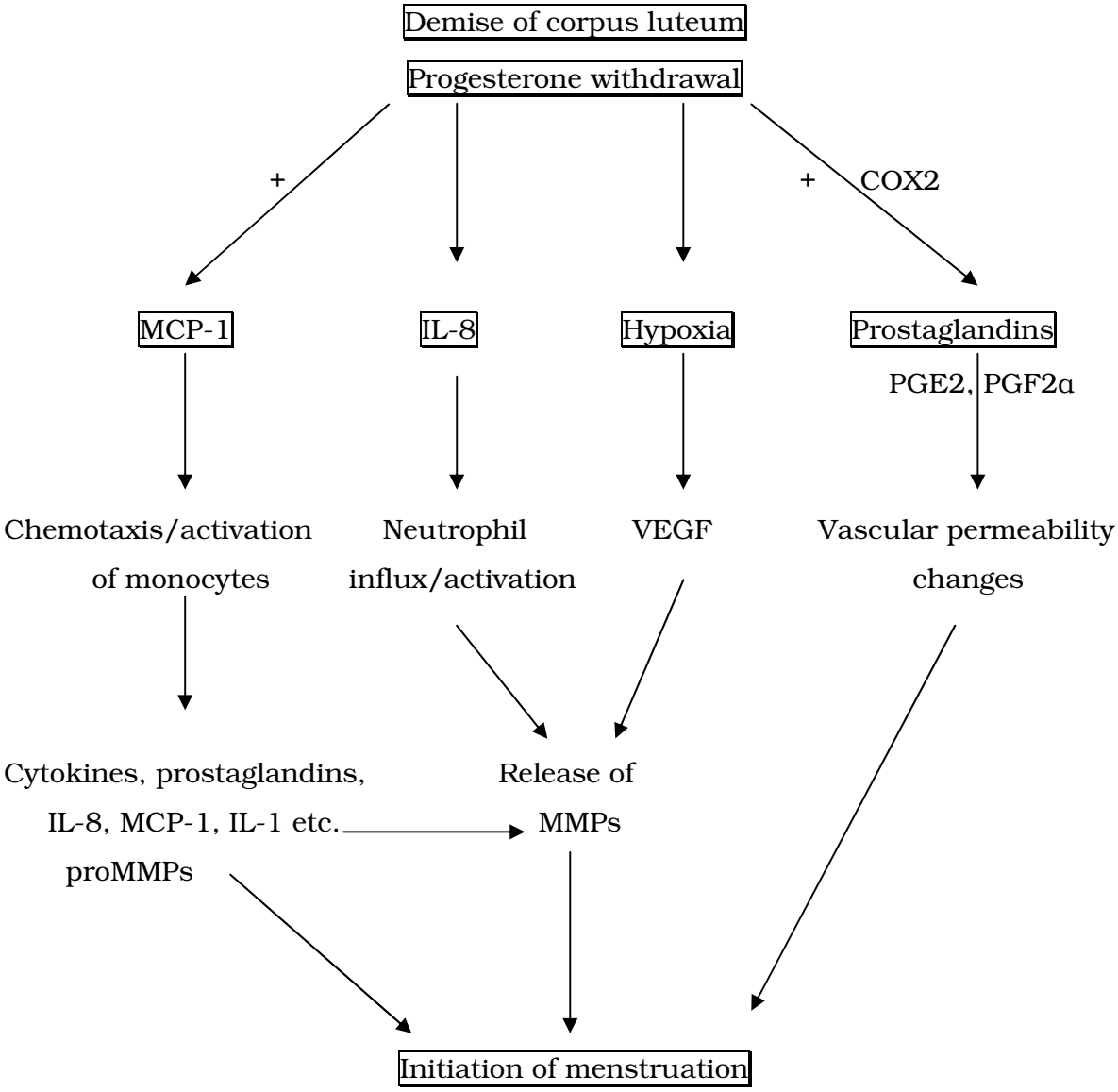


Figure 6: Summary of menstruation hypothesis (268)

C. Immunology of genital tract infections

The understanding of the immune system in the genital tract is of critical importance when considering the increasing prevalence of STDs. The World Health Organisation (WHO) estimated a global incidence of 340 million curable STDs in 1999 compared to 250 million cases in 1990 (1532). Especially in the developing world limited access to diagnosis and treatment causes STDs to be the second leading cause of healthy years of life lost by women of reproductive age (336).
In the United States, it is estimated that approximately 18.9 million new cases of STD infections occurred in the year 2000, of which 9.1 million (48%) were among young persons aged between 15 and 24 (1487). Among them, three STDs, HPV, trichomoniasis and chlamydia, accounted for 88% of all new STI cases (1487).
The epidemiology of each STD is different and dependent of many factors including individual behaviour, social conditions, pathogen characteristics and access to treatment. In the following, the latest developments and current studies on the most essential STDs should be discussed with an emphasis on immune responses and chances for vaccination.

1. Viral infections of the genital tract

a) Herpes simplex virus

The family of human herpesviridae includes the herpes simplex virus type 1 and 2 (HSV-1 and HSV-2) which are of essential clinical importance in gynecology and obstetrics (Table 20).

Alpha-herpesviridae	Herpes simplex virus 1 Herpes simplex virus 2 Varicella zoster virus
Beta-herpesviridae	Cytomegalovirus Human herpes virus 6 Human herpes virus 7
Gamma-herpesviridae	Ebstein-Barr-Virus Human herpes virus 8

Table 20: Overview of human herpesviridae (790)

HSV is an enveloped, linear, double-stranded DNA virus whose only known hosts are humans. HSV-1 and HSV-2 are distinguished by antigenic differences in their envelope proteins. There are 11 glycoproteins in HSV, which are inserted into the envelope of the virus and perform different biological functions (Figure 7).

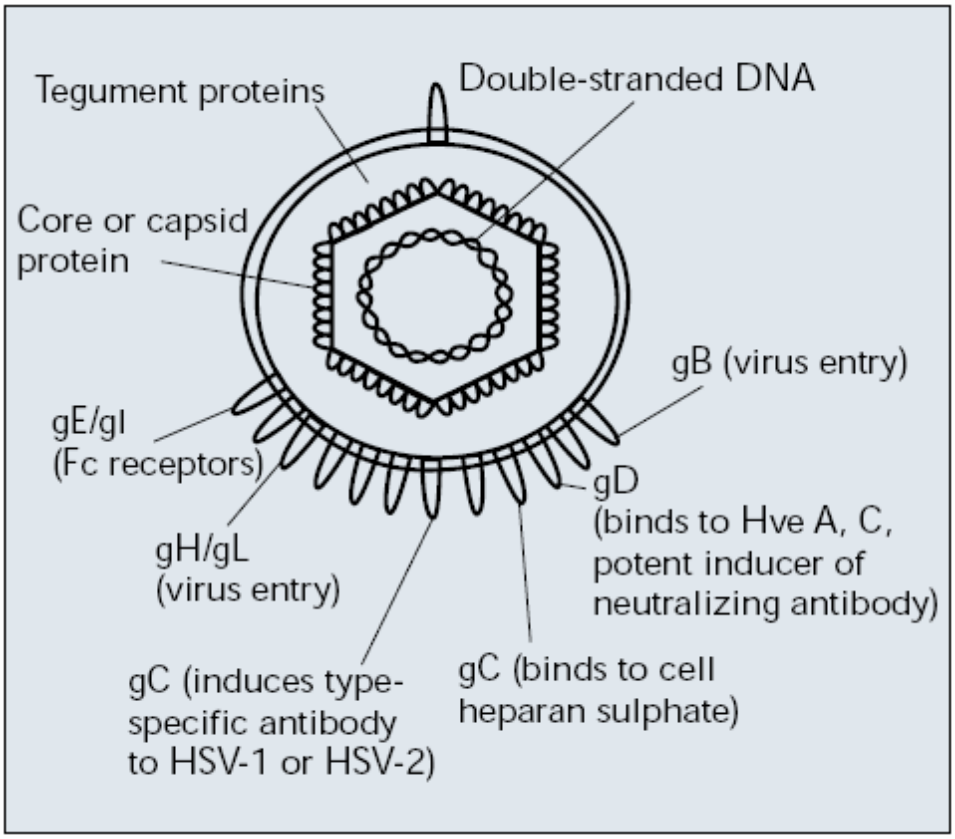


Figure 7: Structural elements and their biological functions of a HSV virion, adapted from Cunningham and Mikloska (279)

Typically, HSV infects ECs at mucosal surfaces or abraded skin. HSV-1 normally is associated with oral infections and HSV-2 with genital infections, but either type can infect a person anywhere on the skin. HSV-2 is usually sexually transmitted and is clinically manifested in the genital or adjacent area as primary or recurrent infection. HSV-1 is becoming an increasingly common cause of primary genital infection but is less commonly a cause of recurrent infection.

The natural history of HSV infection includes an acute or subclinical first-episode mucocutaneous infection, establishment of viral latency in the dorsal root ganglion, and subsequent reactivation. With reactivation, the virus travels back down the nerve root to create a mucocutaneous outbreak, or it may produce no detectable symptoms (1499).

Genital herpes infection caused by HSV-2 is one of the most prevalent STDs worldwide and is the most common cause of genital ulcers. The percentage of HSV-2 infections varies between 6% and 50% among different populations (1337). Genital herpes is more common in women than in men, with approximately one in four women versus one in five men having specific antibodies to HSV-2 (416).
Subclinical viral shedding has been documented in more than 80% of HSV-2-seropositive persons who report no lesions (1465). Only 10 to 25% of persons who are HSV-2 seropositive report a history of genital herpes, which suggests that most infected persons have unrecognized symptomatic or completely asymptomatic infections. However, once patients are told of their positive antibody status, more than 50% identify clinically symptomatic recurrences that previously were ascribed to other conditions. It is thought that viral shedding in persons who are unaware of their infection is responsible for at least 70% of HSV transmission (1465).
There is a concerning relationship between HIV and genital HSV infection because the interaction of HSV-2 and HIV-1 may result in more efficient transmission of HIV-1 and an increased rate of HIV replication during HSV reactivation (1466).

Although oral treatment with aciclovir can reduce the severity of infections, development of a vaccine to prevent or control HSV-2 infections in the genital tract would greatly contribute to preventive health care. To achieve this goal, it is important to understand the host defense mechanisms that are available at genital mucosal sites to protect against this STD.

(1) Innate immune responses in genital herpes infections

The role of immune factors in the control of HSV infection, especially recurrent lesions after viral reactivation, appears complex. There is a general difference between immune responses to primary and recurrent disease, which is clinically demonstrated in the much longer median duration of lesions and viral shedding in initial infection. There is a mean of 21 days for lesions and 10 days for viral shedding in primary infection compared to 10 days for lesions and 4 days for viral shedding in recurrent infections (279).
Extensive studies have identified responses that are important in limiting viral replication and spread to uninfected cells (347). These include IFN type 1 responses which are produced within hours after infection, and neutrophils which are found within 24 hours after vaginal infection and secrete TNF-α. Moreover, there are macrophages, NK cells and submucosal DCs which have been shown to capture

HSV-2 antigen and stimulate T cell activation in regional lymph nodes (1584). Before, basic factors of innate immune responses have also been implicated in the protection against genital HSV infection.

Acidic pH

The acidic environment of the healthy human vagina with a pH of 4.0 to 4.5 contributes to the first line of innate immune defenses in the genital tract. Therefore, earlier studies already developed acid-buffering compounds as candidate microbicides and tested anti-HSV activity provided by acidic pH (1218).

MasCasullo and colleagues found out recently that acidic buffers with a pH ≤ 4.5 irreversibly inactivated HSV-1 or HSV-2 and reduce viral yield at least 1000-fold in vitro whereas exposure to pH of 5.0 has only little effect (880).

In the next step, a vaginal acid-buffering formulation with pH 3.5 named *AmpHora* was tested in a murine model (440). *AmpHora* significantly protects mice from genital herpes when challenged with the virus delivered in human seminal fluid compared to mice that received a placebo gel.

Antimicrobial peptides

Antimicrobial peptides with their subgroups α- and β-defensins and cathelicidines have a broad spectrum activity against different microorganisms and are also presumed to contribute to antiviral activity in the genital tract.

HNP1-3 have been demonstrated to protect human cells from HSV *in vitro* (1557). Further studies indicated that synthetic HNP-4 is less active than HNP1-3 in protecting against HSV and that the EC defensins HD-5 and HD-6 also inhibit HSV-2 infection (880).

Data showed lately that CVS obtained from healthy women by CVL inhibits HSV infection (633). There was a reduction of HSV-2 infection of at least 90% if cells were cultured with CVL from healthy women compared to a control group. The anti-HSV activity of CVL was independent of age, vaginal pH and the presence of HSV antibodies in serum which suggests that CVS contribute to innate resistance to HSV-2 infection. The same study indicated that this anti-HSV activity correlated with the concentration of HNP1-3 in the fluid (633). Both CVL samples and HNP1-3 interacted with virus and prevented virus entry after binding.

Preliminary work failed to demonstrate any *in vitro* anti-HSV activity for synthetic HBD-1 and -2 but further studies are needed to assess whether HBD in cervical secretions contribute to intrinsic anti-HSV activity. The only known human cathelicidin, LL-37, has also not been tested for its anti-HSV activity (880).

These findings suggest that CVS contribute to innate resistance to HSV-2 and identify defensins as contributors to this activity (Table 21).

α-defensins	**Antiviral activity against HSV-2**	**References**
HNP1-3	Yes	1557, 633
HNP-4	Less	880
HD-5	Yes	880
HD-6	Yes	880
β-defensins		
HBD1-2	No; further studies needed	880
Cathelicidins		
LL-37	Not tested	880

Table 21: Antiviral activity of antimicrobial peptides against HSV-2

Although SLPI is known to protect human macrophages and CD4+ T cells from HIV-1 infection (907), not much has been found out about the influence of SLPI in protection against HSV. Preliminary studies showed that SLPI inhibits HSV-2 infection *in vitro,* but HSV-2 significantly decreases SLPI in cervical cell culture supernatants, implying that the virus may downregulate this antiviral protein (880).

Lactoferrin was shown to inhibit HSV-1 infection *in vitro* by interfering with the binding of glycoprotein C to cell surface heparin sulphate receptors (869). This may explain only little anti-HSV-2 activity of lactoferrin as binding of HSV-2 to cells is primarily mediated by glycoprotein B (221). SP-D was lately demonstrated in the genital tract mucosa, and may play a role in inhibiting HIV transmission as well as in protecting against other STDs (921).

Complement

As another factor of innate immunity, complement recognizes infectious agents using three different molecules. These are C1q, mannose binding lectin (MBL), and C3, which trigger the classical, lectin, and alternative pathways of complement activation, respectively. All three of these molecules are detected in vaginal secretions from healthy women (1104).

Notably, HSV has evolved several strategies to evade immune attack by the classical and alternative complement pathways, which include inhibiting activities mediated by C3, C5, and properdin. Specifically, glycoprotein C binds C3 and its activation products, C3b, iC3b and C3c, and accelerates the decay of the alternative

complement pathway C3 convertase (429). In a murine genital tract model, viruses deleted in HSV-1 glycoprotein C are less virulent (835).

Less is known about the role played by MBL and the lectin pathway as a host defense against genital herpes infection. Recent studies indicate that MBL binds to HSV-2 *in vitro* (432). Interestingly, MBL deficiency increases the generalized susceptibility of an individual to infectious diseases (432), and increased susceptibility to HIV infection in MBL-deficient individuals has been described (441).

DCs

HSV-1 has been shown to productively infect immature DCs with progressive loss of DCs and is associated with downregulation of CD1a, CD40, CD54 (ICAM-1) CD80 and CD86, which may lead to delayed activation of T cells and allows more time for replication of HSV type 1 in epidermal cells. Thus, this may be yet another novel strategy of immune evasion (932).

In contrast, **Linehan et al.** showed in a murine model that HSV-2 infection of the epithelium induces activation of the phenotype and function of the neighboring uninfected submucosal DCs (821). Intravaginal inoculation of mice with HSV-2 led to a rapid recruitment of submucosal DCs to the infected epithelium.

Subsequently, DCs harboring viral peptides emerged in the draining lymph nodes and were found to be responsible for the stimulation of IFN-γ secretion from HSV-specific CD4+ T cells (1584).

These results demonstrate a role for submucosal DCs in the generation of protective Th1 immune responses to HSV-2 in the vaginal mucosa, which is also supported by work from **King et al.** (711). LCs from the vaginal epithelium did not migrate to the draining iliac lymph nodes after intravaginal HSV-2 infection. LCs may be inhibited from performing antigen-presenting functions as a result of the lytic destruction of the epithelial layer. This notion is supported by the progressive reduction of the number of LCs in draining lymph nodes after HSV-2 infection (1584).

MasCasullo et al. have recently obtained similar results using immature human monocyte–derived CD11c+ DCs which showed limited productive viral replication and increased expression of CD86, CD83, and HLA DR as well as release of proinflammatory cytokines and chemokines after exposure to HSV-2 (880).

A recent study by **Nordström et al**. showed that blood DCs infected with HSV-2 activated CD8+ T cells which again blocked CD4+ T cell proliferation (1027). These DC can transform CD25- CD8+ T cells into Treg cells that block both antigen-specific and allogeneic CD4+ T cell activation *in vitro*.

NK cells

Several recent studies have also shown a role for NK cells, NK T cells and IL-15 as regulatory factor in an innate immune response to HSV.

Mice lacking IL-15 or NK/NK T cells are significantly more susceptible to intravaginal HSV-2 infection than control mice (58). The lack of NK and NK T cells in these mice, and as a result, lack of early IFN-γ response impairs the innate immune response against HSV-2. A following study by the same group indicated that IL-15 also has direct antiviral activity independent of NK/NK T cells in mediating innate defense against HSV-2 infection (453).

A number of examples of NK cell deficiencies in humans have been reported and are associated with an increase in herpes virus infections as well (1053).

Neutrophils

The role played by neutrophils in the innate immune response to genital herpes in human beings is not exactly known.

In a murine model, depletion of neutrophils before HSV-2 intravaginal inoculation did not increase the incidence of infection, suggesting that the small population of resident neutrophils was ineffective in preventing infection by a viral pathogen (946).

However, neutrophils did help in virus clearance from the genital mucosa after primary infection. The mechanisms by which neutrophils mediate antiviral activity are not well understood. They bind HSV virions or HSV-infected cells *in vitro* and kill them by oxygen-dependent or -independent systems (880). Furthermore, neutrophils may mediate antiviral activity through release of antiviral cytokines such as TNF-α, IFN-α, IFN-γ or oxygen and nitrogen metabolites (182).

TLRs

As mentioned earlier, the recognition of infectious pathogens like HSV-2 by the innate immune system relies on TLRs. TLR2 and TLR9 seem to be involved in cell signaling in response to HSV. TLR9 mediates HSV-2 induced IFN-α secretion from DCs (840) and HSV-1 also elicits inflammatory cytokine secretion through TLR2 (756).

A summary of the innate immune responses of the female genital tract against HSV shows Figure 8.

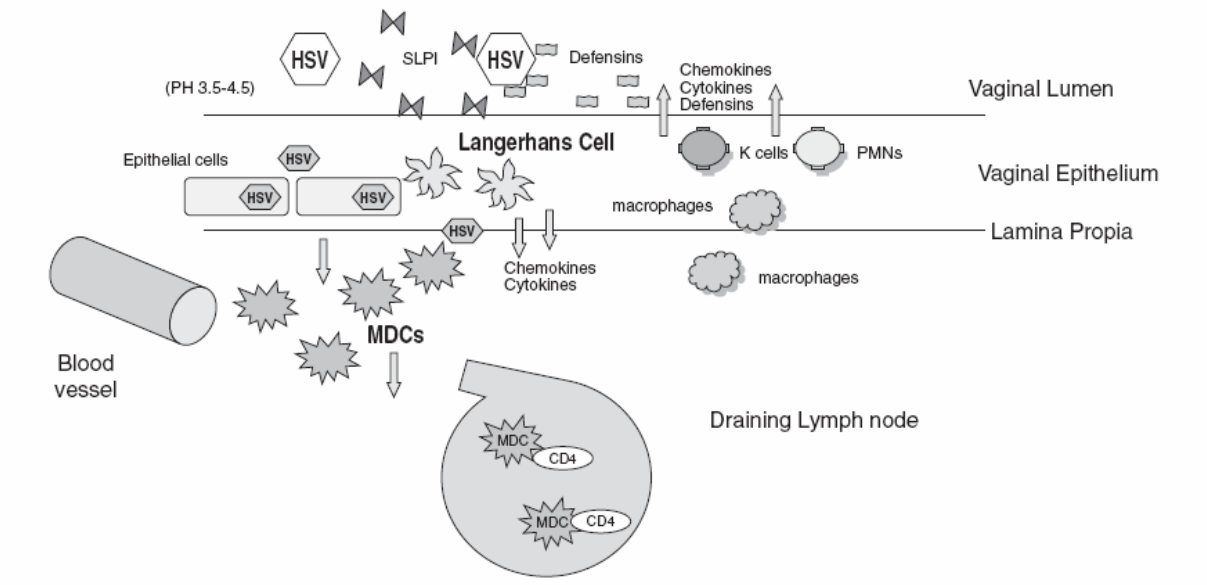


Figure 8: Mucosal innate immune response to HSV in the female genital tract. Viral particles may be inactivated by acidic pH and substances secreted into the vaginal lumen including defensins, SLPI, or complement. Resident immune cells including ECs, DCs, NK cells, and PMNs respond to viral exposure by killing virus or virally infected cells directly or by releasing cytokines and chemokines. These initial responses trigger the recruitment of additional immune cells, notably monocyte-derived CD11c+ DCs which endocytose viral antigens, mature, and then migrate to the draining lymph node to stimulate CD4+ T cells and initiate the adaptive immune response. Adapted from MasCasullo et al. (880)

(2) Specific immune responses in genital herpes infection

T lymphocytes

For decades, the questions about T cell subtypes and the specific role of neutralizing antibodies in HSV infection have been the dominating issues.

The longer prevalence of herpetic lesions in AIDS patients as well as the high frequency in patients after organ transplantation in the 1980s indicates the dominant influence of T cell responses rather than the persistence of neutralizing antibodies in recurrent infections (1301). Moreover, only very high levels of neutralizing antibodies can prevent the axonal spread of HSV-1 to epidermal cells *in vitro* contributing to control of HSV spread and shedding (936).

Mice depleted of CD4+ and CD8+ T cells prior to genital HSV-2 infection shed virus for a prolonged period, confirming a role for T cells in virus clearance (1087).

Early murine studies of the immunology of HSV infection suggested that IFNs and macrophages were an important part of the initial immune response and that the most important protective specific T-lymphocyte response was mediated by CD8+ lymphocytes. However, immunohistology of biopsies of human recurrent herpetic lesions revealed a sequence of immune cell infiltration beginning with CD4+ lymphocytes and macrophages in the first 2 days around the infected epidermal cells which also develop strong HLA-DR expression (281). This was followed by an influx of CD8+ lymphocytes, which normalized the balance between CD4+ and CD8+ lymphocytes. The CD4/CD8 ratio is not restored to that of the blood until after 2 days, indicating an early CD4+ and a later CD8+ lymphocyte influx (281).

A murine model of genital HSV infection by **Milligan et al.** was utilized to examine the local T cell response in the genital mucosa and the draining genital lymph nodes (944). HSV-specific T cells with a higher rate of CD4+ and Th1-like T cells were first detected four days after vaginal HSV inoculation in the genital lymph nodes, followed by their appearance in the genital tract one day later. Also experiments by **Kuklin et al**. with T cell subtype knockout animals and depletion with T cell subset-specific monoclonal antibody indicated that immunity following vaginal HSV challenge was principally dependent on the function of CD4+ T cells (753).

It was found out that HSV is able to downregulate MHC class I on the surface when infecting epidermal keratinocytes, which is a mechanism of immune evasion that allows the infected cell to escape scrutiny by cytotoxic CD8+ lymphocytes (733). The immediate-early viral protein of HSV, infected cell protein 47 (ICP 47), complexes with and inhibits the human transporter associated with antigen presentation (TAP) on the surface of the endoplasmic reticulum in humans but not in mice (1424). TAP is responsible for transporting small antigenic viral peptides into the lumen of the endoplasmic reticulum, where it assembles into the MHC class I peptide complex before transport to the cell surface. Inhibition of TAP inhibits the assembly of MHC class I peptide and recognition by CD8+ cytotoxic T lymphocytes (544).
However, IFN-γ, which is produced by CD4+ lymphocytes in the lesion, partly restores MHC class I expression on the surface of infected cells by the cellular production of TAP and also stimulates MHC class II expression (1427). Specific activated CD4+ lymphocytes are able to recognize infected epidermal cells and probably uninfected cells expressing MHC class II. This may result in the production of more IFN-γ or destruction of infected cells by CD4 T lymphocytes,

which is normally mediated by CD8 lymphocytes (281). The restoration of MHC class I expression on infected epidermal cells allows recognition by CD8 cytotoxic T lymphocytes, which are then apparently able to destroy the remaining infected epidermal cells.
A study by **Posavad et al.** demonstrated HSV-specific CD8+ precursor CTL in HSV-1 and -2 seropositive persons with recurrent genital herpes (1140).

A summary of the adaptive immune mechanisms with T cell priming in genital herpes infection gives Figure 9.

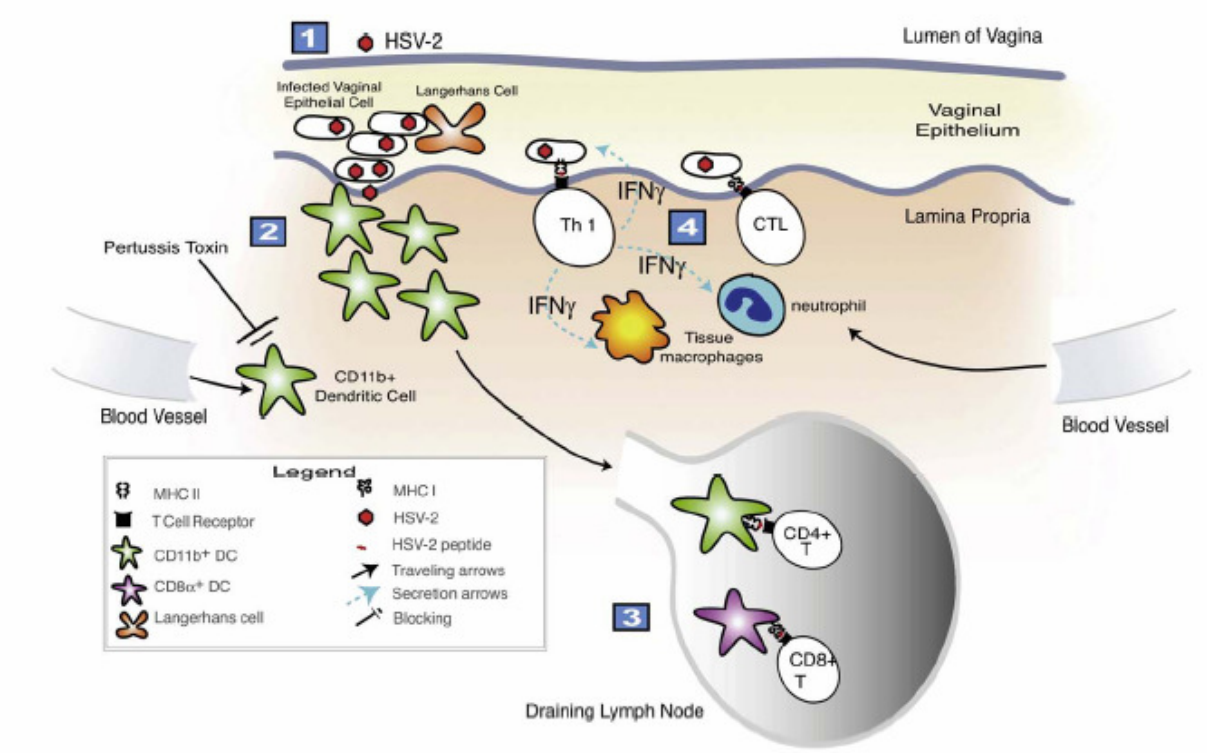


Figure 9: T cell priming in genital herpes infection, adapted from Iwasaki (604) (1) The virus replicates within the infected keratinocytes which results in the induction of signals necessary for the recruitment of the CD11b+ DCs to the submucosa beneath the infected ECs. (2) This signal likely involves chemokines, since pertussis toxin treatment of mice at the time of infection prevents DC accumulation at the infected sites. Once recruited, submucosal DCs can take up virus antigens from the infected keratinocytes and migrate to the draining lymph nodes. (3) By the time the CD11b+ DCs enter the lymph nodes, they assume an activated phenotype, expressing higher levels of costimulatory molecules such as CD80 and CD86. Within the lymph nodes, the virus antigen-loaded CD11b+ DCs can stimulate CD4+ T cells, which undergo differentiation to Th1 cells. It is possible that CD8+ T cells are also stimulated within the lymph nodes, by a separate group of DCs expressing CD8a. Once activated, the effector Th1 and CTLs exit the lymph nodes and migrate back to the vaginal mucosa, where they mediate clearance of infected keratinocytes. (4) The IFN-γ secreted from the Th1 cells has multiple effector functions, including upregulation of MHC class II molecules on the infected keratinocytes, making these cells susceptible to recognition by CD4+ effector cells. Some of these CD4+ T cells may act as CTLs, and eliminate infected cells. The IFN-γ secreted from the effector Th1 cells may also activate phagocytes such as tissue macrophages and neutrophils directly or via chemokines, resulting in the clearance of infected cells.

B lymphocytes

Studies among B cell-deficient mice have been used to further illustrate the contributions of humoral and cellular immunity in protection against vaginal infection in the HSV-2 mouse model (509, 1088). In this model, progesterone-treated adult mice are inoculated intravaginally with a thymidine kinase (TK)-deficient, attenuated strain of HSV-2. The deletion in the TK gene, which is required for the reactivation of the latent HSV-2, renders the virus only infectious during the primary mucosal infection without the neurovirulence associated with the wild-type (WT) virus.

Although B cell-deficient mice in these studies developed an early transient genital inflammation upon primary infection with TK-deficient-HSV-2 (509), during secondary challenge of immune mice with the WT-HSV-2, all of the mice cleared the infection completely (1088). Further, mice immunized with TK-HSV-2 were completely protected from subsequent WT-HSV-2 challenge (509, 1088). These results also indicate that B cells are not the critical APCs required to activate T cells following TK– HSV-2 infection.

Igs

Despite the fact that S-IgA was the predominant Ig in progestin-treated mouse vaginal mucus, nearly all specific viral antibody in HSV-immune mice was of the IgG isotype (1079). This is in agreement with the findings of **Milligan et al**. who also found predominantly IgG antibodies to the virus (943). However, these observations did not eliminate the possibility of contribution of vaginal IgA in immune protection against HSV-2, since ELISA titers of specific antibodies do not necessarily predict functional virus neutralization (1092).

The protective role of antibodies in vaginal secretions of mice that were immune to vaginal challenge with HSV-2 was further investigated by neutralization and passive transfer (1082). These neutralization studies were carried out by incubation of WT-HSV-2 in antibody preparations *in vitro*, followed by inoculation into vaginae of nonimmune mice.

The results showed that HSV-2 was effectively neutralized by both unfractionated antibody and by purified IgG from immune vaginal secretions, but not by purified S-IgA from immune secretions or by unfractionated antibody from nonimmune mice. The protective effect of IgG *in vivo* was further investigated by passively transferring purified serum IgG from immune and nonimmune donors to nonimmune recipients before vaginal challenge infection (1082). Immune IgG

significantly reduced the percentage of vaginal epithelium infected, shed virus protein concentrations in the vaginal lumen, and illness scores, even though the viral antibody titers in the serum and vaginal secretions of recipient mice at the time of challenge, were only 29% and 8%, respectively, of those in actively immunized mice.

Although Igs do not play a critical role during secondary challenge with HSV-2 in mice immunized with TK-HSV-2, passive transfer of immune IgG can significantly reduce local virus replication (1082). Further, neutralizing antibodies may be important in the prevention of viral shedding from neuronal axons to the epidermis during reactivation (936). The importance of IgG in HSV immunity is evidenced by the immune evasion mechanism employed by the virus. The HSV genome encodes glycoprotein E and I, which together comprise a high-affinity Fc receptor expressed by infected cells for the purpose of absorbing and inactivating anti-HSV IgG (998).

Data indicate that IgG antibody in vaginal secretions of immune mice provides early protection against vaginal HSV challenge infection whereas S-IgA contributed very little to protection (1082, 1085).

Concerning the origin of IgG, observations suggest that the titer of viral IgG in vaginal secretions of mice that were intravaginally immunized with HSV-2 may be higher than can be accounted for by passive transudation from serum (1079).

The relative contributions of humoral and cell-mediated adaptive immunity against HSV-2 infection in the mouse model have recently been studied. Antibody is supposed to mainly act early during immune resistance to challenge infection, whereas cell-mediated immunity primarily acts later (1088). An early role for antibody is consistent with the presence of neutralizing IgG in vaginal secretions of immunized mice (1082) whereas memory T cells, on the other hand, require several hours to secrete substantial amounts of IFN-γ in the vagina in response to the challenge antigen and may require even longer developing cytolytic activity (1091).

Women with symptomatic genital herpes have antibodies to HSV-2 of both IgA and IgG isotypes, as well as IgM in CVS (59). Another study aimed at detecting HSV antibodies and neutralizing activity in CVS of women seropositive for both HSV-1 and HSV-2 and to stratify the HSV-2-specific antibody activity according to their HSV-2 DNA genital shedding status (894). HSV-specific binding antibodies were detected at rates of nearly 70% (IgG) and 51% (IgA), with similar proportions of Igs to HSV-1 and HSV-2. The presence of detectable HSV-specific antibodies was inversely associated with HSV-2 DNA genital asymptomatic shedding and a subset

of these women (17%) had functional neutralizing activity against HSV-2 in their CVS.

(3) The role of cytokines

Both HSV-specific CD4+ and CD8+ T cell cytotoxicity have been demonstrated *in vitro* and in lymphocytes cloned from recurrent herpetic lesions *ex vivo* (728). *In vivo*, CD8+ lymphocyte cytotoxicity is presumably dependent upon upregulation of MHC class I by IFN-γ secreted from earlier infiltrating CD4+ lymphocytes. Furthermore, the mechanism for the enhancement of CD8+ T cell cytotoxicity by adjuvants in this system appears to be via increased levels of IL-12 (935). The specific infiltration of monocytes and CD4+ and CD8+ lymphocytes in sequence associated with few B lymphocytes suggests a leukocyte-specific chemotactic stimulus rather of β-chemokines than of α-chemokines which are chemoattractant for neutrophils but not for monocytes and lymphocytes.

Parr et al. have shown in the mouse model that after HSV-2 inoculation a rapid synthesis of IFN-γ by lymphocytes and macrophages was observed which occurred only in immune/challenged but not in non-immune/challenged mice indicating that it required memory T cells (1092). Data indicate that virus-specific memory T cells in vagina of immune mice encounter challenge virus antigen, rapidly secrete IFN-γ, leading to T and B cell recruitment into the vagina. Thus, IFN-γ seems to be an early and important mediator of T cell immunity in the HSV-2 infection.

The high concentration of IFN-γ in herpetic lesion fluid also suggests a Th1 pattern of cytokine response (934). The Th2 pattern leads to predominantly neutralizing antibody secretion by B cells whereas a Th1-pattern leads to production of the antiviral cytokines, IFN-γ and TNF-α, and the activation of cytotoxic CD8+ lymphocytes, through IL-12 and IFN-γ. A Th1 pattern of cytokine induction is important in the control of persistent virus infections since only CTLs are able to eradicate infected cells, and IFN-γ and TNF-α can purge infected cells of viral proteins (487). A Th2 pattern leading to neutralizing antibody production may prevent cell-to-cell transmission of the virus.

Concerning the presence of cytokines, on the first day of the lesion, high concentrations of IL-1β and IL-6, moderate concentrations of IL-1α and IL-10, and low concentrations of IL-12 and β chemokines were found; levels of MIP-1β were

significantly higher than levels of MIP-1α and RANTES. At day 3, the concentrations of IL-1β, IL-6, and MIP-1β were lower, whereas the levels of IL-10, IL-12, and MIP-1α remained similar, and the level of TNF-α was now detectable (934).

These cytokines may play a role in early recruitment, activation, and IFN-γ production of CD4+ cells in herpetic lesions. β-chemokines attract monocytes and T lymphocytes into the lesions, in particular, MIP-1β for CD4+ lymphocytes and IL-12 entrains CD4+ lymphocyte secretion to a Th1 pattern. Macrophages probably also secrete IL-12, IFNs and other cytokines within the lesion. Immune processes in a recurrent herpetic lesion show Figure 10.

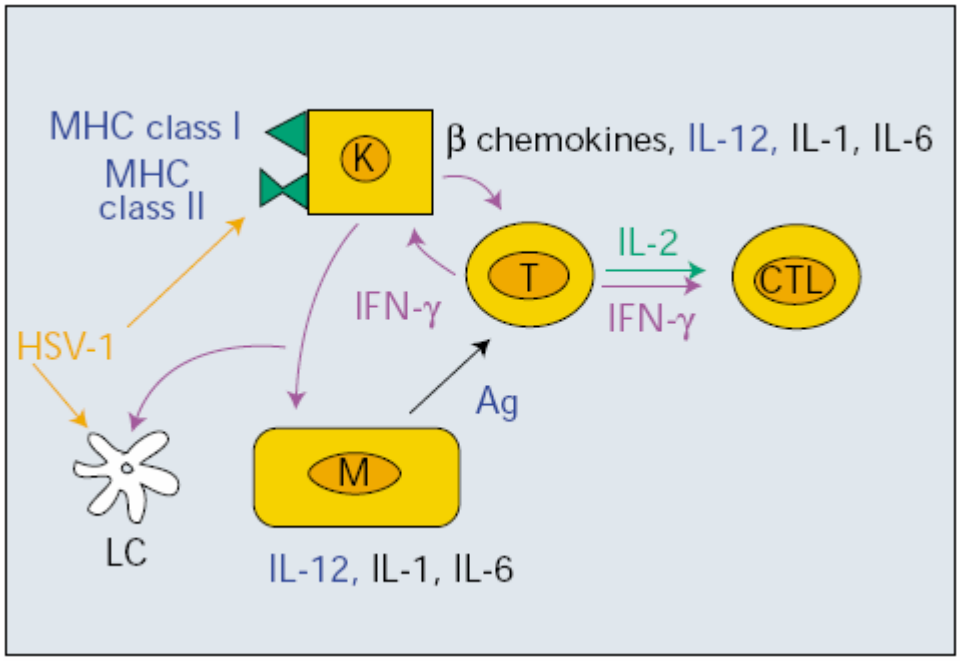


Figure 10: Immune processes and *in vivo* secretions of cytokines and chemokines in herpetic lesions; M=macrophage, K=keratinocyte, adapted from Cunningham and Mikloska (279)

(4) New immunologic therapeutic aproaches

Resiquimod

Current treatment of genital herpes focuses on the direct inhibition of viral replication. The nucleoside analogs, in particular aciclovir, dominate the treatment recommendations for genital herpes (240). Most antiviral therapy is aimed at the treatment or suppression of recurrent episodes. Both episodic and long-term suppressive therapies are prescribed, but nucleoside analogs have no long-term

effect on the disease; genital herpes episodes recur at the pretreatment rate if therapy is stopped.

However, given the limitations of current therapy directed against viral replication, a regimen utilizing an immune modulator may be more acceptable to patients. Therefore, the development of the immune response modifiers (IRMs) as a potential new treatment option for genital herpes should be briefly presented.

IRMs, as for example Imiquimod and Resiquimod, are synthesized ring-structured nucleoside structures (942). They induce certain immune cells to produce cytokines and thus, initiate the immune response which can result in antiviral or antitumor effects. Especially Resiquimod was shown to stimulate the development of Th1 immune responses in guinea pig models of genital herpes (942). Resiquimod stimulates the secretion of IFN-a, TNF-a, IL-6, IL-8 and IL-12 from various cells including DCs, monocytes and macrophages. Resiquimod induces the functional maturation of DC, LC and B lymphocytes into effective APCs and it promotes the development of B lymphocytes into plasma cells (Figure 11).

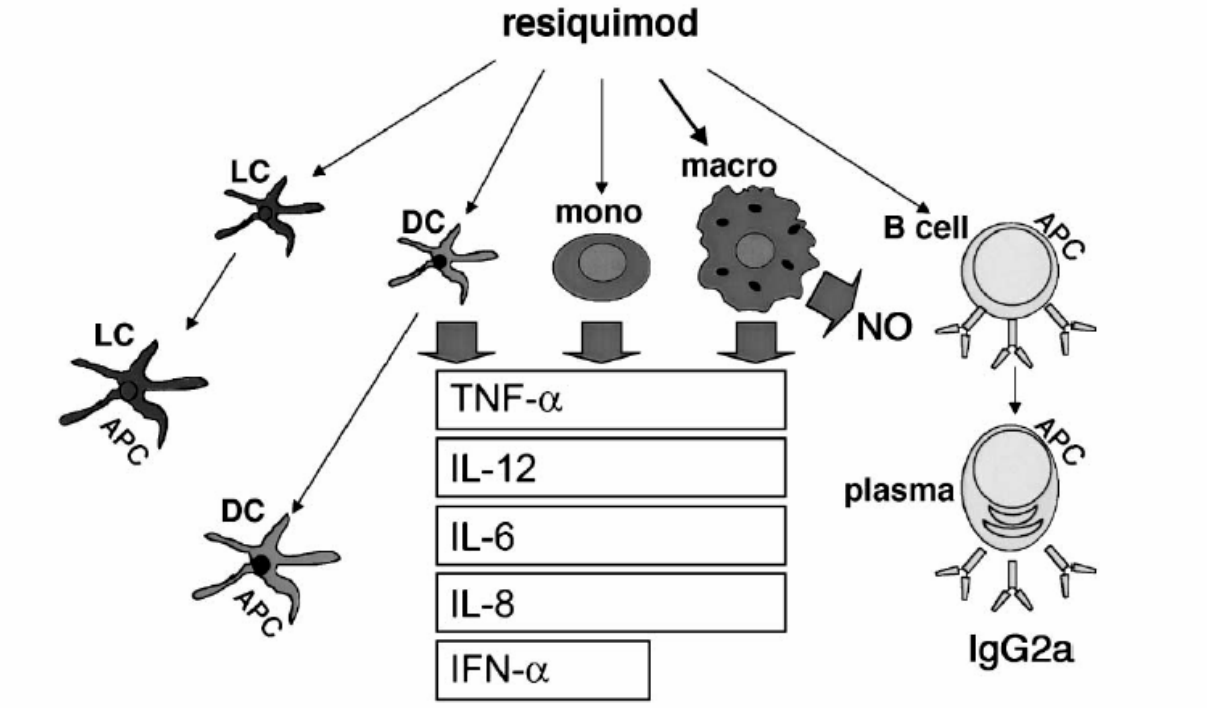


Figure 11: Stimulation of immune responses by the IRM Resiquimod, adapted from Miller et al. (942) Resiquimod stimulates the secretion of IFN-a, TNF-a, IL-6, IL-8 and IL-12 from various cells including DCs, monocytes (mono) and macrophages (macro). Resiquimod induces the functional maturation of DC, LC and B lymphocytes into effective APCs and promotes the development of B lymphocytes into plasma cells and the subsequent secretion of IgG2a. Resiquimod also stimulates nitric oxide (NO) production from macrophages

Resiquimod was shown to inhibit herpetic lesions and delays genital herpes recurrency in the guinea pig model (112). The decrease in recurrency was correlated with an increase of the *in vitro* IL-2 produced by peripheral mononuclear cells in response to HSV antigen but not with IFN levels or circulating anti-HSV-2 antibodies.

The rationale for the use of resiquimod as a treatment for genital herpes is to stimulate the secretion of certain key cytokines, e.g. IFN-a, IL-12 and TNF-α from specific cell types of the innate immune response in order to establish the HSV-specific Th1 acquired immune response.

In a randomized vehicle-controlled Phase II clinical study on 52 patients with a history of six or more recurrences per year, topical application of resiquimod reduced recurrences of genital herpes even after cessation of dosing (1333). The median time to the next recurrence was 169 days in the resiquimod-treated group in comparison to 57 days in the vehicle-treated group. The percentage of patients without a recurrence during the 6-month observation period after dosing was 6% in the vehicle-treated group compared to 32% in the resiquimod-treated group.

This apparent continued post-treatment benefit of resiquimod could be an important improvement to current treatments for recurrent genital herpes, such as oral anti-HSV nucleosides, which do not appear to have post-treatment benefit. However, Phase III clinical trials were suspended due to the lack of efficacy of resiquimod gel in recurrent genital herpes lesions (1333). It may have a comeback as a vaccine adjuvant for viral infections that require a strong Th1 immune response.

CpG oligodeoxynucleotides

Also recently, local vaginal delivery of CpG containing oligodeoxynucleotide (ODN), a synthetic mimic of bacterial DNA and TLR9 agonist, holds substantial promise as a strong inducer of innate immunity against genital herpes infections in the animal models of the disease.

As mentioned, TLRs are responsible for recognition of conserved molecular structures named PAMPs such as LPS, bacterial DNA and viral double-stranded RNA in microbes. Interaction between CpG motifs in microbial DNA and TLR9 in APCs stimulates the responding cells to produce proinflammatory cytokines such as IFN-γ and IL-12 through the Toll/IL-1-receptor signaling pathway and to activate B cells for proliferation and antibody production (748). Among PAMPs, synthetic

analogues of bacterial DNA, termed CpG ODN, have shown a great promise in mobilization of protective immunity against different pathogens (508).

Harandi et al. demonstrated in a mouse model that a single intravaginal dose of CpG ODN induces production of Th1-type cytokines IFN-γ, IL-12 and IL-18, CC chemokines RANTES, MIP-1α and MIP-1β and CXC chemokines IP-10 and MIP-2 in the genital tract mucosa as well as a dramatic increase in the total cell numbers of B cells, NK cells, NKT cells and T cells in the genital lymph nodes (507).

Studies have proven a protective effect of prophylactic delivery of CpG ODN against primary genital herpes in mice and have showed that the observed protection can lead to the development of a HSV-2 specific memory response affording sterilizing immunity against reinfection (55). Also a therapeutic effect of topical application of CpG ODN on genital herpes was demonstrated in both mouse and guinea pig models of the disease (1149).

(5) Vaccination strategies

Despite antiviral therapy, HSV-2 is a suitable target for a vaccine because the virus causes lifelong infection and significant medical morbidity. A vaccine has the potential to reduce HSV acquisition, disease severity and the number of neonatal herpes cases. However, efforts to develop vaccines to protect women against HSV-2 and other sexually transmitted pathogens would be facilitated by a better understanding of the immune mechanisms that protect the female reproductive tract against such infections.

Vaginal versus nasal/parenteral immunization

In the progestin-treated adult mouse model by **Parr and Parr** (1092), intravaginal inoculation of WT-HSV-2 caused infection of the vaginal epithelium followed by lethal neurological illness. Intravaginal inoculation of an attenuated strain of TK-depleted HSV-2 caused epithelial infection but did not lead to neurological illness (1086). Importantly, vaginal infection with the attenuated strain induced protective immunity to subsequent lethal challenge with WT-virus. These observations indicated that intravaginal immunization with the attenuated virus induced mucosal immunity in the vagina that prevented reinfection.

However, many have studied immunization at IgA-inductive sites including the intestine, nasopharynx, and pelvis at least partly with a view toward the induction of S-IgA responses in the female genital tract. **Parr** compared nasal and vaginal

immunization using attenuated HSV-2 for protection against vaginal infection with WT-HSV-2 (1081). Compared to vaginal immunization, nasal immunization neither increased IgA plasma cell numbers in the vagina nor elicited a higher IgA titer in vaginal secretions. Vaginal immunization increased the number of vaginal IgG plasma cells and the secretion/serum titer ratio of IgG, indicating a local production of virus-specific IgG.
Both T and B lymphocyte numbers were rapidly increased by about 20-fold in the vagina after virus challenge in immune mice but not in nonimmune mice and lymphocyte recruitment was mediated by IFN-γ (1091). Data clearly indicate that the IFN-γ secreted by memory T cells in the vagina of immune mice after virus challenge was responsible for rapid recruitment of large numbers of additional T and B lymphocytes to the vagina (1091).
Also the expression of endothelial addressins in the vagina and their regulation by IFN-γ in immune mice after vaginal inoculation with HSV-2 was investigated (1089) as lymphocyte recruitment into tissues requires the presence of adhesion molecules on vascular endothelial cells. ICAM-1 and VCAM-1 may be involved in the rapid IFN-γ-mediated recruitment of lymphocytes to the vaginal mucosa of immune mice.

Another aspect is that the titer of viral IgG in vaginal secretions of mice that were immunized in the vagina with attenuated HSV-2 may be higher than can be accounted for by passive transudation from serum (1079). The number of IgG plasma cells in the vaginae of immunized mice was markedly higher than in nonimmune mice, suggesting that IgG may be produced locally in vaginae of immune mice (1086).
This is also an important issue considering the route of immunization in vaccination strategies that would best protect against reinfection. The results summarized above indicate that vaginal immunization of mice with attenuated HSV-2 elicits a strong protective immune response in the vagina consisting of cell-mediated immunity, IFN-γ, and viral IgG antibody in vaginal secretions.

The question is if parenteral immunization with attenuated HSV-2 would protect against vaginal challenge infection as effectively as vaginal immunization. One of the paradigms of mucosal immunity is that immunization at a mucosal surface provides stronger immunity against challenge at that surface than does parenteral immunization. However, the basis of enhanced immune protection at sites of mucosal immunization is generally thought to be the local production of specific S-

IgA antibody, and vaginal immunization with HSV-2 induces mainly IgG viral antibody.

Parr and Parr have therefore compared immunity resulting from local immunization in the vagina to that induced by parenteral immunization with attenuated HSV-2 [1083]. Interestingly, vaginal immunization induced sterilizing immunity against challenge with a high dose of WT-virus, whereas parenteral immunizations protected against neurologic disease but did not entirely prevent infection of the vagina.

Vaginal immunization caused 86-fold and 31-fold increases in the numbers of IgG plasma cells in the vagina at 6 weeks after immunization, whereas parenteral immunizations did not increase plasma cell numbers in the vagina (1083). Vaginal secretion/serum titer ratios and specific antibody activities in vaginal secretions and serum indicated that IgG viral antibody was produced in the vagina and released into vaginal secretions at 6 weeks and 10 months after vaginal immunization but not after parenteral immunizations. In contrast to plasma cells, the numbers of T and B lymphocytes in the vagina were increased similarly by vaginal challenge with HSV-2 in both vaginally and parenterally immunized mice.

Thus, local vaginal immunization with attenuated HSV-2 increased the number of IgG plasma cells in the vagina and increased vaginal secretion/serum titer ratios 3.0- to 4.7-fold higher than in parenterally immunized groups but appeared to cause little if any selective homing of T and B lymphocytes to the vagina.

HSV protein targets for vaccine construction

As mentioned above, studies have shown that recurrent disease is prevented by virus-specific Th1 cytokines, i.e. IFN-γ, and activated innate immunity. Th2 cytokines, i.e. IL-10, and regulatory (suppressor) T cells downregulate this immune profile, thereby allowing unimpeded replication of reactivated virus and recurrent disease. Accordingly, an effective therapeutic vaccine must induce Th1 immunity and be defective in Th2 cytokine production, at least of IL-10 (66).

In different approaches the most immunogenic HSV proteins were tried to be identified as possible targets for vaccines (Figure 12).

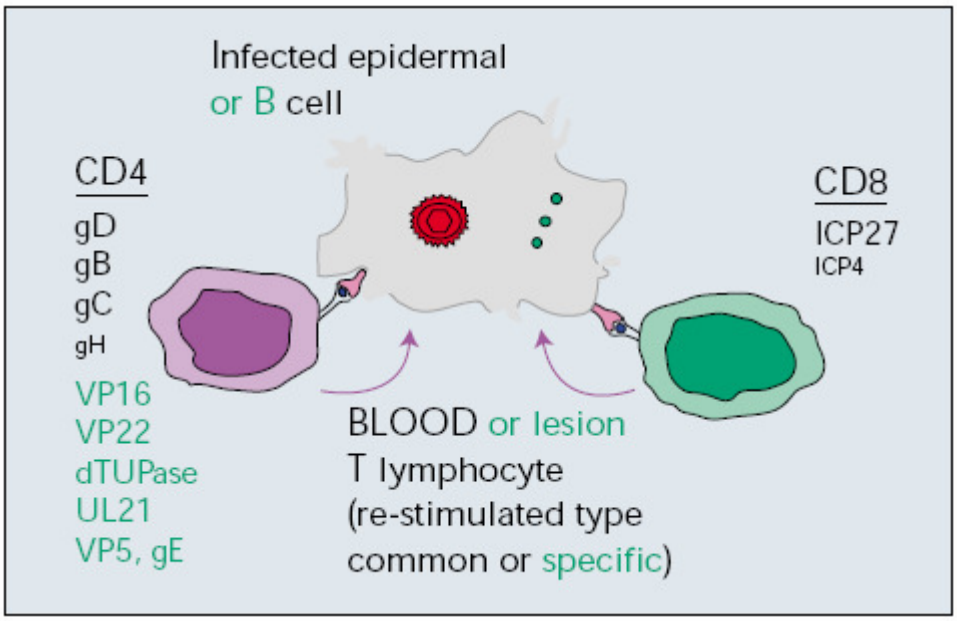


Figure 12: HSV protein targets for CD4 and CD8 T cells as potential vaccine candidates; ICP=infected cell protein, g=glycoprotein, UL=unique long, VP=viral protein, adapted from Cunningham and Mikloska (279)

One approach was directed at identifying the Th2-polarizing proteins so that they can be deleted from a potential vaccine. The functionally independent protein kinase (PK) domain of the HSV-2 large subunit of ribonucleotide reductase which is known as ICP10 has Th2-polarizing activity. In infected cells, ICP10PK upregulates the Th2 cytokines IL-6, IL-10, and IL-13, and downregulates RANTES, which is a chemoattractant for Th1 but not Th2 cells (66).
Significantly, ICP10PK is required for virus replication and latency reactivation (1318), suggesting that its deletion will interfere with virus replication and latency establishment while reducing or eliminating Th2 polarization and toleragenic potential.
Therefore, the vaccine candidate ICP10ΔPK with deleted ICP10PK was developed. It was shown to be growth and latency compromising (1318) and in the mouse, ICP10ΔPK vaccination inhibited HSV-2 replication and provided virtually absolute protection from fatal and cutaneous HSV-2 disease (65).

Other approaches using blood lymphocytes taken from infected patients and restimulated with whole virus *in vitro* identified the viral glycoproteins gD and gB as key targets for the activation of CD4+ T cells (933) and viral ICP27, a protein present only at early stages in infected cells, as the key target for activation of CD8+ T cells (74). Another method is to clone T lymphocytes out of current herpes simplex

lesions and react them against B lymphocytes infected with HSV-1 and -2 and combinations thereof. With this method, several tegument proteins were identified as major T cell targets (730).

Based on these targets, the original goal was to develop a **prophylactic vaccine** that induces local mucosal and systemic immunity which prevents infection and, thereby, virus transmission. The objective of a prophylactic vaccine is to induce sterilizing broad and durable immunity effective at all portals of HSV entry.
A vaccine providing effective immunity against HSV must produce a response more powerful than the response produced by natural infection. This goal was predicated on the belief that neutralizing antibody and activated T cells at mucosal surfaces are the likeliest means to prevent infection, and its construction targets the viral glycoproteins D and B, which are involved in cell entry. However, it is becoming increasingly evident that the original vaccination goals may be unrealistic. Accordingly, the more recent goals of prophylactic vaccination are to prevent or reduce the clinical symptoms of primary infection and to shift the titer of virus necessary to give a primary infection and establish latency (1339). In this context, efforts focus on the selection of ideal adjuvants and the definition of optimal immunization route and protocol (66).

Sununit HSV-2 vaccines

Subunit HSV-2 vaccines using glycoproteins B and D prepared from infected cells (1336) or immune-stimulating complexes (ISCOM) consisting of glycoproteins (956) provided protection from lethal HSV infection and decreased severity and frequency of disease in animal models.
In humans, a vaccine consisting of glycoproteins B2 and D2 with the adjuvant MF-59 by Chiron was only transiently effective in two double-blind, placebo-controlled phase III studies that assessed prevention of HSV-2 infection. The first enrolled 531 persons and the second 1862 individuals who were at high risk for HSV-2 infection. During the first 5 months of the studies, the acquisition rate of HSV-2 among vaccine recipients was 50% lower than in the placebo group, but after one year the overall efficacy was only 9% (254).
In both studies, the vaccine failed to reduce the likelihood or severity of symptomatic disease. Interestingly, the HSV-2 antibody titers did not differ between vaccinated, uninfected and vaccinated, finally infected individuals. These results suggest that neutralizing antibody alone is not sufficient to protect against genital HSV-2 infection and that the vaccine failed to induce critical cell-mediated immune

responses against HSV-2 infection. Possible explanations for the loss of efficacy could be that the protective immune response was only short time or that initial protection was lost with frequent exposure to the virus (1339).

In another trial, a vaccine consisting of glycoprotein D and a mixture of alum and 3-deacylated monophosphoryl lipid A (3-dMPL) as adjuvant by GlaxoSmithKline was tested (1338). In a phase I trial, the vaccine was well tolerated and induced both cellular and humoral immune responses (1339). In two double-blind, placebo-controlled trials with 847 and 2491 partners of HSV-2 infected individuals, respectively, the vaccine`s efficacy was 38% in seronegative male and female in study 1 and 42% in seronegative females of study 2 (1338). This vaccine was effective in preventing symptomatic genital HSV-2 disease in women in about 73% if they were initially HSV-1 and HSV-2 seronegative. The vaccine was shown to induce both gD-specific neutralizing antibodies and a Th1 cell-mediated immune response.

This is the most promising approach of creating a vaccine preventing genital herpes which is currently further investigated in another trial on 7550 HSV-2 seronegative women.
The partial effectiveness of this vaccine compared to the Chiron vaccine suggests that the adjuvant may be critical in facilitating the induction of protective immune responses (Figure 13). The MF59 adjuvant induces a Th2-pattern of response (1313), whereas the MPL adjuvant induces a Th1-pattern of cytokine response. However, *in vitro* studies have shown that MPL alone is not sufficient to induce CD8-lymphocyte cytotoxicity. MPL plus the Quil A derivative, QS21, significantly enhanced cytotoxicity, via induction of IL-12 from APCs and IFN-γ from T lymphocytes (935).

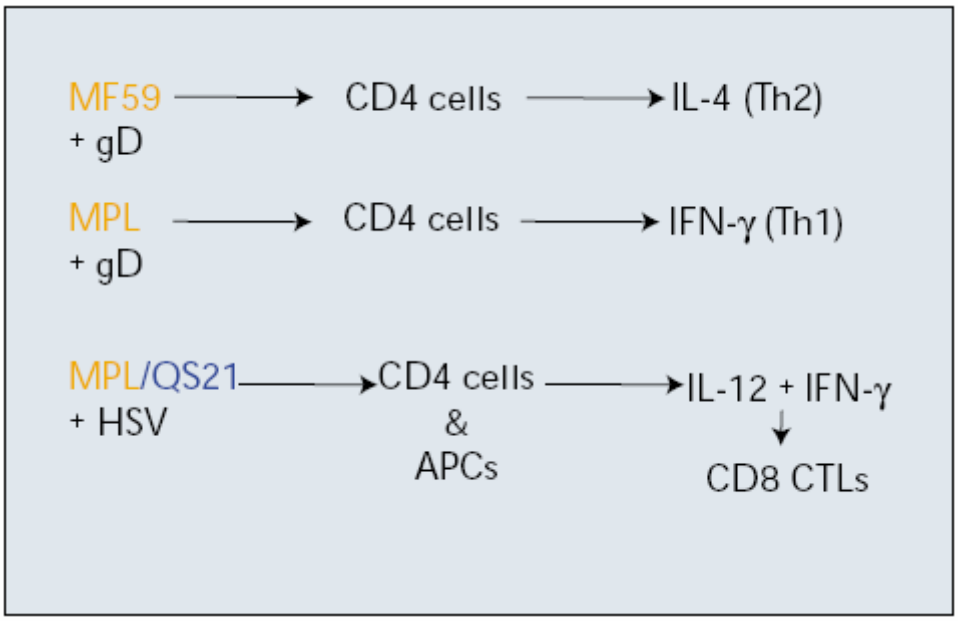


Figure 13: Postulated mechanisms of action of adjuvants for HSV vaccines, adapted from Cunningham and Mikloska (279)

Live attenuated HSV vaccines

Attenuated live virus vaccines cause a primary infection but do not reactivate after establishing latency in ganglia. Two strategies were used to develop live HSV-2 vaccines. The first strategy used viral vectors that express HSV glycoproteins.

R7020, constructed from a HSV-1 strain by replacing a deleted portion with a fragment of HSV-2 genome encoding glycoproteins D, E, G and I, was poorly immunogenic and caused adverse events in clinical trials despite reduced latency results in animal models (912).

Viral DNA vaccines

Concerning viral DNA vaccines, results are inconsistent. Plasmid DNA encoding glycoproteins D and/or B or the immediate-early protein ICP27 induced protective immunity in some animal models whereas immunity was incomplete in other models (136, 449).

Therapeutic vaccines aim to prevent HSV recurrence or minimise disease severity and duration and reduce transmission. A vaccine must augment the host`s specific immune responses but as immune mechanisms controlling outbreaks seem to be different from those required to prevent initial infection, therapeutic vaccines will have to boost different immune responses to that of a prophylactic vaccine (1339).

Therapeutic vaccines probably need to stimulate strong virus-specific cell-mediated immune responses. The challenge in developing a therapeutic vaccine is to identify the HSV antigens that induce a greater protective response than infection with the whole virus. Therapeutic vaccines could be an alternative to antiviral therapy as well.

The above mentioned ICP10ΔPK has shown therapeutic activity in phase I and phase II clinical trials with a reduced recurrency rate, total days of disease and disease severity compared to placebo group (179). At 6 months after treatment, HSV-2 recurrences were completely prevented in 37.5% of the vaccinated patients but were prevented in none of the placebo-treated patients. Vaccinated patients who experienced disease had a significantly lower frequency of episodes, reduced severity of episodes and a lower mean total illness days relative to the placebo group.

The second strategy used to develop live vaccines was to generate mutants rendered nonvirulent by the deletion of one or more genes while retaining the viral glycoproteins previously identified as immunogenic targets.
An HSV-2 mutant deficient in glycoprotein H, known as DISC (disabled infectious single cycle), reduced HSV-2 replication and provided protection from HSV-2-induced disease (904). DISC also caused a 36% reduction in recurrent lesions in the guinea pig when given systemically but not when administered by the mucosal route (904). In phase I clinical trials done by Cantab, now Xenova/GSK, DISC was well tolerated and induced neutralizing antibody and lymphoproliferative T-cell responses. 83% of the vaccine recipients also developed HSV-specific CTL. However, clinical endpoints were not met in phase II trials to assess DISC's efficacy as a therapeutic vaccine, and further development was halted (66).

An overview of double-blind placebo-controlled clinical trials on different therapeutic HSV vaccines gives Table 22.

Therapeutic vaccines	n (vaccine/ placebo)	Results
Non-specific live vaccines		
Bacillus Calmette-Guerin (BCG) (339)	83/72	Mean rate of recurrence 0.528/month vs 0.392/month (placebo) No influence on duration of lesions
Whole inactivated virion vaccines		
Whole virus, formalin-inactivated (689)	16/23	Fewer recurrences in 70% vs 76% of placebo group
Whole virus, heat-killed (Lupidon G/H) (1490)	28/34	Cure/reduced disease severity/prolongation of interval length in 80% vs 30% of placebo group
Whole virus, heat-killed (Lupidon G/H) (881)	142/50	Reduced number and duration of recurrences Increased interval length and reduced length of active disease
Inactivated subunit vaccines		
Subunit, lectin-purfied (758)	18/24	Fewer recurrences in 43% vs 35% of placebo group
Skinner vaccine, formalin-inactivated (1315)	148/144	Reduced number of recurrences in women Reduced number of lesions per recurrence in men
Recombinant glycoprotein vaccines		
gD2-alum vaccine (1360)	98	24% fewer clinical and 36% fewer culture recurrences per month vs placebo group
gD-gB-MF59 vaccine (1361)	101/101	No effect on recurrence rate Reduced symptom duration/new lesion formation/lesion duration
Disabled infectious single cycle (DISC) virus vaccines		
TA-HSV2 vaccine (1339)	483	No significant differences between vaccine and placebo group

Table 22: Results of therapeutic human HSV vaccination trials

b) Human immunodeficiency virus

HIV-type 1 is the etiological agent of AIDS, a disease characterized by progressive immune deterioration and disappearance of CD4+ T cells from peripheral blood and lymphoid organs.
It is estimated that over 40 million people currently live with HIV-1, most of them in sub-Saharan Africa and Asia, and that about 28 million have already died of the pandemic. As the mean incubation period from seroconversion to AIDS is about 8 to 10 years and many HIV-infected persons are unaware of their infection status, the chance for them to further disseminate HIV through sexual contact is very high. Therefore, HIV/AIDS has the potential to become an even more serious health problem than it already is today.

On the African continent, women living with HIV/AIDS make up 60% of the number of HIV-infected people (1531). Heterosexual transmission of HIV-1 is the major route of infection on a worldwide basis and accounts for 70-80% of new infections. Although female-to-male transmission of HIV can occur, the vast majority of cases of 80% involve transmission of virus or virus-infected cells from male to female (571). Nonetheless, only one in 200 to one in 1000 encounters results in productive infection which emphasize the effectiveness of structural and cellular barriers to virus entry (479). Genital tract infection may also be linked to mother-to-child transmission of HIV.

As the genital mucosa is the site of initial contact with HIV-1 for most exposed individuals, study of the virus from the genital tract is critical for the development of vaccines and therapeutics. It is currently not clear which factors contribute to the establishment of HIV-1 infection within the female reproductive tract. The establishment of HIV-1 infection seems to be dependent on the virus amount, the presence of other genital tract infections and the effectiveness of immune systems in the reproductive tract.

In recent years, significant progress has been made in understanding anti-HIV immunity in the vagina by using the simian immunodeficiency virus (SIV)/rhesus monkey model of heterosexual HIV transmission (937).
Both HIV and SIV are retroviruses of the lentivirus family. They have a high degree of nucleotide sequence homology and similar organization of viral genes. It has been shown that vaginal inoculation of SIV in rhesus macaque results in systemic

infection (937). The reproductive physiology of rhesus monkeys is remarkably similar to that of humans with both having menstrual cycles with an average length of 28 days. Also, the similarity of the immune cell populations of the human and rhesus macaque lower female reproductive tract suggest that the biology of the closely related SIV and HIV viruses is likely to be very similar in their respective species. Thus, the rhesus macaque has become a widely accepted animal model for studying anti-HIV immunity in the female genital tract.

(1) HIV transmission and shedding in the genital tract

HIV-1 is present as free virus and virus-infected cells in semen from HIV-1 infected men (1101) and is deposited within the vagina in close proximity to the cervix during sexual intercourse. Which cells become infected and how the virus replicates is still not exactly clear. Also the mechanisms of viral transmission within the female reproductive tract and the mode of viral spread to the periphery are not well understood.

Although HIV can be recovered from the vagina of women who have had a total hysterectomy, most genital virus arises from the cervix and possibly the upper genital tract (378, 251). The proximity to the vaginal lumen of cervical stroma lymphocytes, which compose the genital-associated lymphoid tissue, probably contributes to both HIV shedding and to susceptibility to mucosal transmission. The ectocervix is seen as a likely first site of contact with HIV-1 following heterosexual transmission, and expression of HIV receptors and coreceptors is likely to correlate with susceptibility to viral infection.

HIV receptors

The envelope glycoprotein gp120 of HIV-1 binds to cell-surface receptors on target cells. The primary receptors are CD4, which is mainly expressed on a subset of T cells and on macrophages (614), and galactosyl ceramide (GalCer) (249).

Furthermore, it is evident that the chemokine receptors CCR5 and CXCR4 which are expressed by most leukocyte subsets and ECs function as HIV-coreceptors and are important for HIV infection of cells (110).

There are T cell line-tropic strains of HIV which are specific for CXCR4 and can infect continuous CD4+ T cell lines and primary CD4+ T cells. Macrophage-tropic strains are specific for CCR5 and can infect primary macrophages and primary CD4+ T cells (110). The CCR5 ligands MIP-1α, MIP-1β and RANTES block infection

by macrophage-tropic strains of HIV-1 and similarly, the CXCR4 ligand stromal cell-derived factor-1 blocks infection by T-tropic strains of HIV-1 (110). However, the designation of HIV-1 phenotype is revised to indicate coreceptor usage. Accordingly, HIV-1 variants are designated as either X4 (CXCR4-specific) or R5 (CCR5-specific).

It has been shown that HIV-1 infects viable tissue sections and isolated cells from both the lower and upper female genital tract suggesting that both ECs and submucosal leukocytes may be targets for initial HIV-1 infection (571). Moreover, it was also demonstrated that uterine EC lines can be productively infected with X4 strains of HIV-1 and that these cells express CD4, GalCer and CXCR4, but not CCR5. CCR5 and CXCR4 expressing primary human uterine ECs are able to internalize both X4 and R5 strains of HIV, but can only become productively infected by X4 strains (61).
HIV-1 strains that utilize the CXCR4 chemokine receptor for infectivity are able to undergo reverse transcription, integration, viral DNA transcription and viral release whereas viral strains that utilize CCR5 do not undergo these early replicative events and are only released unmodified from these cells (570).

Therefore, **Asin et al.** suggest three potential mechanisms of virus transmission in the female genital tract (61). One mechanism seems to occur only with X4-tropic strains of HIV-1 and supports the development of a productive viral infection by the EC. Infection with the R5 strain of HIV-1 in which replication is not supported appears to lead to a gradual release of unmodified infectious virus. In addition, HIV-1 infection is transmitted after cell-to-cell contact between the infected ECs or stromal fibroblasts and the susceptible target cell. An alternative mechanism of HIV infection of the lower female genital tract involves the transcytosis of endosome-internalized HIV through the epithelium barrier, without EC infection (558). This approach may allow HIV to transverse directly to the submucosa, where it can infect susceptible immune cells.

To characterize the frequency of potential targets of HIV infection within the female genital tract, a study of the expression of HIV receptors and coreceptors on ECs and leukocytes from the ectocervix was performed (1558).
Results demonstrated expression of CCR5 and CD4 on basal and parabasal epithelium, and a clear compartmentalization of chemokine receptor expression between the squamous mucosa and submucosal stroma (Figure 14, Table 23). CCR5- and CXCR4-expressing leucocytes were found exclusively in the submucosal

stroma adjacent to the basal lamina. GalCer was expressed by cells of the parabasal and cornified layers, and as such is likely to be the only HIV receptor readily accessible to virus. Moreover, studies show that CD4+ and chemokine receptor-expressing cells are proximal to the lumen in the dermal papillae. Given the phenotypes present in the papillae, it is proposed that these structures serve as a potential first site of encounter with infectible leucocytes and, given the large number of HLA-II positive macrophages within the stromal papillae and HLA-II positive DCs as APCs present, may represent an antigen sampling structure for the ectocervix and vaginal mucosa.

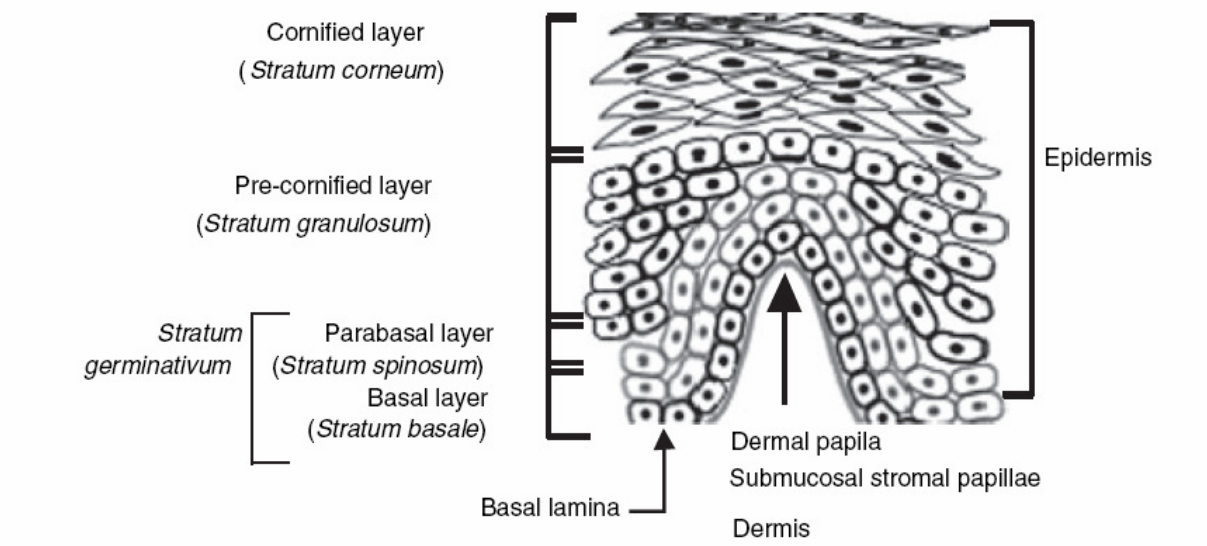


Figure 14: Structure of squamous epithelium in the ectocervix; adapted from Yeaman et al. (1558)

HIV receptor/coreceptor expression on ectocervical ECs in proliferative phase/secretory phase of menstrual cycle, respectively				
	Basal	**Parabasal**	**Midzone**	**Superficial**
CCR5	++/+	+/+	—	—
GalCer	—	+++/+++	+/+	+++/+++
CD4	++/+	++/+	—	—
CXCR4	—	—	—	—

Table 23: HIV receptor/coreceptor expression in the human ectocervix in different phases of the menstrual cycle (1558)

The lack of CCR5-, CXCR4- and CD4-expressing cells accessible to HIV in the lumen of the ectocervix contrasts with previous findings of HIV-1 receptor and

coreceptor expression in the uterus where all three of these receptors are expressed on the luminal aspect of the glandular and columnar epithelium (1561).

Mediators of HIV transmission

Enhanced detection of HIV in CVL is often correlated with decreased CD4+ count and increased viral load (979). Additional correlates of enhanced HIV in the female genital tract include oral contraceptive use, pregnancy, cervical ectopy and STDs (Table 24).

The exact mechanism of STD-mediated enhanced shedding of HIV is still unknown. Enhanced recruitment of activated immune cells, disruption of the epithelium barrier, reduction of T-helper and CTL function, which are all associated with STDs, may contribute to this STD-mediated enhancement of HIV detection in the female genital tract (27). Inflammation associated with STDs may also lead to increased proinflammatory cytokine production such as IL-1, IL-6 and TNF-α, which can potently upregulate HIV replication (1130).

Associations between HIV and STDs
Increased shedding/replication of HIV as a result of the local inflammation produced by the STD ▪ Recruitment of activated (HIV-susceptible) immune cells (e.g. CD4+ T cells) ▪ Increase of proinflammatory cytokine production
Increased susceptibility to HIV as a result of the macroscopic/microscopic breaks in mucosal barriers caused by the STD
Higher prevalence of STDs among HIV infected individuals as a result of common risk factors for both infections
Increased susceptibility to STDs due to immunosuppression associated with HIV infection

Table 24: Causes for increased predisposition of individuals with other STDs to HIV and vice versa

Bacterial vaginosis (BV) is also associated with increased transmission of HIV. Cultured bacteria such as mycoplasma or streptococci from CVL of HIV-positive women with BV have been shown to induce HIV-1 expression *in vitro* (28). These studies point to a direct role of BV-associated microorganisms in the induction of HIV replication, which may increase genital tract viral load and possibly impact horizontal and vertical transmission of HIV. BV also causes an increased vaginal pH, which may prolong the survivability of HIV in this lower genital tract microenvironment.

Additional mediators of HIV transmission may also exist. There has been described an HIV-inducing factor isolated from the CVL of women, independent of HIV-serostatus, that potently upregulated the expression of HIV (29). This factor induced HIV-LTR transcription in a NF-κB-dependent pathway and is closely correlated with abnormal vaginal pH and BV (1046). This HIV-inducing factor in the genital tract of women may have a role in enhanced replication or horizontal or vertical transmission of HIV.

Compartmentalization

One of the main questions in understanding HIV replication in the genital tract is whether HIV is locally produced within the female genital tract or whether the virus is circulated through peripheral/tissue-infected cells to the genital tract. Therefore, the question of compartmentalization, the occurrence of distinct, yet phylogenetically related HIV-1 genotypes within different anatomic sites, is a very important one. Mucosal-associated lymphoid tissues may provide a source of HIV infection in the female genital mucosa but limited studies have addressed this compartmentalization question.

Viral sequence analysis of HIV isolated from the periphery and the genital tract of infected women in one study indicates that these viruses are similar between the blood and the CVL (1282).

However, other studies supported compartmentalization between genital tract and periphery (1588). **Zhu and colleagues** demonstrated that HIV-1 variants in genital secretions of chronically infected transmitters differed from those in the blood and variants in cells differed from those in cell-free plasma, indicating sequence heterogeneity as well as compartmentalization of the virus in different body sites. Some of those differences can be attributed to differences in the techniques used to detect HIV-1 in the lower genital-tract compartment and the significantly higher short-term variations in HIV-1 load in the genital tract compartment than in that of blood. Another study documented significant differences in the mean number of glycosylations on viruses derived from the genital tract and plasma, underscoring the importance of considering HIV-1 and immune response in the genital tract when designing vaccines (688).

In addition, by quantifying the proportion of R5 and X4 viruses in each site, it was found that coreceptor usage often varied significantly between genital tract and plasma. Moreover, the study demonstrated a significant association between higher CD4+ cell counts and compartmentalization of both viral genomes and density of

gp120 glycosylation sites, suggesting that the immune response influences the development of viral genotypes in each compartment.

(2) Innate immune responses in HIV infections

Antimicrobial peptides

HNP1-3 inhibited both laboratory and clinical isolates of HIV *in vitro* (1476) and notably, although it is not as well characterized, HNP-4 shows even greater anti-HIV activity than HNP1–3 and was more effective in protecting human PBMC from infection by both R5 and X4 HIV-1 strains (1539).

HBD-2 and -3 inhibited HIV-1 replication and downregulated CXCR4 expression (1157). Interestingly, a recent study showed a significant correlation between a single-nucleotide polymorphism in the untranslated region of the DEFB1 gene, which probably regulates the gene expression of HBD-1, and the risk of perinatal HIV infection supporting a potentially important role for defensins in innate immunity of HIV infection (138). However, further studies have to further elucidate their role in HIV immunity in the female genital tract.

Another factor that is likely to control HIV infection within the female reproductive tract is SLPI. SLPI protects human macrophages and CD4+ T cells from HIV-1 infection (907), and more recent studies demonstrated that SLPI blocks HIV infection through interactions with a cellular target, annexin II, a cofactor for macrophage HIV-1 infection (849). There is mounting evidence suggesting that SLPI may be an important host defense. Higher SLPI concentrations in vaginal fluid samples correlate with a reduced rate of perinatal HIV transmission (1123).

DCs and macrophages

Several reports have identified DC, NK cells, resting and activated CD4+ T cells and macrophages as the earliest cell populations to become positive for SIV- and HIV-RNA following a nontraumatic exposure to the virus (1558). DCs are considered important target cells in HIV infection and transmission. In SIV-infected rhesus macaques, infected DCs appear first in the mucosa and within 18 hours they are in the draining lymph nodes where they efficiently transmit the virus to CD4+ T cells (574).

Cervical biopsies from HIV-positive women have shown significantly reduced levels of LCs but increased numbers of macrophages (13). The epithelium of the cervix from HIV+ subjects showed a significant increase in both numbers of macrophages

(CD68+) and proportions of activated macrophages (CD68+ HLA-DR+) compared to healthy persons which could act as APCs for lymphocytes. The stroma contained increased proportions of inductive (D1+) and suppressive (D1+ D7+) macrophages but decreased effector phagocyte (D7+) proportions and LCs.

In HIV-infected individuals, viral infection of DCs isolated from peripheral and lymphoid tissues has been demonstrated (1317). Immature DCs (iDCs) as well as LCs express both CD4 and the HIV chemokine coreceptors CCR5 and CXCR4 at their surface. Their surface receptor DC-SIGN is also thought to be one of the receptors to bind and internalize virus prior to its transmission to CD4+ T cells (442). However, they are infected with lower efficiency than CD4+ T cells and macrophages. In contrast, mature DCs (mDCs) do not efficiently replicate the virus and may therefore represent an important reservoir of latent virus infection (1317). Once bound, HIV is internalized and retains its infectivity for several days, a time also required for migration of DCs to regional lymph nodes, their differentiation to mDCs and the transfer of virions (transinfection) of CD4+ T cells (770).

However, several functional impairments and a reduction of DCs have been reported. *Ex vivo* cultured DCs from HIV-infected persons are impaired in their ability to stimulate T lymphocyte proliferation (420). NK cell function and numbers are also impaired in terms of cytolytic activity, mainly by the capacity to secrete CCR5-binding chemokines. This results in impaired elimination of infected cells and limited virus replication (420). However, further studies need to be done to evaluate these findings and if they can also be transferred to the genital tract mucosal immune system.

(3) Specific immune responses in HIV infection

CTLs

CTLs have been shown to be the major means by which an immune response eliminates systemic viral infections. In studies with mice, it has been shown that virus-specific CTLs, generated in the genital lymph nodes, can participate in effective genital immune responses against a sexually transmitted viral pathogen, for example HSV (897).

Systemic HIV-1 specific CD8+ T lymphocytes have been associated with protection against HIV-1 infection and improved host control immune control of HIV-1 (906). In the acute phase of infection, the CTL response initially follows the rise of HIV in the blood and when that response reaches a peak the virus level falls (Figure 15). In the following, there is an inverse relationship between CTL response and virus load

(1040). Quantification of the early T cell response can be made with HLA tetramers (Figure 15). HIV-specific CTLs produce cytokines such as IFN-γ, TNF-α, and the chemokines MIP-1α, MIP-1β and RANTES which can suppress viral replication (906). However, less than 15% of HIV-specific CTLs contained perforin which may result in poor target-cell death. Analyzing their surface glycoproteins compared to CMV-specific T cells, HIV-specific CD8+ T cells may be immature rather than end-stage effectors.

The cellular immune response to HIV, mediated by T lymphocytes, thus seems strong but fails to control the infection completely. HIV undermines this control by infecting key immune cells, thereby impairing the response of both the infected CD4+ T cells and the uninfected CD8+ T cells. The failure of the latter to function efficiently facilitates the escape of virus from immune control and the collapse of the whole immune system (741).

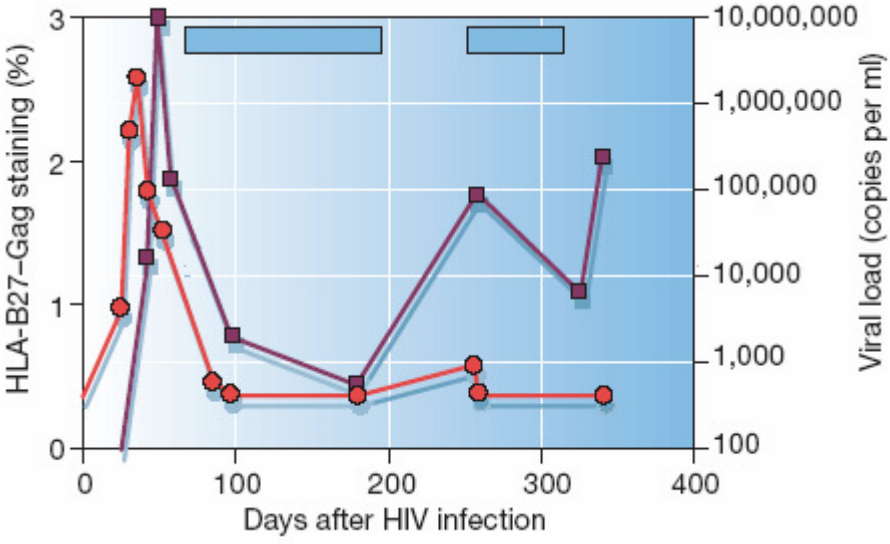


Figure 15: Early CTL responses (mauve line) and virus load (red line) – further observations were influenced by the initiation of antiretroviral drug therapy (blue bands); adapted from McMichael and Rowland-Jones (906)

However, animal models have suggested that it may be mucosal CD8 lymphocytes that play a particularly important role in protecting against mucosal virus challenge. Therefore it is important to assess the function as well as the frequency of HIV-1-specific CD8+ cell responses in the genital tract.

A study showed that SIV-specific CTL activity is present in the CD8+ T cell population in the vaginal epithelium of SIV-infected animals (827). The SIV-specific CTL in the vaginal epithelium may be recognizing SIV-infected DCs present in the

vaginal epithelium of acute and chronically infected rhesus macaques (574). The generation of antiviral CTL activity in vaginal IEL appears to be part of the normal immune response to infection with SIV and HIV.
It is also possible that SIV-specific CD8+ CTL are homing to the vaginal epithelium as part of a normal pattern of lymphocyte recirculation, unrelated to the presence of virus infected cells (938). The immune cell population in the ectocervical and vaginal epithelia are immunophenotypically similar, and it is likely that similar CTL activity is present in the CD8+ T cells in the ectocervical squamous epithelium.

A similar population of anti-HIV CD8+ CTL has been found in brushings of the endocervical canal from HIV-infected women (993) and in the genital tract of highly exposed persistently seronegative sex workers (668). Genital HIV-1-specific CD8+ cell responses of HIV-positive women were present at similar or higher levels than in the blood (669). In a study by **Ahmed et al.**, HIV-positive women had significantly increased numbers of CD8+ lymphocytes resulting in reversal of the CD4/CD8 ratio compared with the control group which is in keeping with systemic events (13). However, the elevated numbers of CD8+ cells seen in the ectocervix of HIV-positive women demonstrates the active recruitment of nonresident CD8+ lymphocytes rather than an expansion of the resident intraepithelial population.

As elsewhere, these cells do not appear to be capable of mounting an adequate immune response to the virus. There was a significant increase in the proportion of activated CD8+ HLA-DR+ and CD4+ HLA-DR+ lymphocytes, but not in CD8+ TIA-1+ cells (13). The monoclonal antibody TIA-1 identifies cytotoxic granules in cells. Although the majority of CD8+ T cells express TIA-1 in low-risk women, the lack of a significant increase in the proportions of CD8+ TIA-1+ cells in HIV-infected women provides circumstantial evidence that there is no increase in the cytotoxic capacity of these CD8+ cells. The increased proportion of activated CD4+ HLA-DR+ cells seen in the female genital tract despite declining CD4 T-cell numbers suggests that this mucosal immune system of HIV-positive women exists in a more activated state than that in seronegative individuals.

Anti-HIV antibodies in genital tract secretions

Anti-HIV antibodies in vaginal washing samples include antibodies from serum transudates, local vaginal production and cervical mucus. Anti-HIV antibodies have been found in CVS of seropositive individuals. Cervicovaginal Igs were significantly increased with IgG as the predominant isotype of anti-HIV antibodies in CVS of

HIV-1 infected women (101) and SIV-infected rhesus macaque (939). Low levels of anti-HIV-1 IgA associated with large increases in anti-HIV-1 IgG in CVS are common in HIV-infected women. **Lü** found a significantly higher HIV-1-specific IgG activity than that of IgA in CVS, saliva and breast milk of HIV-infected individuals (833). Correlation studies suggested that IgG, IgM, and HIV-1-specific IgG in CVS is mostly serum derived. CVS IgA seems to be both locally produced and serum derived, while IgA, IgM, and HIV-1-specific antibodies in saliva and breast milk are mostly locally produced.

In the rhesus macaque, the relative levels of anti-SIV IgG and IgA in the serum seem to reflect the relative levels of anti-SIV IgG and IgA in vaginal secretions (939). This is most easily interpreted as an indication that the bulk of anti-SIV antibody in vaginal secretions is due to serum transudation. However, the results in hysterectomized animals are consistent with anti-SIV Ig production in the vaginal mucosa. Thus it seems that both local production and transudation of serum antibodies are sources of anti-SIV antibodies in the vaginal secretions.

There is the hypothesis that in frequently HIV-exposed but uninfected individuals, HIV-specific mucosal antibody responses may exist and play a role in resistance to HIV. It has been published that HIV-1-resistant sex workers show the presence of HIV-specific IgA in their genital secretions (670) whereas other studies described the absence of HIV-specific antibodies in genital secretions of HIV-resistant sex workers (337). So far, a condition that mimics the reports of HIV infections that produce genital anti-HIV antibodies without exposures of a systemic immune response could not be reproduced in monkeys (938).

(4) Cytokine profiles

Proinflammatory cytokines may stimulate replication and spread of HIV.
Belec et al. determined TNF-α, IL-1β and IL-6 concentrations in paired serum and CVL from 45 HIV-negative and 50 HIV-positive women to evaluate to what extent the female genital tract represents a source of proinflammatory cytokines in normal conditions and during the course of HIV infection (102). Expression of cytokine mRNA in cervicovaginal fluid and proportion of inflammatory cells was increased in advanced stages of HIV infection. Levels of TNF-α and IL-6 correlated positively with the homologous serum cytokine levels in HIV-infected women. A transudation of cytokines from serum to the cervicovaginal fluid may have occurred in HIV-infected

patients, but local production of cytokines was assessed by the detection of cytokine mRNAs (102).

Anderson et al. also described elevated levels of IL-1β and MIP-1α in genital secretions of HIV-infected persons compared to uninfected women which suggest an inflammatory state of the genital tract (35).

Corwley-Norwick and colleagues evaluated IL-2, IL-10 and IL-12 concentrations in cervical secretions of female adolescents in order to determine how cytokine levels are influenced by infection with HIV and coinfection with other sexually transmitted pathogens (271). Compared with HIV–negative patients, HIV-positive patients had higher concentrations of IL-10. Coinfection of HIV and HPV predicted the highest IL-10 concentrations; coinfection of HIV, human papillomavirus, and other sexually transmitted pathogens predicted the highest IL-12 concentrations. In contrast, HIV infection, HPV infection or infection with other STDs is not associated with differences in IL-2 concentrations.

The data indicate that concomitant infection of the genital tract with HIV and other viral, bacterial, or protozoan pathogens influences the local concentrations of some immunoregulatory cytokines.

In the review of **Fauci**, he summarized that IL-2, TNF-α, IL-1, and IL-6 can upregulate HIV replication, whereas INF-α, TGF-β, IL-10, and β-chemokines (MIP-1α, MIP-1β, RANTES) can downregulate HIV (380). The balance between HIV-inducing and HIV-inhibiting cytokines may impact the viral load in the mucosa and subsequently, the sexual transmission of the virus.

(5) Impact of hormones and menstrual cycle on HIV

Female hormones may impact HIV replication not only in the genital tract but also in the periphery. Steroid hormones can bind to hormone-responsive elements within the long terminal repeat (LTR) of HIV which leads to an upregulation of HIV transcription (450). Recent studies using the SIV-macaque model of vaginal infection indicated that progesterone implants enhanced HIV transmission, presumably by thinning of the vaginal wall (878), whereas estrogen inhibited HIV infection, inversely, by thickening of the vaginal wall (1320).

The impact of the menstrual cycle on genital tract shedding of HIV is controversial. **Reicheldorfer et al.** reported that genital tract HIV-1 RNA levels from CVL fluid and endocervical cytobrush specimen were highest during menses and lowest immediately thereafter (1183). The menstrual cycle had no effect on blood levels of

HIV-1 RNA. **Benki et al.** investigated the association between hormone fluctuations during the menstrual cycle and HIV-1 RNA shedding in cervical and vaginal secretions (108). A significant positive correlation between serum levels of progesterone and serum levels of HIV-1 RNA was detected. The lowest levels of cervical virus levels were present at the midcycle surge in LH, which was followed by an increase in virus levels that reached a maximum before start of menses.
However, other studies were unable to detect a menstrual cycle pattern to HIV genital tract shedding (978). Variation in assay methods and small number of women sampled in previous studies may account for this discrepancy. The length of the menstrual cycle also seems to be independent of HIV serostatus (511).

Wang et al. investigated the influence of hormonal contraception on genital tract shedding of HIV-1 (1473). Women demonstrated a significant increase in the prevalence of HIV-1 infected cells and a slight increase in HIV-1 RNA detection in CVS after initiating hormonal contraception whereas no changes were observed in concentrations of HIV-1 RNA. This may have implications for the HIV-infectivity of women using hormonal contraception.

Expression of HIV-1 receptors and coreceptors in the female genital tract varies as a function of menstrual cycle stage, suggesting that sex hormone levels may influence a women`s susceptibility to HIV-1 infection. The expression of HIV-receptors and -corecptors was evaluated on uterine epithelia at different stages of the menstrual cycle (1561). CD4, CCR5 and CXCR4 were found on glandular and luminal ECs. Both CD4 and CCR5 expression on uterine ECs was high throughout the proliferative phase of the menstrual cycle; CXCR4 expression increased gradually during the proliferative phase. During the secretory phase of the cycle, CD4 and CCR5 expression was reduced whereas CXCR4 expression remained elevated. Expression of GalCer on endometrial glands is higher during the secretory phase than during the proliferative phase.
This variation in receptor expression suggests that receptors are regulated by estradiol and progesterone and that a woman`s susceptibility to HIV infection may vary due to this hormonal regulation of HIV receptor expression. Progesterone also causes a decrease in IL-2-mediated induction of the two main coreceptors for HIV entry, CCR5 and CXCR-4, on activated T cells, leading to reduction in HIV infection (1449).
The results presented by **Yeaman et al**. suggest that, in contrast to the uterus, expression of CD4, CCR5, CXCR4 and GalCer in leukocyte populations from the

ectocervix do not greatly vary with the stage of the menstrual cycle (1558). Moreover, ECs from tissues at early and mid-proliferative stages of the menstrual cycle express CD4, although by late proliferative and secretory phases, CD4 expression was absent or weak. In contrast, GalCer and CCR5 expression on ectocervical ECs was uniform in all stages of the menstrual cycle (Table 23).
A study among 55 HIV-positive women with CD4 count <350 cell/µl examined over the course of 8 consecutive weeks has found that vaginal IL-1β, IL-2, IL-4, IL-6, IL-10, MIP1β, TGF, and TNFRII are all increased during menses whereas peripheral cytokines were not altered (26). Increased cytokine levels during menses of HIV-positive women may be correlated with the increased numbers of granulocytes and macrophages in the genital mucosa at menses (102) which in the case of HIV may be hyperactivated, leading to enhanced cytokine production.
In HIV-seropositive women with advanced HIV disease and a CD4 count <200 cells/µl, TNFα, IL-1β and IL-6 are enhanced in comparison to patients with early HIV disease and a CD4 count >500 cells/µl or HIV-seronegative women (1280). Phenotypic and functional analysis of systemic lymphocytes was not altered by the menstrual cycle as evaluated from HIV-positive or HIV-negative women (26).

c) Human papillomavirus

HPV is a heterogenous group of DNA viruses from the Papovaviridae family. With its at least 100 different genotypes it can infect and replicate in skin epithelium (cutaneous HPV) and in mucuos membranes (mucosal HPV), and induce epithelial proliferation resulting in warts (1055). More than 40 anogenital genotypes have been associated with STD, which makes HPV the most common viral STD in the world.

Two out of three people having sexual contact with an HPV-infected partner will develop an infection within the next months which will be asymptomatic in almost three out of four cases (1417). Most HPV infections regress spontaneously with HPV DNA persisting for about 6-12 months in the genital tract and spontaneously disappearing in the majority of patients.

The correlation between genital HPV infections and cervical cancer was first documented in the early 1980s by the study group of **Harold zur Hausen**, a German virologist (458). HPV infection is now generally accepted as being involved

in the development of anogenital precursor neoplasia, i.e. cervical, cervical glandular, vulval, vaginal and anal intraepithelial neoplasia. Approximately 30% of high-grade CIN will progress to invasive cervical carcinoma over a period of 10-20 years which makes persistent HPV infection the major risk factor for developing cervical carcinoma (867, Figure 17).

HPV types 16, 18, 31, 33, 35, 45, and 58, and about eight to ten other minor types, are oncogenic and are found in almost all cervical cancer biopsy samples and in 90% of high-grade intraepithelial precursor lesions (Figure 16). HPV types 16 and 18 are the most commonly detected HPV types in biopsy samples (1340).

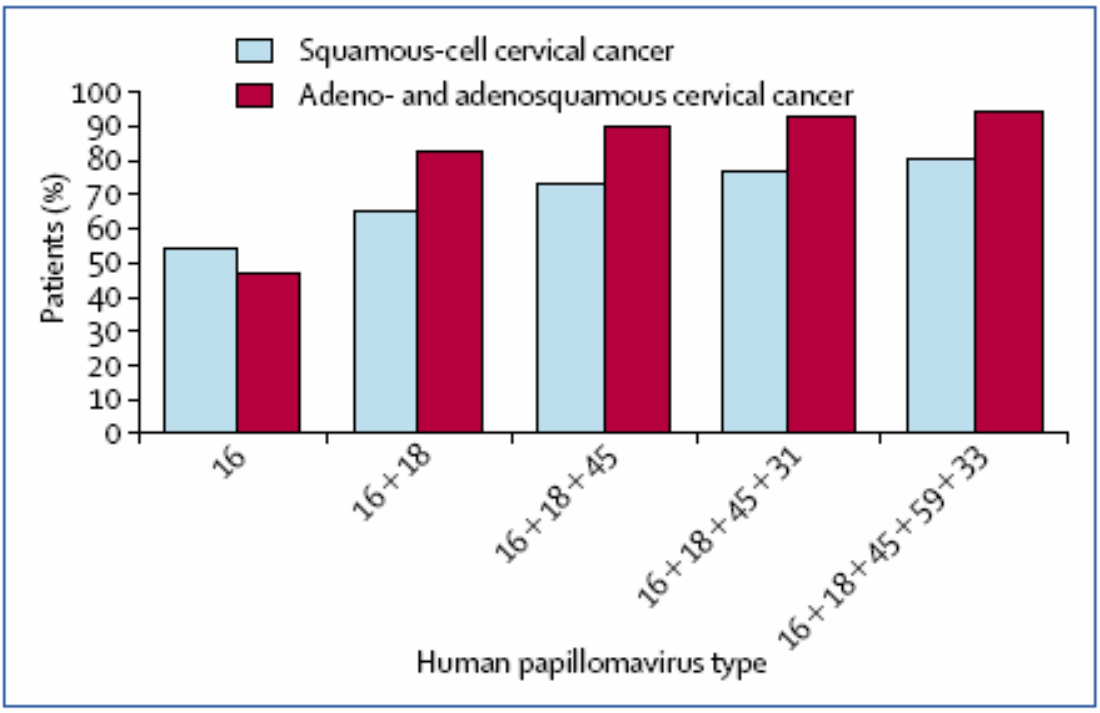


Figure 16: Prevalence of different HPV types in cervical cancer biopsies, adapted from Stanley (1340)

HPV types which infect the genital tract can be classifed into three groups (831, Table 25).

The low-risk group includes HPV 6 and 11 which are commonly associated with condylomata accuminata and low-grade squamous intraepithelial lesions (LSIL or CIN I).

The high-risk group includes HPV 16, 18, 45 and 56 which are commonly found in patients with high-grade squamous intraepithelial lesions (HSIL or CIN II/III) or invasive carcinoma of the cervix, vulva, anus or penis.

The intermediate risk group is associated with high-grade CIN but less commonly with invasive cancer; this group includes HPV 31, 33, 35, 51 and 52 (867). Low risk types remain extrachromosomal or episomal whereas the genomes of high risk HPV types 16 and 18 are found integrated into the cellular host DNA in most human cervical carcinomas (1359).

Risk type	HPV type	Association with
Low risk	HPV 6 / 11 / 41 / 42 / 43 / 44	Condylomata accuminata Low-grade CIN (CIN I)/LSIL
Intermediate risk	HPV 31 / 33 / 34 / 35 / 39 / 51 / 52	High-grade CIN (CIN II-III)/HSIL
High risk	HPV 16 / 18 / 45 / 51 / 52 / 56 / 58 / 59 / 61 / 62 / 64 / 66 / 67 / 68 / 69 / 70	High-grade CIN (CIN II-III)/HSIL Invasive carcinoma of cervix, vulva, anus or penis

Table 25: Classification of HPV types according to oncogenic potential and clinic

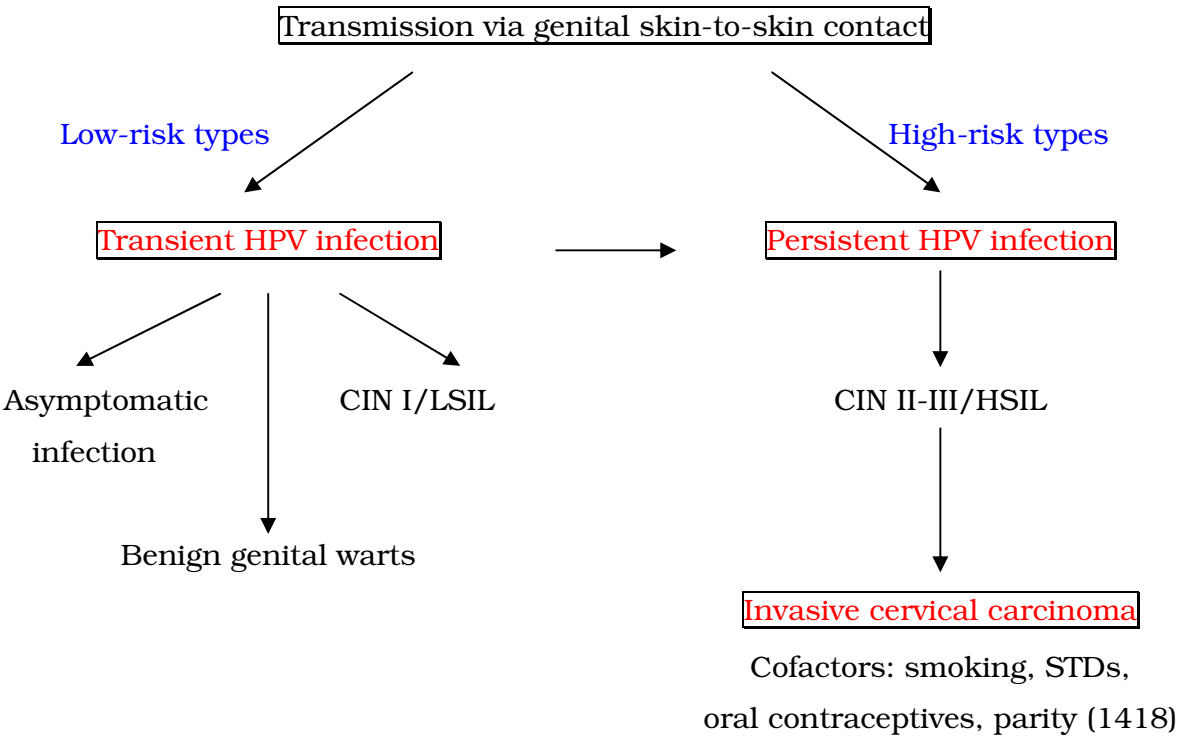


Figure 17: Etiology of cervical carciogenesis as the result of HPV infection

Cervical carcinoma constitutes a major public health problem worldwide with 500,000 new cases per year. It is the most common female malignancy in developing countries and second most common in western countries following

breast cancer. The latest cancer statistics for the year 2006 reported 9,710 estimated new cases and 3,700 estimated deaths of cervical cancer in the USA (620). Despite optimal management with options like radical surgery, radiotherapy and chemotherapy, the overall 5 year survival for patients with cervical carcinoma is still only 58% (867). This suggests a need to develop novel approaches not only to treatment, for example immunotherapy, but also the strong need to develop prophylactic or therapeutic vaccines.

(1) Composition and mechanism of HPV infection

The HPV genome

The HPV genome can be divided into a coding and a noncoding region. The noncoding region, the long control region (LCR), contains the origin of viral DNA replication and the enhancer/promoter elements regulating the viral transcription.

The coding region consists of open reading frames (ORFs) and encodes the early (E) and late (L) viral proteins.

The late genes encode structural proteins, the viral capsid proteins (L1 and L2) which selfassemble into the viral capsid interacting with a receptor of the target cell facilitating entry of the viral DNA.

The early proteins E1 and E2 are involved in regulation of viral transcription and DNA replication. The E6 and E7 genes of high-risk HPV types encode for oncoproteins that can immortalize human keratinocytes (1102). This potential appears to be limited to high-risk types, because E6 and E7 from HPV6 or HPV11 are nontransforming.

E6 will bind, inactivate, and degrade the host`s oncosuppressor protein p53, which results in loss of p53-induced apoptosis and G1 arrest of the cell cycle (691). The binding of E7 to retinoblastoma gene product (Rb) will lead to the transcriptional deregulation of cell-cycle control and results in uncontrolled cell proliferation (210). When viral DNA integrates into the host genome (malignant transformation), it will cause successively the disruption of the E2 ORF, loss of E2 protein expression, the overexpression of E6 and E7, uncontrolled cell proliferation, and in the end oncogenic transformation of the cell (1417).

HPV genome is usually present in an episomal (circular and nonintegrated) configuration in CIN, whereas in invasive cervical cancer the genome is commonly integrated into the host DNA (572). HPV DNA integration appears to be the critical event in the development of cervical neoplasia, since HPV E6 and E7 are conserved intact and show persistent and increased expression in carcinomas.

Molecular pathogenesis

HPV penetrates the suprabasal cells in the cervical epithelium and tightly maintains a program of viral transcriptional repression of its late genes L1 and L2, which are potentially the most powerful immunogens that HPV synthesizes (1059). This repression allows escape from immune surveillance and recognition. This is different from certain animal models where papillomavirus infections are rapidly eradicated due to the expression of L1 and L2 in all layers of the infected epithelium, which attracts more immune effector cells to the infected area.

As HPV progresses through the layers of the epithelium, the replicative program of its genes changes in an orderly fashion. The HPV early proteins E6 and E7 are produced through most of the phases of the HPV life cycle, making them better candidates for therapeutic vaccines. On the other hand, the HPV L1 and L2 late proteins are not produced until the virus is located in the most superficial layers of the epithelium, correlating with the assembly of infectious virions and their release from the epithelium with the desquamated infected superficial cells (1059).

Human HPV infections are exclusively intraepithelial and, theoretically, HPV attack should be detected by the APC of squamous epithelia, the LC. The activated LC should then migrate to the draining lymph node, processing HPV antigens en route, and present antigen to naïve T cells in the node. The T cells should then differentiate into armed effector cells, migrate back to the infected site, and destroy the infected keratinocytes (1342).

(2) Mechanisms of HPV to evade the immune response

HPVs, unlike other recurrent or persistent human viruses such as influenza or Epstein-Barr-Virus, do not provoke strong humoral or cellular immune responses (328). The diagnosis of HPV still relies on the cytological detection of cellular abnormalities and the histopathological confirmation of epithelial lesions. Also the chronic nature of HPV infection, especially with oncogenic types, suggests that the virus has apparently evolved to avoid the mammalian immune response.

A number of immune evasion mechanisms have been proposed (427, Table 26).

Low profile

HPV infection per se does not elicit any major damage likely to evoke the principle innate immunity danger signals (1351). The virus infects only ECs, encodes non-

secreted proteins expressed at low levels, with virus production in cells, which are sloughed off at the end of their lifespan. There is no viraemia and the infected cells are not lysed, limiting the production of antigens for systemic presentation.

The infectious cycle of HPV is itself an immune evasion mechanism inhibiting host detection of virus. The virus replication cycle is conducted within the maturing keratinocyte (KC) and mature virions escape from the infected epithelial surface within desquamating KCs, so there is little local or systemic presentation of HPV antigens to the immune system by professional APCs during infection.

HPV replication and release do not cause cell death, since the differentiating KC is already programmed to die which does not present a danger signal to the immune system. Thus, for most of the HPV infectious cycle, there is little or no release of the proinflammatory cytokines important for DC activation and migration into the local milieu, and the essential signals required for immune responses in squamous epithelia are absent (755).

Another level of immune evasion derives from the tropism of genital HPVs for KCs. Unlike so-called professional APCs such as DCs, KCs have low levels of HLA class I and class II molecules, and costimulator molecules such as B7.1 (427). Exposing CD4+ T-cells to KCs in vitro has lead to non-responsiveness, a mechanism which could be operating in vivo to render HPV-specific T-cells functionally inert (427).

Immunosuppression by early proteins

However, even in the absence of viral-induced cytolysis and cell death, HPV-infected KCs should activate secretion of type 1 IFNs, IFN-α and IFN-β. As most DNA viruses, HPVs have evolved mechanisms for inhibiting IFN synthesis and signaling by downregulating IFN-α-inducible gene expression (1016). HPV 16 E6 and E7 oncoproteins directly interact with components of the IFN signaling pathways (78, 814).

It is also apparent that these viral genes regulate other factors likely to influence the survival of virus infected cells including TGF-β2 as important components of any local inflammatory response (1017). Another strategy for immune evasion stems from the ability of HPV 16 E6 to downregulate the IFN-promoting factor IL-18 (224). Other immunosuppressive actions of HPV early proteins include the inhibition of EC interactions with DCs by E6 (884). E6 and E7 further inhibit the activity of the important MCP-1 (722).

Thus, activity of E6 and E7 provides the molecular basis for promoting viral persistence and avoiding innate immunity and the consequential activation of adaptive immunity.

E5, another early protein of HPV, prevents the efficient pH-dependent processing of peptidic antigens (171). Other relevant influences include the modulation of antigen processing pathways through E5-mediated MHC expression (1578)

Downregulation of HLA

The further level of immune evasion is also related to the KC in the aspect that HLA class I molecules on HPV-transformed cells are often expressed at a low level or are absent altogether (248). It is not known if this is of functional consequence for CTLs, but the downregulation of certain HLA-B alleles does correlate with a poorer prognosis for some patient populations with cervical dysplasia (130).

The MHC molecular expression is usually upregulated by cytokines associated with an inflammatory response, so this will be suboptimal in an HPV infected target cell. However, the genetics of HLA may also influence susceptibility to HPV infection or ability to clear the virus and thus avoid persistence, which is the key risk factor for progression (543).

Activation of T cell subsets

Another potential mechanism of immune evasion is the suppression of immune responses by HPV-transformed KCs, through the production of either inhibitory cytokines (238) or proteins that can inactivate stimulatory cytokines (1319). HPV-transformed KCs might resist CTL-killing either by producing proteins that interfere with CTL-lytic mechanisms or by inducing apoptotic cell death in the CTLs themselves (1570).

It is possible that HPV has evolved to exploit the endogenous tissue responses utilized by innate immunity to disfavour induction of a more threatening Th1 response which would favour the development of CTLs. Indeed, it has been shown that in CIN lesions there is a relative downregulation of TNF-α by the epithelium and upregulation of the Th2 cytokine IL-10 compared to normal cervix (980). The migrating LC may thus be inappropriately activated, skewing any subsequent immune activation of T cells, which might include the induction of anergic or Treg cells.

Recent studies have documented a predominant Th2 polarity of tumor infiltrating lymphocytes in human cervical cancer (1294) and the draining lymph node in cervical cancer appeared to have an increased proportion of Treg cells (379).

Immune evasion by HPV-transformed cells can also be considered at the level of the effector T-cells. Immune responses in patients with advanced cancer are often diminished as a result of disease or treatment. One mechanism that has been

proposed for their decreased immune responsiveness is the downregulation of signaling components of the TCR such as CD3 zeta chain (739). These alterations were associated with reduced cellular functions such as the production of TNF.

Suppression of LCs

Disturbances in the afferent phase of the immune response are suspected. The analysis of inflammatory infiltrates in cervical dysplasia has revealed reduced numbers of LCs in CIN II and III lesions (1454). This may result in changes in cell surface E-cadherin expression in the basal and suprabasal layers. For example, E6 can down regulate epithelial E-cadherin expression, which could modulate their contacts with LCs not allowing optimal antigen capture or activation necessary for the initiation of anti-viral T cell responses (884). In addition, the change in tissue architecture is more permissive for the expansion and spread of immortalized and/or transformed cells (1351).

Fausch et al. explored the interaction of HPV with LCs which are the first APCs that the virus comes into contact with during infection (384). In contrast to DCs, LCs are not activated by HPV virus-like proteins (VLPs), which is illustrated by the lack of upregulating activation markers, secreting IL-12, stimulating T cells in a mixed lymphocyte reaction and inducing HPV-specific immunity and migrating from epidermal tissue. Since DCs or LCs are generally accepted as being the most efficient APCs of the immune system their lack could probably result in an inefficient primary immune response (1418).

Moreover, capsid entry is usually an activating signal for DCs, but there is evidence that LCs, unlike stromal DCs, are not activated by uptake of HPV capsids, a phenomenon that would inhibit both LC migration and maturation, and the priming of the immune response against the capsid proteins (385).

Mechanisms of HPV to evade host`s immune response		Results in
Low profile	KC as priviledged site for HPV infection ▪ Low HLA I/II expression ▪ No lysis of infected cells ▪ No viraemia	Little local/systemic HPV antigen presentation by APCs Little release of proinflammatory cytokines Resistance to CTL-mediated lysis
Early proteins	▪ Interference with IFN pathways ▪ Downregulation of activity of MCP-1/IL-18/TGF-β2 ▪ Interference with MHC II antigen maturation on KC	Limitation of HPV antigen presentation by LC Inhibition of cytokine release
T cells	▪ Downregulation of TCR signaling components ▪ Predominance of Th2 lymphocytes and Treg cells ▪ Downregulation of TNF-α ▪ Upregulation of IL-10	Resistance of KC to CTL-mediated killing Reduction of cell-mediated immunity
LCs	▪ Reduction in number ▪ Inhibition of EC-DC interaction	Interference with antigen presentation

Table 26: Mechanisms of HPV to evade the host`s immune responses, resulting in increasing viral persistence

(3) Cell-mediated immunity in HPV infection

As HPV transmission and subsequent infection is a local event in the lower female genital tract, local immunity seems to be of crucial importance in the outcome of an HPV infection. The local immune status might determine whether the virus infection will be cleared or will be persistent, finally resulting in the development of cervical neoplasia.

Although a very high percentage of young sexually active women are positive for genital HPV (555), most HPV infections are subclinical in immunocompetent patients or manifest intermittently as self-limiting warty lesions. Most HPV infections are transient with a median duration of about 8 months which means that HPV is successfully attacked by the immune system after transmission (549). A small fraction of the lesions, however, will progress to CINs and if left untreated, to invasive and metastatic carcinomas (899).

The peak prevalence of genital HPV infections is soon after the onset of sexual activity in women. Thereafter, the prevalence declines, indicating that long-term protection can be acquired. Supportive to this is the fact that up to 65% off all CIN1 lesions in women between 15 and 34 years of age will regress (1260).
Cell-mediated immunity is likely to be important as in immunocompromised individuals like HIV-infected women and organ transplant recipients, the presence of HPV infections and anogenital neoplasia is higher than in the general population (32). In addition, HIV-1-infected patients have lesions that progress more rapidly (390). Furthermore, the regression of HPV-induced warts is associated with lymphocytic infiltration in both patients with genital warts (245) and animal papillomavirus models (1273).

Clues to the nature of the cellular immune response to HPV infection have come from immunohistologic studies of spontaneously regressing genital warts (245).
Nonregressing genital warts are characterized by a lack of immune cells at the site of infection; the few intraepithelial lymphocytes are CD8+ cells, and mononuclear cells are present mainly in the stroma.
Histologic examination of regressing genital warts reveals a large infiltrate of both CD4+ and CD8+ T cells and macrophages in the warts` stroma and epithelium. The infiltrating lymphocytes express activation markers, the cytokine milieu is dominated by proinflammatory cytokines such as IL-12, TNF-α, and IFN-γ, and there is upregulation of the adhesion molecules required for lymphocyte trafficking on the endothelium of the wart capillaries (245). This is characteristic of a Th1-based immune response.

Moreover, the detection of HPV-specific circulating T cells in healthy individuals substantiates the role of a cell-mediated response (1492) as well as the detection of anti-HPV IgG1 and IgG2, IFN-γ and IL-4 in individuals with cleared infection (316).
The role of specific or nonspecific cell-mediated immunity in the clearance of HPV is not exactly known, largely because immune parameters after primary HPV infection have not been studied to a large extent.
In women with preinvasive cervical neoplasia, the spontaneous regression of lesions occurs in approximately 30% of cases (867). The question if HPV-specific immunity influences this process and if the development of cancer results from a failure of the immune system is still unclear.

The investigation of the role of natural immunity to HPV infection is problematic as HPV infections can be transient and often asymptomatic. Studies have been carried out largely on patients with established HPV-associated disease and have focused on the oncogenic HPV types, in particular responses against the viral transforming proteins, E6 and E7, the only viral antigens constitutively expressed in transformed cells. Also studies have focused on systemic rather than mucosal immunity, so a substantial component of the host response against HPVs could be missed (867).

The link between HPV16 and cervical carcinoma opens up the possibility of immune T-cell intervention, either against the preinvasive lesions from which tumors arise, or against the virus antigen-positive tumor cells themselves. This requires a better understanding of the spectrum of T-cell responses induced by HPV16 antigens during the course of natural infection, and of their role in disease clearance and progression.

CD4+ T cell responses

There are several studies providing information about CD4+ T-cell responses to HPV16 where proliferative responses to either peptides or soluble HPV proteins have been demonstrated in patient groups.

In early studies, PBMCs from patients with a history of skin warts were able to proliferate *in vitro* against crude wart antigen preparations but also PBMCs from individuals with no history of warts responded specifically to the same wart antigen preparations (276).

Serological T cell responses against peptides derived from HPV 16 E7 and HPV 16 L1 were not, however, significantly associated with HPV disease as T cell responses were also seen in healthy asymptomatic controls (846, 845). It is not clear if the results in healthy control group represent *in vitro* artefacts or memory responses based on a prior exposure to HPV.

However, results suggested that a Th1 pattern of response was predominant among healthy adults but reduced in women with cervical dysplasia (845). Indeed, immunohistological studies have indicated that regression of HPV-infected lesions is associated with a Th1 response (1343).

De Gruijl et al. examined T cell proliferative responses to HPV16 E7 peptides in women with mild dyskariosis and demonstrated the strongest T cell responses in

women with persisting HPV infection and progressive disease (99% reactive) compared to those who cleared the infection (44% reactive) (314).
Other studies on HPV 16 E7 peptides have shown that the T cell responses correlated with both the stage of disease and the presence of HPV 16 DNA (643). A following study by **Kadish et al**. on women with CIN I or II found out that cell-mediated responses to E7 peptide significantly correlated with disease regression and resolution of viral infection within 12 months (644).
In summary, these studies suggest a relationship between T cell responses against HPV and cervical disease, with a decreased response in cancer patients and an increased response in women with high-grade CIN and viral persistence.

De Jong et al. showed that failure of immune system to eliminate HPV was reflected by the absence of type 1 T cell immunity against HPV 16 E2 and E6 in patients with cervical lesions (307).

CD8+ T cell responses

The role of naturally occurring CTLs in mediating the regression of HPV-related disease has not been proven. There has been an increasing interest in CD8+ CTLs as vehicles for immunotherapy in human cancers, using either vaccines capable of inducing CTLs or adoptive therapy protocols which has been based on clear demonstrations of anti-tumor immunity (867). The expression of E6 and E7 proteins by HPV-transformed cells provide attractive tumor-specific targets for immunotherapy.

The search for human memory CTL responses was initially difficult because of the lack of well-defined viral reagents for *in vitro* testing.
HPV16 E2-, E6- and E7-specific CTLs can be detected in patients with previous (1000) or ongoing HPV infections (1022) using whole HPV proteins to restimulate CTL responses, either in a soluble form or expressed by recombinant viral vectors. Results suggest that naturally occurring HPV E6- and E7-specific CTLs do exist in patients with HPV-associated disease, for example HPV16 E7-specific CTLs that exist in the peripheral blood of women with high-grade CIN and cervical carcinoma, but are extremely rare (1565).

The difficulties of obtaining memory T-cell responses from patients have resulted in the use of DCs to generate primary responses. HPV-specific CTL responses could be generated from healthy asymptomatic donors (990) and cervical cancer patients

(1255) using DCs pulsed with recombinant HPV 16 or HPV 18 proteins. The successful processing and presentation of exogenous HPV antigens *in vitro* raises the possibility that antigens released from HPV-infected cells during the disease process or by surgical procedures could be stimulating HPV immunity *in vivo* (867).

CTL responses in asymptomatic subjects appear to be rare, suggesting that CTL responses are associated with HPV-induced disease.
Preliminary studies have not, however, addressed whether CTLs have a role in the prevention, control or treatment of disease. Furthermore, although CTLs have been operationally defined as HPV specific, based on their reactivity against recombinant HPV proteins, few as yet have been shown to recognize tumor cells.

(4) Antibody responses in genital HPV infection

Circumstantial evidence suggests a role for the serologic immune system, for example antibodies to capsid proteins, besides the cellular immune system in the clearance of HPV infections.

The generation of serum neutralizing antibody is observed in most, but not all, infected individuals (714) and is directed against conformational epitopes on the L1 protein displayed on the outer surface of the intact virus particle. Serum neutralizing antibody levels following natural HPV infections, even at peak titers, are low (1456). This probably reflects the exclusively intraepithelial infectious cycle as well as the production of virus particles in the superficial ECs distant from APCs and patrolling macrophages. These factors limit antigen uptake, delivery to the lymph node, and presentation to naïve B and T cells.

As mentioned, the majority of women with cervical HPV-16 infections generate a systemic IgG Ab response to the major HPV-16 capsid protein as detected by ELISA, using VLPs as antigens (714). Systemic IgA and IgG responses against HPV-16 VLP were more frequently observed in women with HPV DNA than in the controls and the presence of HPV-16 VLP-specific IgG in plasma is correlated with persistent viral infection (131).
In contrast, systemic IgG response to HPV-16 E7, as determined by peptide-based ELISA, is correlated with viral clearance in a subset of CIN (131). However, the presence of serum Abs to HPV-16/18 VLP L1 as well as antigens E6 and E7 is not correlated with occurrence or prognosis of cervical cancer (800, 1303).

So far, few studies have been reported on the local antibody response to HPV.
IgA antibodies against HPV capsids from HPV 6, 11, 16, 18, 31, 33 and 35 could be demonstrated in cervical mucus from patients with cervical neoplasia and controls (1478).
In other studies, local antibodies have been detected against E2, E7, L1, and L2 of HPV 16 in cervical secretions and in CVL of patients with cervical neoplasia, patients with condylomata and women with normal cervical cytology (342). IgA against HPV 16 E2 was found in 49% of the cervical secretions from patients with CIN whereas IgA against E2, E7, L1, and L2 from HPV 16 was observed in 15-32% of the secretions in healthy women. Local IgG against HPV 16 E2 was reported in 46% of patients with CIN and in 15% of healthy controls (342).
A recently published study showed IgA, IgG, and S-IgA antibodies to HPV 16 capsids in cervical samples in, respectively, 11%, 24%, and 9% of the subjects (496).
Bard et al. quantified IgG, IgM, IgA and S-IgA in CVS of healthy and HPV-infected women (76). IgG, IgA and S-IgA were significantly higher in CVS of HPV-infected patients with a lower S-IgA/IgA ratio compared to the control group which suggest that HPV could be responsible for an increase in local production of non-secretory IgA.

Another study by **Nguyen et al**. analyzed systemic as well as mucosal IgA and IgG responses to HPV-16 oncoprotein E7 in women who underwent radical hysterectomy for cervical cancer (HCC) compared to those in women who underwent loop excision for cervical dysplasia or had hysterectomy for other reasons (1020).
It was demonstrated that the levels of HPV16 E7-specific IgA and IgG were relatively, but not significantly higher in sera of patients undergoing HCC as compared to those determined in patients of other noncancerous groups. These results indicate that serum antibodies to oncoprotein E7 were not a specific marker for diagnosis or prognosis of cervical cancer which is in agreement with other studies on large populations (800).
Another interesting finding in this study was that the local mucosal immune response, as determined by levels of HPV-16 oncoprotein E7-specific IgA in genital tract of women with cervical cancer, was downregulated (1020). Gene expression profiling also indicated downregulation of genes associated with Ig synthesis including gene encoding the heavy chain of IgA2 in cervical tumor tissue as compared to that in the normal tissue of the same patient.

(5) Vaccination strategies

Compared to developing countries, considerably lower incidences of cervical cancer are observed in West Europe, USA and Japan probably due to the widely available screening in these regions. Screening methods comprise the conventional Papanicolaou test, liquid cytology and HPV DNA testing (641, 1417).
Unfortunately, no trials have shown reduction of cancer incidence in populations where HPV-testing was added to cytologic screening and currently, despite all the screening and treatment facilities, the incidence of invasive cervical cancer remains stable or even increases (550).

The role of HPV in cervical carcinogenesis and their cellular immune response have created expectations for prevention. A primary preventive strategy which also includes vaccination aims to reduce the incidence of disease and generally targets the entire population without symptoms or disease.

Two types of HPV vaccines are currently being developed. First, there are therapeutic vaccines which induce cellular immunity targeted against ECs infected with HPV and induce regression of precancerous lesions or remission of advanced cervical cancer. Second, there are prophylactic vaccines inducing virus-neutralizing antibodies protecting against new but not against established infections. The prophylactic vaccines are focused on the generation of neutralizing antibodies against L1 and in a lesser extent on L2. In therapeutic chimeric vaccines, the targets are E6 and E7, which have no share epitopes with human cellular proteins; therefore, the risk of inducing an autoimmune response is theoretically eliminated (1349).

Prophylactic vaccines

Most viral vaccines are based on an attenuated form of the virus. But the development of an attenuated HPV vaccine is not possible, as there is no effective culture system. Secondly, such a vaccine would be ethically unacceptable, because it would expose healthy subjects to potentially harmful viral oncogenes.
The observation that the major papillomavirus capsid protein L1 has the intrinsic ability to selfassemble into VLPs when expressed in specific expression vector systems in the absence of other gene products has provided a major technical advance in the field (713).

VLPs contain empty virus capsids containing the major HPV capsid antigen and, possibly, the minor capsid antigens, but lack viral DNA. Therefore, they mimic the natural structure of the virion and are immunogenic but not harmful as they do not contain the viral genome.

VLPs were found to bind very well to human and mouse immune cells that expressed markers of APCs such as MHC class II, CD80 and CD86, including DCs, macrophages and B cells (289). DCs were found to internalize and present VLP-derived antigens to CD4+ and CD8+ T cells *in vitro*, suggesting that DCs initiate immune responses to the VLP *in vivo* (805).

In a study by **Lenz et al.** results showed that both monocyte-derived DCs and plasmacytoid DCs bind and acquire papillomavirus VLP, but only monocyte-derived DCs undergo phenotypic activation after exposure to the VLP and induce primary T cell responses (804).

Together with the observation that cutaneous LCs are not activated by papillomavirus VLP (385), these findings underscore the heterogeneity of DC populations and strongly suggest that monocyte-derived DCs are the principal cells in the T cell response to VLP observed in individuals vaccinated with VLP (1125). However, HPV16 VLP induced plasmacytoid DCs to secrete IFN-α and IL-6, both cytokines that play a role in the generation of antibody responses, as well as TNF-α and IL-8 (804).

In several animal papillomavirus models the parental injection of VLPs elicited high titres of type-specific neutralizing antibodies and subsequent protection against virus challenge (823) which made L1 VLPs clear candidate immunogens for prophylactic vaccination in humans.

Other studies have shown oral, intranasal, or intravaginal vaccination to be more effective in inducing mucosal immunity and also in producing serum IgG antibodies of higher affinity than parental vaccination routes (300).

At present, several vaccines have been developed and tested in clinical trials. The vaccines are generally well tolerated and highly immunogenic. Current clinical data indicate that prophylactic vaccines are very effective against new persistent infections and the development of cervical intraepithelial lesions. The protection is type specific but the follow-up of the vaccination trials is still short.

So far, there have been several published phase I/II clinical trials using HPV VLP vaccines which present encouraging results (Table 27).

Authors	Trial phase /patients	Vaccine	Results
Evans et al. (2001) (371)	Phase I n=65	HPV11 L1 VLP	Well tolerated; induction of neutralizing antibodies and T cell response
Harro et al. (2001) (513)	Phase I n=72	HPV16 L1 VLP	Well tolerated; highly immunogenic
Koutsky et al. (2002) (742)	Phase II n=1533	HPV16 L1 VLP	Overall infection rate 0.6 vs 6.3 in placebo group
Ault et al. (2004) (63)	Phase I n=40	HPV18 L1 VLP	Induction of neutralizing antibodies
Harper et al. (2004) (512)	Phase I n=1113	HPV16/18 L1 VLP	Well tolerated; high efficacy in incident and persistent HPV16/18 infections
Villa et al. (2005) (1456)	Phase II n=277	HPV6/11/16/18 L1 VLP	High efficacy in persistent HPV6/11/16/18 infection

Table 27: Clinical phase I/II trials on prophylactic HPV vaccines

The overall trial results show a good safety profile and an almost universal induction of high titers of virus-specific antibody by VLP-based vaccines. The dominant antibody responses induced by VLP vaccines are of the IgG1 subclass and have been shown to be neutralizing by a variety of surrogate neutralization assays. 100% of vaccinees in the per-protocol cohort were protected against persistent infection with the homologous HPV type, whereas the placebo group had persistent infections with both HPV and CIN (512, 742).

The most important characteristics of the two clinical trials on HPV VLP vaccines by GlaxoSmithKline and Merck are shown in Table 28.

Features	GlaxoSmithKline	Merck
Vaccine type	Bivalent HPV 16/18 VLP L1	Monovalent HPV 16 VLP L1
Concentration	20µg HPV 16, 20µg HPV 18	40µg HPV 16
Application	0,5ml i.m. 0, 1 and 6 months	0,5ml i.m. 0, 2 and 6 months
Vaccinees/ placebo	560/533, age 15-25years	768/765, age 16-23years
Requirements	No history of cervical lesions Few sexual partners	No history of cervical lesions Few sexual partners
Duration	Up to 27 months	Up to 48 months
Efficacy	Prevention of incident infection 92% Prevention of persistent infection 100% Prevention of pre-invasive lesions 100%	Prevention of incident infection 91% Prevention of persistent infection 100% Prevention of pre-invasive lesions 100%
Seroconversion	100%	100%
Specific titers	50 times greater for HPV 16 80 times greater for HPV 18 (compared to natural infection)	60 times greater (compared to natural infection)
Reference	Harper et al. (512)	Koutsky et al. (742)

Table 28: Characteristics and results of two large randomized, placebo-controlled trials on HPV VLP vaccines by GSK and Merck, adapted from Franco et al. (421)

Further follow-up is needed to see whether the antibody levels remain after decades or whether booster vaccination is needed (1417). The duration of protection of vaccine-induced immunity measured by antibody titers has not been evaluated and needs to be established. Encouraging data presented on the antibody response to HPV L1 VLP vaccines suggests only a slight decline in the high serum antibody titers over a 6-to-8-months evaluation period (1417). There is also the possibility that vaccinations could lead to selection of HPV types, subtypes, or variants that would be unaffected by the vaccine.

In a study by **Pinto et al.**, innate and adaptive immune system cytokine responses induced by HPV-16 L1 VLP in whole blood cultures from individuals receiving the vaccine or placebo before and after vaccination were evaluated (1124). Cytokine profiles from whole blood samples clearly discriminated between vaccine and placebo recipients and between pre- and post-vaccination responses. Significant

increases in Th1-, Th2- and inflammatory cytokines were observed in whole blood assays following vaccination.

Arising problems in HPV vaccine development

Some further problems should be briefly discussed here.

Nardelli-Haefliger et al. determined whether HPV immunization results in specific antibody levels in cervical secretions of women who had been immunized with HPV16 VLPs (1004). They additionally examined the influence of the menstrual cycle and oral contraceptive use on these levels.

All participants developed detectable titers of anti-HPV16 VLP IgGs in their cervical secretions after immunization; however, cervical titers of specific IgG and total IgGs and IgAs among participants in the contraceptive group were relatively constant throughout the contraceptive cycle. In contrast, the cervical titers of specific IgG and total IgGs and IgAs among participants in the ovulatory group varied during the menstrual cycle, being highest during the proliferative phase and decreasing approximately ninefold around ovulation (1004).

Another consideration is the preferred delivery route for vaccine administration in order to induce effective mucosal immune responses (1349).

The vaccination should induce a local mucosal immunity in form of S-IgA in the lower genital tract in addition to systemic immunity to adequately prevent HPV infection (300). Mucosal antibodies are induced by systemic delivery of VLPs. Obviously, a single dose with mucosal delivery would be the preferred route for vaccine delivery; if nasal or oral delivery can induce antibodies still has to be further investigated.

In another study by **Nardelli-Haefliger et al**. women were vaccinated with escalating doses of HPV16L1 VLPs via nasal nebulisation, bronchial aerosolisation, or a combination of intramuscular and aerosol vaccination (1003).

The alternative routes of vaccination were well tolerated and many of the volunteers who received aerosol vaccinations exhibited serum antibody titers that were comparable to those induced by intramuscular vaccination. A mucosal immune response was induced by aerosol vaccination as demonstrated by the induction of anti-HPV16 VLP IgA secreting cells in PBMC and S-IgA in secretions.

The choice and the number of HPV types included in the vaccine is an important issue as there are geographic differences in the prevalence of HPV types involved in CIN and cancer.

In order to be effective for at least 80% of the population, the vaccines should theoretically consist of VLPs of the four or five most common types of HPV of that country or region but combining multiple types in one vaccine may be problematic (989, 1417). A pentavalent vaccine with VLPs of HPV types 16, 18, 45, 31, and 33 would potentially prevent 83% of all cervical carcinomas whereas a heptavalent vaccine that also included types 52 and 58 could potentially prevent 87% of the overall cervical cancer burden internationally (989).

Villa and colleagues presented results of a double-blind, placebo-controlled efficacy trial of a quadrivalent (HPV types 6, 11, 16, and 18), aluminium-adjuvant vaccine in young women negative for HPV (1456). As in the previous reports, peak antibody concentrations were much higher in vaccinees than in seropositive non-vaccinated individuals at seroconversion, and these concentrations remained higher 36 months after vaccination, when antibody titres in the vaccinees had decreased. In this study, no patients in the vaccinated group had CIN, whereas in the placebo group, seven had CIN and four had external genital warts.

Finally, all trials to date of HPV vaccines have enrolled women, but genital HPV infections are mainly sexually transmitted and men will also need to be vaccinated if the whole population is to develop immunity.

Therapeutic vaccines

Therapeutic vaccines for HPV are at a much earlier stage of development than their prophylactic counterparts. In contrast to preventive vaccines, HPV therapeutic vaccines would need to include some antigenic determinants derived from the early HPV proteins as E6 or E7 rather than the late proteins that VLPs use to self-assemble (1059).

For therapeutic vaccines L1/L2 would be a poor target because of its restricted expression in patients already having cervical neoplasia whereas both E6 and E7 proteins are continuously expressed in cervical lesions and tumors. This has proved to be a much more challenging task in terms of biodelivery and response.

E7 is probably the best studied; it induces protective cellular immunity against cervical dysplasia (644) and prevents tumor growth in animal models (14). Anti-E7 antibodies have been correlated with more advanced stage of disease (414) and worse prognosis (1455), but do not seem to denote an effective host immune response against cervical cancer. Indeed, it seems that immune responses in

patients with cervical cancer are marginal and functionally ineffective, making this a less attractive group for immunomanipulative strategies. Given that E6 and E7 expression increases as the HPV infection progresses in establishing a dysplastic process, they seem ideal targets for immune attack (1059).
Therapeutic vaccines consisting of E6 and/or E7 have been tested in patients and have proven to be safe and effective against benign warts, however, they have had limited therapeutic effect so far in cases of cervical cancer (425).

In general, there are several broad categories of therapeutic vaccine strategies: chimeric VLPs, peptides, proteins, nucleic acid–based, and cell-based.
The major challenge in infected patients remains the targeting of already infected basal cells that do not express capsid antigens. Therefore, **chimeric VLP** vaccines with structural viral L1 or L2 proteins and functional E proteins are being developed (986).
In preclinical data, these chimeric VLP vaccines elicited both neutralizing antibodies to the VLP and T cell responses to L1 and E7. Chimeric VLP comprised of a fusion of HPV16 E7 to the L1 or L2 capsid proteins have been shown to initiate a potent E7-specific CTL response in vaccinated mice. The vaccine was found to be sufficient to protect mice against challenge with an E7-containing tumor cell line (288).
The reason behind this vaccine`s high immunogenicity is a result of the interaction of chimeric VLPs with potent APC residing at or near the vaccination site which are able to induce an efficient antigen-specific immune response after interaction with naïve CD8+ T cells (1223).
These vaccines could therefore be prophylactic and therapeutic and would eliminate any breakthrough infection that escaped antibody neutralization (435).

Nieland et al. reported on the results of a randomized, double-blind, placebo-controlled, clinical trial with HPV16/E7 chimeric VLPs (1341). HPV DNA was cleared in vaccinees and placebo group although there were more responders in the vaccinees. Serum IgG to HPV16 L1 developed in all vaccinees but not in placebo recipients whereas only some vaccinees showed CTLs. Antibody responses and CTL generation, however, did not correlate with clinical outcome.
Chimeric capsomeres may be more stable and less expensive than chimeric VLPs and **Gissmann et al**. showed that HPV16 L1/E7 capsomeres were immunogenic, generating both anti-L1 antibody and E7-specific CTLs in mice (1341).
In another study, DCs and LCs are activated by three different therapeutic vaccination strategies including heterologous papillomavirus VLPs and HPV VLP

immune complexes (383). DCs and LCs incubated with these VLP upregulated surface activation markers and increased secretion of IL-12 p70 and IL-15. The activated cells are then able to initiate an immune response against chimeric VLP-derived antigens.
This shows that also other therapeutic vaccination strategies based on using heterologous chimeric VLP or chimeric HPV VLP immune complexes may be more effective in generating an immune response against HPV-induced diseases such as cervical cancer.

In animal models, CTLs can be generated using E6 and E7 **peptide-based** vaccines that are protective against subsequent challenge with lethal doses of E6- and E7-containing tumors (389). Stimulation of peripheral blood lymphocytes from patients with HPV16-positive cervical cancer with synthetic HPV-16 E7 peptide generates specific CTLs capable of tumor recognition and lysis of cervical cancer cells (25).
A clinical trial using a peptide-based vaccine was performed on 18 patients with high-grade dysplasia. Ten of 16 subjects mounted primary CTL responses to the E7 peptides, and a complete clinical response was observed in three subjects (984).

Protein-based vaccinations have been used in early-phase clinical trials. A randomized, placebo-controlled phase I study with E6/E7 fusion protein plus adjuvant was tested in women with CIN (426). Antibody and CD8+ T cell responses were significantly greater in immunized subjects than in placenbo recipients.

DNA vaccines usually involve intramuscular injection of plasmid DNA or DNA delivery into the epidermis via gene gun (853). Polynucleotide and recombinant viral vaccines encoding non-structural viral proteins show therapeutic efficacy in animal models and are candidate immunotherapies for established low-grade benign genital infections.
Immunization of rabbits with with the non-structural viral proteins E1 and E2 induced a CD4+ T cell response; fewer papillomas developed and they regressed more rapidly than those in non-vaccinated animals (1277). The canine oral papillomavirus (COPV) model has provided evidence that immunization with codon-modified early proteins will be effective in preventing the development of lesions postexposure to virus. Animals challenged orally with COPV and immunized subsequently with with a COPV E2 polynucleotide vaccine remained free of oral warts (962). Immunization of rabbits with established papillomas with an E1, E2,

E6, and E7 gene cocktail reduced the development of carcinoma by 75% and suppressed papilloma growth substantially (502).
In high grade intraepithelial lesions there are only two possible antigenic targets, E6 and E7. Tolerance to viral antigens, modulation of cytokine milieu and downregulation of MHC class I alleles on the neoplastic KCs are associated with progressive CIN and possibly invasive cancer, which pose strong barriers for immunotherapies (1344).
It is possible and also supported by few studies that there is a spectrum of responses to therapeutic vaccination ranging from complete through partial up to no regression of the clinical disease.
For example, studies with a recombinant vaccinia virus encoding modified E6/E7 genes of HPV 16 and 18 in patients with high grade vulval intraepithelial neoplasia showed complete clearance in only less than 10% of the patients, partial regression in at least 50% and no clinical change in 10-30% (299). Lesion regression did not correlate with T cell responses in all cases. This method for delivering antigenicity using a DNA vaccine involves the use of viral vectors as delivery carriers into the body.

Vaccines designed to elicit CTLs specific for the HPV oncoproteins E6 and E7 show immunogenicity and inhibition of tumor growth in transplantable tumor models in rodents (1344). However, human HPV-induced tumors have been largely refractory to the approaches successful in rodents. All the vaccines tested have been safe, well-tolerated and have induced T cell responses which did not necessarily correlate with clinical responses (666, 1191).

A previous trial with a L1 DNA vaccine evidenced only weak immune responses to HPV (350). Recently, a modified version DNA vaccine targeting HPV6 L1 and L1-E7 was constructed and tested, to determine if this DNA vaccine would elicit an immune response in mice (822).

In summary, other potential delivery systems for HPV immunogens besides recombinant viral vectors (666, 139) include recombinant bacterial vectors such as *Listeria monocytogenes* (1279), viral DNA (229), proteins (426, 1277) and peptides alone (984) or tagged to immune adjuvants such as CpG oligonucleotide (215) and DCs (1026); some of these experimental strategies have already even been tested clinically with promising results (1351).

An overview of the different strategies with candidate biologics for HPV vaccines gives Table 29.

Virus-like particles (L1 based)	Prophylactic
Chimeric virus-like particles (both late and early proteins expressed)	Prophylactic/Therapeutic
Viral DNA	Therapeutic
Fusion proteins	Therapeutic
Recombinant viral vectors	Therapeutic
Recombinant bacterial vectors	Therapeutic
Peptides (alone or with adjuvants such as CpG oligodeoxynucleotide)	Therapeutic
Dendritic cells	Therapeutic

Table 29: Candidate biologics for HPV vaccines, adapted from Padilla-Paz (1059) and Steller (1349)

2. Bacterial infections of the genital tract

a) Gonorrhea

Gonorrhea is another example for a STD and is caused by *Neisseria gonorrheae*, a gram-negative diplococcus. It is an exclusive human pathogen that primarily infects the urogenital epithelia but can also lead to infection of other EC surfaces.
Gonococcal infection is with over 60 million cases a major global health problem with highest incidences in less developed countries (444). However, more than 300,000 cases are reported to the Centers for Disease Control and Prevention in the United States each year but an estimated 600,000 people are infected (197).

Gonococcal infections usually remain localized to the sites of primary infection, the male urethra and the female cervix. Gonococcal cervicitis often remains asymptomatic but subsequently tends to spread to the upper genital tract inducing chronic complications (353). Ascending infection occurs in up to 45% of infected women and can result in pelvic inflammatory disease (PID) which can cause infertility and ectopic pregnancies due to fallopian tube blockage (1330). Repeated infections are rather common, which suggests antigenic variations in the organisms or an ineffective immune respose to infection.

Furthermore, the gonococcus-induced increase in the local expression of viral RNA together with the loss of mucosal integrity due to an acute inflammatory response is associated with increased susceptibility to HIV-1 infection (211). Despite the presence of effective antibiotic treatment these health concerns demand the need for further investigation on the mechanisms of the infection in order to develop a vaccine against gonorrhea.

(1) Mechanisms of N. gonorrheae to evade the immune response

Virulence factors

Only recently it has been shown that *N. gonorrheae* can invade mucosal ECs and are intracellular during human infection (39).

A repertoire of virulence factors have been identified and allow this bacterium to successfully adapt to variable microenvironments within its sole human host and are thought to play a role in EC invasion. These virulence factors are responsible for gonococcal evasion of the human immune system and explain the tendency to chronificate. These factors consist of outer membrane constituents such as porin, pili, opacity-associated outer membrane proteins (Opa), reduction modifiable protein (Rmp) and lipooligosaccharides (LOS) (353).

Pili seem to modulate host cell signaling mechanisms to aid gonococcal epithelial invasion and participate in forming an initial attachment with host cells (1372). They may also provide a mechanism by which nonmotile gonococci are able to colonize and to ascend mucosal surfaces through their ability to exhibit twitching motility (1469). The transmembrane protein CD46 involved in regulation of complement activation has been demonstrated to serve as a receptor for gonococcal pilus in human cells (653).

Opa proteins are thought to contribute to the cellular trophisms exhibited by gonococci and are divided into two classes based on their ability to differentially recognize host cell surface molecules (353). Opa_{50} recognize host cell heparin sulphate proteoglycans and Opa_{52} recognize carcinoembryonic antigen-related family of cell adhesion molecules (CEACAM or CD66).

LOS play a role in attachment of the pathogen to epithelial tissues and is a target for bactericidal antibodies found in normal human serum (160). Variations of LOS molecule structure in the outer membrane of *N. gonorrheae* are observed within and

between strains which results in constant antigenic variation. This spontaneous conversion of oligosaccharide determinants can change the manner in which the gonococcus associates with host tissues and, hence, can potentially alter the course of gonococcal disease.

Porin, a water-filled channel through which small molecules traverse the gonococcal outer membrane, is thought to play multiple roles in potentiating disease caused by *N. gonorrhoeae*. Porin molecules trigger variable functional responses within host cells depending upon the particular porin and the host cell type under study. A unique feature of gonococcal porin is its ability to translocate into eukaryotic cell membranes and form a voltage-gated channel (1484).
By demonstrating the ability of porin to induce apoptosis in ECs (985) it is proposed that porin plays a role in the cytotoxicity observed in fallopian tube organ culture and in the shedding of ECs which occurs *in vivo* during mucosal infection (353). In contrast, gonococcal infection of primary human male urethral ECs results in antiapoptotic events (116). It is hypothesized that the enhanced survival of the urethral EC may allow the bacterium to proliferate within an intracellular, protective environment and, consequently, promote gonococcal colonization. Porin may also facilitate the cytoskeletal rearrangements required for actin-mediated entry of the gonococcus into its target host cell (1493).

These virulence factors together with the high heterogeneity and adaptability with repeated phase and antigenic change may be a way to downregulate the functional immune response of the host. Pathogens use at least two basic strategies to survive the host`s immune response. Some avoid provoking specific host defenses and others induce immune responses but possess the ability to evade the consequences. The high rate of reinfection despite the presence of antigonococcal antibodies leads to the assumption that *N. gonorrhoeae* evades the host's immune response.
Indeed, *N. gonorrhoeae* possess several mechanisms which could potentially thwart the effects of immune responses directed toward this organism *in vivo*, including hypervariation of surface antigens, resistance to complement- mediated bacteriolysis, and the production of IgA1 protease (530).

Resistance to complement-mediated bacteriolysis

Gonococci possess several potential mechanisms to avoid complement-mediated bacteriolysis, including sialylation of LOS, induction of blocking antibody to Rmp and binding of complement downregulating proteins such as C4bp to particular

porin domains (1229). However, it seems unlikely that complement-mediated, IgG-dependent bacteriolysis operates at mucosal surfaces, where a fully functional complement system is not usually present (1396) and IgA may interfere with complement activation. Both IgA and IgG in genital secretions have been shown to inhibit adherence of gonococci to ECs (1428).

IgA1 protease

Vaginal washes from patients infected with *N. gonorrhoeae* have been found to contain IgA1 protease activity demonstrable *in vitro* and were able to cleave exogenous IgA1 in a manner suggestive of IgA1 protease activity (120). However, despite the presence of substrate, and contrary to expectations, **Hedges et al.** did not detect any evidence of IgA1 cleavage fragments by gonococcal IgA1 protease in cervical mucus or vaginal wash samples (530). Nevertheless, all of the clinical isolates of *N. gonorrhoeae* infecting these patients produced IgA1 protease *in vitro*.

These apparently contradictory results may be explained by two linked hypotheses. First, *N. gonorrhoeae* may not be present in the lumen or on the mucosal surface in sufficiently high numbers but rather may colonize a subepithelial niche (530). This hypothesis is supported by the lack of significant local immune or cytokine responses in women infected with *N. gonorrhoeae*. Therefore, the lack of detectable IgA1 protease activity in cervical mucus, in addition to the lack of local host responses, may simply be due to small numbers of bacteria at that site.
Secondly, *N. gonorrhoeae* may require IgA1 protease for survival within as well as outside the host tissues.
In addition, there is recent evidence that the lysosomal-phagosomal protein LAMP-1 on ECs is cleaved by neisserial IgA1 protease (820). Growth of gonococci within ECs was enhanced by cleavage of LAMP-1 which may contribute to intracellular gonococcal survival and facilitate escape from antibodies and complement.

Differences of gonococcal infection in male and female

Interestingly, there are important differences between gonococcal genital infection in men and women and *N. gonorrhoeae* has evolved variable pathogenic mechanisms to ensure its survival in the distinctly different microenvironments found within the male and female (uro)genital tracts (353).
In male urethral epithelium, the interaction of gonococcal LOS with the asialoglycoprotein receptor present on the urethral EC mediates invasion and results in production of the cytokines IL-1, IL-6, IL-8 and TNF-α (516, 514).

Cytokine release contributes to the usually symptomatic nature of gonococcal disease in men and is accompanied by a large influx of PMNs, which in turn contribute to the observed cytokine release and inflammation. The interaction of gonococci with PMNs is mediated by the interaction of Opa gonococcal proteins and CEACAM host cell proteins (1192). The specific interactions occurring between these two families of proteins may dictate specific host cellular responses and the survival or death of phagocytosed gonococci. The PMN response to gonococci is further modulated by gonococcal porin, which inhibits PMN degranulation and the production and release of toxic oxidants from the host cell to the extracellular milieu. Transmission of gonococci to a sexual partner is then partly aided by the ability of gonococci to bind to human sperm which also were found to express asialoglycoprotein receptor (515).

In contrast to the inflammatory response generated predominately with gonococcal infection of the male urethra, 50 to 80% of women with lower genital tract *N. gonorrhoeae* infection are asymptomatic, and 70-90% of women with disseminated infection lack signs of genital tract involvement (1330).
The clinical findings that there is neither an antibody response nor elevated local cytokine levels in women with gonococcal infection are consistent with the ability of the gonococcus to evade and subvert host immune function (531).
Cervical epithelia provide a source of alternative pathway complement activity, yet, at a level comparable to only approximately 10% of that observed for human serum (1440).
Within minutes of infection of primary cervical ECs, complement protein C3b is deposited on the lipid A portion of gonococcal LOS and is rapidly inactivated to iC3b (354). This is supported by the predominance of iC3b in comparison to C3b on the surface of clinically isolated gonococci (1460).
Analysis of clinical biopsies obtained from women with culture-documented gonococcal cervicitis and infection studies performed with primary human cervical ECs indicate that complement receptor 3 (CR3) on female genital epithelia serves as the primary receptor for *N. gonorrhoeae* adherence to and invasion of the ectocervix and endocervix (355).
Binding of gonococcal pilus to the I domain of CR3 probably allows the gonococcus to overcome the electrostatic repulsion between its own cell surface and that of the cervical cell and may juxtapose the gonococcus at the cervical cell surface, where complement concentrations would be expected to allow efficient opsonization for the subsequent intimate adherence of iC3b, i.e. converted C3b, and gonococcal porin to

the I-domain. Binding of the gonococcus to CR3 requires the cooperative action of iC3b bound to the gonococcal surface in conjunction with gonococcal porin and pilus (355). This ligand binding to the I-domain of CR3, however, does not invoke a proinflammatory response in professional phagocytic cells and cellular fate of gonococci is not clear (353).

Interestingly, menses is associated with an increased risk to women for PID and for disseminated infection (1373). C3 production by the cervical epithelium exhibits cyclic variability, and the highest levels of C3 are detected during menses (521). Additionally, a correlation can be made between the presence or the absence of Opa and the site of gonococcal infection. Opa- gonococci are predominate within the fallopian tubes and in the cervix at the time of menses while Opa+ gonococci are found predominately within the male urethra and within the cervix at the time of ovulation (353).

Ascent to the upper female genital tract might be facilitated through the ability of gonococci to exhibit twitching motility, in conjunction with hormonal changes which influence the mucosal epithelium and the expression of complement and molecules serving as gonococcal receptors within the female genital tract (353).

Microscopic analysis of tissue biopsies indicates that the expression of CR3 progressively decreases in an ascending manner from the ectocervix to the fallopian tubes (354). But expression of the lutropin receptor (LHr) which might serve as gonococcal receptor in upper genital tract epithelia (1331) increases in an ascending manner to fallopian tubes with highest levels at menses (521). The presence of LHr on the human uterus, placenta, decidua, and fetal membranes may partly contribute to the fact that the increased risk of spontaneous abortion associated with *N. gonorrhoeae* infection is due to a gonococcus-LHr interaction occurring on deciduas and placental membranes (1190).

(2) Immune responses to gonococcal infection

Igs

Early studies using enzyme immunoassay determined the presence of IgM, IgA and IgG antibodies to gonocoocal pili antigens in serum (930). In all Ig classes, a significantly higher mean antibody activity and a higher percentage of positive sera were found in men and women with *N. gonorrhoea* than in controls. The magnitude of antibody response was higher among infected women than men, especially in the IgM class. Another study found serum and local IgG and IgA to be produced against

several antigens during goncoccal infection, although the quantity of antibody was greater in serum (601).

As mentioned earlier, recent preliminary evidence indicated, however, that while antigonococcal antibodies were detected in infected patients, the levels of both serum and antigonococcal antibodies in genital secretions were surprisingly low (532).

A following study measured the concentrations of total IgA1, IgA2, IgG, and IgM in cervical mucus, vaginal wash, and serum samples from volunteers without demonstrable infection, from volunteers in whom only *N. gonorrhoeae* was detected, and from volunteers infected with other pathogens (*C. trachomatis* or *T. vaginalis*) with or without *N. gonorrhoeae* (531).

There were no differences between the concentrations of total IgA1, IgA2, IgG, and IgM in genital tract secretions in patients with different STDs compared with non-infected women. Moreover, levels of IgA1, IgA2, IgG, and IgM antibodies specific for *N. gonorrhoeae* MS11, a widely studied gonococcal strain, in female mucosal secretions and serum were found at low levels in both uninfected and infected women. IgA1 antibody levels in serum, but not in secretions, were higher in female patients infected with *N. gonorrhoeae* than in noninfected patients while the levels of IgG and IgM antibodies in serum and secretions were not different between gonococcus-infected and noninfected patients (531).

A history of previous infections with *N. gonorrhoeae* did not alter the antibody levels in patients with a current infection except for arising levels of serum IgA1 antibody. These results further support the possibility that repeated infections with *N. gonorrhoeae* are common because there is little development of immune memory and therefore only minimal levels of protective immunity.

One potential explanation for the paucity of antibody responses to *N. gonorrhoeae* in uncomplicated genital tract infections may be related to the absence of organized mucosa-associated lymphoid tissue, such as the Peyer`s patches of the small intestine, which are recognized as major sites for the uptake and processing of antigens leading to generalized disseminated mucosal immune responses as described earlier.

Although in some studies local vaginal antibody responses were recorded, intravaginal immunization in humans appeared to be inefficient in inducing either circulating or generalized mucosal antibody responses compared to oral or nasal immunization (743). In contrast to the genital tract, the rectum contains lymphoid

follicles resembling Peyer`s patches that likely serve as an inductive site of the common mucosal immune system. In addition, it has been suggested that these sites may preferentially supply specific antibody-secreting cell precursors to the adjacent genital tract which shares the same lymphoid drainage (272). Therefore, it seemed likely that persons infected at both the rectum and genital sites might be expected to display enhanced antibody responses to the infecting organism, both in the genital tract and perhaps also in remote secretions.

Due to the prevalence of rectal infections with *N. gonorrhoeae* in the mentioned study it could be examined whether more pronounced antigonococcal antibody responses were generated by gonococcal infection at a site known to contain organized inductive lymphoid tissue.

There was a small effect of rectal infection on the levels of isolate specific IgG in cervical mucus but overall only little difference in antibody levels in patients with cervical compared with cervical and rectal infections was found, suggesting that rectal infection was no more efficient than the genital tract infection for inducing humoral responses to *N. gonorrhoeae* (531).

A recent study by **Pantelic et al**. demonstrated that *N. gonorrheae* via their Opa protein has the ability to suppress antibody production by killing CEACAM1-expressing B cells (1075).

Cytokines

This relative paucity of antibody response provokes the question as wether there is a cytokine response to gonococcal infection as it is seen in female urinary tract infection and experimentally infected men (1166).

Therefore, the levels of IL-1, IL-6, IL-8, IL-10, and TGF-β in sera and genital tract secretions from women with gonococcal cervicitis and other genital infections were examined (532). Surprisingly, the local levels of all these cytokines in genital secretions were not elevated in women with gonococcal cervicitis compared with levels of uninfected persons. In contrast, serum IL-6 levels, but not IL-8 and IL-1 levels were significantly elevated in gonococcus-infected women.

Serum, but not local, IL-1 and IL-6 levels were elevated in patients concomitantly infected with *Trichomonas vaginalis* or *Chlamydia trachomatis* in addition to *N. gonorrhoeae* compared with levels in patients infected with any single organism (532).

However, in contrast, **Fichorova et al**. have reported increased IL-1, IL-6, and IL-8 expression in similar studies performed with immortalized vaginal, endocervical and

ectocervical epithelia (396). Experiments with whole gonococcal lysates revealed that the IL-8 and IL-6 response by cervical and vaginal ECs was not restricted to the interactions with viable gonococci.
Similarly, a recent study has shown that viable *N. gonorrhoeae* is not essential for proinflammatory response by innate immune cells, since mature human macrophages generate an array of cytokines and chemokines in response to purified gonococcal surface antigens (861).
These findings suggest that gonococcal components can stimulate proinflammatory responses, which are independent of either gonococcal metabolic activity i.e. viability or entry into the host cells.

T lymphocytes

Gonorrhea typically correlates with a transient reduction in T cell counts in blood, and these populations recover when gonococcal infection is resolved. Opa proteins have been shown to bind CEACAM1 expressed by primary CD4+ T cells and suppress their activtion and proliferation (134).

On the basis of the absence of cytokines and the low levels of antigonococcal antibody detected during uncomplicated cervical infections by *N. gonorrhoeae* it is proposed that in addition to their ability to evade the consequences of immune responses, gonococci either fail to induce, or possibly actively suppress, the host`s immune and inflammatory responses.

(3) Development of vaccines

As *N. gonorrheae* is an obligate human pathogen, the development of a vaccine has been hampered by the unavailability of a convenient and simple animal model. Attempts to infect or colonize the genital tracts of different animal species have been unsuccessful (49).
Certain vaccines have been evaluated in human males but earlier prototype gonococcal vaccines have shown limited or no protection against reinfection with *N. gonorrhoeae* despite the generation of serum antibody responses against the vaccine antigens. A trial with gonococcal pilus vaccine, which has been shown to be safe as well as antigenic and resulted in the production of specific antibodies, failed to protect men (1429, 132).
A second vaccine consisted of porin protein as a systemic immune response to porin protein was shown after endocervical and urethral infection (244). This

vaccine failed to provide protection (1193) but it was later recognized that the vaccine was contaminated with Rmp. Rmp leads to production of blocking antibodies capable of preventing the function of bactericidal antibodies against porin.

This suggests that vaccination endeavors should therefore be directed toward exploiting novel concepts and strategies of mucosal or systemic immunizations. Examples include nasal immunization, which has been shown to generate antibody responses in genital secretions, and which has been studied to determine if it could elicit an immune response capable of preventing vaginal colonization of gonococci in a mouse model (1127). Bacterial clearance was significantly faster for mice immunized intranasally with gonococcal outer membrane preparations than control mice. The development of systemic and local vaginal antibodies directed mainly against a number of these outer membrane proteins was induced.

However, **Jerse** has developed a more improved mouse model of gonococcal female genital tract infection (621) which will facilitate testing of topical microbicides and experimental vaccines for mucosal gonococcal infection (1332).

b) Chlamydia trachomatis

Chlamydia trachomatis is one of three major species within the genus *Chlamydia* and an etiologic agent for several common genital tract syndromes such as urethritis, cervicitis and PID in women as well as urethritis and epididymitis in men. Genital tract infection with *C. trachomatis* is often chronic and is associated with few symptoms and a scant inflammatory exudate.

Despite continous improvement of screening and treatment programs, genital chlamydial infection is with approximately 4 million new infections per year the most common bacterial STD in the United States and with about 90 million new cases per year worldwide a major health problem (1543). The prevalence rates for chlamydial infection among sexually active individuals range from 3% to 25% (683). The highest rates of chlamydial infection occur among adolescent women who are also at the greatest risk to develop complications arising from untreated infection.

It is an obligate intracellular bacterium and therefore, efficient antimicrobial therapeutics have to achieve adequate intracellular concentrations. Most patients

are free from infections after a 2 or 3 weeks treatment with tetracyclines or macrolides (443).
However, approximately 70% of initial chlamydial infections remain asymptomatic (187), which results in the lack of seeking medical help and in further spreading of the disease. *Chlamydia* specifically infects ECs in the reproductive tract where the organism ascends from the cervix to the fallopian tubes (1527). The lack of antibiotic treatment and the ability of *Chlamydia* to evade immune defense mechanisms results in persistent fallopian tube infection. An ongoing infection eventually leads to scar formation and occlusion of the fallopian tubes which consequently results in pregnancy loss, infertility or ectopic pregnancy.

In order to fully appreciate the development of a local immune response against *Chlamydia*, it is important to understand its life cycle with respect to host-parasite interactions.
Chlamydiae have a unique developmental cycle among obligate intracellular bacteria that involves two distinct morphological forms called the elementary body (EB) and the reticulate body (RB) (90).
The infectious but metabolically inactive EB particle enters host cells by first binding to a number of proposed ligands on *Chlamydia* which induces the internalization of the pathogen. EBs are finally differentiated to RBs within the cell and start replication. After the developmental cycle is completed after 40 to 72 hours, RBs differentiate into infectious EBs which are released from the host cell and infect neighbouring cells after having reached a certain density (1527). The most common cellular host in the reproductive tract is the superficial columnar EC.

With exception of the *Chlamydia* serovars L causing lymphogranuloma venereum, infections are mainly local. *Chlamydia* exits the apical end of the EC preventing the spread of infection to cells underlying the basement membrane. Currently there have been detected 18 serovars of *C. trachomatis* based on immunoepitope analysis using monoclonal antibodies directed against the major outer membrane protein (MOMP) of chlamydiae (1475).
Most efforts at elucidating the pathophysiology of chlamydial infections have focused on two protein antigens (596). The first is MOMP which is almost certainly involved in the earliest interactions of this organism with leukocytes during the course of natural infection. MOMP, an immunodominant molecule, constitutes almost 60% of the outer membrane protein of *C. trachomatis* (170) and appears to evoke a protective humoral response to infection (636). A second protein produced

by pathogenic strains of *C. trachomatis* is the heat shock protein-60 (hsp-60), which induces inflammatory changes in trachoma (972).

(1) Early cell-mediated immune response to Chlamydia

The immune-mediated eradication of chlamydiae from the genital mucosa appears to occur in two distinct phases and most likely by different mechanisms (1112).

Neutrophils

One early mechanism that appears to reduce the number of organisms soon after infection is an influx of neutrophils.

Shortly after the vaginal inoculation of guinea pigs with *Chlamydia*, neutrophils were found in the uterine horns and oviducts (1171). *In vitro* experiments have shown that neutrophils have the capability to destroy *Chlamydiae* (1182). Also in mice that were depleted of neutrophils by antibody treatment the number of organisms isolated from the genital tract were approximately 10-fold greater the day after infection (82). In addition, a greater number of mice that were depleted of neutrophils were culture positive during the first week of infection compared to controls.

However, neutrophils were not critical for eradication of *Chlamydiae* since all mice were able to resolve the infection within the same time frame. Thus, neutrophils appear to play a role in reducing the initial amplification of *C. trachomatis* and possibly limit the spread locally within the genital tract.

NK cells

NK cells also seem to play an important role in the initial control of chlamydial infection.

Tseng et al. reported that mononuclear cells isolated from the genital tract of infected mice demonstrated YAC cell cytotoxicity *in vitro* which is a measure of NK cell function (1431). This response peaked as early as 3 days after vaginal inoculation. Although the antibody-induced depletion of NK cells in mice did not reduce the number of pathogens isolated from the genital tract within the first week after infection, continued depletion throughout the course of infection resulted in delayed clearance of *Chlamydiae*.

In experiments performed by the enzyme-linked immunospot assay, high numbers of cells producing IFN-γ were found in the genital tract, concomitant with resolution

of the infection; however, in addition, an increase in IFN-γ-producing cells which were CD4-negative was seen early in the infection (1431).
Also, **Hook and colleagues** showed that both IL-12 and IL-18, which synergise to stimulate NK cells to produce IFN-γ, are produced following the infection of DCs and ECs by live *C. trachomatis* (563).

DCs

Stagg and colleagues reported the recruitment of cells with a DC phenotype in the genital tract during *Chlamydia* infection of mice (1334). These cells expressed the costimulatory molecules CD86 and CD40 and stimulated allogeneic T cells, suggesting that these mononuclear cells are a population of APCs and that they may play a role in clearing antigen and protecting against inflammatory disease.

(2) Specific immune response to chlamydial infection

The knowledge of the processes that influence the induction of an acquired immune response against *Chlamydia* within the genital mucosa is still limited.

Although *C. trachomatis* primarily utilizes ECs to complete the developmental cycle, the organism can also enter other cell types such as DCs and monocytes.
In monocytes, chlamydial EBs were found to colocalize in lysosomal compartments containing MHC class I molecules and, upon activation with IFN-γ, in vesicles containing both MHC class I and II (1043). Thus, during a chlamydial infection, the production of both CD8+ and CD4+ specific T cells can occur.
In DCs, the internalized bacteria appeared in lysosomes expressing MHC class II molecules. These cells most likely play a role in initiating a Th1 immune response needed to eradicate the organism.
Recently **Zhong et al**. reported that *C. trachomatis* infection of APCs reduced the ability of these cells to stimulate T cell proliferation through defects in antigen processing but not presentation (1586). This has been shown to be mediated via a chlamydial protein that induces degradation of transcription factors that control IFN-γ-induced expression of both MHC class I and II antigens.

While the ability of *Chlamydia* to manipulate the host cell response to infection obviously is a survival advantage, it may also contribute to observations that immunity to *Chlamydia* is weak, resulting in persistent or recurrent infection.

CD4+ T cells

The first studies implicating a role for T cells in clearing infection in the local genital mucosa were performed in nude mice (1172). **Rank and colleagues** showed that infection in nude mice was chronic and persisted over 200 days whereas normal mice could resolve the infection within 18 to 21 days after vaginal inoculation. These findings have been confirmed by showing that the transfer of CD4+ or CD8+ lymphocyte lines specific for chlamydial antigens could clear infection in nude mice where the CD4-enriched cell line was much more efficient (1165).

Morrison and colleagues showed that mice lacking MHC class II molecules were unable to clear *Chlamydia* from the genital tract (971). In addition, mice with disrupted CD4 expression also showed significantly delayed clearance.

Cain et al. investigated the local mononuclear cytokine response by examining IFN-γ and IL-4 production (167). The biphasic appearance of IFN-γ-producing cells at 1 and 3 weeks after infection was attributed on the one hand to NK cells at week 1 and on the other hand to the time when T cell proliferative responses against chlamydial antigens and the influx of CD4+ lymphocytes were observed (682). In contrast, only small numbers of IL-4-secreting cells could be found throughout the course of infection. The dominant production of IFN-γ-producing cells was also observed in draining iliac lymph nodes as well as the mesenteric lymph nodes and spleen during the course of infection.

Recently, it has been shown that a *Chlamydia*-specific CD4 Th2 clone is unable to clear organisms from the genital mucosa of nude mice for up to 2 months after infection (523).

In addition, it has been found that if the immune response was manipulated to favor a Th2 response, the local clearance of organisms was delayed. For example, the absence of IL-6 during a *C. trachomatis* respiratory infection did prolong the clearance of organisms from the lung and resulted in higher titers of antichlamydial IgG1 antibody in the serum (1506). The authors also noted increased levels of IL-10 in these mice and were able to restore a dominant Th1 response by administering anti-IL-10 during infection.

Thus, from these studies it appears that a predominately Th1 CD4+ lymphocyte response is required to clear *Chlamydiae* from the local genital mucosa.

CD8+ T cells

Over the past few years reports have been published examining the role of chlamydial-specific CD8+ lymphocytes in the resolution of genital infection.

Cytotoxic killing of chlamydial-infected cells was demonstrated in targets transfected with ICAM-1 (92) and **Starnbach et al**. were able to demonstrate classical MHC class I restricted cytolysis from the spleens of infected mice (1345).

IFN-γ

However, the majority of the antichlamydial activity for both CD4+ and CD8+ lymphocytes is associated with the production of high levels of IFN-γ. This was initially demonstrated *in vivo* by administering anti-IFN-γ to mice prior to vaginal inoculation which interfered with the clearance of *Chlamydia* from the genital mucosa (1167).

In addition, a protective CD4+ Th1 clone was shown to produce high levels of IFN-γ and TNF-α (593) whereas **Starnbach et al**. also demonstrated that the ability of a cytotoxic CD8 cell to eliminate *Chlamydia* infection was largely due to the production of IFN-γ in a series of experiments (683).

The bacteriostatic effects of IFN-γ itself were demonstrated in murine models *in vitro* where chlamydial growth inhibition was mediated by the induction of NO (892). Although NO inhibits chlamydial replication *in vitro* and is induced following infection (1112), it does not contribute to the resolution of genital infection *in vivo* (592).

Another possible role for IFN-γ *in vivo* may include the activation of local macrophages which may be necessary to stimulate antichlamydial activity from local Th1 lymphocytes within the genital mucosa (683).

While IFN-γ clearly plays an important role in immune-mediated killing of *Chlamydia in vivo*, studies in IFN-γ-/- mice have revealed an additional, IFN-γ-independent mechanism for eradication of *Chlamydia* from the genital tract. The large majority of chlamydial burden in the genital tract is cleared in IFN-γ -/- mice (1112) and these mice did not develop dissemination of the organism following a second vaginal inoculation (260). In the respiratory model, results showed that immunity in IFN-γ -/- mice was associated with increased levels of TNF-α and GM-CSF in the lungs (1507).

Adhesion molecules

However, not only high concentrations of cytokines as IFN-γ delivered in close proximity to infected cells, but also activation and cell-to-cell contact for the inhibition of growth by a specific CD4+ T cell clone is required (683). When considering the extensive surface area of the genital tract mucosa and the small numbers of CD4+ lymphocytes present there during infection (680), the result would be that immune effector cells must be directed to infected ECs in order to be effective at eradicating the infection. The recruitment of leukocytes into tissue sites depends on interactions between adhesion molecules on ECs and integrin receptors on the leukocytes.

Predominantly CD4+ T cells with a peak at 3 weeks after infection were recruited to the genital mucosa following chlamydial vaginal inoculation in mice (680). **Kelly et al**. showed that in the genital tract of mice, recruitment of CD4+ cells is mediated through the interactions between homing receptors on CD4+ T cells such as α4β7 and adhesion molecules ICAM-1, VCAM-1 and MadCAM-1 (524, 680). The latter were both temporarily induced in genital tract of mice due to chlamydial infection.
Kelly and colleagues also reported that during *Chlamydia* infection, cellular recruitment differed in the upper genital tract, i.e. oviduct and uterine horn, compared to the lower genital tract, i.e. cervix and vagina (682).
It was found that CD4+ cells were recruited mainly to the upper genital tract during a primary genital infection with mouse pneumonitis (MoPn), a biovar of *C. trachomatis*, whereas neutrophils and monocytes were recruited to all regions of the genital tract. This was unexpected given that *Chlamydia* initially infect ECs in the cervix and then ascend to the oviducts.
The study group also discovered a differential regulation of adhesion molecules on endothelial cells in the lower tract compared to the middle or upper tract. ICAM-1 was found to be expressed in the lower tract of uninfected mice; VCAM-1 and MAdCAM-1 were induced early after infection in the lower genital tract. In contrast, no adhesion molecules were expressed in the upper genital tract of uninfected mice, but all three were induced later in the course of infection which correlates with the appearance of CD4+ cells in these tissues (682).

Other data suggest that the IFN-γ-inducible protein-10 (IP-10) and monokine induced by interferon gamma (MIG) are responsible for recruiting Th1 cells to the genital tract during infection. The binding of these chemokines to specific receptors activates integrin homing receptors on leukocytes, which in turn facilitates

adhesion and directed migration within the tissue parenchyma. Focusing on chemokines that would attract Th1 cells, it was found that protein levels of IP-10 and MIG were elevated in upper but not lower genital tract early after infection (891).

These data showing differential chemokine expression in the upper and lower genital tracts support increasing evidence that the inflammatory response in the lower genital tract may be prematurely terminated even in the presence of an active *C. trachomatis* infection.

(3) Cytokine and chemokine response to chlamydial infection

Several studies *in vivo* and *in vitro* have shown the release of proinflammatory mediators including IL-1β, TNF-α, IL-6 and IL-12 upon chlamydial infection (683). However, studies demonstrated that *C. trachomatis* can enter host ECs without inducing the immediate production of inflammatory cytokines (1174).

Peak levels of IL-8 were delayed until 2 to 4 days after the initiation of infection suggesting that neither physical entry of *C. trachomatis* nor cell surface contact with molecules such as chlamydial LPS were capable of inducing IL-8 secretion. Transcription of IL-8 mRNA was also delayed and was first observed 2 hours after infection, most likely through the translocation of NF-kB. These data suggest that the initiation of the chlamydial developmental cycle is responsible for inducing IL-8 secretion (1174).

Other proinflammatory cytokines such as IL-1, IL-6, and GM-CSF were also released following chlamydial infection of EC lines as well as primary endocervical ECs. However, unlike other invasive bacteria, the entry of *Chlamydia* was not responsible for the cytokine release (1369).

Rasmussen et al. proposed the relatively small size of infectious EBs compared to other invasive bacteria as one factor allowing *Chlamydia* to enter without stimulation of cellular cytokine production (1174).

Ingalls and colleagues found that chlamydial LPS has a weaker stimulatory ability compared to other LPS-containing bacteria, for example gonococcal LOS (596). Others illustrated the ability of live *Chlamydia*, but not of heat-killed, to induce IL-1β, TNF-α and IL-6 from a monocyte cell line (114).

Belay et al. analyzed the dynamics of chemokine and chemokine receptor expression in genital mucosae during genital chlamydial infection in a murine

model to determine how these molecular entities influence the development of immunity and the clearance of infection (100).
The study revealed an increase in the levels of expression of RANTES, MCP-1, IP-10, MIP-1, and ICAM-1 which are involved in the recruitment of Th1 cells after genital infection with the *C. trachomatis* agent of mouse pneumonitis. Peak levels of expression of RANTES, MCP-1, and MIP-1 occurred by day 7 after primary infection, while those of IP-10 and ICAM-1 peaked by day 21. After 6 weeks and resolution of infection the expression of these molecules as well as of chemokine receptors CCR5 and CXCR3 decreased but was upregulated again after secondary infection.

While *in vitro* studies have revealed that *Chlamydiae* can enter ECs without signaling the immediate production of proinflammatory cytokines, a similar result is also seen in the genital mucosa *in vivo*.
Peak levels of TNF-α were not seen until 5 days into the infection of *C. trachomatis* after the pathogen was inoculated into the mouse vagina (297). Overall, the local cytokine response appears to be weak and does not follow serum levels.
Hedges and colleagues examined IL-1, IL-6, IL-8, IL-10, and TGF-β in genital secretions of women with bacterial STD. The genital secretions of women with *C. trachomatis* or *N. gonorrhoeae* were not consistently elevated compared to uninfected controls (532).
However, increased levels of IFN-γ were found in endocervical secretions compared to uninfected women (51), and TNF-α expression was found in cultures of fallopian tubes that were infected *in vitro* with *C. trachomatis* (64).

Reddy et al. reported that all Th1- and Th2-cytokines analyzed in their study, i.e. IFN-γ, TNF-α, IL-10 and IL-12 were upregulated. As both CD4+ and CD8+ cells contribute to the production of and IL-10, these results confirm again that CD4+ and CD8+ lymphocytes may be important for local regulation of Th1/Th2 responses in the genital tract during *C. trachomatis* infection (1179). Thus, the predominant cytokines produced following chlamydial genital infection in animal models are also found in humans.

APCs play a central role in shaping the cytokine profile of an immune response to *Chlamydia* through production of IL-12, IL-18, and IL-10. Both IL-12 and IL-18 are important for producing Th1 responses and are induced following infection with *Chlamydia* (834). IL-12 appears to play a dominant role over IL-18 since the

chlamydial burden was only slightly increased following infection in IL-18 knockout mice. Anti-IL-12 prolongs the course of infection (1112) while IL-12 administration shortens infection (575). Finally, IL-10 appears to reduce the frequency of IFN-γ-producing T cells and resulted in an increase in chlamydial burden (589).

Darville et al. found that depletion of TNF-α in genital secretions early after infection did not alter chlamydial burden or tissue pathology following vaginal inoculation of guinea pigs (298). However, there was an increased influx of inflammatory cells in TNF-α-depleted animals. Surprisingly, earlier studies had found that TNF-α displays antichlamydial properties *in vitro* (1291).

The following table shows a summary of the different immune parameters and their functions in primary and secondary chlamydial infection (Table 30).

Immune effectors	Role in immunity against genital Chlamydia infection	Importance
Factors of adaptive immunity		
CD4+ T cells	Th1 cytokines (IFN-γ, TNF, IL-2) Eradication of infection Protective immunity	Obligatory
CD8+ T cells	Th1 cytokines (IFN-γ, TNF, IL-2) Synergistic role together with CD4+	Contributory
B cells/Igs	Faster clearance of infection	Contributory; obligatory in reinfection?
Factors of innate immunity		
DCs/ Macrophages	Phagocytosis and antigen presentation, stimulation of T cells, cytokine costimulation, ADCC	Obligatory
NK cells	Production of IFN-γ	Contributory
Neutrophils	Reduction of amplification and focal spread	Contributory
Cytokines/ Chemokines	Immunostimulation	Obligatory

Table 30: Summary of relevant immune parameters in immunity against C. trachomatis infection, adapted from Igietseme et al. (590)

(4) Persistence of chlamydial infection

A number of studies have documented that within a year after treatment of a previous *Chlamydia* infection, individuals have persistent or recurrent infections at a rate of 13% to 26% (683). In fact, **Burstein et al**. have recently estimated that the mean time to reinfection is 6 to 7 months in a sexually active adolescent population (163).

Batteiger and colleagues noted that nearly one third of individuals became reinfected with the same serovar and that this was associated with continued contact with the same partner (87). As an explanation, selective immune pressure has been postulated to induce point mutations in the MOMP which have been shown to abrogate the binding of antibodies to epitopes that can neutralize infectivity *in vitro* (782).

Given the frequent reinfection with *Chlamydia* in humans, the question arises as to whether protective immunity against chlamydial infection can develop.

There are several studies that support this concept. For instance, the incidence of chlamydial infection was significantly reduced in both women and men with a history of STDs (665). In addition, older women with an equivalent number of sexual contacts became infected less often than younger women (50). Also, immunosuppressed women with HIV infection had both an increased prevalence and risk of reinfection (695).

However, the immunity that develops appears to last for only a short time. The rate of reinfection significantly increased in those individuals whose last reported *Chlamydia* infection was more than 6 months previous, as compared to those with more recent infection (665).

Animal models of chlamydial genital infection exhibited short-term immunity against subsequent infection (1169).

Using a second vaginal inoculation of *Chlamydiae* at various times after resolution of the primary infection, guinea pigs demonstrated immunity against reinfection with a shortened course of infection and reduced numbers of viable *Chlamydiae* when the animals were challenged 30 days later. However, if the guinea pigs were reinfected on day 77, the level of immunity was reduced. In addition, a protective response was associated with the more rapid recruitment of CD4+ cells to the genital tract upon reinfection (680).

Studies have also observed a more rapid increase in the number of venules expressing adhesion molecules when mice were challenged shortly after the resolution of infection (682).

Peak levels of adhesion molecules were seen 7 days after a challenge infection in the lower genital tract, similar to the kinetics following a primary infection. In the oviducts and uterine tissues, however, the number of venules expressing VCAM-1 peaked 3 days after infection compared to the peak on day 21 in VCAM-1-positive venules observed following a primary infection.

A reason may be that low numbers of anti-chlamydial memory T lymphocytes recirculate through the genital mucosa following resolution of infection. Upon activation with chlamydial antigens, these lymphocytes could release cytokines and chemotactic factors to facilitate the rapid recruitment of CD4 lymphocytes.

It is likely that cellular immunity is primarily responsible for clearing the initial infection; however, it appears that antibody plays a role in protection from reinfection.

It was shown that IFN-γ receptor knockout mice resolved infection only after a prolonged time period while high levels of antichlamydial IgA and IgG antibodies were present in genital secretions (631). Antibodies against *Chlamydia* are found in genital secretions and serum after infection (1170) and the resolution of a primary infection coincides with the appearance of antichlamydial antibody in genital secretions.

A few proposed antibody-mediated mechanisms of control include neutralization of the entry of *C. trachomatis* into ECs; opsonization, which enhances the uptake of *Chlamydia* by macrophages; and antibody-dependent cell-mediated cytotoxicity via antichlamydial IgA (683).

A less intense infection was noted in guinea pigs that were challenged as long as 825 days after a primary infection which was correlated to serum IgG levels but not IgA levels in genital secretions since IgA antibodies were undetected 75 days after infection (88).

Recently, the study by **Morrison and Morrison** in antibody-deficient mice demonstrated that antibodies contribute to a high degree to immunity to chlamydial genital tract reinfection, and that antibody-mediated protection is highly dependent on CD4+ T cell-mediated adaptive changes that occur in the local genital tract tissues during primary infection (973).

However, in a study by **Johansson and Lycke** in B cell-deficient mice, long-term protection in the genital tract against *C. trachomatis* infection is conveyed by IFN-γ-

producing CD4+ memory T cells, which appear to be maintained in the absence of antibodies and local antigen deposition (629).

One serious problem with repeated chlamydial genital infections is, as already mentioned above, tissue damage followed by tubal scarring, chronic salpingitis and distal tubal obstruction.
Studies proposed the hypothesis that at least some component of the immune response in form of a delayed-type hypersensitivity reaction could be responsible for causing tissue damage.
By characterizing the inflammatory infiltrate, **Patton** found only mononuclear infiltrates during repeated infection compared to both mononuclear and polymorphonuclear infiltrates after a primary infection (1098).
Moreover, a lack of *Chlamydia*-specific T lymphocytes may enhance tubal pathology. Studies observed oviduct ectasia, hydropsalpinx, and marked acute inflammatory infiltrates in mice that lacked an anti-chlamydial CD4+ lymphocyte response (1112).

Recurrent or chronic chlamydial infections, which are associated with damage to the reproductive tract, may result from an insufficient number of antichlamydial Th1 cells or possibly factors that interfere with the effector mechanisms of Th1 cells, for example IL-10.
Yang and colleagues found that *Chlamydia* was eradicated more rapidly following infection in IL-10 knockout mice and that antichlamydial Th1 responses were enhanced in IL-10 knockout mice (1555).
IL-10 is a potent counterregulatory cytokine that suppresses Th1 immune responses by many mechanisms such as inhibiting IL-12 production from APCs or suppressing transcription of the chemokine IP-10, which attracts Th1 CD4 cells (683).
Another factor that may dampen Th1 responses during *Chlamydia* infection is the antiinflammatory cytokine TGF-β which is elevated in the genital tract late in the course of MoPn infection in mice [207].

In vitro, the production of most chlamydial components is extremely downregulated when *C. trachomatis* is in its persistent, non-replicative form. However, synthesis of chlamydial heat shock protein (c-hsp60) is upregulated under these conditions (93).
Immunity to this protein which only results from a persistent infection seems to contribute to the development of tubal occlusion and adverse pregnancy outcome.

A study showed that detection of antibody to c-hsp60 did not correlate with a positive *C. trachomatis* genital culture but with tubal occlusion and PID (352, 150). There is a human hsp60 (h-hsp60) homologue to c-hsp60 which is expressed by ECs of the decidua and embryo during early pregnancy (1527). Therefore, a chlamydial fallopian tube infection can induce the development of autoantibodies to h-hsp60 and in women already sensitized to c-hsp60, the exposure to h-hsp60 will reactivate the c-hsp60 lymphocytes. This may lead to immune rejection of the embryo (1527).

A study by **Kinnunen et al**. has shown that T lymphoytes derived from salpingeal tissues of patients with tubal factor infertility show specific reactivity with c-hsp60 (712). However, immunizing mice with both c-hsp60 and h-hsp60 could induce IL-10 production in mice (1562). Thus, bacterial hsp60 may trigger a Th2 cell response during infection, which could suppress a concurrent Th1-driven response.

(5) Chlamydial vaccine development

The development of a safe and protective vaccine against chlamydial infection is still ongoing.

In the 1960s, a vaccine for trachoma was developed and tested in humans but the protection was short-lived and induced immunopathologic scarring in a few individuals (683).

T cell epitopes appear to be found in association with many HLA alleles and for the most part are directed against nonpolymorphic constant regions of the MOMP. Thus, cellular immunity could be generated to nonvariable segments of the organism for protection against an array of serovars. Support for this approach has been generated in mice where infection with MoPn provides subsequent protection against human serovars of *Chlamydia trachomatis* (1164).

Different routes of immunization have been examined for stimulating protective immune responses against chlamydial infection within the reproductive tract. Thereby it is possible to eliminate disturbances on immune responses coming from reproductive hormones, for example.

Pal et al. have reported that *Chlamydia* were recovered from the upper genital tract of significantly more mice when infected during the luteal phase with increased levels of progesterone and estradiol than follicular phase (1062). **Kaushic and colleagues**, however, concluded in their study of a rat model that progesterone

increases and estradiol decreases susceptibility to intrauterine chlamydial infection (673).

In the mouse model, various mucosal versus parenteral routes of immunization were compared.

In mice which were given live *Chlamydia* by mucosal routes, i.e. oral, intranasal, and vaginal, the course of infection was significantly shortened and of less magnitude compared to mice injected subcutaneously with live *Chlamydia* (681).

Examination of the cytokine profile produced after immunization showed a dominant Th2 response in the draining iliac lymph nodes following parenteral immunization, whereas a dominant Th1 response was noted in mice immunized via mucosal routes.

Igietseme and colleagues further showed that mice first given live *Chlamydia* intranasally or vaginally had greater levels of IFN-γ in the genital tract after a challenge infection compared to mice immunized via a subcutaneous route (594).

Oral immunization has been shown to elicit antibodies in other mucosal tissues, such as the reproductive tract and mammary glands, as well as the intestine. However, the intranasal route was found to produce the greatest amount of IgA and IgG in genital secretions for the longest period of time. For instance, both IgA and IgG, specific for the immunizing antigen, could be found in genital secretions following immunization via the nasal, oral, rectal, and vaginal routes. However, both the nasal and vaginal routes consistently produced the greatest increase in IgA and IgG compared to other routes in humans (743).

Igietseme et al. also reported that immunization via the nasal route elicited the highest level of antichlamydial IgG antibody titers in the genital tract even in comparison with a vaginal immunization (594). Moreover, immunization by the nasal route has been shown to protect mice from infertility following a challenge infection (1063).

The induction of a protective cell-mediated immune response also appears to depend on whether a live infection initiates the immunizing response.

In the guinea pig model of chlamydial genital infection, protection following parenteral immunization was observed (1168). Chlamydia-specific IgG and IgA levels were comparable in the serum and genital secretions among vaginal, oral, and parenteral immunization routes when live EB were used. Moreover, guinea pigs could be protected from reinfection following a parenteral immunization with UV-inactivated *Chlamydia* which is most likely due to the production of a protective antibody response (1168).

In contrast, immunization of mice with UV-inactivated *Chlamydia* provided no protection against a genital infection (681).

These data show that to develop a protective vaccine many factors in addition to the antigen must be considered, such as route of immunization or form of antigen. A summary of different approaches to create a protective and safe vaccine against chlamydial infection shows Table 31.

Immunmodulatory approach	**Vaccine effect**	**Research status**
Selection of antigens with T and B cell epitopes	Induction of required T and B cell response	In progress
Multisubunit vaccines	Furnishing adequate B and T cell epitopes for elevated immune responses	In progress
Use of delivery vehicles (adjuvants, vectors)	Boost of immune responses; vehicles for subunits	In progress
Modulation of cytokine/chemokine expression	Upregulation of Th1 cytokines; downregulation of Th2 cytokines	Experimental stage
Modulation of costimulatory factors	Induction of high T cell response	Experimental stage
Vaccine targeting to specific inductive sites	Mucosal immunity	Active

Table 31: Vaccine design strategies in development against chlamydial infection, adapted from Igietseme et al. (590)

c) Bacterial vaginosis

For a normal vaginal flora after menarche, estrogen provides the conditions for survival of lactobacilli again which is the dominant vaginal flora in the female adult. There are various different species of lactobacilli with the dominant species *L. crispatus, L. gasseri, L. jensenii* and *L. iners* in an asymptomatic vagina (1395). The healthy vaginal flora is characterized by high concentrations of this *Lactobacillus* spp. (10^8 colony forming units/ml) and a pH of less than 4.7 (874). During menopause, there is a mixed flora with a considerable portion of mycoplasma and anaerobic bacteria (419).

The roles of the lactobacilli species are to produce lactate for acidification of the vaginal mucus, to produce bacteriocines, hydrogen peroxide, a bisurfactant and organic acids, and furthermore to compete with pathogenic organisms for space, receptors and nutrients.

The vaginal epithelium and its mucus layer help to regulate the intrinsic bacterial and mucosal defense systems (874). The vagina is lined with squamous epithelium, whose thickness directly correlates to estrogen concentration. Glycogen supplied by the vaginal ECs supplies metabolic fuel to for the generation of lactic acid by lactobacilli, thus enabling a low pH. This low pH, along with adequate concentrations of hydrogen peroxide, controls potential overgrowth of other commensal flora such as *Gardnerella vaginalis* and *Candida albicans*. Apparently, a low estrogenization of the vaginal mucous membrane will enhance the growth potential of flora, which is normally encountered in small quantities in the vaginal mucus (419).

Some decades ago, **Gardner and Duke** reported aspects of the so-called *Haemophilus vaginalis*-vaginitis (438) which is now renamed BV. They proposed *Haemophilus vaginalis*, which is now *Gardnerella vaginalis*, as the single cause for BV.

Today it is generally known that BV significantly alters the vaginal ecosystem and a profuse mixed flora with anaerobic and facultative anaerobic bacteria besides depletion of *Lactobacillius* spp., especially those that produce hydrogen peroxide, is established. The vaginal flora in BV includes *G. vaginalis, Prevotella* spp., *Mobiluncus* spp., *Corynebacterium spp.*, viridans streptococci, coagulase-negative staphylococci, *Enterococcus faecalis*, *Atopobium vaginalis* and *Mycoplasma hominis* (419, 874). But there is not only a qualitative, but also a quantitative change with bacterial concentrations about 100 to 1000 times higher than normal. Production of fatty acids by these anaerobic bacteria leads to an elevated pH in the vagina which itself promotes anaerobic growth again.

BV is the most prevalent form of vaginal infection in women of reproductive age, affecting about 3 million women in the United States per year, and is the most common etiology of vaginal symptoms leading women to seek for medical care (720). Symptomatic BV, which only accounts for 60% of all cases, causes abnormal amounts of a malodorous vaginal discharge deriving from degraded vaginal mucus.

Symptoms for a vaginitis including vaginal pruritus, burning or dyspareunia, however, are rare and visible local inflammation signs are absent (969). BV is clinically diagnosed by the Nugent criteria which quantify the number of lactobacilli relative to BV-associated morphotypes (1033) or the Amsel criteria (Table 32).

Vaginal pH greater than 4.5
Homogenous vaginal discharge on examination
Detection of fishy odor on addition of potassium hydroxide to vaginal fluid
Presence of significant clue cells in vaginal smear (>20% of total vaginal ECs)

Table 32: Amsel criteria for BV, of which at least three of four should be present (34)

(1) Pathogenesis and implications of BV infection

What influences the susceptibility to BV of some women is currently not clear. Prepuberty and postmenopause are rarely associated with BV which has led to the proposal that vaginal microbial changes may be due to hormonal variations during the menstrual cycle (870). It is also considered that BV is a genetically determined miscolonization of the vagina (969) or may be another example of an STD (970).

One virulence factor in bacteria associated with BV is a haemolytic toxin produced by *G. vaginalis*. Women who lack an adequate IgA immune response against this toxin are said to risk BV (189).
Another hypothesis respecting the pathogenesis is that hydrolytic enzymes degrade the mucin and thus damage the protective vaginal mucosa. In comparison with healthy women, women affected by BV are also known to have significantly higher vaginal concentrations of the hydrolytic enzymes sialidase and proline dipeptidase. Proline dipeptidase is produced by both *Mobiluncus* spp. and *G. vaginalis.* Sialidase, which is produced by, for instance, *Prevotella* spp., is thought to be an important virulence factor in BV pathogenesis, not only because it leads to lysis of the mucin, thus aiding the adherence of bacteria, but also through its ability to counteract a specific IgA defense against *G.vaginalis* toxin (193). It was furthermore suggested that a high sialidase activity in BV cases gives rise to a significant risk of prematurity.

A causal relationship between BV and the acquisition of HIV has been supported by studies evaluating HIV genital shedding in BV and from prospective studies examining the risk of HIV infection associated with abnormal vaginal flora (1265).

Presence of BV was associated with a sixfold increase of in quantity of HIV shed relative to normal flora (285).
Relations between BV and the acquisition of HSV-2 have also been reported (220).

Data strongly support the fact that BV is significantly associated with adverse sequelae related to the upper genital tract.
During pregnancy, BV increases the risk of preterm delivery and low-birth-weight children (548), first trimester miscarriage among women undergoing in vitro fertilization (IVF) (1163), chorioamnionitis and other infections (1305). Screening for BV and treatment has been considered a strategy to reduce the rate of preterm birth (1215).
In nonpregnant women, BV increases the risk for posthysterectomy infections and PID (1500) as well as the risk for acquiring *Neisseria gonorrheae* or *Chlamydia* (1501).
The correlation between BV and infection in the upper genital tract is believed to be due to the fact that an abundant vaginal overgrowth of BV-associated bacteria makes ascending infections in the genital tract more likely.
Considering the concept of vaginal inflammation, a balanced and appropriate immune response is thought to be crucial to clear infectious agents through the production of antimicrobial agents, phagocytosis and clearance of microorganisms. Individuals differ in their ability to mount an inflammatory response; some are hyperresponders with an excessive local and systemic response, others are hyporesponders with the risk of an overwhelming infection (1307).
In this context, there is a hypothesis to understand the relationship between changes in the vaginal flora, inflammatory response and clinical outcome. Hyporesponsive mothers may no be able to control microbial challenge which results in ascending intrauterine infection and clinical chorioamnionitis. Hyperresponders develop an excessive local immune response, clinical symptoms of vaginitis and be at risk for preterm delivery.
To underline this hypothesis, **Simhan** reported that women with low concentrations of cytokines IL-1β, IL-6 and IL-8 in vaginal fluid in early pregnancy are more likely to develop chorioamnionitis than those without low concentration of these cytokines. Also, it is agreed that women with elevated cytokines are at risk for preterm delivery (1215). More data concerning the circumstances of preterm delivery associated with infections such as BV should be further discussed below in the chapter on disturbances in maternal-fetal interactions.

(2) Innate and specific immune responses in BV

BV is generally regarded as a noninflammatory vaginal condition and inflammatory signs are rare in BV-positive women.
Activation and recruitment of neutrophils is one of the main components of innate immunity in general against microbial and viral infections in the mucous membrane of the genitalia. However, the number of vaginal neutrophils in most BV-positive patients is not increased with respect to healthy women (192, 195).
The anti-inflammatory and antimicrobial SLPI, which is produced by mucosal ECs, is present at lower levels in vaginal fluid when BV exists (341).

Also production of local proinflammatory cytokines, which was shown to be increased in genital tract infections with *Candida* or *T. vaginalis* (411, 1287), for example, was investigated in BV.
In contrast to neutrophils, the cytokine level of IL-1β in CVS was found to be higher in BV-positive patients (192), which presumably indicates a host response to microbial products.
In a trial of the Munich study group on 45 symptomatic BV patients and 36 asymptomatic controls, the proinflammatory cytokines IL-6, IL-10 and the antiinflammatory IL-12 in vaginal secretions were measured (1471). Results showed no significant difference in levels of all three cytokines between the two study groups.
These findings are consistent with results from other studies. **Wennerholm et al**. did not find an association between IL-6 concentrations and BV but a significant increase of IL-1α and IL-8 in BV-positive pregnant patients (1494).
Mattsby-Baltzer et al. also failed to demonstrate a significant rise in concentrations of IL-6 and TNF-α in pregnant BV-positive women compared to controls whereas vaginal levels of IL-1β were increased (890).
In a study by **Yudin et al**., oral and vaginal metronidazole treatment of BV-positive patients has been shown to reduce cervical mucus levels of IL-6 as well as IL-1β and IL-8 (1569). In the group of patients with persistent BV, however, the metronidazole treatment did not result in a cytokine reduction.
Another recent study of the Munich study group determined the levels of the proinflammatory cytokines IL-1α and IL-1β, and the antiinflammatory cytokines IL-5 and IL-10 in vaginal secretions of BV patients and healthy women (37). They found significantly increased levels of IL-1β and significantly decreased levels of IL-10 in BV patients compared to healthy women.

IL-8 is a potent chemotactic and activating factor for neutrophils that has been detected in vaginal fluid of women with BV and other vaginal infections (1494). A recent study showed that impairment of IL-8 induction in women with BV is associated with low levels of vaginal IgA against haemolysin produced by *G. vaginalis*, low number of leukocytes, and high microbial hydrolytic enzyme activities (191).

IL-8 does not, however, seem to be correlated with the presence of BV in the same distinct manner. Recent findings indicate that microbial hydrolytic enzymes could be responsible for dampening the expected proinflammatory response cascade after IL-1β increase (195). The impairment of IL-8 increase may explain the absence of neutrophil increase in most women exposed to a massive anaerobic vaginal colonization.

Recent findings have demonstrated that low concentrations of IL-1β were associated with low levels of anti-*G.vaginalis* hemotoxin IgA in vaginal fluid of women with BV (190) suggesting a necessary association between the innate and adaptive immune responses for protection from ascent of pathogens to the upper genital tract.

Sturm-Ramirez et al. proposed that increased IL-1β during BV could upregulate local HIV replication through activation of the LTR promoter region and therefore partly explain the mechanism how BV enhances HIV transmission (1368).

A problem is that some forms of abnormal vaginal flora are neither normal, nor can they be called BV. Such forms of abnormal flora have been termed “intermediate flora” in some studies, or have been included with BV in other studies (334, 335).

However, also **Taylor-Robinson et al.** proposed to make a difference between the classic finding of BV and an “intermediate” vaginal fluid (1403). Abnormal vaginal flora comprises a wide range of changes, which include not only BV but also other less well-characterized conditions.

An interesting study was performed by **Donders et al.** which raised the question if inflammation really exists with BV or if it is a separate entity. They tried to exactly define the changes in abnormal vaginal flora to differentiate between BV and a vaginitis (335). In opposite to BV with no signs of local inflammation, some women with abnormal vaginal flora showed signs of a vaginitis including redness, pruritus and burning pain. This was referred to as a condition characterized by a decrease in lactobacilli, high vaginal pH, absent clue cells and the presence of leukocytes which Donders called “aerobic vaginitis”. Women with “aerobic vaginitis” were reported to have decreased levels of lactic acids and a local inflammatory response with high concentrations of vaginal IL-1β, IL-6 and LIF (334).

A recent work by the Munich study group investigated 102 patients with a symptomatic vaginal dysbiosis, similar to the picture of an aerobic vaginitis of the Donders study, and 50 healthy asymptomatic controls (955). In women with vaginal dysbiosis, the proinflammatory cytokines IL-1α and IL-1β were significantly increased whereas IL-2 and IL-4 were significantly lower in this group compared to healthy controls.

In a study on 193 women attending outpatient clinic for vaginal discharge problems, vaginal swabs were investigated for pathogens, lactobacilli and leukocytes, as well as vaginal secretions for IL-4, IL-10 and IL-12. Preliminary results proposed tendencies to an association of high vaginal levels of IL-12 with high leukocyte numbers and a high total BV-related pathogen count, whereas low levels of IL-10 were correlated with high total and BV-related pathogen counts (**G. Anton, personal communication**). High vaginal IL-10 levels were therefore supposed to be associated with healthy women whereas high levels of IL-12 were positively correlated with infection and inflammation.

3. Candidiasis

Vulvovaginal candidiasis (VVC) is a common mucosal fungal infection caused in about 80-90% by the opportunistic pathogen *Candida albicans*, a commensal organism in the gastrointestinal and reproductive tracts (1323, 1324). Other causative agents are *Candida glabrata, Candida krusei* and others (915).

It affects an estimated three out of four women at least once during their reproductive age and a significant percentage of those women (5-10%) experience recurrent VVC (RVVC) (585). An overview of different clinical forms of vaginal candida infection gives Table 33.

Asymptomatic form	Colonization, lack of symptoms
Acute form	Burning, itching, vaginal discharge Vaginal erythema and edema
Persistent form	Persistence after antifungal therapy
Recurrent form	Recurrence after antifungal therapy CRVVC: ≥4 episodes/year, caused mainly by *C. albicans*, but increasing rates of *C.glabrata* and non-albicans-strains

Table 33: Different clinical forms of vaginal candidiasis

Several exogenous predisposing factors such as pregnancy, oral contraceptives and uncontrolled diabetes mellitus are known to cause acute episodes of VVC (1324). However, RVVC seems to be idiopathic. Chronic RVVC (CRVVC) is clinically defined as at least four episodes per year in the absence of any predisposing factors (408).

Unlike symptomatic oropharyngeal candidiasis of HIV-positive subjects, which seems to be closely dependent upon both the severity of cellular immunodeficiency and reduced CD4+ cell counts, symptomatic VVC is common in women, regardless of HIV serostatus and immunodeficiency (1498). Recurrences seem to be caused by persistent yeast in the vagina, rather than by reinfections, as demonstrated by the isolation of the same karyotypically identical strains in relapses (1450).

Acute VCC can be treated topically with nystatin, imidazole or amphotericin B or systemically with fluconazole or itraconazole (915). Antifungal therapy is highly effective for individual attacks of VCC and RVCC but does not prevent recurrence. Thus, RVVC is presumed to result from some local innate and/or acquired dysfunction in the normal protective immune response most healthy individuals acquire from early exposure to *Candida albicans*.

a) Cell-mediated immunity in Candida infection

Cell-mediated immunity by T cells and cytokines, specifically a Th1 type response, is considered to be the predominant host defense mechanism against mucosal candidiasis and is thought to play a role in maintaining the organism in its commensal state at those sites as well.
This is evidenced not only by the high incidence of *Candida* infections in HIV-positive persons with reduced cell-mediated immunity (397) but also by a similar prevalence under other conditions of T cell immunosuppression, as for example corticosteroid therapy (725).

Several experimental studies show that Th1-type cell-mediated immune responses are associated with resistance against systemic and mucosal *C. albicans* infections, whereas Th2-type responses are associated with susceptibility to infection (1214).
Recently, the murine model of VCC has been used to study host immune responses against *C. albicans*. In these models, the most important requirement for a persistent infection is a state of pseudoestrus (401). In the absence of estrogen

treatments, the infection is short-lived with a low fungal burden in the vagina (406). However, the local vaginal immune responses in *Candida* infection are not clearly understood so far.
Clinical as well as experimental estrogen-dependent murine model studies examining *C. albicans* vaginitis show that although *Candida*-specific systemic Th1-type responses are clinically present or experimentally induced during a vaginal infection, these responses appear to have no effect on the infection (405, 407).
Further experimental studies using the murine model show that partial protection against infection can be achieved, but without involvement of systemic Th1-type cell-mediated immunity (404). Through clinical and experimental studies it has become apparent that the host response to a *C. albicans* vaginal infection is more dependent on local immunity than the response observed in the systemic circulation (405, 404).

Other studies evaluated the role of local vaginal associated Th1 cell immunity. Data to assess vaginally-associated T cells showed no changes in the percentage or composition of local phenotypically distinct T cells (404) during primary or secondary *C. albicans* vaginal infection, and also showed no evidence for systemic T cell infiltration into the vaginal mucosa during infection (403).
By that it can be concluded that if T cells are responsible for any host defense in vaginal *Candida* infection, they exert their protective effect without significant changes in number and composition.

In the absence of any clear exogenous predisposing factor, the risk of clinical infection has therefore been correlated with a partial T cell dysregulation, which might be exacerbated by the hormonal balance present during the follicular phase.
Indeed, a significant reduction in the proliferation of peripheral blood lymphocytes from women in the follicular phase with RVVC has been reported, following their *in vitro* stimulation with *Candida* cells (255). These women had a depleted T cell pool and, in response to *Candida* stimulation, showed significantly reduced IFN-γ-production which is normally a hallmark of anti-*Candida* protection (1523).
However, in at least partial contradiction with these data, clinical evidence demonstrated that *Candida*-specific cell-mediated immunity at the periphery is normal in women with RVVC (408) and is also remarkably similar both in HIV-positive subjects with oral candidiasis and in HIV-negative subjects (801).

Experimentally, both humoral and cellular protective anti-*Candida* responses at vaginal level are totally uncoupled to those occurring at the periphery (310), suggesting either an immunological independence, or compartmentalization of the vaginal mucosa, or a differential regulation of T-cell recruitment between different regions of the genital tract (682). Interestingly, murine vaginal T cells are phenotypically distinct from T cells in the blood (409). However, *Candida*-specific cell-mediated immunity was expressed by vaginal T cells in rats immunized against *Candida* after healing a primary infection (310), and vaginal T cells, particularly the CD4+ subset, transferred protection to naïve rats (**Santoni G et al., unpublished data**). These apparently contradictory data raise the issue of whether the data from experimental models of vaginal infection can be extrapolated to human disease.

In contrast to a limited response to *Candida* at the murine vaginal mucosa, mice given an experimental *Chlamydia trachomatis* genital tract infection exhibit a CD4+ T-cell infiltrate into the genital tract (680). This Th1-type response is critical for the resolution of infection and indicates that T cells can reach the genital mucosa and provide substantial protection. A study by **Kelly et al**. addressed whether or not a dual infection with *C. albicans* and *C. trachomatis* could enhance the local recruitment of a cell-mediated host response against *C. albicans* and facilitate clearance of the infection (679). The result was that a concurrent *Candida* and *Chlamydia* infection could not accelerate or modulate the anti-*Candida* cell-mediated response. These results suggest that host responses to these two genital tract infections are independent and not influenced by the presence of the other.

b) Cytokine responses in candidiasis

Concerning cytokines, clinical evaluation of Th1/Th2 cytokines in vaginal lavage fluid of women with RVVC showed no differences in comparison to control women without a history of vaginitis (406).

Taylor and colleagues therefore investigated the cytokine production associated with the elicitation of Th1- (IL-2, IL-12, IFN-γ) and Th2- (IL-4, IL-10, TGF-β) type responses in the vagina and draining lymph nodes during an experimental *C. albicans* vaginal infection in mice (1398).

It was found that in naïve animals TGF-β1 production was at least two-fold higher at the protein level and ten-fold higher at the mRNA level than the other cytokines evaluated, while most of the Th1 and Th2 cytokines were present at extremely low

levels. These high levels of TGF-β1 were further increased as a result of pseudoestrus and/or infection, and furthermore, the levels of TGF-β in naïve or infected mice were significantly higher in the vagina compared to other areas of the genital tract. Finally, TGF-β1 predominated as well in the draining, but not in non-draining lymph nodes during infection (1398).
Of the evaluated Th1/Th2 cytokines, TGF-β1 had the greatest likelihood of impacting the vaginal microenvironment and the local immune response, which may be suggestive of the newly classified Th3-type response based on the predominance of TGF-β1 and the virtual absence of changes in other Th-type cytokines during infection (810).

During the last years, especially study groups around **Weissenbacher et al**. and **Witkin** emphasized the importance of cytokines in vaginal mycotic infections.
Witkin reported that patients with CRVVC showed a decreased *in vitro* proliferative response of lymphocytes compared to patients with candidiasis which showed an increase in vaginal lymphocytes (1525). The cause is the amplified production of prostaglandin E2 by macrophages which consequently inhibits production of IL-2 and therefore the proliferation of lymphocytes, thus inhibiting a Th1 immune response. Due to the impaired lymphocyte responses, *C. albicans* can proliferate and initiate a clinical infection.
Prostaglandin E2 production can arise as a consequence of a vaginal allergic response due to local release of histamine. The allergen could be a semen constituent, an environmental product or a component of *C. albicans*. The result of a vaginal allergy can thus result in the weakened immune response against vaginal infections (1524). Epitopes of *C. albicans* enolase were already detected as binding sites for IgE antibodies (602).
Earlier studies already proposed allergic reactions to *Candida* in the sense of a local vaginal hypersensitive immune response and suggested an allergic cause for chronic and recurrent symptoms (1197, 1217).
In patients with CRVVC, *Candida*-specific IgE were detected in vaginal secretions, which further emphasizes the theory of a local vaginal hypersensitivity (1525).
In a study of **Weissenbacher et al**., 104 women with clinical symptoms of a CRVVC and 44 healthy controls were investigated (1489). *Candida* was only detected in cultures of about 30% of all symptomatic patients, with PCR in about 42% of all symptomatic patients.
The level of IL-4, IL-5 and IL-13 were identified, but there was only detected a significant increase in the antiinflammatory IL-4 compared to the controls (1488).

In addition, prostaglandin E2, whole IgE and *Candida*-specific-IgE was investigated. Prostaglandin E2 and the *Candida* IgE were significantly higher in comparison with the control group, while whole IgE showed no significant increase.
A further study concentrated on the comparison of 150 healthy women and 74 women with vaginal *Candida*-infection with respect to IL-8 and glucose in vaginal secretions (486). Healthy women not only showed higher concentrations of physiological lactobacilli, lower levels of neutrophils and a significantly lower presence of gram-positive cocci but also higher titers of IL-8. With respect to glucose levels, there was no difference between these two groups. An explanation could be that higher vaginal levels of the proinflammatory IL-8 in healthy persons serve as expression of an activated immune state, or that IL-8 may be inhibited in women with vaginal infection or downregulated in a kind of feedback-mechanism.

c) Antibody responses in candidiasis

The efficacy of humoral immune mechanisms and the role of antibodies against *C. albicans* has been a controversial subject for decades (1134).
It is usually assumed that antibodies play little or no role in defense against this infection. This is based on the fact that the disease is not more common or more clinically serious in subjects with Ig disorders; and that subjects with candidiasis often have high titres of anti-*Candida* antibodies in their plasma, not different from other uninfected subjects (859).

The fact that VVC and RVVC are much more common than Ig disorders clearly mandates that the infection also occurs in normal antibodies responders, but certainly does not mean that these subjects produce the correct antibodies in the correct place. Protective antibodies with specificity to a limited number of yeast epitopes might not necessarily be produced at protective levels, and some relevant yeast epitopes might not be expressed constitutively (859).
Several earlier studies reported that antibodies are not protective against candidiasis but in the 1960s, however, reports indicated that antibodies against *C. albicans* might be protective against experimental disseminated candidiasis (983).
Evidence from experimental settings of rodent models suggests that antibodies against specific virulence factors of the fungus play an important role in protection. This indicates that antibodies and cell-mediated immune responses are both active

players in the host-fungus relationship and can contribute to the host protection against yeast (1134).
In a study by the group of **Weissenbacher et al.**, 184 symptomatic patients with CRRVC and 46 healthy controls were investigated for detection of vaginal Candida IgG and IgA antibodies and vaginal Candida in culture or PCR (**Anton G**, **personal communication**). In the patient group, 33% were *Candida*-positive in PCR or culture and in the control group 0%. Healthy controls were mostly negative for vaginal *Candida*-specific IgA and IgG (96% and 85%, respectively). Of the CRRVC patients with *Candida*, 70% were positive for *Candida*-IgG and 43% were positive for *Candida*-IgA. Therefore, the question arises if *Candida*-IgG is probably a better marker for *Candida*-positive CRRVC.

Finally, it is important to consider the extracellular nature of the infectious agent, and its possession of several virulence traits, many of which are in principle inhibitable by suitable antibodies. Indeed, protective antibodies against well-defined virulence factors of the fungus have been reported. Furthermore, anti-idiotypic killer antibodies (KAbs) have been described that carry the internal image of a yeast killer toxin (KT) from a selected strain of the yeast *Pichia anomala* (*Pa*KT), thus functionally mimicking it (859). KTs are glycosylated proteins secreted by self-immune yeasts, which exert a microbicidal activity against sensitive microorganisms characterized by the presence of specific KT cell wall receptors (KTR).
On the basis of previous observations, and through the exploitation of new molecular technologies, three different possible immunotherapeutic strategies have been developed based on protective antibodies, KAbs and therapeutic vaccines, as discussed below.

d) Effect of reproductive hormones

Use of oral contraceptives and hormone replacement therapy were shown to predispose women for VCC (1324). Clinical observations show that VVC most often occurs in women during the luteal phase of the menstrual cycle, when estrogen and progesterone levels are elevated (654). In contrast, premenarchal and postmenopausal women who do not receive hormone replacement therapy, rarely suffer from VVC (1323).

Estrogen and progesterone have been shown to inhibit aspects of both innate and acquired immunity at the systemic or local level, including *Candida*-specific human peripheral blood lymphocyte responses (654) or neutrophil anti-*Candida* activity (1024) *in vitro*. Furthermore, *in vitro Candida*-specific lymphocyte responses were reduced in women during the luteal phase of the menstrual cycle concomitant with increased serum-induced germination of *C. albicans* (654).

Although estrogen-dependent experimental rodent models of *C. albicans* vaginal infection are used for many applications, the role of reproductive hormones and their limits in the acquisition of vaginal candidiasis remain unclear.
A study by **Fidel and colleagues** examined the effects of estrogen and progesterone on several aspects of an experimental infection together with relative cell-mediated immune responses (401). Results showed that near-physiologic concentrations of estrogen were as capable as supraphysiologic concentrations of sustaining experimental infections induced by a wide range of *C. albicans* inocula.
Additionally, it was found that a persistent infection with high rates of infection could equally occur if estrogen treatments were initiated several days before or after inoculation (401). The requirement for a maintained state of pseudoestrus was confirmed by the rapid clearance of the infection when the estrogen treatments were removed. Finally, estrogen was found to reduce the ability of vaginal ECs to inhibit the growth of *C. albicans*. In contrast to estrogen, progesterone treatment alone could not support an experimental vaginal infection for any significant period of time (401). In fact, vaginal fungal burdens in progesterone-treated animals were lower than those in untreated animals.
This may have been due to a lack of or reduced influence by endogenous estrogen. Interestingly, progesterone had no effect on the titers of *C. albicans* in the vagina, rates of infection, or chronicity of the vaginal infection in the presence of estrogen. Furthermore, estrogen and progesterone treatment of mice had no effect on *Candida*-specific systemic cell-mediated immunity, i.e. *in vitro* proliferation of lymph node cells in response to *Candida* antigens.

Taken together, estrogen is predicted to be the primary factor in the susceptibility to vaginitis during the luteal phase of the menstrual cycle, despite higher concentrations of progesterone than estrogen during that time (401). This is also consistent with the lack of prevalence of *Candida* vaginitis in women taking progesterone contraceptives. On the other hand, one may speculate that it is the peak levels of estrogen during the short ovulatory phase of the menstrual cycle that

precipitate the vaginal infection and that the symptomatic infection does not fully present itself until the luteal phase. Similarly, one would predict that despite high levels of progesterone during pregnancy, the high incidence of vaginitis in pregnant women is more likely due to estrogen.

e) Immunotherapeutic strategies

The first immunotherapeutic strategy is based on protective antibodies against immunodominant candidal antigens. Many candidal virulence factors such as adhesins and the enzymes aspartyl proteinases can stimulate the immune system to generate an immune response (169). Several studies tested antibodies against specific candidal proteins which were protective against natural and experimental disseminated candidiasis. Protective antibodies against disseminated candidiasis and vaginal infection were elicited by a vaccine composed of liposome-encapsulated *C. albicans* surface MANNAN (503). An IgM monoclonal antibody (mAb) specific for β-1,2-mannotriose, an acid-labile component of the cell wall phosphomannoproteins acting as an adhesin, protected normal inbred, outbred, severe combined immunodeficient and neutropenic mice against disseminated or vaginal experimental candidiasis (503).
Passive transfer of vaginal fluids from animals clearing a primary *C. albicans* infection conferred significant protection against vaginitis in naïve rats. This protection was associated with the presence of both anti-mannan and anti-Sap antibodies, mostly of the IgG and IgA isotypes (183). However, the mechanisms of protection by these antibodies against *Candida* infection are still unclear.

The second immunotherapeutic approach is based on KAbs. KAbs exert a remarkable candidacidal activity *in vitro* and confer significant immunoprotection against infection in systemic and mucosal animal models of candidiasis. KAbs functionally mimic *Pa*KT by interacting with a putative *Pa*KT receptor expressed on the yeast cell wall. The mimicry of the yeast killer phenomenon through the Id network has allowed the development of exclusive models of Id vaccination and anti-Id therapy, which could represent new approaches to the control of systemic and mucosal candidiasis (1135). Direct use of the toxic and instable *Pa*KT is not possible but a marked systemic or mucosal immunoprotection mediated by KAbs has been induced by parenteral or vaginal Id vaccination using a *Pa*KT-neutralizing mAb in mice and in rats (1136).

An active immunization with immunogenic and protective microbial antigens represents a hallmark strategy to prevent or fight infectious diseases. To date, no effective prophylactic or therapeutic vaccine has been developed against candidiasis, although studies are in progress in animal models (312). Because of the antigenic complexity of *Candida*, it has been difficult to define the putative protective antigens to be considered for an effective vaccine. Moreover, the human commensal nature of the fungus, and the consequent natural 'immunization' against it, poses several obstacles for the feasibility of a prophylactic vaccine. More realistic might be the generation of therapeutic vaccines.

In a study by **Han et al**. it was reported that immunization with the L-mannan vaccine in mice induced an increased resistance to VVC (503). A recombinant form of the mannoprotein which was identified as the major target of T cell response in humans is investigated as active immunogen in mice and rats (771).
Recently, **Elahi and colleagues** reported of a therapeutic vaccine in a murine model of oral candidiasis (360). They have used this model to identify regulatory and effector molecules of T cell activation as parameters of induced immunity. Oral but not systemic immunization with the blastospore yeast form induced clinical immunity with a shift in parameters of cytokine response characterised by an early and sustained production of both IFN-γ and IL-4 from antigen-stimulated cervical node T lymphocytes.
As studies have demonstrated the importance of the Th1 cytokine pattern in the anti-*Candida* immune response and in the vaginal mucosa, suggesting a possible beneficial role of cytokine-anti-cytokine therapeutic strategies, a role for some cytokines typical of a Th1 response, has been considered. However, no evidence for a beneficial outcome of a pure cytokine or anti-cytokine treatment has yet been reported. Some antifungals, such as amphothericin B, have direct immunomodulatory properties (1353), suggesting that a combination of chemotherapy with cytokines and antibodies might have great potential for the treatment of RVVC.

4. Trichomoniasis

Vaginal infections caused by the parasitic protozoan *Trichomonas vaginalis* are among the most common conditions found in women attending reproductive health facilities. The prevalence of trichomoniasis in young women attending American

STD clinics for routine care typically approaches 25% up to 48% in certain populations, for example African American women (68, 1328).
The WHO has estimated that this infection with 170 million cases per year accounts for almost half of all curable STDs worldwide (186). Based on WHO estimates, it is one of the two most common STDs in the United States with approximately 7.4 million new cases in 2000 (186, 1487).
Symptoms of trichomoniasis in women include vaginal discharge, odour, irritation, and pruritus; however, about half of all women infected with *T vaginalis* are asymptomatic (1113). The pathogenesis of *T. vaginalis* is not well understood, also in respect to factors causing symptomatic versus asymptomatic infections in women. The extent of the inflammatory response to the parasite may determine the severity of the symptoms.

Although survival on fomites is documented, the organism is thought to be transmitted almost exclusively by sexual activity. Epidemiologically, *T. vaginalis* infections are also commonly associated with other STDs, particularly BV (322, 613). Unlike other STDs, which have a higher prevalence among adolescents and young adults, the rates of trichomoniasis are more evenly distributed among sexually active women of all age groups, further strengthening its potential utility as a sensitive marker for high-risk sexual behavior.

Acquisition of HIV has been associated with trichomoniasis in several African studies, possibly as a result of local inflammation often caused by the parasite. **Leroy et al**. found a significant difference between the prevalence of trichomoniasis among a cohort of HIV infected and non-infected pregnant women in Rwanda, with 20.2% versus 10.9%, respectively (806). In a prospective study by **Laga et al**., incident trichomoniasis was significantly associated with HIV seroconversion among a cohort of women in Zaire in multivariate analysis (774). **Buve et al**. reported significantly higher rates of vaginal trichomoniasis among women residing in high HIV prevalence cities than in low HIV prevalence cities and suggested that trichomoniasis may be an important factor in determining rates of HIV (165).
Moreover, trichomoniasis is associated with adverse pregnancy outcome, such as preterm birth and premature rupture of placental membranes, which should be further elucidated in the chapter on infections and preterm birth.

a) Innate immune responses in trichomoniasis

Current understanding of immunity to *T. vaginalis* has come largely from observations of responses in human patients and experimentation using *in vitro* models and animal models of the related species, *T. foetus*.
The host immune responses to *T.vaginalis* infection are reported to be usually low and variable. Moreover, natural infection seems to produce immunity that is only partially protective, since the reinfection rate of patients can be 36% on follow-up (1021). Factors that influence the host inflammatory response are not well understood but may include hormonal levels, the coexisting vaginal flora, and the strain and relative concentration of the organisms present in the vagina.

(1) TLR

While it is clear that *T. vaginalis* infection can induce an inflammatory response in the female genital tract, the type of receptors involved in host recognition of the pathogen has not yet been established.
Zariffard et al. recently determined if infection with *T. vaginalis* activates cells through TLR4 (1572). Genital tract secretions from infected women stimulated TNF-α production by cells with functional TLR4 but significantly less by cells that are unresponsive to TLR4 ligands. Secretions collected after clearance of infection also induced significantly lower responses by cells with functional TLR4. TNF-α responses were not reduced by Polymyxin B and did not correlate with β2-defensin levels, indicating that stimulation of cells was not through LPS or β2-defensin.
These studies show that *T. vaginalis* infection results in the appearance of substances in the genital tract that stimulate cells through TLR4, suggesting a mechanism for the inflammation caused by this infection (1572).

(2) The role of neutrophils

Buchvald et al. reported that vaginal inflammatory leukocytes in 47 patients with urogenital trichomoniasis were almost exclusively PMNs, and their concentration was positively correlated with the number of trichomonads in vaginal exsudate (152). Also patients with a clinical picture of severe mucosal inflammation had significantly higher vaginal exsudate leukocyte concentrations and viability than

those without inflammatory signs. Groups of PMNs surrounding large trichomonads are able to fragment them and phagocytose the pieces (1186).

Little is known about the exact mechanism of how neutrophils accumulate or mediate the initial inflammatory response after acute *T. vaginalis* infection. The parasite was shown to secrete proteins, for example excretory-secretory product (ESP), that are chemotactic to PMNs (1077).
It is also rationally presumed that leukocyte-derived chemoattractants may be generated during the infection. Chemoattractants which are reported to be involved in the inflammatory response include leukotriene B4 and IL-8.
Leukotriene B4 can be found in high levels in vaginal discharges of symptomatic patients (1286) which could be due to a release from *T. vaginalis* or from neutrophils induced by the interaction of trichomonads and humoral immunity.
In a study by **Shaio et al**. results indicated that humoral immunity could further promote the interaction of neutrophils with *T. vaginalis* and augment the inflammatory response through the amplification of leukotriene B4 production (1285). Specific IgG augmented leukotriene B4 production by neutrophils, possibly mediated by Fcγ receptor.

(3) Complement

Of the known chemoattractants involved in the inflammatory response of *T. vaginalis* infection, complement components have been documented to play a role in the activation of neutrophils as well (1186).
Shaio et al. reported that human neutrophils in combination with serum were able to kill *T. vaginalis* (1284). This serum was shown to have specific IgG for *T. vaginalis* which facilitates neutrophil killing by an IgG-enhanced classical component pathway. However, also antibody-independent alternative complement pathway activation provided C3 for *T. vaginalis* to facilitate neutrophil killing (1284).
The importance of the alternative complement pathway in host defense against *T. vaginalis* was investigated *in vitro* (185). Kinetic studies using immunofixation following electrophoresis showed that both a strongly and weakly virulent strain of *T. vaginalis* activated murine serum C3.
In vivo studies on mice showed that the presence of C5 is also a significant factor in innate host resistance to primary infection with a strongly, but not a weakly virulent trichomonad strain (185). Also the previous findings of **Rein et al.** and

Demes et al. suggested a role for the alternative pathway activation of complement and the trichomonocidal effect of menstrual blood complement (1186, 320).

(4) Nitric oxide and RNI

It has become increasingly clear that the oxidant-antioxidant balance is essential for immune cell function. Neutrophil and macrophage phagocytosis can also stimulate other cellular processes including the respiratory burst whereby increased cellular oxygen uptake results in the production of antimicrobial agents, killing *T. vaginalis* by O_2-dependent mechanisms (724).
Rein et al. detected that PMNs were able to kill *T. vaginalis in vitro* under aerobic conditions which speaks for the importance of oxidative microbicidal systems (1186).
The aim of a study by **Malla et al**. was therefore to assess the RNI production in animals experimentally infected with *T. vaginalis* isolates from symptomatic and asymptomatic women (865). The mean concentration of RNI in vaginal tissue of mice infected with isolates from symtomatic women was significantly higher than that of vaginal tissue of mice infected with isolates from asymptomatic women while it was less in the vaginal washes and plasma of mice infected with isolates from symptomatic women compared to those infected with isolates from asymptomatic women. This may be due to different macrophage populations with different functional capabilities (865). However, this would suggest a possible role for RNI production in establishing the infection.

b) Specific immunity in trichomoniasis

(1) Antibodies

T. vaginalis infection in humans results in parasite-specific antibodies which can be found in the serum and vaginal secretions of infected individuals.
In earlier studies, **Cogne et al**. detected IgA antibodies in serum of infected patients correlating to their clinical status (242). The presence of systemic IgG antibodies of all subclasses may be related to a local response of IgG-secreting cells but may also represent a systemic response to released antigen.
Wos et al. found IgG, IgM, and IgA antibodies in serum of patients (1536). **Street et al**. detected serum IgG and IgM antibodies and IgG and IgA antibodies in CVS

(1363). Specific antibody responses to *T. vaginalis* antigens in serum and specific local antibodies, both IgG and IgA, have also been identified in women by **Alderete and colleagues** (21, 22).

The experimental mouse model of **Paintlia et al**. showed a significant increase in serum and vaginal IgA levels in mice infected with *T. vaginalis* isolates from asymptomatic women compared with mice infected with isolates from symptomatic women and control mice (1061). A further significant response was also observed in group A and group B mice as compared with the control group. This suggests that specific IgA antibodies might help to protect asymptomatic individuals from severe infection. IgA antibodies may also act as a first line of defense for immune exclusion of the parasite at the mucosal surface.

The latest study by **Yadav et al**. reports anti-Trichomonas IgG, IgM, IgA and IgG subclass antibody responses on different post-infection days in serum and vaginal washes of mice infected intravaginally with *T. vaginalis* isolates from 15 symptomatic and 15 asymptomatic women (1548).
A significant increase in parasite load was observed on the 14th post-infection day (p.i.d.) in mice inoculated with *T. vaginalis* isolates from symptomatic women as compared to asymptomatic women, followed by reduction until the 28th p.i.d. A significant increase in specific IgG and in particular IgG1 and IgM responses was observed on the 14th p.i.d. in serum and vaginal washes of mice infected with *T. vaginalis* isolates from symptomatic women as compared to asymptomatic women. Significant increases in specific IgG, IgM and IgG1 responses was observed on the 14th p.i.d. in serum samples as compared with vaginal washes of mice infected with *T. vaginalis* isolates from symptomatic and asymptomatic women, whereas no significant difference was observed in IgA, IgG2a, IgG2b and IgG3 antibody responses.
The study indicates that specific IgG, particularly IgG1 and IgM, may be playing a role in establishing symptomatic infection (1548).

Four antigenic surface molecules have also been implicated in the adhesion of *T. vaginalis* to vaginal ECs; their expression is being upregulated during attachment to host cells (53). Antibodies to these molecules protected target cells from parasite-mediated cytotoxicity (52), suggesting that anti-adhesion immune responses could be important in *in vivo* protection against the pathogenic effects of *T. vaginalis*.

Alternatively, antibodies specific for soluble parasite molecules such as proteases, cytoactive molecules, or lytic factors such as phospholipases may also be protective (1269). However, proof of the protective nature of antibodies in eliminating infection or limiting pathogenesis *in vivo* has been hampered by lack of an adequate experimental animal model for vaginal infection studies.

(2) T lymphocytes

The study of **Paintlia et al**. revealed a significant increase in the population of total T cells, as well as CD4+ T cells in mice infected with isolates from asymptomatic women compared with mice infected with symptomatic isolates (1061). However, no significant difference was observed in CD8+ cells, whereas a significant difference between the two groups was noticed in the vital ratio of CD4+/CD8+. Also a significant increase in NK cells was observed in animals infected with isolates from asymptomatic women compared with mice infected with isolates from symptomatic women and control uninfected mice, indicating that NK cells might be playing a role in the pathogenesis of this disease.

c) The role of cytokines

The cytokine profile in vaginal cervical tissues of mice infected intravaginally with *T. vaginalis* isolates from 15 symptomatic and 15 asymptomatic women was subject of a study by **Paintlia et al**.

They reported that the concentrations of IFN-γ and TNF-α in vaginal washes of mice infected with isolates from symptomatic and asymptomatic women were significantly higher compared to uninfected mice (1061). Between the two infected groups, IFN and TNF levels were higher in the group of mice infected with isolates of asymptomatic women.

Significantly higher IL-2 production was observed in mice infected with isolates from asymptomatic women compared mice infected with isolates from symptomatic women or the uninfected control group. IL-4 levels were found to be significantly higher when results from both groups infected with isolates were compared with controls (1061).

IL-8 is found in high levels in vaginal discharges of symptomatic trichomoniasis patients. This cytokine has been shown to enhance antimicrobial activities of neutrophils by inducing neutrophil degranulation and respiratory burst.

Shaio et al. investigated the possible role of IL-8 and showed that membrane components of *T. vaginalis* induced blood monocytes to produce large dose- and time-dependent amounts of this cytokine (1287). Amounts of IL-8 were higher than those induced by live trichomonads or ESP. Neutrophil chemotaxis was inhibited by a neutralizing mAb directed against IL-8 itself or partly decreased by a mAb directed against TNF-α, suggesting also a role for TNF-α in the release of IL-8 (1287).

In another study of **Ryu et al**. it was revealed that *T. vaginalis* can induce IL-8 production in neutrophils which may be mediated through the NF-κB and mitogen-activated protein (MAP) kinase signaling pathways (1232). Live *T. vaginalis* produced higher amounts of IL-8 than ESP or *T. vaginalis* lysate. Moreover, GRO-α, another chemoattractant for PMNs, was also produced by neutrophils in response to *T. vaginalis* activation (1232).

NF-κB is implicated in the regulation of inflammatory responses by inducing cytokines such as IL-12 and TNF-α and activating effector mocules of innate immunity including macrophages.

In a study by **Chang et al**., it was investigated whether the inflammatory response of macrophages elicited by *T. vaginalis* infection required NF-κB activation (198). *T. vaginalis* induced a rapid activation of NF-κB in RAW264.7 macrophage during the early stage of adhesion which was not maintained but led to inhibition of the production of the proinflammatory cytokines. Furthermore, *T. vaginalis* infection induced a state of nonresponsiveness to subsequent stimulation with bacterial LPS which suggest that *T. vaginalis* induces an inhibitory mechanism that prevents or delays the immune response of host cells, thereby leading to their apoptotic cell death (198).

d) Vaccination strategies

Systemic immunization of cows with a purified surface antigen of *T. foetus* induced serum and vaginal IgG and IgA antibodies, and intravaginal boosting enhanced the genital IgA response to infectious challenge (253). IgG and IgA antibodies in vaginal secretions were associated with protection or faster clearance of infection. Interestingly, lymphoid aggregates in vagina and uterus in response to infectious challenge were demonstrated in control and intravaginally immunized animals,

which suggestes development of mucosal inductive sites for local IgA responses (253).

Immunity has been difficult to produce *in vivo*, since in humans, repeated infections with *T. vaginalis* do not confer immune protection (1021). Despite this, antibodies can be found in the serum and vaginal secretions of infected individuals and a cell-mediated immune response is also invoked.

Older experimental work using subcutaneous or intraperitoneal injections in mice suggested that antibodies could be protective, and more recent experiments with immunized mice challenged by intravaginal inoculation indeed indicate some protection (2).

Abraham et al. were able to induce immunity to *T. vaginalis* in the mouse model, which may lead to the development of a vaccine. Whole, live trichomonads at different concentrations were injected subcutaneously into mice, first with Freund`s complete adjuvant and then in a booster dose with the trichomonads and Freund`s incomplete adjuvant. The mice were given estrogen and inoculated intravaginally with *Lactobacillus acidophilus* to simulate the conditions in the human vagina; they were then inoculated intravaginally with *T. vaginalis*. Immunized mice had significantly less intravaginal infection and had elevated antibody levels in the serum and vagina compared with naïve control groups (2). Mice that had been infected vaginally, treated, and reinfected vaginally were not protected and did not mount an immune response which suggests that antigen presentation may be crucial for developing protective immunity.

To date, only one vaccine, the Solco Trichovac vaccine, had been produced against *T. vaginalis*. It was prepared from inactive lactobacilli and was thought to work by inducing antibodies to abnormal lactobacilli and *T. vaginalis* without adversely affecting the growth of normal lactobacilli in the vagina (464). However, a lack of antigenic similarity between this vaccine and *T. vaginalis* was shown, which makes this cross-reaction hypothesis unlikely.

D. Immunology in reproductive medicine

1. Immunology of pregnancy

Pregnancy can be described as the symbiosis of two allogeneic individuals which live in intimate contact. Although the fetus provides a panel of MHC antigens derived from both its mother and father it is not rejected by lymphocytes from its mother. The maternal immune system reacts towards the foreign tissue, but it tolerates, supports and regulates its development instead of rejecting it. The formation of the placenta is efficiently controlled ensuring development of the embryo and fetus.
In the course of the discovery of the MHC and it role in transplantation **Medawar** was the first to compare the immunologically privileged nature of the fetus with an allograft in 1953 (908). He already proposed some ideas such as the anatomic separation of mother and fetus, antigenic immaturity of the fetus, and maternal immunosuppression all of which has now been investigated and partly disapproved over the years. However, maternal tolerance of an allogenic fetus is a paradox that still remains a central theme in the field of reproductive immunology.

There are many factors influencing this system which can lead to imbalances and pregnancy disorders such as infertility and abortion. Therefore it is essential to investigate the immunoregulatory processes of pregnancy to prevent and treat these disorders. Knowledge of the structural basis of the placenta and the immunobiology of the deciduas and of trophoblasts are important issues to discuss before coming to the disturbances in this system including preeclampsia, preterm labor or pregnancy loss.

a) Immunology of fertilization

An impending pregnancy must prepare the maternal environment to accept a partially foreign body, a semi-allograft, which occurs in four distinct phases (79). The first is the pre-fertilization period in which the egg is surrounded by the follicular fluid with its immunosuppressive activity (184). This may facilitate the following fertilization process as well as embryo development by minimizing the

maternal immune response to the sperm, which has been shown to express foreign antigens shortly after fertilization.

The second phase comprises fertilization and embryo development until the Morula stage in which the sperm penetrates the egg and becomes immunologically invisible for the maternal system (79). No maternal immune reaction occurs during the process of egg and spermhead fusion, for as long as the egg surface membrane does not change its characteristics. Once this happens, the fertilized egg is rapidly surrounded by the zona pellucida, which wards off maternal immune cells. Further protection against these cells is provided by maternal cumulus cells for the first days after fertilization.

The third phase consists of the blastocyst and pre-implantation trophoblast polarization after the first embryonic cell divisions when the differentiation into the maternal embryoblast and the paternal trophoblast starts (79). The surrounding zona pellucida continues to provide a major protection against the maternal immune system so that the development of maternal immune tolerance to the embryo does not take place until implantation phase, when direct embryo/maternal contact occurs in the uterus.
From the immunological point of view, this is the most vulnerable time for the embryo which is now exposed to maternal endometrial immune cells and cytokines. The uterus is designed to prevent implantation except during a narrow implantation window in which embryonic signals help promote endometrial priming and maternal immune tolerance. The embryo likely plays the significant role in conditioning the maternal environment towards immune tolerance of pregnancy. The arising questions are how this maternal immune tolerance takes place, what embryo-derived elements are involved and where, if these mechanisms are common to all mammals, and how early embryo tolerance can be detected in the maternal organism.

The developing fetus is not directly exposed to maternal blood except in the placenta as the newly formed organ at the fetomaternal interface. The placenta plays the key role in maintenance of local tolerance and allows the mother to accept the embryo during pregnancy. As the fetal side of the placenta, the villous trophoblast forms a continous barrier which physically separates the mother from the fetus but is exposed to maternal immunocompetent cells present in the circulating blood of the intervillous space. Syncytiotrophoblast and villous

cytotrophoblast can also be found in maternal circulation and lung providing additional antigenic stimuli for the mother. Trophoblast is also present in the decidua where they contribute to further stimulating the mother with fetal antigens. This physiological condition of close physical contact between maternal immune system and fetal cells with paternal antigens is unique and normally does not trigger a maternal immune reaction that would lead to fetal death.

Earlier studies suggest that shortly after fertilization, certain changes that favor immune tolerance take place in the maternal environment, possibly due to early pregnancy factor (EPF) and platelet activating factor (PAF) (976, 1035).
EPF is an immunosuppressive factor with growth-regulatory properties which interacts with T lymphocytes and suppresses cellular immune responses and the early expression of cell surface membrane IgG (1156). It was first described more than 20 years ago as a very early serum marker of fertilization (975) and is now known to be essential for the initiation and maintenance of pregnancy, displays the regulated production and pleiotropic function typical of many cytokines and growth factors.
On the one hand, it may prevent sperm penetration and maintain a successful pregnancy but on the other hand, its immunosuppressive activity might cause some adverse impact. Studies have proved that EPF activity is detectable in serum (506), amniotic fluid (1585) and cervical mucus (218) of pregnant women. Therefore, EPF may provide wide clinical applications, such as testing the occurrence of fertilization *in vivo*, predicting the prognosis of pregnancy, and evaluating embryo quality in the *in vitro* fertilization-embryo transfer treatment (218).

As these factors have since been found to be not specific to pregnancy and are found in a non-pregnant state as well, these factors do not appear to be critical in initiating maternal recognition of the embryo. **Barnea** postulated that unique embryo-derived compounds are involved in creating the unique maternal immunological response to the embryo (79).
Shortly after fertilization, the viable embryo starts to emit signals that promote maternal recognition of pregnancy and immune tolerance. Recent studies have found that the cumulus cells surrounding the embryo might be able to serve as a relay system, because they contain active immune cells that secrete cytokines (1118). This proximity between the putative embryo-derived compounds and the maternal immune system would permit rapid diffusion of embryonic signals leading initially to a local immune response, followed by a systemic maternal immune

recognition (79). This conclusion is based on the observation that pre-implantation factor (PIF) peptide expression modulates but do not suppresses the maternal immune system, allowing the mother to maintain her ability to fight diseases.

b) Immunobiology of the trophoblast

As already described, the fetal-placental unit initially is a semi-allograft due to the paternal genetic contributions. Subsequently, there is a maternal immune reaction to the allogeneic pregnancy. The constituents of the maternal immune reaction to the allogeneic stimulus are not different from any other immune reaction and the allogeneic conceptus, the trophoblast, is like all other allogeneic tissue grafts.

However, trophoblast is an unusual cell type (1430). It is extra-embryonic and has several distinctive properties such as the expression of endogenous retrovirus products, oncofetal proteins, and imprinted genes (953). In addition, the DNA in trophoblast is relatively unmethylated. All these properties could have some relevance in interaction with the maternal immune system (1430).
The special characteristic, however, that distinguishes the trophoblast from other tissues is its ability to eliminate abortogenic maternal B cell and T cell responses. The trophoblast induces an immunmodulation and thus actively defends itself from the maternal immune attack. The presence of progesterone, and its interaction with progesterone receptors at the decidual level, appears to play a major role in this defense strategy (1159).

Indeed, trophoblasts can influence the immune system during pregnancy through their expression of soluble and cell surface-associated immunomodulatory molecules.
For example, trophoblasts secrete the indoleamine 2,3-dioxygenase (IDO), which limits the availability of the essential amino acid tryptophan, consequently limiting lymphocyte proliferation (1270). IDO is an enzyme from the tryptophan catabolic pathway that depletes tryptophan in local tissue environments, thereby suppressing proliferation of cells in the vicinity (914). LPS and the inflammatory cytokines IL-1 and TNF-α act synergistically with IFN-γ to further increase IDO expression in human DCs (586) while antiinflammatory cytokines IL-4, IL-10 and TGF-β inhibit IDO expression (852).

It has been shown experimentally that cells at the maternal-fetal interface expressing IDO may establish local microenvironments in which reduced tryptophan concentration precludes T cell proliferation, thus protecting the conceptus from rejection (988).

Clark et al. also hypothesized that regulation of the expression of CD200 plays an immunoregulatory role at the maternal-fetal interface (235). CD200 is another DC-associated molecule which has been shown to contribute to the successful outcome of organ and tissue allografts in mice (466). CD200 is upregulated in rodent transplantation models where successful inhibition of rejection is accomplished, and is believed to signal immunosuppression following engagement of a receptor, CD200R. The investigation of CD200 expression in implantation sites of different strains of mice resulted in an elevated the abortion rate in mice due to anti-CD200, and infusion of a CD200 immunoadhesin reduced the abortion rate (466). CD200 mRNA expression was demonstrated on fetal trophoblast and certain areas of decidua (235). Reduction in CD200 has proven essential for fgl2 prothrombinase triggering of abortions in the mouse model, but the mechanism behind it remain unclear.

There is growing evidence that trophoblast cells are able to recognize and respond to pathogens through the expression of TLRs (4). Normal term placental tissue has been shown to express TLR1-10 at the RNA level (1571). **Holmlund et al**. demonstrated that term syncytiotrophoblast and intermediate trophoblast cells express TL-2 and TLR4 at the protein level (561). In contrast, **Kumazaki et al.** found the TLR4-positive placental cells to be term extravillous and intermediate trophoblasts (754). **Abrahams et al**. observed that TLR2 and TLR4 are highly expressed in first trimester placental tissues (3). The first trimester trophoblast cell populations expressing these receptors are the villous cytotrophoblast and extravillous trophoblast cells while first trimester syncytiotrophoblast cells do not express these TLR and this suggests that the placenta serves as a highly specialized barrier, protecting the developing fetus against infection.

These findings suggest that trophoblast cells may interact with microorganisms present at the implantation site and may be able to initiate an immune response. The trophoblast may thus function as an active member of the innate immune system, as it was once proposed by **Guleria and Pollard** (489).

Trophoblast cells from term placental explants have been demonstrated to produce IL-6 and IL-8 following ligation of TLR2 or TLR4 by LPS (561). Activation of TLR4 by LPS triggers first trimester cytotrophoblast and villous trophoblast cells to generate a classical TLR response, characterized by the increased production of both pro- and antiinflammatory cytokines (3).

Remarkably, in contrast to most other somatic tissues, invasive trophoblast cells lack not only the classical MHC class I but also MHC class II, which might be important in avoiding the attack of maternal T cell-mediated rejection (1158). Among the polymorphic classical class I molecules they only express HLA-C and additionally, the nonclassical HLA-E, HLA-F and HLA-G class I molecules (698). There also have been detected membrane-bound HLA-G1 and soluble HLA-G1 and -G2 in extravillous trophoblast (965).

Increasing evidence suggests that placentally-expressed MHC molecules may play an important role in modulating the innate branch of the maternal immune system. These molecules have several characteristics, which suggests that antigen presentation is not their primary function, including limited tissue expression, relatively short half-life at the cell surface, and limited genetic polymorphism (577).
It is assumed that the non-classical HLA-G class I may contribute to the immunological mechanisms that protect the fetus against maternal alloimmune response (1143). HLA-G expression may promote allograft survival (1219). HLA-G was found to inhibit cytotoxicity by NK cells via HLA-E (696) and is thought to be important when considering the presence of atypical CD16+ NK cells in successful pregnancy and increased presence of classical CD16+ NK cells in pregnancy failure (230). Recent evidence suggests that soluble HLA-G1 is immunosuppressive and induces apoptosis of activated CD8+ T cells and downmodulates CD4+ T cell proliferation (794). Thus, soluble HLA-G1 could also play a role during implantation.

In addition, the trophoblast also protects itself by expression of FasL (582). In mice, FasL is also expressed on uterine glandular ECs and decidual cells in placental trophoblasts. Predominant expression of FasL in mice is found in the uterus which shifts to the placenta at later gestation days during pregnancy (582). Recently, FasL expression was also reported in first trimester and term human placental villi (500). Thus, expression sites of FasL are obviously positioned to induce apoptosis in

maternal Fas positive immune cells, such as NK and T cells, at the fetomaternal interface (582).

Ohshima and colleagues studied the relationship between FasL expression and NK cell infiltration in human placenta during early pregnancy (1042). The findings suggested that a reduction in FasL expression seems to be closely associated with activation and infiltration of maternal NK cells and destruction of uterine glands, resulting in rejection of the foetus (1042). Thus, expression of FasL in the uterine glands and cytotrophoblasts may play a role in the downregulation of the maternal immune response, thereby maintaining pregnancy at early stage.

c) Immunology at the fetomaternal interface

(1) Antigen-presenting cells

APCs are often regarded as the responsible inductors of maternal tolerance against fetal antigens. In their function of presenting antigens to lymphocytes, it could be assumed that they would be ideally located at the fetomaternal interface to present fetal antigens in a tolerance-inducing way (660).

DCs

Especially, the presence of DCs in the maternal decidua has pointed to a biologic role of APCs in a maternal-fetal interaction. DCs of the pregnant human uterine mucosa are likely to regulate immune responses to both uterine infections and placental trophoblast cells (642).

In studies on pregnant rat and mouse uteri the first hints for a possible role of APCs in creating a local environment prohibitive of maternal lymphocyte stimulation against the embryo came up.

Macrophages from the rat uterus were shown to be depleted shortly after implantation (1385) and **Hunt** observed that macrophages from pregnant mouse uteri are immunosuppressive (579). **Bulmer et al**. found a high population of early pregnancy human decidual cells to be HLA-DR+ and concluded that these HLA-DR+ cells mainly belong to the macrophages (158). Later he described that macrophages increase premenstrually and make up to 35% of the decidual leukocytes around implantation (157). In their study on HLA expression by human decidual cells throughout gestation **Lessin et al**. found that 21-32% of maternal decidual cells

were positive for HLA class I and II molecules (808). The class II+ cells were identified as macrophages by the antibody staining. **Mizuno et al.** showed that isolated decidual macrophages are able to present soluble antigens in an MHC-restricted manner but also possess some some suppressive activity for the maternal immune responses (952).
However, another study showed that cultured human decidual stromal cells express HLA-DR and the activation markers CD80 and CD86 but not the classical macrophage marker CD14 and are able to stimulate allogeneic T cells (1047). In recent years, classical mature CD40+ CD45+ CD83+ DCs similar to those of other mucosal surfaces were demonstrated in human endometrium and early pregnancy decidua (659). CD83 has been proved as a suitable and selective cell-surface marker for mDCs.

Also the immature precursors of decidual DCs, DC-SIGN positive HLA-DR+ decidual cells which also stained for CD14 and CD68 as classical macrophage markers, were detected (657). The maturation of CD14+ DC-SIGN+ decidual cells into CD25+ CD83+ mDCs upon inflammatory cytokine treatment resulted in downregulation of antigen uptake capacity and effective stimulation of resting T cells.
A small population of decidual DC expressing DC11c, a marker for myeloid DC, but not expressing other classical leukocyte lineage markers was detected by **Gardner et al.** (439). These decidual DCs comprised about 1.7% of CD45+ cells in the isolates. **Strbo et al**. demonstrated a bidirectional cross-talk between decidual $CD56^{bright}$ NK cells and decidual CD83+ cells, resulting in activation and proliferative response of autologous NK cells (642).
In experiments with mice, **Blois et al**. found that the majority of uterine DCs were of myeloid lineage and that the relative number of CD11c+ uterine cells is not constant during pregnancy and shows an increase occuring simultaneously with the decisive phase of gestation, when implantation takes place (121).

Miyazaki et al. showed that decidual mDCs secrete less of the proinflammatory cytokine, IL-12, than blood monocyte-derived DCs and induce Th2 cells when co-cultured with naïve CD4+ T cells, which suggests an immunosuppressive phenotype of decidual DCs (951).
Yoshimura et al. studied the systemic response of DCs and their cytolytic products and reported that the frequency of myeloid DCs, in the early and middle stages of human pregnancy, was comparable with that of lymphoid DCs, while in the late stage of pregnancy, the frequency of myeloid DCs significantly increased (1563).

They also showed that in the presence of human chorionic gonadotropin (hCG) both peripheral blood DC subsets can be activated to respond to any invading pathogens that affect DC subtypes.
Myeloid DCs can produce IL-12 and IFN-γ in response to hCG, thus IL-12 produced from myeloid DCs can stimulate the production of IFN-γ as a positive feedback loop, while lymphoid DCs might be directly activated by hCG to produce IFN-γ (308).

Macrophages

Macrophages constitute 20-30% of the decidual cells at the site of implantation and unlike NK cells, remain high throughout pregnancy (808). Indeed, macrophages are one of the major cell types in both the maternal and fetal compartments of the uteroplacental unit. In humans, during the first weeks of implantation, macrophages are found in high numbers in the maternal decidua and in tissues close in proximity to the placenta (940), especially in the stroma surrounding the transformed spiral arteries and extravillous trophoblast (963).

This evidence suggests that the innate immune system is not indifferent to the fetus and may have a role not only in host protection to infections, but also as important players in the feto-maternal immune adjustment. Macrophages are also a main source of cytokines and growth factors and contribute to the maintenance of the adequate balance between Th1 and TH2 cytokines at the placental bed (963).

There are also several indications that macrophages are often more closely associated with trophoblast than uterine NK cells, with close associations between macrophages and extravillous trophoblast in decidua basalis and a recent study of rhesus monkey decidua also highlights the close association of macrophages, rather than uterine NK cells with trophoblast (1316).
Although decidual macrophages are activated *in vivo*, as indicated by their expression of HLA class II, CD11c, and CD86 (581), they have recently been reported to express markers such as DC-SIGN that may aid in immune evasion or are associated with macrophage alternative activation, a phenotypic state of immunosuppressive activity (467). Early *in vitro* studies of the suppressive functions of decidual cell mixtures pointed to maternal macrophage production of prostaglandin E2 (584). Decidual macrophages have also been shown to elicit reduced allogeneic and autologous T cell responses when compared to their blood counterparts (952). The question then arises as to how decidual APC are driven into immune inhibitory profiles.

Several studies have indicated that placental HLA-G as product of infiltrating fetal cytotrophoblast cells may suppress APC functions, but reports published to date fail to show that HLA-G is acting specifically at the level of the APC (900). Macrophages and DCs both express the HLA-G receptors, ILT2 and ILT4, which can suppress activation signals in order to induce immunosuppressive activities by APCs.

The dense macrophage-infiltration at the maternal fetal interface suggests that these cells are also involved in specific pregnancy-associated functions, and not only to perform their usual immunological tasks (107). **Hunt et al**. have implied that maternal macrophages assist in the tissue remodeling necessary to accommodate expansion of extraembryonic tissue (583).
Macrophages actively orchestrate apoptosis of unwanted cells during tissue remodeling through cytokines and the influence of hormonal factors. During implantation, apoptosis is important for the appropriate tissue remodeling of the maternal decidua and invasion of the developing embryo. Apoptosis has been described in the trophoblast layer of placentas from uncomplicated pregnancies throughout gestation, suggesting that there is a constant cell turnover at the site of implantation necessary for the appropriate growth and function of the placenta (1175, 1321). In addition, the incidence of trophoblast apoptosis is higher in third trimester villi compared to first trimester placenta, suggesting that increasing placental apoptosis may be involved in the process of parturition.

However, the clearance of apoptotic bodies represents the critical step in tissue homeostasis, preventing the release of intracellular contents, which may cause tissue damage and the possibility to initiate an inflammatory reaction that may have lethal consequences in pregnancy (963).
During the period of implantation and trophoblast invasion with its induction of apoptosis in maternal tissue, numerous macrophages are present at the implantation site which was originally thought to represent an immune response against the invading trophoblast. Instead, it is suggested that macrophage engulfment of apoptotic cells prevents the release of potentially proinflammatory and proimmunogenic intracellular contents that occurs during secondary necrosis (963).
Trophoblast cells are carriers of proteins, which are antigenically foreign to the maternal immune system and if released, as result of cell death, may initiate or accelerate immunological responses with lethal consequences for the fetus.

Therefore, the appropriate removal of dying trophoblast cells by macrophages prior to the release of these intracellular components is critical for the prevention of fetal rejection (963). **Mor et al**. proposed that during normal pregnancy, the uptake of apoptotic cells suppresses activated macrophages from secreting pro-inflammatory cytokines such as TNF-α and IFN-γ and promotes the release of Th-2 type, anti-inflammatory and immunosuppressive cytokines with protective effects on trophoblast survival and immunological tolerance (Figure 18).

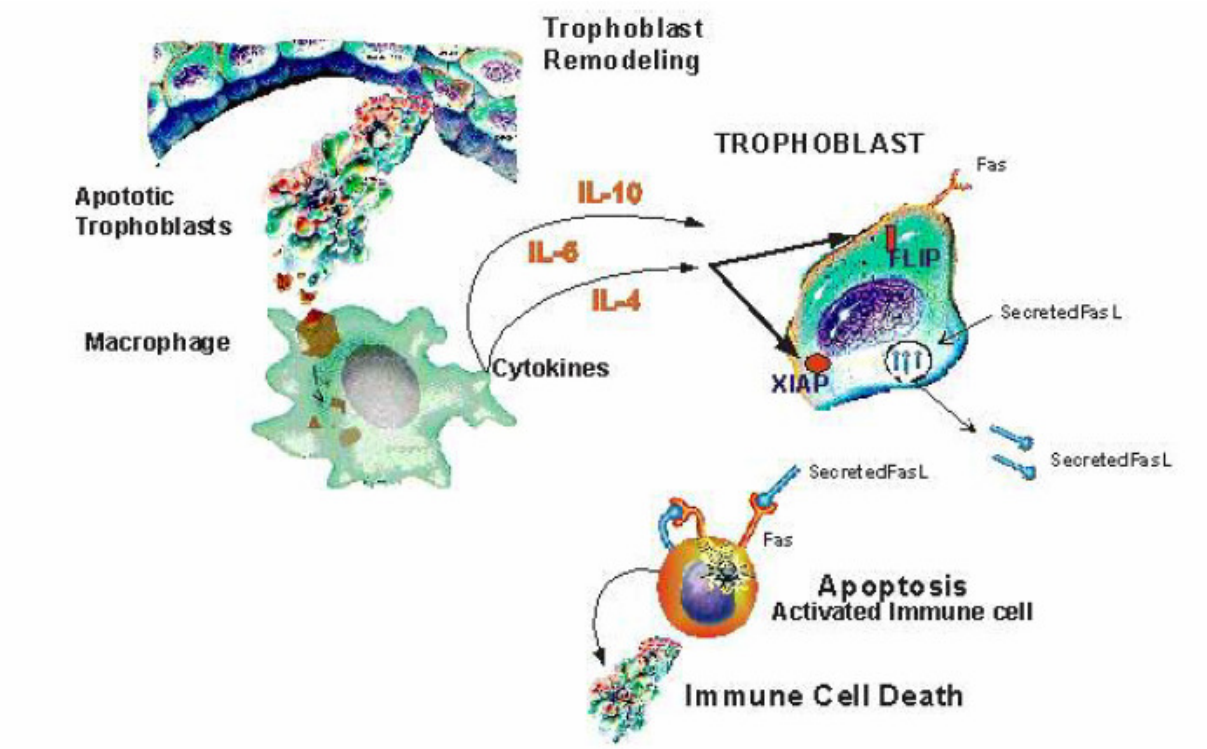


Figure 18: Clearance of apoptotic cells by macrophages in normal pregnancy, adapted from Mor et al. (963)

Changes in the cytokine milieu, owing to elevated levels of apoptotic bodies and inefficient clearance, will result in a proinflammatory microenvironment that in turn may result in changes in trophoblast resistance to Fas-mediated apoptosis and the maternal immune system (Figure 19). Further explanations are to be discussed in the chapter on disturbances in maternal-fetal interactions later.

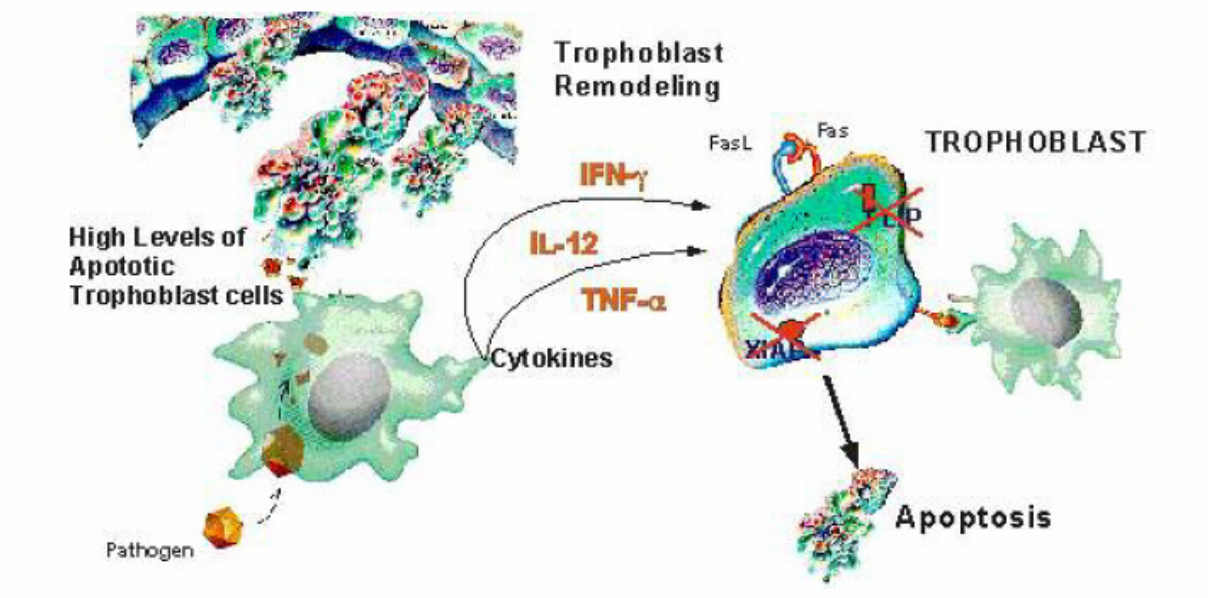


Figure 19: Proinflammatory environment by inefficient clearance of apoptotic bodies in complicated pregnancy, adapted from Mor et al. (963)

(2) Leukocytes

Already in the final part of the menstrual cycle a large number of maternal leukocytes can be found in the endometrium (1119). As the mucosal lining of the uterus is transformed from endometrium in the non-pregnant state to the decidua of pregnancy by the influence of sex hormones, these leukocytes further increase in number and are found in close contact with trophoblast. CD56+ leukocytes make out 70% of the immunocompetent deidual cells. The predominant type of these cells are uterine NK cells (46%), followed by macophages (19%) and T cells (8%), mainly CD8+ T cells whereas B cells are virtually absent (949). In general, cells of the innate immune system seem to dominate this tissue since the levels of T and B lymphocytes are with 1-3% relatively low (808).
Extravillous trophoblast cells interact with maternal immune immune cells at the implantation sie in the decidua basalis, including the abundant NK cells, APCs and T cells.

NK cells

Analysis of the leukocytes in the uterus has shown that NK cells are the predominant population but their total number in the uterine mucosa varies throughout the menstrual cycle with an increase throughout the secretory phase and in the decidua during the early stages of gestation (829).

There is a massive recruitment of NK cells at the embryonic site of implantation which makes them the dominant cell type of maternal immune cells in the decidua basalis in early pregnancy (1158). As gestation proceeds, NK cell numbers decline and at term these leukocytes are absent (954). This reduction of uterine NK cells in later pregnancy remains unexplained.
Phenotypically, decidual NK cells ($CD56^{bright}$ CD16- CD160-) differ from NK cells in peripheral blood ($CD56^{dim}$ CD16+ CD160+) which suggests that either decidual NK cells represent a distinct subpopulation of circulating NK cells or they have undergone some tissue-specific differentiation.

After blastocyst implantation and decidualization decidual NK cells are activated and secrete IFN-γ, perforin and angiogenetic factors in order to control trophoblast invasion through their cytotoxic activity and also initiate vessel instability and remodelling of decidual arteries to increase the blood supply to the fetoplacental unit (1430). Moreover, they take part in regulation of the maternal immune response producing Th2- and Th3-type cytokines, which result in placental augmentation and local immunosuppression (233).

Decidual NK cells express receptors for classical and nonclassical HLA class I which are expressed on the cell surface of or secreted by extravillous trophoblast. The extravillous cytotrophoblast attracts decidual NK cells by producing MIP-1α chemokines so that the HLA class I molecules on the trophoblast interact with MHC class I-dependent receptors present on the surface of decidual NK cells (1158).
These receptors on decidual NK cells comprise four different families with both activating and inhibitory members (700). The specific ligands for most NK cell receptors are the only HLA molecules expressed on extravillious trophoblast, HLA-C, HLA-E and HLA-G (1444).
These are the KIRs for which HLA-C and HLA-G molecules are the ligands, the Ig-like receptor ILT2 which is expressed by 20-25% of decidual NK cells and interacts with HLA-G, the CD160 receptor with its major ligand HLA-C which is expressed on a minor set of decidual NK cells, and the CD94/NKG2 receptor which binds HLA-E.

However, the functions for decidual NK cells in pregnancy are still not exactly clear but there are some hints for a possible control of placental development and pregnancy maintenance. Due to the high abundance of uterine NK cells in the decidua in the first and second trimesters of pregnancy, and their association with

extravillous trophoblast cells it has been proposed that they play an active role in the regulation of trophoblast invasion (155).
Results from *in vitro* studies have proposed the involvement of NK cell receptor-HLA class I interactions in the protection of the trophoblast. HLA-G antigen was thought to be the main factor for the protection of the fetus from decidual NK cell lysis (793). NK cells in decidua express a noncytotoxic phenotyp lacking CD16 and CD160 as the markers of cytotoxicity (1444). Although expression of granzyme A, granzyme B and perforin was detected in decidual NK cells, these cells had a very low potential cytotoxic effect on K562 target cells (155). Moreover, the extravillous trophoblast may not express enough triggering ligands of activating NK cell receptors such as NKG2D and natural cytotoxicity receptors (1158). Trophoblast cells are resistant to cell lysis unless decidual NK cells have been stimulated with IL-2 which is not present in decidua (701). However, no in situ evidence of trophoblast lysis by uterine NK cells in early decidua has been detected yet. Another argument is the exposure of decidual NK cells to progesterone. A mediator of progesterone, progesterone-induced blocking factor (PIBF), was shown to block decidual NK cytolytic activity (787).

As there is considerable interest in the role of cytokines in pregnancy, attention has also focused on uterine NK cell cytokine production. Studies detected transcripts for various cytokines and growth factors including GM-CSF, TNF-α, IFN-γ, IL-10, IL-1β, TGF-β, VEGF and LIF in decidual NK cells (1195, 1239).

Uterine NK cells have also been proposed to play a role in spiral artery transformation. Murine studies on NK cell-deficient mice also showed abnormal decidual vasculature including abnormal thick spiral arteries suggesting an additional role in uterine vascular remodelling (275). These abnormalities could be reversed after injection of allogeneic NK cells. It has also been demonstrated that the major uterine NK cell product responsible for the spiral artery remodelling defects is IFN-γ (275, 57).

These results may be also true in humans as decidual NK cells are closely aggregated around maternal spiral arteries (1430). However, much less is known about the mechanisms involved in spiral artery remodelling in humans but it is likely that they are, at least in part, different from those controlling trophoblast invasion. Early structural changes in decidual spiral arteries, including dilatation and medial disorganization, occur before cellular interaction with trophoblast, but

at a time when uterine NK cells are present (156). Uterine NK cells reduce in number after 20 weeks' gestation when vascular changes are generally complete.

Granulocytes and monocytes

It appears that other components of the immune system, especially the innate immune system, are activated during pregnancy. Monocytes are activated in pregnancy and data showed elevated surface expression of CD11b, CD14 and CD64 antigens on monocytes from third trimester pregnant women (841). In addition, an increased expression of CD11a, CD49d and CD54 on monocytes was also observed from pregnant women.

Granulocytes from pregnant women show increased surface expression of both activation markers and adhesion molecules, for example of CD11b and CD64 (1234). These cells also show increased production of intracellular ROS and enhanced phagocytosis, as compared with nonpregnant women. Also in the study of **Naccasha et al**. baseline intracellular ROS and oxidative burst were higher in both granulocytes and monocytes from pregnant women than in the control group (997).

Together, these data are consistent with the idea that pregnancy is a proinflammatory state. However, granulocytes from pregnant women in other studies had reduced microbial killing activity and chemotaxis as well as a decreased respiratory burst activity and intracellular H2O2 production when challenged (269). In contrast to **Sacks et al**. (1234) there was not found an increase in CD11b expression and there was no upregulation of CD18 or downregulation of CD62L expression, both of which are needed for appropriate priming and activation of granulocytes.

A study by **Luppi et al**. found that during pregnancy the percentage distribution of granulocytes was significantly increased, with a consequent reduction in the percentage of lymphocytes and monocytes as compared with nonpregnant women (841). Data on pregnant women followed longitudinally throughout gestation showed that an increase in the proportion of granulocytes and a decrease in the proportion of lymphocytes appear from the second trimester onward (948).

Neutrophils are located near the placenta where they might phagocytose cellular debris from decidual cells killed by invading trophoblast (392).

T lymphocytes

In early pregnancy, T cells comprise about 10-20% of the leukocytes in the uterine mucosa (1119). Their proportion, however, increases with gestational age followed by a decline in the term pregnant uterus. A significant increase in the frequency of CD8+ T lymphocytes and a decrease in the frequency of CD4+ T lymphocytes were evident in pregnant women (398). Furthermore, an increase in the frequency of CD8+ T cells was observed in pregnant women during labor together with a concomitant reduction in the CD4/CD8 ratio.

Th1- and Th2-type cytokines produced by maternal T lymphocytes present at fetomaternal interface seem to play a role in the development of pregnancy as above mentioned already. In humans, the success of pregnancy seems to be associated with the production of IL-4, IL-10 and M-CSF by T cells (1115). Both IL-4 and IL-10 can inhibit the development and function of Th1 cells and macrophages, thus preventing the allograft rejection.

There is direct evidence that the pregnancy-associated hormones progesterone and estradiol modify the cytokine production pattern of human antigen-specific T cells. Progesterone is a potent inducer of the production of Th2-type cytokines and of LIF and M-CSF production by T cells which is mediated by IL-4 (1116). Estradiol and hCG have both no effect on T cell differentiation to Th1 or Th2 cells (1116). In summary, results suggest a hormone-cytokine-T cell network at the fetomaternal interface with progesterone partly responsible for the T2 switch there. Defects in this network can result in fetal loss.

T cells have also been detected in surrounding cell masses of the oocyte during ovulation, the so called cumulus oopherus. In women with blocked fallopian tubes CD4+ T cells and macrophages were found in cumuli, but only few NK cells (1118). Cumulus T cells produce higher levels of IL-4 and LIF than peripheral blood or ovary specimen T cells. Hormones can also modulate the cytokine profile of these cumulus oopherus T cells. Progesterone produced by cumulus granulosa cells may favour IL-4 production by T cells, which in turn can produce LIF (1118).

T regulatory cells

Treg cells, representing 2–5% of CD4+ cells, are believed to be important in immune tolerance by maintaining natural self-tolerance and negative control of pathological, as well as physiological, immune responses (1243). Recently, **Aluvihare et al**. reported that in mice the absence of CD4+CD25+ Treg led to a failure of gestation

due to immunological rejection of the fetus, suggesting that CD4+CD25+ Treg cells mediate maternal tolerance to the fetus (33).

With only the CD4+ T cell population that expresses high levels of CD25 demonstrates regulatory function in humans, it was first reported in 2003 that decidual and peripheral blood CD4+CD25^{high} T cells increased during early pregnancy (1258). Recently, **Somerset et al**. reported an increase in circulating CD4+CD25+ T cells during early pregnancy, peaking the second trimester and then declining postpartum (1325).

Sasaki et al. reported that the population of CD4+CD25^{high} T cells rose from 6% in the peripheral blood of nonpregnant subjects to 8% in normal early pregnancy subjects, but this elevated CD4+CD25^{high} T cell ratio decreased to a non-pregnancy level in miscarriage cases (1259). They reported also that the population of CD4+CD25^{high} T cells to CD4+ T cells increased to over 20% in early pregnancy decidua, and this population rate decreased to 6% in spontaneous abortion cases.

Shao et al. have focused on a subset of Treg cells, the CD8+ regulatory T cell (1288), which were described originally in the intestine where they are activated by a combination of the nonclassical class I molecule CD1d and a costimulatory molecule of the carcinoembryonic antigen family (31). CD1d and a form of this costimulatory molecule were also expressed by trophoblasts during the early stages of pregnancy (1288). Furthermore, isolated trophoblasts preferentially activated clonal populations of CD8+ T cells. The authors suggest the possibility that, *in vivo*, CD8+ T cell subsets may have a role in regulating B cells, potentially protecting the fetus from deleterious antibody effects (1288).

Figure 20 tries to summarize te interactions between different cells at the human implantation site.

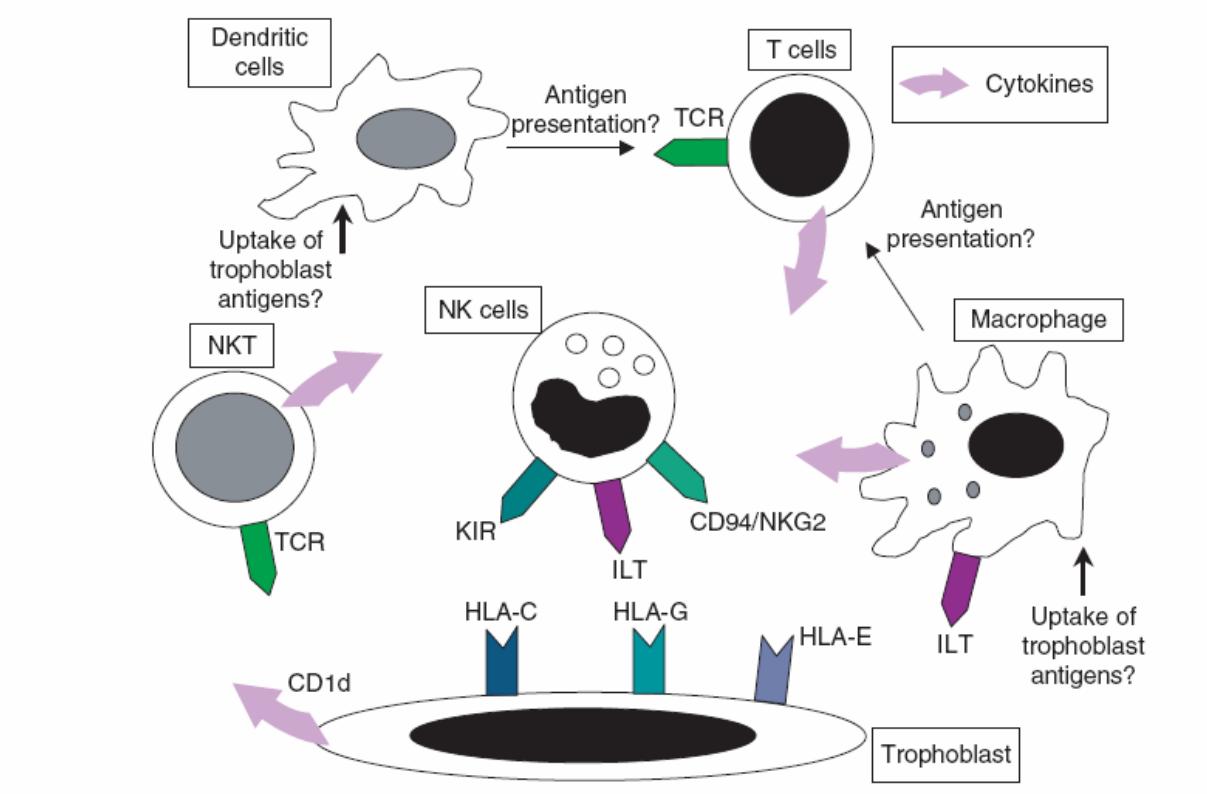


Figure 20: A schematic representation of some of the interactions at the human implantation site. Trophoblast cells expressing HLA-C, HLA-G, HLA-E, and possibly CD1d invade into the decidua. Different leukocytes in the decidua can detect the presence of trophoblast through their various HLA receptors. APCs may take up trophoblast antigens and present them to T cells whose recognition would then result in the production of cytokines from the maternal immune cells. These cytokines contribute to controlling the immune response against trophoblast and may also control trophoblast invasion. ILT=immunoglobulin-like transcript; KIR=killer immunoglobulin-like receptor; TCR=T-cell receptor; adapted from Trundley and Moffett (1430)

(3) Complement system

Complement activation could potentially harm the developing fetus. Recent evidence in murine models has implicated a deficiency of a C3 convertase inhibitor and C3 products in the mechanisms of pregnancy loss (1545). Additionally, fetal injury and pregnancy loss in experimental antiphospholipidantibody syndrome have been attributed to the effects of complement, mainly C5a (454). Therefore, it has been proposed that inhibition of the complement system is an absolute requirement for normal pregnancy (457).
The placental tissue contains a fully organized complement system that is mostly contributed by the complement factors in the maternal blood circulating in placental vessels, although some components may also be produced locally. As the placenta as a newly formed organ undergoes the process of tissue remodelling, the role of the complement system in the clearing of potentially destructive debris products may be essential.

In the placenta, deposits of complement components can be detected in physiological pregnancy whereas at other tissue levels there are usually seen in association with diseases (153). **Faulk et al**. were the first who documented complement components C1q, C4, C5, C6, and C9 in normal human placenta (381). These were found to be associated with some stromal cells as well as in the wall of fetal stem vessels and colocalized with fibrin on trophoblast plasma membranes and perivillous fibrin. C3d and C9, but not C4 were seen associated with trophoblast basement membranes, which suggested that activation pathways leading to complement disposition on trophoblast basement membrane and on perivillous fibrin may be different (1314).
Deposits of the components were also detected on spiral arteries in normal pregnancy with the highest staining for C3d and C9 suggesting that the complement system is likely to be activated through the classical pathway (1491). A humoral immune response leading to complement activation may thus be involved in the physiological changes of spiral arteries in early pregnancy. The terminal C complex as the final end product of all three pathways was also found to localize in the fibrinoid material of basal decidua, chorionic villi stroma and in vessel walls (1405).

Fetus protection against maternal complement activation products is achieved by surface expression of complement regulators that act at different steps in the

activation cascade (153). These regulators including DAF, MCP or CD59 are present in placenta about 6 weeks from gestation until term and syncytiotrophoblast is protected from complement attack by expressing these three regulatory molecules (967). CD59 is distributed on all types of trophoblast while DAF and MCP are preferentially expressed on giant decidual cells (154).
The effect of complement on trophoblast is not necessarily cytotoxic and may result either in impairment or in stimulation of the cell function caused by the membrane attack complex (MAC) (153).

The following shows a summary of the changes in the innate immune system during pregnancy (Table 34).

Monocytes	Increase in number Increase in phagocytosis activity Increase in IL-12 production
Neutrophils	Increase in number Increase in phagocytosis activity
NK cells	Decrease in number Decrase in cytotoxicity Decrease in IFNγ production
Complement system	Increase of C1q, C3, C4, C4d
Acute phase proteins	Decrease of albumin Increase of Caeruloplasmin / Fibrinogen / Globulins/alpha1-antitrypsin/clotting factors

Table 34: Changes in innate immunity during pregnancy, adapted from Herz et al. (541)

(4) Cytokines and growth factors

The local environment of the maternal-fetal interface is characterized not only by the cell types present, but also by the soluble factors produced therein. A wide range of cytokines and growth factors is present in the decidua physiologically including LIF, TNF-, IFN-, IL-1, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-11, IL-12, IL-13, IL-15, IL-16 and IL-18 (205).
They are able to act on lymphocytes and NK cells which express a variety of receptors. The production and effects of these cytokines at the implantation site is important for the regulation of trophoblast cell growth, differentiation and invasion

(1309). APCs and T lymphocytes in the maternal decidua are potentially able to promote the rejection of the fetal allograft, which is first mediated by the recognition of paternal MHC antigens by APCs and then by the activity of effector T cells via the release of cytokines. Therefore, changes in the cytokine production by activated T cells may play an important role in the immunological tolerance of the conceptus.

Th1/Th2 cytokine dichotomy

Placental and decidual tissues from normal pregnancies have been shown to express both pro- and antiinflammatory cytokines (1463). In normal pregnancies, particularly at the maternal fetal interface, antiinflammatory Th2-type cytokines predominate over proinflammatory Th1-type cytokines and, therefore, an appropriate balance between proinflammatory and antiinflammatory cytokines is thought to be crucial for determining the success or failure of a pregnancy (1485).

The evidence for a dichotomous Th response in reproduction originated from murine models, which showed that pregnancy rejection is mediated by Th1-cytokines, whereas a Th2-cytokine response confers protection (1485). There have been only few *ex vivo* studies on cytokine production in human pregnancy, which yielded conflicting results and were unable to detect the expected early Th1/Th2 shift during pregnancy initially (1446).

In 2000, a study by **Lim et al**. demonstrated *in vivo* that women with recurrent miscarriage exhibit primarily Th1 cytokines, whereas healthy women exhibit decreased Th1 cytokines and increased Th2 cytokines which suggests a potential role for a dichotomous Th response in the mediation of subsequent reproductive events (819).

Kruse et al. were then able to detect significantly reduced IL-2, IL-18 and IFN-γ mRNA expression levels already during the first trimester of normal pregnancy. Surprisingly, the mRNA levels for IL-4 and IL-10 also declined during pregnancy but this reduction was only marginal. Calculating the cytokine ratios revealed a shift from a Th1 to a pronounced Th2-type response, which was at the highest level during the second trimester (750).

Other studies showed that proinflammatory Th1 cytokines appear to be potentially harmful to pregnancy since excess production of TNF-α or IFN-γ has been associated with preterm delivery (180). Low concentrations of LIF, IL-4, IL-6 and IL-10 in endometrial tissue and deciduas were described in women with multiple failures of implantation (789) and habitual abortions (1357).

Similarly, low levels of decidual IL-4 and IL-10 have been observed in women suffering from unexplained recurrent abortions and where spontaneous abortion has occurred during the first trimester of pregnancy (181). Moreover, a defect of IL-4 production by decidual CD4+ and CD8+ T cells and a defect of IL-10, LIF and M-CSF by decidual CD4+ T cells was observed in women with recurrent abortions (1115). IL-4, IL-5 and IL-10 were detected at the fetomaternal interface throughout the period of gestation in mice whereas IFN-γ was only present in the first period (1485).

Th2 cytokine activity in pregnancy

CD4+ T lymphocytes of Th2-activity are the source of IL-4, IL-5, IL-6, IL-10, IL-13 and GM-CSF (99).

Already during the luteal phase of menstrual cycle endometrial cells indicate increased mRNA expression for Th2 cytokines including IL-4 and IL-6 compared to Th1 ones such as IL-2, IL-12, and IFNγ (819).

IL-4, for example, is known to be the essential antiinflammatory cytokine for Th2 differentiation and is constantly present at the fetomaternal interface where it is involved in pregnancy-supporting mechanisms (365). IL-4 secreted by endometrium infiltrating lymphocytes stimulates production of LIF in endometrial tissue, another cytokine of great significance for periimplantation period as it together with TGF-β facilitates process of trophoblast invasion (1117), endometrial decidualization (45), regulates interactions between decidual lymphocytes and trophoblast and, together with IL-6, controls angiogenesis inside trophoblastic villi (619).

During labor at term as well as premature labor even when the onset of contractions was not connected with intrauterine infection, increased concentration of IL-6 was found in serum, placental villi, decidua and fetal membranes (1348).

IL-10 is crucial for survival of the fetal allograft and counteracts the effects of inflammatory cytokines (1128). High levels of IL-12 during pregnancy are associated with preterm labor, preterm birth or recurrent abortions (365).

It was also found that peripheral blood lymphocytes of pregnant women in the first trimester secrete "*in vitro*" more Th2, i.e. IL-4, IL-10 and less Th1, i.e. IL-2, IFN-γ cytokines compared to nonpregnant patients (879). Also the number of IL-4 secreting cells rises progressively in the course of pregnancy (887). Pprobably alloantigens localized on trophoblast are the signal for peripheral lymphocytes to Th2 activity which is supported by observation that in further trimester peripheral

lymphocytes of pregnant women differentiated to Th2 cells secreting IL-4 after recognition of paternal alloantigens in mixed lymphocyte culture (358).

Progesterone seems to be another possible inductor of Th2 overactivity during pregnancy. Progesterone influences cytokine network by decreasing Th1 activity of TNF-α in luteal phase endometrial tissue (1117) and by inducing synthesis of TGF-β in endometrial cells. It stimulates lymphocytes for production of PIBF which has potential to intensify production of Th2 cytokines (IL-3, IL-4, IL-10) and to block IL-12 secretion by peripheral blood lymphocytes of pregnant women (624).
Some role in regulation of endometrial Th1/Th2 balance seems to play hCG which stimulated IL-6 and TNFα (1435).

Th1 cytokine activity in pregnancy
CD4+ T lymphocytes of Th1 activity produce mainly IL-1, IL-2, IL-12, IL-15, IL-18, IFN-γ and TNF-α (99). The significance of these Th1 cytokines on the implantation period was defined by examination performed on mice. Components of seminal plasma influenced the expression of chemokines by endometrial cells which activates elements of innate immunity including neutrophils and macrophages homing endometrial stroma and uterine cavity (628). In a local inflammatory reaction, activated neutrophiles secrete ROS and phagocyte cellular debris while activated macrophages become the most important source of Th1 cytokines IL-1β and TNF-α (963). Uterine cavity is cleaned from sperm elements and accompanying microorganisms.

Th1 biased reaction produces an adequate environment for presentation of paternal alloantigens to maternal immunocompetent cells and it also induces changes in number and composition of endometrial leucocytes, activates angiogeneic and growth factors, rebuilds extracellular matrix, activates endothelial and stromal cells, thus preparing maternal tissues for embryo implantation (674). Th1 activity triggers endometrial leucocytes and embryo itself to produce Th2 cytokines, LIF and TGF-β (1503). Th1 cytokines stimulate cytolytic activity of decidual NK cells and lymphokine activated "killer" T lymphocytes which are able to restrict excessive trophoblast proliferation and invasion.
Similar mechanisms have been studied during periimplantation period in humans. Th1 cytokines present inside the uterine milieu as a result of inflammatory response to paternal seminal components can stimulate trophoblastic MMP-9 (816). It can thus enhance its invasive properties and can mediate neoangiogenesis by

inducing VEGF gene transcription (1401). IL-1β together with TNF-α stimulates secretion of LIF which has positive impact od trophoblastic growth and differentiation (431). Embryonic cells are also capable to secrete IL-2 which seems to play a stimulating role for decidual NK cells which together with macrophages by secreting IFN-γ, IL-12 and TNF-α can restrict depth of trophoblastic infiltration, as these Th1 cytokines are potent inductors of trophoblast apoptosis (89).

IL-15 is one of the critical cytokines controlling uterine NK cell cytokine production and cytolytic potential. Moreover, IL-15 has been implicated in differentiation and proliferation of uterine NK cells and plays a possible role in induction of IFN-γ production as a mediator in vascular remodelling during early pregnancy (785). It was detected in nonpregnant endometrium, decidua and placenta but also in uterine macrophages, stromal cells, chorion and amnion (1453).
In a study by **Kitaya et al**., IL-15 was found in glandular ECs and endometrial stroma during the late cycle with highest levels in perivascular cells surrounding the decidual spiral arteries (716). Expression of IL-15 during early pregnancy is most prominent in endothelial cells of spiral arteries. Decidualization elevates IL-15 levels which promote survival of pre-NK cells present in and mobilizing into the uterus (1453). Recent studies demonstrated that IL-15 is essential for the support of NK cell differentiation in the decidualizing uterus (56). IL-15-deficient mice revealed a complete absence of uterine NK cells, poor development of decidua and unmodified spiral structure of arteries.

Progesterone induces IL-15 expression and therefore, when progesterone levels fall as a result of failing pregnancy, the manifestation of apoptotic NK cells could be the result of a decreasing level of IL-15 (828).
IL-15 seems to stimulate GM-CSF production by resting CD56+ NK cells and, together with IL-12, induces IFN-γ and TNF-α as macrophage-activating factors (178). NK cells also produce MIP-1α and MIP-1β after stimulation with IL-15 which may be a mechanism for trafficking of additional NK cells to the site of implantation (122). IL-15 is essential for Th2 cytokine production by NK cells and it stimulates uterine NK cells production of IFN-γ and IL-10 (370).
Concerning cytotoxicity, IL-15 was found to activate cytotoxicity by decidual NK cells (785). IL-15 appears to directly induce upregulation of perforin and FasL expression on human decidual NK cells at the fetomaternal interface suggesting that IL-15-activated decidual CD56+ cells use perforin and FasL-mediated against transformed or infected cells (124).

IL-18 at the fetomaternal interface is produced by the entire decidua on gestation day 4. In murine studies, production starts in the basal proliferative stroma followed by glandular cells in peri-implantation uterus and appears early in murine spongiotrophoblast (1057). IL-18 does not appear early in human villous trophoblast cells but later persists in giant extravillous trophoblast cells and rare activated macrophages (1421).
IL-18 enhances innate immunity and both Th1- and Th2-driven immune responses (1002). IL-18 is essential for its induction of IFN-γ production from Th1 cells, NK cells, B cells and DCs, often in combination with IL-12 (785). IL-18 alone has the potential to induce IL-4 and IL-13 production by T cells and NK cells, and promotes a Th2-mediated response (1002). It also plays a role in regulating GM-CSF production and induction of IL-8 and TNF-α (785). Therefore, IL-18 at the periimplantation site is able to stimulate murin uterine NK cells to produce IFN-γ that plays a key role in vascular remodelling during early pregnancy (274). Therefore, IL-18 might be essential for proper vascularization of the implantation site.

Only few studies investigated the role of IL-18 on the cytolytic potential of decidual NK cells. IL-18 directly upregulates cytotoxic activity of NK cells and CD8+ T cells and induces perforin and FasL receptor expression on NK cells (587). **Tokmadzic et al.** showed that stimulation of decidual lymphocytes with IL-18 increased perforin expression and perforin-mediated cytotoxicity of NK cells (1421).
IL-18 serum levels in pregnant women also show a significant increase from first trimester to labor and remain at high levels until at least day 3 of puerperium (588), which also indicates an important role of IL-18 during implantation.
However, there are also high serum IL-18 levels in women with implantation failure, fetal growth restriction or recurrent abortions (588, 609). An increased level of IL-18 promotes strong NK cell activation and probably excessive IFN-γ production (785). It seems that a tight regulation of IL-18 is important for normal implantation and decidual remodelling in early pregnancy.

Leukemia inhibitory factor

LIF is required for implantation and embryo development. This is shown in studies on mice lacking a LIF gene; they are fertile but their blastocyst fail to implant and do not develop unless the animals are treated locally with LIF (1355). LIF is produced by endometrial ECs, NK cells and T cells. However, LIF expression by glandular epithelium is dramatically downregulated after implantation whereas

expression by leukocytes is upregulated in the decidua. Most of the LIF expression was assigned to NK cells but decidual NK cells in culture did not produce LIF (1289). Therefore, production of decidual LIF seems to be assigned to T cells. LIF secretion is inhibited by Th1 activity cytokines such as IL-12 and IFN-γ *in vitro* (1117).

(5) Humoral immunity in pregnancy – asymmetric antibodies

It has been established that the synthesis of asymmetric antibodies is increased under different physiopathological situations involving Th2 responses including pregnancy (493). These are asymmetrically glycosylated IgG molecules which are affected in their antigen interaction turning them into functionally univalent and blocking antibodies. As a consequence, they are not capable of triggering immune effector mechanisms.

An asymmetric proportion of 10-20% of IgG molecules has been demonstrated in non-immune sera (173). Their existence could either be beneficial or harmful to the host, depending on the self or nonself nature of the antigen (493). They act as protective antibodies if binding self-antigens but when they are specific for the foreign aggressor, for example in chronic infections, they block antigens of the pathogen leading to its survival and chronicity (871).

In murine pregnancy, antibodies with antipaternal specificity, predominantly of the IgG1 subclass, were detected both in serum and on the placenta (1461). In an analysis of the humoral immune response in pregnant females, the predominance of antipaternal blocking IgG antibodies was demonstrated (104). Multiparous women had a marked increase in asymmetric IgG in serum during the first trimester followed by a decrease after delivery. Antibody activity was located in both symmetric and asymmetric IgG in a 1:5 ratio, which indicates the prevalence of asymmetric antibodies in this immune response (864).

(6) The role of progesterone as immunomodulator in pregnancy

The best-studied immunomodulator at the maternal-fetal interface is progesterone, which clearly has a role in survival of the fetal allograft (1376). Numerous studies have demonstrated that progesterone blocks mitogen-stimulated lymphocyte proliferation, improves allograft survival time and modulates antibody production besides affecting other phases of the immune response (1105).

However, the mechanism by which progesterone exerts its immunomodulatory actions in reproductive tissues is unclear but may involve both direct and indirect actions on immune cells. The biological effects of progesterone are mediated by PIBF which is secreted by lymphocytes of pregnant women in the presence of progesterone. PIBF concentrations in pregnancy urine are related to pregnancy outcome as PIBF levels increased up to term in normal pregnancies whereas failed to increase in pathological pregnancies (1376). Therefore, PIBF concentration correlates with positive or negative pregnancy outcome, and premature pregnancy termination is predictable by lower PIBF values than normal.

PIBF affects B cells and induces increased production of asymmetric antibodies which has a regulatory effect on antifetal immune responses during pregnancy. In animal experiments, blockade of the progesterone receptor resulted in reduced PIBF production together with a reduced amount of asymmetric antibodies and elevated fetal resorption rates (677).
PIBF inhibits arachidonic acid release which results in a reduced cytotoxic NK activity and therefore favors a normal pregnancy outcome (1076). NK activity in pregnant women is inversely related to the rate of PIBF-positive lymphocytes. PIBF keeps NK activity at a low level both by controlling IL-12 production and also by inhibiting perforin liberation (786). Decidual NK cells express a high level of perforin; however, they have low cytotoxic activity. *In vitro* studies in decidual cells obtained from elective pregnancy termination suggest that it is PIBF that inhibits the cytotoxicity of NK cells via blockade of degranulation, and thus contributes to the low decidual NK activity (386).

PIBF also alters the profile of cytokine secretion by activated lymphocytes and increases the production of IL-3, IL-4 and IL-10 (386). PIBF seems to induce a Th2-type cytokine production and inhibits IL-12 production by lymphocytes, which was

shown to be pregnancy-protective as an increased lymphocyte IL-12 production correlated with pathological pregnancies and high NK activity (1378).

As a summary of the last chapter, the following figure should elucidate the immunological requirements for the maintenance of a successful pregnancy described above (Figure 21).

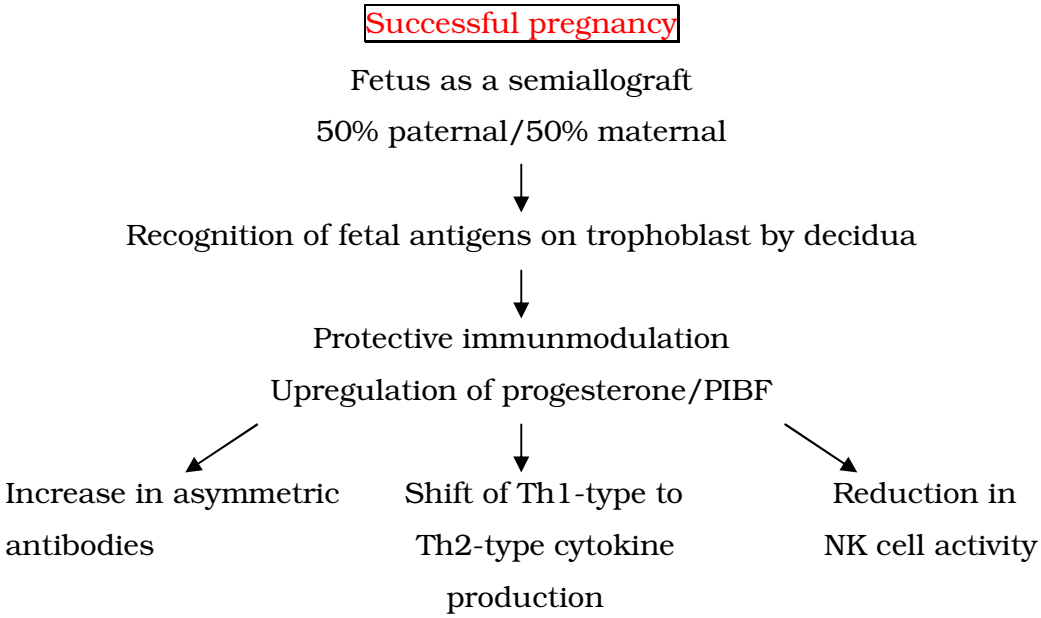


Figure 21: Protective immunomodulatory mechanisms associated with a successful pregnancy, adapted from Druckmann et al. (343)

d) Immunology of labor

Some of the physiological processes involved in labor seem to be mediated by proinflammatory cytokines suggesting that the immune privileges that the fetal-placental unit has enjoyed during pregnancy are revoked at the time of labor. An inflammatory response during labor may also help to remove placental fragments and prepare the uterus for the pathogens that it will undoubtedly encounter during the immediate postpartum period (1105). In the following, the immunological changes during the three important processes in parturition should be briefly described.

The process of cervical ripening appears to be an inflammatory reaction associated with the catabolism of cervical extracellular matrix by enzymes released from infiltrating leukocytes. During cervical ripening and remodelling, IL-8, IL-1β, IL-6

and TNF-α production were found to be increased in the human cervix (1056). IL-1β is produced predominantly by leukocytes, IL-6 by leukocytes, glandular ECs and surface ECs, and IL-8 is produced primarily by leukocytes, glandular ECs, surface ECs and stromal cells (1566). During labor there is an influx in the number of leukocytes in the cervix that is caused primarily by increased numbers of neutrophils and macrophages (CD68+ cells) but not T (CD3+ cells) or B cells (CD20+ cells) (1056).

The ripening of the cervix is induced by proinflammatory cytokines in different ways. IL-1β and TNF-α increase the production of MMP-1, MMP-3, MMP-9 and cathepsin S (1480). IL-1β can act on a number of cell types to increase the production of cyclooxygenase (COX)-2 and prostaglandin E2, the most effective chemical for inducing cervical dilation in women (1105). Prostaglandin E2 may then further stimulate labor by increasing the production of proteinases or indirectly by increasing the permeability of blood vessels for leukocyte trafficking (687).

Besides prostaglandin E2, IL-8 also causes neutrophils from the periphery to migrate towards the cervix and can activate them to release MMP-8 (neutrophil collagenase) and neutrophil elastase that can digest the extracellular matrix produced by cervical fibroblasts. Increased concentrations of G-CSF in the cervix during labor may also stimulate proliferation of the neutrophil subset (1274). Possible roles for IL-6 in the cervix could be to stimulate neutrophils, macrophages or other cells in the local tissues to produce additional proinflammatory cytokines that aid the process of cervical ripening such as prostaglandin E2 or NO (1105). Il-6 has been used as an effective biomarker for predicting labor.
The process of weakening and rupture of the membranes in the region that overlies the cervix also involves a similar proinflammatory process as in the cervix. During labor, the production of IL-8, TNF-α, IL-6, IL-1β, and MMP-9 increases in the membranes (1567, 1546) and decreases levels of TIMPs (1198). TNF-α and IL-1β increase the production of MMP-9 by amnion, but not chorion, explants *in vitro* (44).

The initiation of rhythmic contractions of increasing amplitude and frequency as another component of labor in myometrium is also associated with these cytokine-induced changes. Increased protein concentrations of IL-1β, TNF-α and IL-6 have been detected in myometrium at labor and immunolocalized to the leukocytes (1567). The increased concentrations of leukocytes in the myometrium during labor could be due to increased expression of chemokines such as MCP-1 and IL-8 that

are also increased during labor (1566) and may recruit macrophages and neutrophils to the myometrium.

IL-1β and TNF-α stimulate arachidonic acid release, activate phospholipid metabolism, and increase the production of prostaglandins by the myometrial cells (958, 1419). These effects of IL-1β on myometrial cells are similar to the effects of oxytocin which also upregulates COX-2 and prostaglandin E2 production by myometrial cells (957). IL-1β and TNF-α can also increase the production of MMP-9 by myometrial cells, which may be important for detachment of the placenta (1212).

e) Disturbances in maternal-fetal interaction of the immune system

The following chapter deals with pathological conditions during pregnancy including preeclampsia, intrauterine groth retardation (IUGR), preterm labor (PTL) and recurrent spontaneous abortion (RSA).

There are several histological similarities between IUGR and other pregnancy disorders such as preclampsia and RSA which leads to a common aetiology of all these pregnancy disturbances (885). An increased decidual cellular immunity limiting trophoblastic invasion and leading to failure of placentation has been reported to be involved in the pathogenesis of several pathologic conditions in pregnancy such as spontaneous abortion, preeclampsia and IUGR. Probably pregnancy complications as IUGR, abruptio placentae and fetal death are all clinical signs of placental ischemia and inflammation (885) as the following diagram should illustrate (Figure 22).

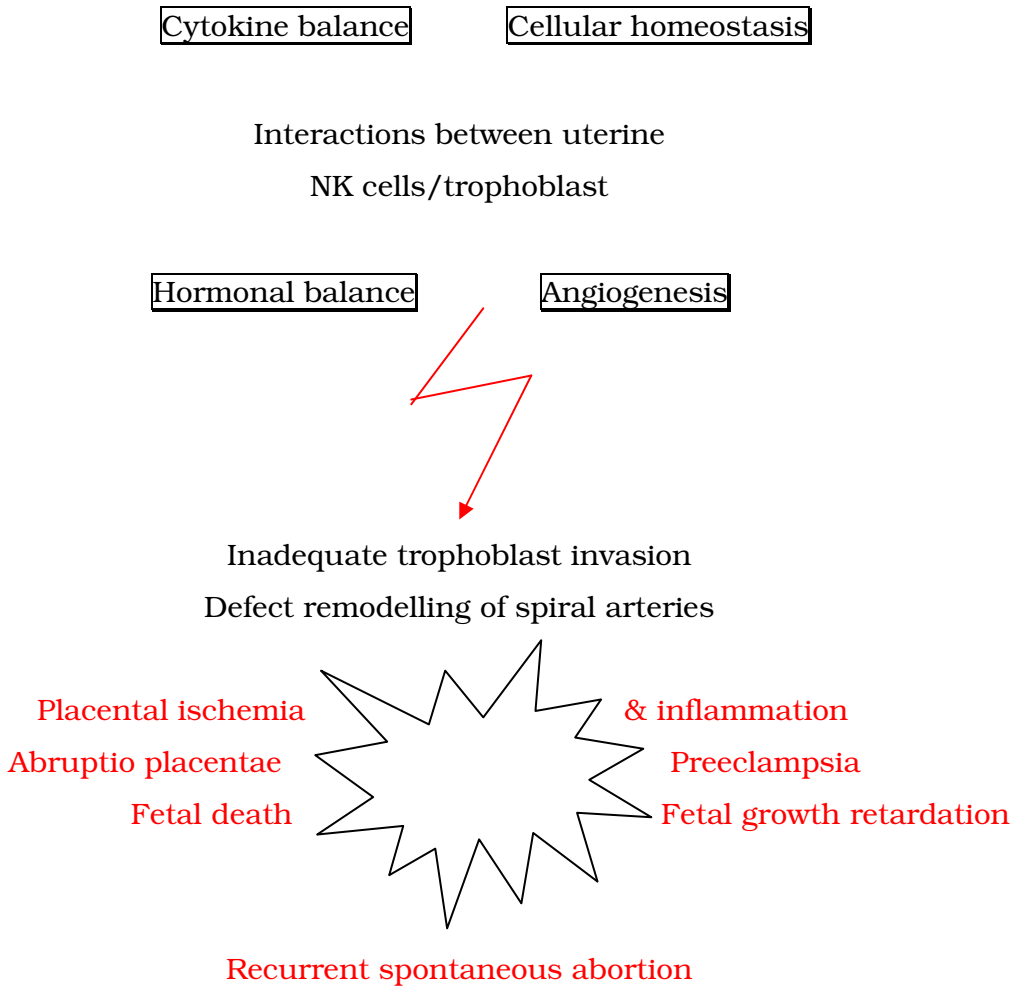


Figure 22: Mechanism of placental development in pathological pregnancies, adapted from Matthiesen et al. (885)

(1) Immunologic aspects of preeclampsia

Preeclampsia is a placenta-dependent disorder with both local intrauerine and systemic anomalies resulting in maternal and fetal morbidity and mortality (885). It affects about 3-7% of all pregnancies (1202) and is often detected in the second half of pregnancy, but most probably it has its onset during early pregnancy.

As histological features such as restrained trophoblast invasion, placental ischemia and vasculitis are similar to other pregnancy complications including IUGR and RSA, a common immune etiology with local subclinical inflammation at the placental bed and systemical immune responses in maternal circulation has been suggested (885).
However, for several years there has been suggested an endothelial cell dysfunction as pathophysiological denominator (1203). Thus, a two-stage process is proposed

with an excessive maternal inflammatory response, probably against foreign fetal antigens, results in an impaired trophoblast invasion, a defective spiral artery remodelling and a reduced placental perfusion at first (1203). As a consequence, placental hypoxia and infarction lead to the release of inflammatory cytokines and placental fragments into the maternal circulation. This finally ends in systemic vascular endothelial disruption and eventual clinical manifestation of preeclampsia (317).

Preeclampsia is likely, at least in part, the consequence of an abnormal maternal immune response to antigenic challenge by the fetoplacental allograft.
Already in the 1970s, studies showed that histological changes in the placental beds of preeclamptic women resembled those of acute allograft rejection (717). Several epidemiological evidence also point convincingly toward an immunogenetic basis for preeclampsia. Although exposure to paternal antigens through a prior pregnancy has a protective effect against development of preeclampsia, exposure to new or different paternal antigens as a result of change in paternity is associated with an altered risk of preeclampsia (813).
Consistent with these findings, this hypothesis of immune maladaptation contends that certain reproductive practices such as barrier contraception and oocyte embryo donation that minimize maternal exposure to seminal fluid are associated with increased risk of preeclampsia (1208).

Macrophages

Mor et al. proposed that in pregnancy pathologies like preeclampsia an increase in the levels of trophoblast apoptosis, possibly a result of infection, may initiate an inflammatory event that will further promote trophoblast cell death preventing normal trophoblast invasion, spiral arteries transformations and fetal survival (963).
In pregnancies complicated with preeclampsia or IUGR, activated macrophages secrete proinflammatory cytokines such as TNF-α and IFN-γ and induce apoptosis in extravillous trophoblast. This hypothesis is supported by a recent report of **Pijnenborg et al**. who found a higher incidence of cell clusters secreting TNF-α, probably macrophages, in the placental bed of patients with severe forms of preeclampsia (1122).
Studies of placental bed specimens demonstrated changes in the distribution of macrophages during pathologic conditions such as preeclampsia (1188).

While in normal pregnancies macrophages are located in the stroma surrounding the transformed spiral arteries and extravillous trophoblast, in preeclampsia macrophages are located within and around the spiral arteries separating them from the trophoblast cells.

In addition, it was reported that macrophages residing in excess in the placental bed of preeclamptic women are able to limit extravillous trophoblast invasion of spiral arteries segments through apoptosis mediated by the secretion of TNF-α (1188). Whereas in normal pregnancies macrophages function as support cells by facilitating trophoblast invasion through the placental bed macrophages seem to function as a barrier for trophoblast invasion and differentiation by inducing trophoblast apoptosis and therefore preventing spiral arteries transformation in pathologic conditions (Figure 23).

Increased trophoblast apoptosis would increase the amount of trophoblast debris, syncytical knots, which leak into the maternal circulation and generate a systemic endothelial activation as seen in preeclampsia (1256). Trophoblast debris can activate TNF-α and IL-12 production from moncytes *in vitro*, which further pushes the systemic immune response towards excessive inflammation instead of a normal immune activity.

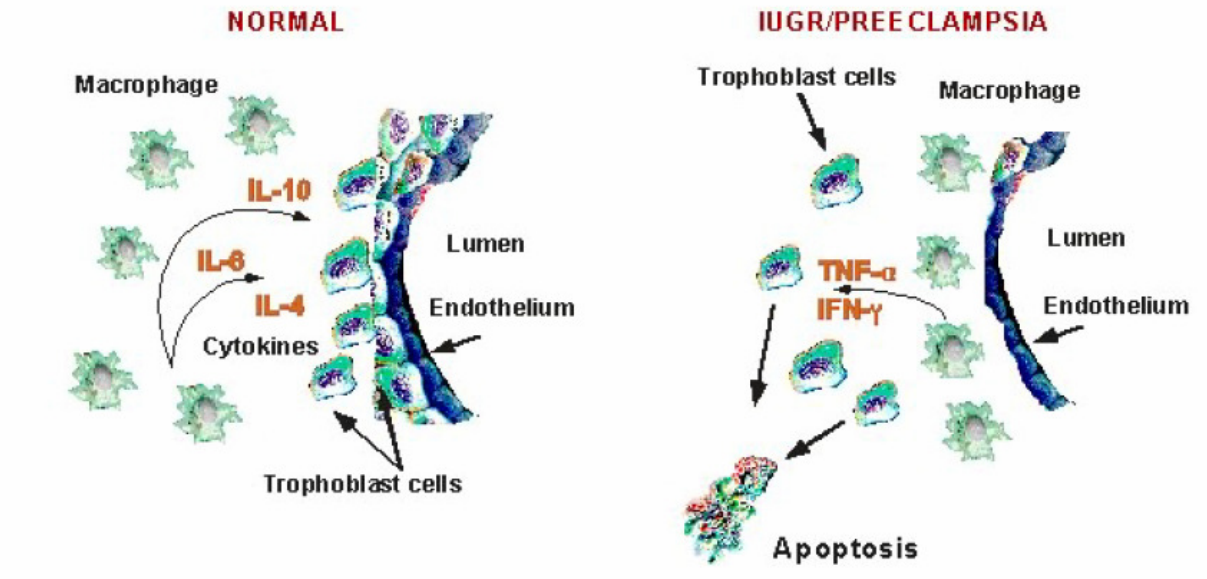


Figure 23: Difference in macrophage distribution in normal and complicated pregnancies with preeclampsia and IUGR, adapted from Mor and Abrahams (963)

Lymphocytes

An increased number of classical cytotoxic NK cells was described in third trimester decidua of preeclamptic patients (1335). Preeclamptic pregnancies are also characterized by an increase of CD8+ decidual T lymphocyte percentage (1335). This would be consistent with a dominance of Th1 cytokine activation which stimulates NK cells and CTLs.

In preeclamptic patients, the T cell activation marker HLA-DR is extremely high expressed on CD8+ T cells (1242). **Wilczynski et al**. characterized lymphocyte subsets in decidua of patients with preeclampsia and reported an increased percentage of CD3-/CD56+CD16+ NK cells and cytotoxic CD8+/CD28+ T cells and a decreased percentage of T CD3+, B CD19+, and suppressor/inducer T CD4+/CD45RA+ lymphocyte subsets (1504). Increased levels of activated/memory cells (CD4+CD45RO+ and CD4+CD29+) are interpreted as T cells activated by antigens. These changes within suppressor/inducer and cytotoxic T lymphocyte subsets suggest that systemic immunological deviation towards suppression seen during normal pregnancy is absent not only in peripheral blood but also locally in decidua of preeclamptic women.

Cytokines

As preeclampsia is often regarded as a syndrome of an excessive inflammatory response even similar to that during septic shock (1234), the question of the role of superantigen involement with a predominant Th1-type cytokine production has arised (842).

Endothelial dysfunction in preeclampsia is found to be associated with excessive release of Th1 cytokines like TNF-α, IL-1, and IL-8, and the Th2 cytokine, IL-6, which has proinflammatory properties (1459). The imbalance of Th1- and Th2-type cytokines has been found in peripheral lymphocyte and placenta in preeclampsia, for example by **Saito et al**. who found increased TNF-α, IL-2 and IFN-γ in PBMCs from preeclamptic patients compared to controls (1242). **Wilczynski et al**. also found a shift to Th1 activity with elevated IFN-γ levels and low IL-6 and IL-10 secretion in third trimester decidua of preeclamptic patients (1504).

IFN-γ secreted by activated T cells activates specialized uterine NK cells with regulatory properties for physiological trophoblast invasion in decidua. IFN-γ affects the Th1/Th2 cytokine balance again and increases the proinflammatory potential. High levels of IFN-γ, together with TNF-α, can lead to apoptosis of trophoblast (954).

IFN-γ upregulation could also involve superantigen-like stimulation of decidual lymphocytes by an infectious factor (842). IL-12 stimulates the production of IFN-γ which activates lymphocytes and neutrophils. In an inflammatory environment, macrophages secrete high levels of IL-12 that stimulate IFN-γ secretion by NK cells, thereby inhibiting angiongenesis (1240). Increased serum concentrations of IL-12 in peripheral blood of preeclamptic women have been observed (296) which points to a local dysregulation in IL-12 production.

Interestingly, **Reimer et al**. reported an upregulated leptin expression in placental tissue of preeclampsia (1185). Leptin is an obesity regulating protein that also induces the production of large amounts of IFN and IL-2, and decreases IL-4 production (830).
However, a study by **Omu et al**. has shown the dichotomous role of IL-4 in pregnancy (1048). Normotensive pregnancy was associated with high increase in IL-4 in the first half of the pregnancy, but in the second half of pregnancy and puerperium, high levels of IL-4 are associated with preeclampsia. This may be a compensatory attempt to control or balance the effect of proinflammatory cytokines like TNF-α.
In conjunction with an overexpression of TNF-α in placenta and in plasma, as it is observed in preeclampsia, an enhanced plasma and placental expression of IL-1 has been reported (885). Both cytokines promote functional and structural changes in endothelial cells including oxidative stress, complement activation and microthrombosis which all are present in preeclampsia.

The role of TGF-β in trophoblast invasion might be also dysregulated in preeclampsia (174). An increase of placental oxygen during the second physiological invasion normally results in a decrease of TGF-β, which prevents excessive trophoblast invasion. In case of hypoxia, TGF-β does not decrease which results in insufficient trophoblast invasion. However, **Lyall et al**. found no changes in expression of TGF-β in placenta or placental bed in preeclampsia compared with normal pregnancy which has put the impact of an overexpression of TGF-β on trophoblast invasion under question (847).

Elevated levels of IL-18 in preeclampsia, especially in the HELLP syndrome, have been reported as well (588). IL-18 induces IFN-γ production and diverts Th1 predominant immunity when IL-12 is present (1044).

IL-10 as antiinflammatory cytokine promotes the termination of Th1 rejection reactions against the fetal-placental unit. A deficiency in IL-10 expression in placenta and decidua were observed during preeclamptic pregnancy which was interpreted as a modified immune balance consistent with inflammatory responses in preeclampsia (540). However, in some preeclampsia cases there were seen high peripheral and placental levels of IL-10, which might be a compensatory response to elevated levels of IFN-γ, TNF-α, and IL-12 (992).

Sakai et al. showed recently that serum levels of granulysin, a cytotoxic granule protein of NK cells and CTLs, were significantly elevated in preeclamptic patients compared to nomal pregnancies (1244). Levels were also associated with mean blood pressure, percentage of peripheral blood Th1 cells and Th1/Th2 ratio. Serum granulysin would thus be a useful marker to evaluate the Th1/Th2 balance in preeclampsia (1244). In the search for an early marker of preeclampsia, **Eneroth et al.** found increased expression of soluble IL-2 receptor in plasma of first trimester pregnancies that later developed preeclampsia (368).

Other immunological mediators of endothelial cell injury

Also important for the pathogenesis of preeclampsia are other mediators of inflammation such as ROS which are generally found to be increased (885). In case of preeclampsia, the balance between antioxidants and free radicals is disturbed. Free radicals and levels of lipid peroxidation are increased in preeclampsia and probably evoke systemic endothelial activation and increased TNF-α production (1468).

Autoantibodies may also contribute functionally to vascular and placental dysfunction associated with preeclampsia (1402). Studies have confirmed the presence of higher levels of antiphospholipid as well as anti-endothelial cell antibodies in serum of preeclamptic women compared to controls (664). Some authors found that autoantibodies against angiotensin II type 1 receptor are present in sera of preeclamptic patients (311).

(2) Immunology of preterm labor and preterm birth

PTL and preterm birth, defined as labor or birth before 37 weeks gestation, is preceded in 30% by preterm, premature rupture of membranes (PPROM), which is defined as membrane rupture prior to 37 weeks gestation (1105). Previously, all babies that were born less than 2500 g were considered to be premature but it was

revealed that many of these infants were actually delivered at term but were small because of decreased fetal growth (IUGR).
PTL is probably the final common pathway of a number of pregnancy complications due to causes such as infection, smoking or coagulation disorders (839). Inflammation at the maternal-fetal interface, mediated by proinflammatory cytokines, is considered as the main common component to these conditions, which results in fetal loss of immunological privileges.

Infection and preterm birth

According to literature, about 50% of spontaneous preterm births are associated with ascending genital tract infection (826). The earlier in pregnancy at which spontaneous preterm labor occurs, the more likely it is due to an infection and the earlier in pregnancy at which abnormal genital flora is detected, the greater the risk for a subsequent infective adverse outcome (781).
Different kinds of infection significantly predispose to spontaneous preterm birth (Table 35).

Intrauterine infection, clinical or subclinical	Mostly polymicrobial
Lower genital infection	BV, Trichomoniasis
Distant infection	Periodontitis

Table 35: Kinds of infection with examples predisposing to preterm birth

Multiple studies have led to the hypothesis that ascending lower genital tract infection can result in preterm labor.
Bacterial entry into the decidua is followed by recruitment of leukocytes and cytokine production which in turn leads to prostaglandin synthesis in amnion, chorion, decidua and myometrium and MMP synthesis by chorion and amnion (676). MMPs are involved in cervical ripening and degradation of fetal membranes while trigger of prostaglandin synthesis results in cervical dilatation, uterine contractions and even greater entry of microbes.
In the following, aspecs of different lower genital infections and intrauterine infection should be briefly discussed.

Long considered a “minor” STD with few associated complications, infection with *T. vaginalis* has recently been implicated as a cause of preterm delivery in several studies.

In a large multicenter study, trichomoniasis was significantly associated with low birth weight, premature rupture of membranes, and preterm delivery (259). Similarly, **Minkoff et al**. also documented a significant correlation between trichomoniasis and premature rupture of membranes with an incidence of this complication at term of 27.5% in women with trichomoniasis versus 12.8% in those without (950).
A more recent randomised treatment study has, however, lent controversy to this area. **Klebanoff et al**. reported the results of a study designed to prevent preterm labor among women with trichomoniasis (718). Pregnant women with asymptomatic trichomonal infection were randomised to placebo versus treatment with metronidazole. Unexpectedly, women in the treatment group were found to have higher rates of preterm birth than those who received placebo.

As mentioned above, BV increases the risk of preterm delivery and low-birth-weight children (548), first trimester miscarriage among women undergoing IVF (1163), chorioamnionitis and other infections (1305). BV is associated with a twofold increased risk of preterm birth, presenting the greatest risk when detected before 16 weeks` gestation (802).
Screening for BV and treatment has been considered a strategy to reduce the rate of preterm birth (1215) but recent meta-analyses revealed no reduction in overall preterm birth with routine screening and treatment for BV except probably for women with a history of preterm birth (803, 898).
However, results from two recent trials with intravaginal or oral application of clindamycin in early gestation resulted in a decreased incidence of preterm birth suggest that early treatment of BV before 20 weeks` gestation may prevent preterm birth (780, 1433).

Other genital tract infections found to be associated with increased preterm birth rate are *C. trachomatis* and *N. gonorrheae.*
The Preterm Prediction Study found that chlamydial infection detected at 24 weeks` gestation doubled the risk for preterm birth (36). However, trials to treat *C. trachomatis* during pregnancy have failed to demonstrate a consistent reduction in preterm birth rate (875). Gonorrheae was associated with a 2.9-fold increased risk of preterm birth (363).
Other vaginal colonization including *Candida*, *U. urealyticum* and group B streptococci have not found to be associated with increased risks for preterm birth (176, 258, 719).

Intrauterine infection was early found to be another frequent cause of PPROM and PTL due to pathologic studies of placentas (1230). Signs for chorioamnionitis were found in 94% of placentas of infants delivered at 21-24 weeks compared to 5% of all placentas.

The main mechanism of this infection is ascending microbial invasion by lower genital tract organisms which could produce local inflammation, i.e. subclinical chorioamnionitis leading to PPROM, PTL and possibly preterm birth. **Simhan et al**. found a strong association of elevated vaginal pH and vaginal neutrophils with early third-trimester PPROM (1306).

Depending on which laboratory technique is performed, prevalences of intra-amniotic infection in the setting of preterm labor range from 0-24% to 30-55% (451, 721).

Many infections are polymicrobial. *Ureaplasma urealyticum*, *Mycoplasma hominis*, group B streptococci, *G. vaginalis* and gram-negative bacteria such as *Escherichia coli* have been identified as pathogens commonly associated with PTL (445, 1030, 1235).

Cytokines

The administration of bacteria or bacterial products to rodents or primates during late pregnancy causes PTL that is preceded by increased production of proinflammatory cytokines including TNF-α and IL-1β. Treatment with IL-1β was able to mimic the effects of bacteria by causing PTL, suggesting a causal role for this cytokine in infection-induced PTL (71).

Studies both in primates (478) and mice (1216) have demonstrated that intraamniotic or systemic maternal infusion of IL-1β readily induced preterm labor and delivery which suggests the possibility that fetal or maternal conditions leading to elevated levels of IL-1β or other proinflammatory cytokines might precede and predict the later occurrence of preterm labor.

Many bacteria involved in ascending infection produce phospholipases A2 and C, proteinases and endotoxins activating placental, decidual, amnion and fetal membrane cells (1306). This in turn may stimulate these cells to production of proinflammatory cytokines and prostaglandins which play an important role in the initiation of parturition, especially in the cases related to chorioamnionitis. Cytokines such as TNF-α and IL-1β may originate from trophoblast cells as well as fetal or maternal macrophages as cultures of amnion, chorion, and decidual cells produce proinflammatory cytokines such as MIP-1, IL-6 and IL-8 in response to

bacteria or bacterial products (345, 346, 1187). However, it is also possible that macrophage-derived cytokines can act on receptors on placental cells, which augment the production of proinflammatory cytokines at the maternal-fetal interface (1105).

In a further study by **Perni et al**. the differences between singleton and twin gestations in immune mediators in midtrimester amniotic fluid were investigated (1108). Concentrations of IL-1β, TNFα and IL-4 were increased in amniotic fluid from twins, which may contribute to the increased rate of PPROM and spontaneous preterm birth in twin populations.
Increased concentrations of free radicals such as proinflammatory NO and prostaglandin E2 in amniotic fluid are also associated with intrauterine infection (573). Furthermore, a COX-2 inhibitor blocked LPS-induced PTL in mice (1245).

Antiinflammatory effects of IL-10 and progesterone
IL-10 may be helpful in reducing the incidence of PTL and the resulting neonatal morbidity.
Administration of LPS to pregnant rats between gestation day 14 and 17 causes extensive IUGR, fetal death and low birth weight which is associated with increased production of TNF-α and NO in the placenta and increased numbers of apoptotic cells (1200). However, coadministration of IL-10 to LPS-treated dams improved fetal outcome by restoring birth weight and decreasing placental TNF-α, NO and apoptosis (1200). Intravenous administration of IL-10 also prevents preterm birth caused by intrauterine infusion of LPS in rats (1407).

Progestins have also been shown to be effective in preventing PTL and delivery (1105). Administration of pharmacological, but not physiological levels, of progesterone to mice delayed PTL in response to intrauterine injection of *E. coli* (552).
The following shows a model for the immunological mechanisms involved with PTL and PPROM (Figure 24).

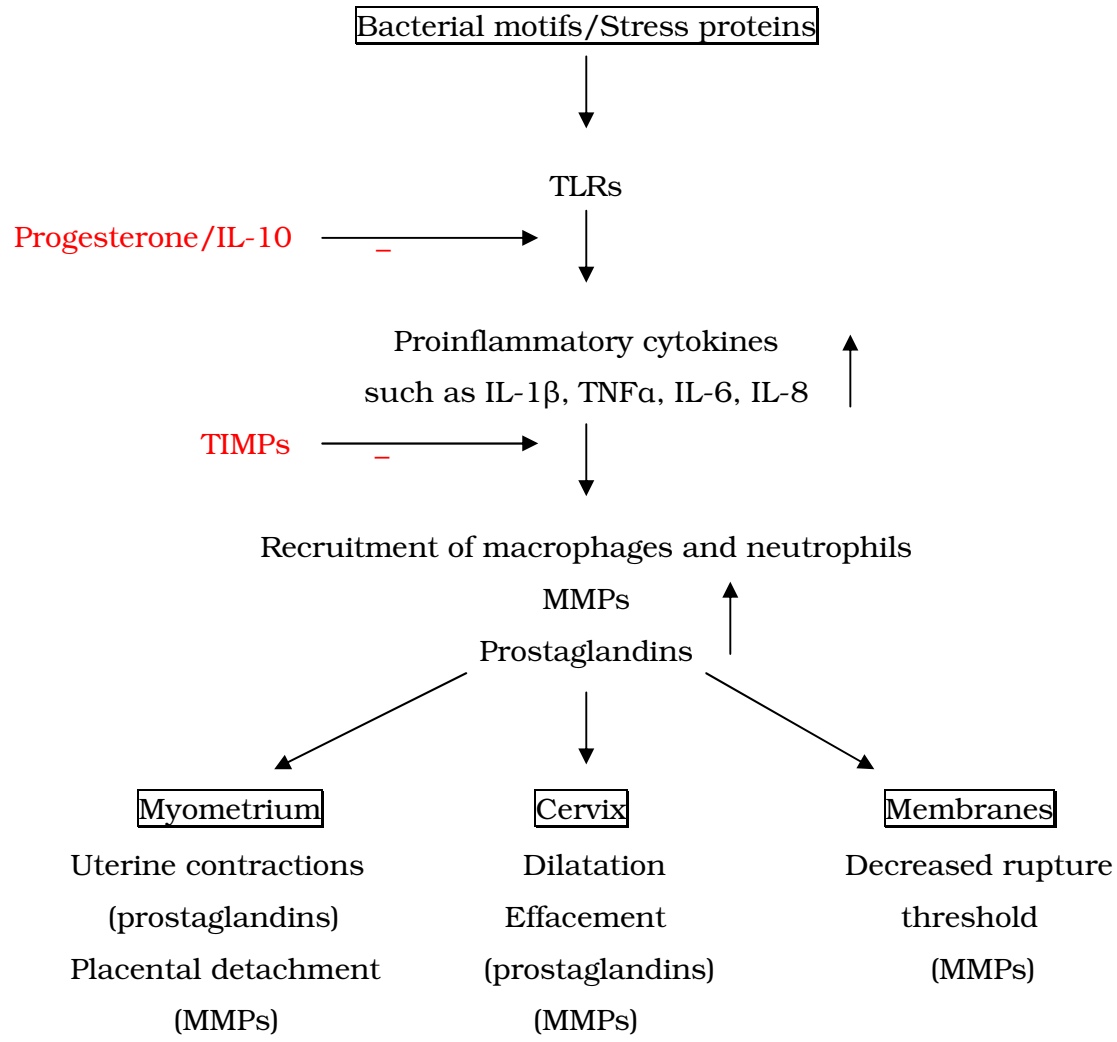


Figure 24: Cascade of immunological events in the pathomechanism of PTL and PROM, adapted from Peltier (1105)

(3) Immunology of fetal growth retardation

IUGR is another main cause of perinatal morbidity and mortality. It is a heterogeneous condition that includes abnormal situations, most of them related to placental insufficiency and physiological but misunderstood events leading to healthy small-for-gestational-age babies (84). However, there are many reports studying the role of immunological mechanisms in the genesis of spontaneous abortion and preeclampsia, but only a few about IUGR.

An increased production of antibodies and a chronic activation of the lymphoid system similar to those found in other placental disorders seem likely in women with IUGR. **Selvaggi et al**. found that patients with IUGR presented numbers of circulating lymphoid cells positive for cytoplasmatic IgM, IgG, and IgA at least 10-

fold higher than in normal pregnancies (1272). **Matthiesen et al**. reported higher levels of B lymphocytes in women with IUGR compared to women with normal pregnancies (886) as well as **Bartha et al**. who found a higher absolute number and percentage of B cells in women with IUGR (83).
There have been only a few studies on the subpopulations of peripheral lymphocytes in IUGR. The percentage of circulating CD4+ cells and CD4/CD8 ratio were found to be decreased while the percentage of CD8+ cells is increased in women with IUGR with respect to normal pregnancies (886, 947).

Bartha et al. found in their study that serum TNF-α is increased in women with IUGR and placental insufficiency but normal in those with IUGR and normal placental perfusion (84). It was suggested that elevations of TNF-α could be a specific phenomenon of certain subsets of IUGR, identifying cases with placental dysfunction.
Holcberg et al. reported that increased TNF-α secretion in placentas of intrauterine growth-restricted fetuses was related to enhanced vasoconstriction of the fetal placental vascular bed (560). In addition, when they perfused normal placentas with the vasoconstrictor angiotensin II, TNF-α production was increased which supports the finding of elevated TNF-α levels in cases of IUGR with increased umbilical artery resistance (84).
Heinig et al. determined cytokine mRNA expression in term human placentas of patients with IUGR and found higher IL-1α as well as higher PDGF-A and -B levels compared to normal placentas but no clear correlation of these differences with clinical data (535).

f) Immunologic aspects in early pregnancy loss

Spontaneous miscarriage (abortion) occurs in 15% of all clinically recognised pregnancies, primarily in the first trimester, and it is the most common complication of pregnancy. While most are sporadic and non-recurrent, RSA, defined as two consecutive or more than three spontaneous abortions prior to the 20th week of gestation, occurs in approximately 1 to 3% of women with diagnosed pregnancies (457).
Approximately 50–60% of spontaneous pregnancy losses can be explained by chromosomal anomalies of the foetus, infectious aetiologies, maternal endocrinology or anatomical comorbidity, but still 40–50% remain unexplained (343). A

substantial portion of these unexplained cases could be attributable to an abnormal, abortogenic immune response of the mother towards potential antigens on the foetus.

There is considerable evidence that pregnancy is recognised by the maternal immune system. For example, already 20 years ago antibodies with paternal specificity have been found in sera from multiparous women (96) and immunosuppressive activity has been described in supernatants of oocytes after fertilisation (303). As early as 1977, it was suggested that HLA-matching between parents was associated with spontaneous abortion (738), a hypothesis confirmed in a more recent 10-year prospective study (1038). Only these couples could have histocompatible fetuses, i.e. fetuses whose HLA alleles do not differ from from maternal alleles.

To study the role of immune cells and molecules in the etiology of RSA, it would need placental tissue during the first trimester of human pregnancy which is clearly not possible (776). Various alternative approaches have been adopted instead including the analysis of immune cell populations and cytokines in the peripheral blood of women with RSA and normal fertile women either before pregnancy or at the time of miscarriage.
Moreover, studies use endometrial tissue obtained from women with RSA and normal fertile women in the periimplantation period in the non-pregnant state, and placental tissue obtained at the time of miscarriage from women with a history of RSA, from women with spontaneous abortions and from women requesting terminations of normal pregnancy (776). In the following, the most important approaches concerning immunologic aspects in RSA are mentioned.

(1) Complement system

Evidence collected over the last few years suggests that dysfunction of the innate immune system, including uncontrolled complement activation, may cause fetal loss (188). Complement inhibition is an absolute requirement for normal pregnancy which has been demonstrated by the finding that deficiency of Crry, an intrinsic complement regulatory protein, in utero leads to progressive embryonic lethality in mice (1545).
Although the presence of complement components in the villi and in decidua is a common finding in physiological pregnancy, the amount of proteins deposited in

these areas increases substantially in pathological pregnancies. Preeclampsia is an example of pathological condition associated with marked deposition of both early and late complement components, as reported by **Sinha et al**. (1314). However, complement is also thought to play a pathogenetic role in RSA following the observation that Crry deficiency in mice is associated with fetal loss and C3 deposition (1545). There is some indication that an abnormal expression of complement regulators may account for the occurrence of abortions in patients with a history of RSA (455).

The involvement of complement at placental levels is also suggested by the finding of increased deposition of C3 and TCC on the wall of decidual vessels in patients with RSA (**Radillo et al., unpublished data**). An interesting observation was that the complement activity progressively declined prior to fetal loss in about 25% of patients with RSA (284). The trophoblast tissue collected from hypocomplementemic patients was responsible for complement consumption through the alternative pathway and had a reduced level of DAF as compared to the tissue obtained from patients with normal complement activity.

Beside the reduced expression of the complement regulatory molecules, antibodies directed against surface antigens on trophoblast may also be implicated in complement activation in patients with RSA (456). Antiphospholipid antibodies (APA) are the antibodies that are more frequently encountered in these patients and are dealt with in details further down in the next section. However, APA are not the sole antibodies found in these patients. During the screening of a large number of sera obtained from primary and secondary aborters that did not contain APA, **Tedesco et al**. were able to detect complement-fixing antibodies that were found to be cytotoxic for syncytiotrophoblasts in 10-20% of the sera (1404).

(2) Cell-mediated immune mechanisms

NK cells

NK cells as the predominant leukocyte population in decidua during early pregnancy may play an important role in RSA when too high in concentrations (1111). High concentrations of classical CD56+ CD16+ NK cells have been found in the uterus of women with abortions (773).

Studies have shown increased numbers of CD56+ cells among a general increase of various lymphocyte populations (CD4+, CD8+, CD14+, CD16+/CD56+) in the non-

pregnant endometrium of women with RSA, and lower numbers were seen in women with RSA who subsequently had a live birth compared with those who miscarried (1153).
This is in contrast to a flow cytometric study which showed similar numbers of CD56+ cells in the endometrium of women with RSA and control subjects, although the women with RSA did have increased numbers of endometrial $CD56^{dim}$ CD16+ cells compared with control subjects (772). However, a decreased number of decidual CD56+ NK cells are reported in the placental tissue from spontaneous miscarriages in RSA women compared with tissue from spontaneous miscarriages in women without RSA and women requesting termination (1150).

Moreover, normal human pregnancy is characterized by low peripheral NK cell activity whereas spontaneous pregnancy termination is linked to increased NK cell activity (1377). Some women with RSA show an abnormal cellular immune response with a marked increase in peripheral CD56+ CD16+ NK cells (1111). **Perricone et al**. also showed increased peripheral blood NK cells, both in absolute and in percentage values, in RSA pregnant and non-pregnant women (1111). A significantly increased number of circulating CD56+ NK cells was found in RSA women who miscarried compared with RSA women who delivered (1365). Moreover, high preconceptional peripheral NK cell activity in women with RSA is found to associate with subsequent abortion (38).

However, it is not the presence of NK cells that is detrimental to the trophoblast as it is able to resist NK-mediated lysis *in vitro* (1377). In fact, NK cells are able to induce trophoblast lysis and cause foetal loss if they are converted to lymphokine activated killer cells by IFN-γ, TNF-α and IL-2 (340). As NK cells are not only the target of cytokines, but also producers of proinflammatory cytokines such as IFN-γ, this can result in the initiation of a vicious circle.
Interations of between trophoblast HLA-C, HLA-E and HLA-G with inhibitory receptors like KIR and CD94/NKG2 in NK cells normally appear to block NK cell cytotoxicity against trophoblast cells. A previous study has shown that NK cells in peripheral blood of women with RSA expressed higher levels of the activation marker CD69 whereas significantly less of the inhibitory receptor CD94 compared to fertile controls (1032).
Ntrivalas et al. further investigated the expression of inhibitory and activating receptors in peripheral NK cells of women with RSA and found an imbalance with increased expression of CD161-activating receptor and decreased expression of

CD158-inhibiting receptors (1031). A similar result found **Yamada et al**. when investigating peripheral blood NK cells form women with RSA (1552).
Recently **Haviid et al**. investigated the HLA-G polymorphism in couples with RSA and compared their results with normal fertile couples (522). Although no significant difference were found in the distribution of HLA-G alleles between controls and RSA couples, 15% of the women who aborted carried the HLA-G*0106 allele compared to 2% of those that did not. **Aldrich et al**. also evaluated the role of HLA-G polymorphisms in 113 women with RSA and found a significant association with increased risk for RSA (24).

Macrophages

No significant differences have been found in the number of macrophages in first-trimester decidua from women with RSA either with chromosomally normal and abnormal fetuses, or in first-trimester decidua from spontaneous abortions and controls (1150). However, an increase in the number of macrophages in the non-pregnant endometrium of women with RSA, together with an increased number of endometrial macrophages in RSA women who subsequently miscarried compared with those who had a live birth has been reported (1153).

Lymphocytes

Studies have shown no differences in numbers of CD3+ T cells as the second most abundant population in endometrium/decidua in the peripheral blood of RSA and normal fertile women prior to pregnancy (1549). In contrast, one study has shown a significantly decreased number of CD3+ T cells in the peripheral blood during pregnancy in women with RSA who subsequently miscarried compared with those who had a live birth and normal pregnant controls (766). Two recent studies have investigated a subpopulation of CD3+ T cells that also express the CD56+ uterine NK cell marker and have shown a decrease in the number of CD56+CD3+ cells in the peripheral blood prior to pregnancy (1549).
No differences in the numbers of CD3+ T cells in endometrium from RSA and control women have been reported (772); neither were there any differences in numbers of CD3+ cells in early pregnancy decidua from normal fertile women and women with RSA (1150) and in decidua from normal pregnancies and after spontaneous abortion (1448). However, a decreased number of CD56+CD3+ cells in the decidua of women with RSA compared with control women has been reported (1549).

T cells can also be classified according to protein components of their TCR. The majority of peripheral blood T cells express αβ, but γδ T cells are found in EC layers where they are thought to play a role in preventing the invasion of infectious agents. Extensive studies by **Clark and colleagues** have suggested that these different populations of T cells play important roles in successful pregnancy outcome in mice, with αβ cells being important immediately after implantation, and decidual γδ T cells being important in preventing RSA (231). In abortion-prone mice the production of IL-10 and TGFβ2 by Vγ1.1δ6.3 T cells, which infiltrate into the decidua on day 8.5, has been shown to be important in preventing miscarriage (40).

In humans, the ratio of specific subpopulations of peripheral blood γδ T cells, Vγl4Vδl and Vγ9Vδ2, is reported to be different in pregnant women with a history of RSA compared with controls (1374). Although several reports have suggested the presence and importance of γδ T cells in the human decidua (1374), other investigations have shown that most human decidual T cells are αβ positive, with only 5 to 10% of T cells expressing γδ (1447).

As in older studies (222), any significant changes in the CD4/CD8 ratio in RSA women were not observed (606). In a recent study 15% of RSA women had an increased CD4/CD8 ratio (1365). These patients had normal levels of CD4+ T cells but low or absent suppressor/cytotoxic CD57+ CD8+ T cells suggesting lack of suppression, i.e. activation.
The activation status of both T cells and CD56+ has been investigated by measurement of expression of CD25 as T-cell activation marker and CD69 as NK-cell activation marker. In a study by **MacLean et al**., levels of CD25 in peripheral blood obtained from women with RSA in the first trimester of pregnancy were higher than those of healthy pregnant and healthy non-pregnant women (854). An increased number of CD25+ cells have been shown in the first-trimester decidua of women with RSA with chromosomally normal fetuses compared with decidua from elective terminations and women with RSA with chromosomally abnormal fetuses (1150).

During normal pregnancy, the absolute leukocyte number rises due to increased numbers of granulocytes with unchanged numbers of lymphocytes and monocytes (886). The proportion and number of B cells (CD19+) were found to be significantly increased in the first trimester of pregnancy in RSA women compared with normal pregnant controls (606).

Fas–FasL interaction

The critical role of Fas/FasL interaction between activated mother`s immune cells and trophoblast during pregnancy leads to apoptosis of activated immune cells that may block alloreactive responses and prevent the pregnancy failure (1246). This mechanism is generally regulated by the two specific proteins bcl-2 and bax that can promote or inhibit apoptosis process. However, the decreased expression of bcl-2 and increased expression of bax in the deciduas are among of the characteristic features of pregnancy failure in women with RSA (736). Hence reduction of Fas or FasL during pregnancy may be associated with fetal loss in women with RSA.

(3) Cytokine and growth factors

The Th1/Th2-bias

Concentrations of Th1 type cytokines, both at the maternal-fetal interface and in PBMC, are higher in women with unexplained habitual abortion. A Th1 type reaction in the maternofetal interface mainly triggers the inflammatory response with increase of IFN-γ, TNF-β, IL-2 and TNF-α, which contribute to trophoblast toxicity and failure of pregnancy in women with RSA (545). These Th1 type cytokines may damage the placenta directly or indirectly via the activation of certain immune cells.

In addition, Th-1 type cytokines induced NK cells and lymphokine activated killer cells activity whereas Th-2 type reaction triggers the secretion of noninflammatory cytokines (IL-3, IL-4, IL-5, IL-10 and IL-13] during normal pregnancy that promotes the success of pregnancy by alloantibody induction, counter inflammation and suppression of the NK cell activity. Th1 and Th2 cytokines are mutually inhibitory and a shift towards a Th1 bias tends to further downregulate Th2 reactivity (343).

It has been shown that stimulation of PBMC with autologous placental cells results in production of Th2 cytokines by women at labor and Th1 cytokines by patients at spontaneous miscarriage (1159). Compared to controls, **Lee et al**. found that the secretion of the Th1 cytokine from the immune cells was increased in RSA patients, which was associated with trophoblast apoptosis (799). There was a positive correlation between apoptosis and increased IFN-γ-levels of PBMCs.

These results suggest that women with evidence of Th1 immunity to trophoblast stimulation undergo apoptosis of trophoblast cells, offering a potential pathological mechanism of TH1-mediated reproductive failure (799). Higher serum levels of IL-6

and IL-10 were detected in women at normal delivery than in patients with RSA at the time of abortion, and increased concentrations of TNF-α are detected in this group of women with RSA compared to those with successful pregnancy (862). Some studies have evaluated women at the time of abortion, during pregnancy or at labor and these reports have also shown lower levels of IL-6 in RSA patients compared to healthy women (1159).

Daher and colleagues detected significantly higher levels of IFN-γ and a trend toward increased TNF-α in whole blood cultures from nonpregnant RSA women as compared to nonpregnant controls but no significant difference in IL-6 and TGF-β1 was detected between the two groups (290).

Under experimental conditions, the outcome of pregnancy can also be influenced by modulating the cytokine balance. Administration of TNF-α, IFN-γ or IL-2 to normal pregnant mice caused abortion whereas anti-TNF-α reduced the resorption rate in a murine model of spontaneous immunologically-mediated abortion (204).

IL-12

IL-12 has been shown to be present in significantly elevated levels in the serum of pregnant women with habitual abortion (1509). It significantly augments the cytolytic activity of lymphocytes against trophoblast cells and the administration of IL-12, along with IL-18, to pregnant mice has also been shown to result in pregnancy loss (991).

GM-CSF

IFN-γ has been shown to inhibit the secretion of GM-CSF that promotes the growth and differentiation of the trophoblast during normal pregnancy as compared to pregnancy in women with RSA (1110). Studies in mice have also suggested that GM-CSF and CSF-1 are important in successful pregnancy outcome (1133). Implantation is compromised in CSF-1 mutant mice which have both a lower rate of implantation and fetal viability, both of which can be restored to normal by administration of exogenous CSF-1 (1133). Decreased expression of utero-placental CSF-1 mRNA has also been shown in mice with spontaneous and induced pregnancy loss compared with control mice (468).

TGF-β

TGF-β is produced by Th3 cells and has been suggested to play an essential role both in promoting and in limiting placental development (597). TGF-β2 also induces decidual antiproliferative activity and is reduced in deciduas from women with RSA

at the time of miscarriage compared to women at first trimester with legal pregnancy termination (798).

Ogasawara et al. have reported significantly higher levels of TGF-β1 both in nonpregnant and pregnant women with RSA compared to nonpregnant and pregnant controls, respectively (1039). They suggested that TGF-β1 is necessary for pregnancy development but may also represent a risk factor for recurrent abortions.

The role of TNF-α in early embryonic death

TNF-α is synthezised throughout the female reproductive tract as well as in placenta and embryo practically at all stages of development (1406). Data suggest that this multifunctional cytokine is involved in triggering immunological pregnancy loss, i.e. death of embryos owing to failure of defense mechanisms preventing rejection of the semiallogenic fetoplacental unit (1420).

The injection of TNF-α into pregnant mice resulted in embryonic death and moreover, in a mouse model with high incidence of embryonic death, experiments have revealed an elevated level of TNF-α in supernatants of decidual cell cultures (204).

TNF-α also influences the preimplantation development of embryos. Embryo transfer studies addressing the *in vivo* development of blastocysts that were exposed to TNF-α revealed that TNF-α treatment caused a 17% decrease in proportion of implanted embryos and that the proportion of embryos that died after implantation was 40% higher in the TNF-α pretreated group compared to controls (1542). Results suggest that TNF-α acting on blastocysts mainly decreases their ability to differentiate into fetuses after implantation rather than their ability to implant in the uterus (1424).

Uterine cells may also serve as targets for the toxic effect of TNF-α. Experiments in the mouse uterine EC line WEG-1 revealed that TNF-α had a dose- and time-dependent cytotoxic effect on these cells while stimulating apoptosis (1068). **Monzon-Bordonaba et al**. also demonstrated that TNF-α may be involved in pathological processes leading to pregnancy loss by disturbing normal trophoblast endocrine function (961).

There have also been conducted several experiments showing the involvement of TNF-α in the pathogenesis of stress-induced early embryonic death. An elevated TNF-α expression was observed in the uterine epithelium, stroma, and in giant and spongiotrophoblast cells of mouse placenta exposed to DNA-damaging

cyclophosphamide (470). In mice exposed to ultrasonic sound stress, TNF-α-producing cells at the fetomaternal interface appeared to be activated and increased their TNF-α production (41). In a study with diabetic mice, there was observed a much higher decrease in pregnancy rate in severly diabetic TNF-α +/+ mice than in TNF-α-deficient mice (1425).

Activation of thrombotic events

Recent studies have suggested that the way in which Th1 cytokines bring about pregnancy loss is via upregulation of a newly described procoagulant, fg12. Fg12 converts prothrombin to thrombin, which in turn leads to deposition of fibrin and activation of polymorphonuclear leukocytes that can destroy the vascular supply to the placenta (232).

In mice, anti-fg12 antibodies completely prevent spontaneous abortion and dramatically reduce the effects of TNF-α and IFN-γ on abortion rates (234), while TNF-α and IFN-γ have been shown to upregulate the production of fg12 by both fetal trophoblast and maternal decidua (235). In humans, increased expression of fg12 in trophoblast cells from failing pregnancies with chromosomally normal embryos, but not in trophoblast tissue from chromosomally abnormal embryos, have also been reported (723).

(4) Humoral immunity – alloimmune and autoimmune antibodies

The majority of unexplained RSA cases is found to be associated with certain autoimmune and alloimmune antibodies that may play major role in the immunologic failure of pregnancy and may lead to abortion (1071). Indeed, infertility and recurrent miscarriages can be a manifestation of subclinical autoimmune disease, and a variety of autoantibodies have been found at increased frequencies in women with pregnancy failures.

Autoimmune abortions are characterized by maternal autoimmune reactions where antibodies or autoreactive cell target decidual or trophoblast molecules and affect placental and fetal growth. Alloimmune abortions are characterized by impaired aternal immune reactions against paternally derived molecules on trophoblast resulting in rejection of the fetus.

Autoimmune factors

Autoimmune factors represent the immunologic response of the mother to a pregnancy that can cause fetal rejection in 30% of women with RSA (1071).

Recently **Yamada et al**. have reported different types of APA, including Lupus anticoagulant, anticardiolipin β2-glycoprotein IgG/IgM/IgA, anticardiolipin IgG/IgM/IgA, anti-phosphatidylserine prothombin IgG/IgM, and anti-phosphatidylethanolamine IgG/IgM in women with RSA (1550).

As phospholipid molecules are normal components of cell membranes holding the dividing cells together, the production of antibodies to phospholipid molecules may inhibit the development of placenta at the materno-fetal interface (1071). These antibodies can specifically damage the inner wall of the blood vessel, which allows blood cells to stick to the site of the injury and cause blood clot formation. The combination of blood clots and constricted blood vessels may impair blood supply to the fetus and placenta resulting in complete fetal demise or growth retardation in women with RSA (1457).

In a study over 20 years by **Makino**, he observed that anti-phosphatidylethanolamine antibodies and anti-annexin antibodies were more common factors in RSA patients than were anticardiolipin antibodies in terms of induction of recurrent pregnancy losses (863). In a study by **von Landenberg et al**. the association of IgG antiprothrombin antibodies with pregnancy loss and in particular early pregnancy loss in cohort of patients with antiphospholipid syndrome was detected (1462).

Women with RSA of unknown etiology have a higher incidence of antinuclear antibodies (ANA) which indicated that there may be an underlying autoimmune process that affects the development of the placenta and can lead to early pregnancy loss (1365).

Bussen et al. found that the incidence of antithyreoid antibodies (ATA) in women with RSA appears to be significantly increased compared to controls (164). Women with ATA double their risk of miscarriage as compared with women without these antibodies (164). Increased levels of thyroglobulin and thyroid microsomal (thyroid peroxidase) autoantibodies are associated with an increased miscarriage rate, and as many as 31% of women experiencing RSA are positive for one or both antibodies. Risk of fetal loss increases to 20% in the first trimester of pregnancy and there is also an increased risk of post-partum thyroid dysfunction.

A high percentage of women with RSA were also shown to have anti-endothelial cell antibodies, which suggests that the migration of endovascular trophoblast may be inhibited by anti-endothelial cell antibody in women with RSA (1221).

Alloimmune factors

The investigation that trophoblast expresses MHC antigens on its surface which when recognized by maternal immune system triggers some alloimmune mechanism essential for the development of maternal immunotolerance has led to finding that RSA of unknown etiology could be attributed to the following alloimmune characteristics that elicits weather the fetus will survive or reject during pregnancy (1071).

In normal pregnancy, the maternal immune system usually recognizes the paternal HLA as different from its own and induces the expression of several alloantibodies including antipaternal cytotoxic antibodies (APCA), antiidiotypic antibodies (Ab2) and mixed lymphocyte reaction blocking antibodies (MLR-Bf) that may coat and protect the fetus from the cytotoxic maternal immune responses (1071).

However, several investigators reported that absence or decreased expression of APCA (1054), Ab2 (603) and MLR-Bf (6, 1072) might lead to abortion in women with RSA.

Hasegawa et al. have reported less than 8.7% APCA positivity in women with poor pregnancy outcome (517). **Agrawal et al**. have also demonstrated that APCA were present only in 8.5% of women with RSA as compared to 33-46% in women with normal pregnancy (11).

Anti-anti HLA antibodies (Ab2) and seemingly clonotypic antibodies recognize alloantigens receptor on T-lymphocytes and induce the suppression in alloimmune response during normal pregnancy (1071). The prevalence of Ab2 in women with RSA as well as in women with normal pregnancy was evaluated by **Pandey et al**. (1070). They found a 30% Ab2 positivity in women with normal pregnancy as compared to none in women with RSA which indicated that role of Ab2 is important for maintenance of pregnancies.

The same group also observed a similar fetoprotective effect of MLR-Bf (1069). **Tamura et al**. found MLR-Bf positivity in 82.4% women with normal pregnancy as compared to 10% of women with RSA (1394). They also demonstrated that a blocking effect of MLR-Bf was inhanced as the pregnancy progresses and once it is developed may also be helpful with subsequent pregnancies. A study on time-kinetics of MLR-Bf during the course of a successful pregnancy showed maximum levels during the first trimester and a progressive decline through the subsequent trimesters and post-delivery (11).

Symmetric antibodies

Approximately 80% of the IgG specifically reactive to endometrial antigens are symmetric antibodies which can be cytotoxic in women with habitual abortion; in healthy pregnant women, the percentage is much lower at approximately 25% (343). According to a recent study, women with a history of recurrent abortions have significantly lower proportions of asymmetric IgG antibodies (3% of total IgG) compared with healthy pregnant women (29% of total IgG), nulliparous and multiparous women.

In addition, amongst antibodies reactive with endometrial antigens, recurrent aborters had significantly lower proportions of asymmetric antibodies than healthy pregnant women (351). Some investigators consider that the lower levels of the non-precipitating asymmetric-type of antibodies in RSA patients that would allow immunological tolerance are the major determining factor for unsuccessful pregnancy (351).

(5) The role of progesterone in recurrent abortion

The immunmodulatory effects of progesterone in the maintenance of pregnancy were already described in the last chapter. Chronic exposure of immunocompetent maternal cells to allogenic embryonic antigens progressively increases the number of PR on maternal lymphocytes, whereas cases of spontaneous abortion and preterm labor are associated with decreased numbers of maternal PR-positive immune cells (485).

A specific T cell subpopulation, the γ/δ TCR+ cell, is likely to play a role in recognising embryonic antigens as they are able to recognise unprocessed antigens without MHC (1374). In the decidua, the proportion of γ/δ TCR+ cells significantly increases and is under hormonal control; 90% of these cells express PRs and overlap to a high degree with the CD56+ population. **Heyborne et al**. showed that trophoblast recognition is TCR-dependent and is mediated by the hsp60 reactive Vγl subset of γ/δ T lymphocytes (542). Within this subset, specific differences in the ratio of Vγl4Vδl and Vγ9Vδ2 cells in peripheral blood have been detected between healthy pregnant women and recurrent aborters (1374). Signaling via the Vγl4Vδl receptor induced a Th2 response whereas activation of the lymphocytes via the Vγ9Vδ2 receptor resulted in increased IL-12 production and NK cell activity.

The immunomodulatory effect of progesterone by the stimulation of PIBF, as mentioned above, leads to the inhibition of NK cell activity and induction of the Th2 response, especially IL-10 (485). Both progesterone and its stereoisomer

dydrogesterone are thought to inhibit the activity of NK cells at the foeto-maternal interface in humans via PIBF. PIBF production by lymphocytes is induced by both progesterone and dydrogesterone in a dose-dependent manner and can be inhibited by equimolar concentrations of RU-486 (mifepristone) (485). The absence or decreased expression of PIBF during pregnancy in RSA patients may cause abortion (343).

Inhibiting the effects of progesterone by receptor blockade, or neutralising PIBF effects with a specific antibody, significantly reduced the production of asymmetric antibodies in mice as well (1375).
Progesterone associated endometrial proteins or placental derived glycodelin found in epithelial glands of endometrium, endometriotic tissues, ECs of umblical cord, human fallopian tube, decidua amniotic fluid, normal and ovarian tumors also act as immunosuppressive agent during pregnancy (1587). Low levels of these proteins might lead to RSA and termination of pregnancy.

To conclude, the following diagram is supposed to summarize the immunological mechanisms leading to early pregnancy loss in women with RSA (Figure 25).

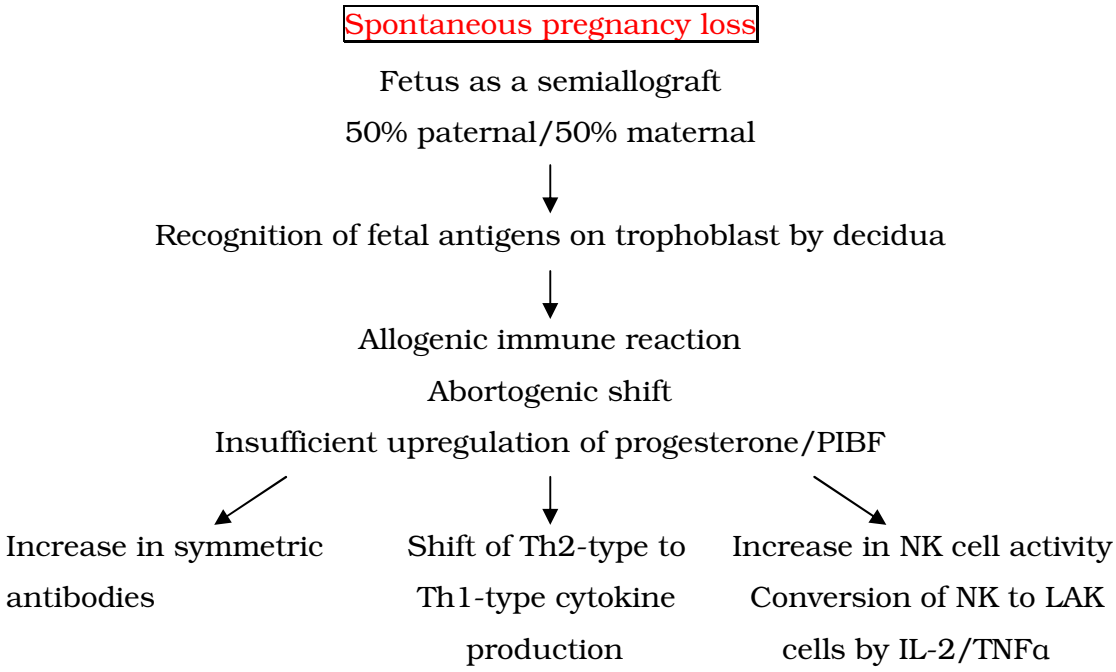


Figure 25: Abortogenic immune mechanisms associated with pregnancy loss, adapted from Druckmann et al. (343)

(6) Influence of stress on pregnancy-related immune mediators

Since a well-balanced interaction of nervous, endocrine and immune system is crucial for the maintenance of successful pregnancy, recent observations on stress-triggered pregnancy failure have been recently reviewed by **Arck** (43).
The exposure of pregnant mice to a defined stressor during the periimplantation period has been shown to induce neurotransmitter substance P-mediated activation of T cells, mast cells and macrophages in the uterus, resulting in decreased levels of TGF-β2 and increased secretion of TNF-α (41).
One recent study revealed a positive correlation between increasing stress scores and number of decidua basalis mast cells, CD8+ T cells and TNF-α expression in women with RSA (42). However, no significant differences between individuals with lower or higher stress scores could be observed with respect to decidual CD56+ NK cells and CD3+ T cells. It is proposed that the increase in CD8+ decidual cells observed in miscarriage patients with higher stress scores may reflect an attempt to abrogate the imminent or present failure of pregnancy (43).

Interestingly, the increase in maternal stress perception has recently been shown to cause a decrease of the pregnancy supporting hormone progesterone and subsequently PIBF (624). PIBF is produced by peripheral CD8+ T lymphocytes of pregnant women and recent findings on immune parameters in stress-scored miscarriage patients further suggest an involvement of CD8+ cells (42).

2. Immunological infertility

Infertility is defined as a failure to conceive after 12 months of unprotected intercourses (1148). The causes of infertility which affects about 18-20% of couples in reproductive age include endocrine, tubal, uterine, cervical, and male factors. Unexplained infertility occurs when no cause of infertility can be identified after full clinical investigation of both partners. Recent studies in humans suggest that an unexplained reproductive failure can be influenced by some immunological abnormalities, similar to the causes described for RSA (225).

Immunological infertility is considered to be an incapability to conceive due to immunological disturbances in the reproductive tract (747). Reproductive failure and infertility in association with autoimmune diseases has been recognized for

decades but only recently has it been demonstrated that certain characteristic autoantibodies are involved.
Autoantibodies, such as APA, ATA, and ANA are considered to be responsible not only for recurrent miscarriages and some pregnancy complications but also for infertility. Antisperm antibodies (ASA) which can affect the transport of sperm cells, the process of capacitation and acrosome reaction as well as the fertilization, post-fertilization and preimplantation processes can also be also associated with reproductive failure (893).

The following lines try to give an overview of the most important disorders in immunological infertility, the presence of ASA, anti-ovarian antibodies (AOA) and the group of non-organ-specific antibodies including APA. Further, the latest approaches in treatment options in immunological infertility and abortion are outlined. To start with, a short summary of immunological infertility in males is given.

a) Immunological infertility in males

Although the clear focal point of these explanations is lying on the immunology of the female genital tract with its entire implications, one should also consider the importance of influences of the male reproductive system, especially in the aspect of immunological infertility. Therefore, the following lines briefly discuss the basic facts on male genital tract immunology and on male genital tract infections as a possible cause for infertility.

(1) Immunology of the male reproductive system

The testis is one of few organs in the human body which is capable of sustaining foreign grafts over a period of time without evidence of rejection (526). This “immunological privilege” is derived from the need to prevent immune responses against meiotic germ cells expressing nonself antigens which first appear long after the establishment of self-tolerance during puberty.
However, innate and adaptive immune responses are not generally impaired in the testis as proven by the capability of inflammatory responses in the testis to infections (529). Also immune cells are found in considerable numbers within the interstitial compartment of the normal human testis (359, Table 36). Following

macrophages which are the second most abundant cell type next to Leydig cells, mast cells can be found as well. There is a small number of lymphocytes whereas there is no clear evidence for the presence of NK cells, DCs and granulocytes.

Macrophages	++	**NK cells**	?
Mast cells	+	**DCs**	?
Lymphocytes	(+)	**Granulocytes**	?

Table 36: Immune cells in the normal human testis

Macrophages in the testis have considered being potential effector cells activating innate immune responses and inflammation. Testicular macrophages also express MHC class II antigens indicating a capacity for antigen-presentation to CD4+ Th cells (529). However, their ability to produce proinflammatory cytokines such as IL-1, IL-6 and TNF-α was reduced in rats compared to macrophages of other origin (690). Probably, resident macrophages in the testis mainly exert antiinflammatory activities (528). Similar to mast cells, macrophages also seem to be involved in the local regulation of testicular functions.

Only few lymphocytes were detected in testicular tissue whereas the lining epithelium showed more CD8+ than CD4+ T cells and the intertubular tissue more CD4+ than CD8+ T cells (359).

Immunotolerance in the testis has long been explained on the basis of the fact that Sertoli cells can mechanically segregate all germ cell autoantigens by means of the so called blood-testis barrier (1103). However, tissue barriers and mechanical sequestration are important but not sufficient to protect male germ cells from autoimmune attack as circulating antibodies and and T cells can enter and soluble sperm antigens can exit at the level of the rete tesis (359).

The presence of an immunosuppressive activity in testicular fluid was confirmed by a study which showed that Sertoli cells secrete molecules capable of inhibiting proliferation of lymphocytes, especially B and T lymphocytes (410). These lymphocytes drastically reduced the production of IL-12 which shows that Sertoli cells contribute to the maintenance of of imunological privilege in the testis.

Expression of FasL by Sertoli cells has also been implicated in maintaining testicular immune privilege. CD8+ T cells migrating into the testis are capable of mounting an immune response against foreign tissue grafts but undergo apoptosis

at an increased level via upregulation of Fas on their surface (291). However, the role of FasL in the immune privilege in the testis remains controversial.
Local anergy of T lymphocytes may also play a key role. Constitutive expression of MHC molecules is found in the testis whereas costimulatory molecules such as ICAM-1, CD80 and CD86 are absent which would leave naïve T cells refractory to antigen-specific activation (1237).

Resident macrophages as well as nonimmune testicular cells have been shown to produce both pro- and antiinflammatory cytokines such as IL-1, IL-6, TNF-α, and TGF-β even in the absence of inflammation (527). The overlap between testicular functions including steroidogenesis and spermatogenesis and immune regulatory functions of these cytokines could help to understand testicular immune privilege and the processes leading to inflammation-mediated damage in the testis (1267). Consequently, local upregulation of cytokine expression during infection or injury may contribute to the disruption of testicular function and fertility.

(2) Influence of male genital tract infection on fertility

In men, infection and inflammation of the reproductive tract including the testes are widely accepted as important etiological factors of infertility.
Invasion of microorganisms into the male genital tract has been frequently shown to be associated with impaired sperm function and thus, persistent infertility (675). Pathological bacterial strains present in semen may act directly on sperm cells causing agglutination of motile sperm (959), reducing ability for the acrosome reaction (734), and also causing alterations in cell morphology.

Sanocka et al. just recently described a statistically significant deteriorated semen volume, sperm concentration, motility, morphology and vitality in ejaculated samples of patients with genital tract infection with *Escherichia coli*, *Ureaplasma urealyticum* and *Staphylococcus aureus* in comparison to healthy controls (1253). Moreover, the inflammatory process in the genital tract may lead to deterioration of spermatogenesis and obstruction of the seminal tract (675), worsening characteristics of semen and sperm density. Microorganisms trigger a local inflammatory reaction, activating leukocytes and inflammatory mediators such as cytokines and ROS, which are known to be important in the aetiology of male infertility.
In general, genital tract infection may still worsen male infertility.

Sanocka et al. compared the influence of bacteriospermia on the anti-oxidant status and proinflammatory cytokine levels in seminal plasma of infertile males with genital tract infection to those of fertile controls with genital tract infection (1252).
Results suggested that urogenital infection in the latter phase in normozoospermic patients may lead to the predominance of antioxidant levels in seminal plasma, creating a positive environment for the recovery of complete function of sperm cells whereas genital tract infection in infertile patients may cause a pro-oxidant overbalance in semen that may additionally impair the fertilization ability of spermatozoa and worsen the prognosis for future fertility (1252). Also, the significantly enhanced IL-6 concentration in the seminal plasma of normozoospermic patients may indicate that normozoospermic semen recovers better from infection whereas in semen of infertile patients leukocytes may be an additional factor worsening the fertility prognosis.

The impact of *Chlamydia trachomatis* as one of the most common STD on semen quality and its role in male infertility is still controversial, probably due to different study designs (465). It is thought that up to 50% of men infected with *Chlamydia* might be asymptomatic and in those with symptoms, the most common presentation is urethritis (465). Most upper genital tract infections in young men, including epididymitis as a cause for infertility, are also attributable to *Chlamydia*.
Data suggest that a *C. trachomatis* infection of the male reproductive system may contribute to the formation of ASA (1527). Antichlamydial antibodies in women`s sera correlated with detection of IgA antibodies in the male ejaculate and not with anti-chlamydial antibodies in the corresponding men`s sera (1526). The presence of anti-chlamydial antibodies in semen has been correlated with the development of autoimmunity to spermatozoa.

Overall, *in vivo* studies of *C. trachomatis* in men have provided conflicting evidence as to whether it is associated with reduced fertility. By contrast, *in vitro* studies show that coincubation of spermatozoa with *Chlamydia* causes a significant decline in numbers of motile sperm and results in premature sperm death possibly due to chlamydial LPS (361).
It appears from the literature that there is no association between male infertility and genital infections caused by *C. trachomatis*, however, prospective longterm investigations regarding the fertility of men after acute infections with *C. trachomatis* are lacking (746). The effect of *C. trachomatis* infections on male fertility

seems to be related to the quality of the immune response of the host. Thus, the cell-mediated immunity must be study more accurately (465). No doubt exists that the female partners carry the main risk of chlamydial infection of the genital organs as *C. trachomatis* infection of the female genital organs may be deleterious to female fertility.

Chronic inflammatory reactions following acute orchitis are characterized by multifocal lymphocytic infiltrates and tubular changes which finally result in complete disruption of spermatogenesis as reflected by testicular atrophy and persistent infertility (1268). A symptomatic orchitis due to bacterial or viral pathogens is considered to be rare but a high prevalence of asymptomatic testicular inflammatory reactions could be demonstrated among infertile men.

Only few studies have investigated testicular inflammatory reactions among infertile males. Tissue specimen from asymptomatic patients with impaired fertility showed a testicular infiltration of lymphocytes in about 56% of the cases, predominantly CD4+ and CD8+ T cells, mast cells and non-resident CD68+ macrophages (1268). The degree of infiltration correlated with characteristic signs of tubular damage. These testicular inflammatory reactions in infertile men were associated with significantly reduced testicular volume and impaired spermatogenesis (1268). This indicates a significant disturbance of the local immunoregulation and testicular immune privilege. The dysbalance of locally produced cytokines towards a proinflammatory profile, impairment of Sertoli cell function and subsequent breakdown of the blood-testis barrier appear to be important features of testicular inflammatory reactions which finally result in deterioration of spermatogenesis and infertility (410). The pattern of lymphocyte infiltration and concomitant damage of seminiferous tubules also supports the concept that activation of autoreactive T cells is involved (1268).

b) Autoimmune processes in infertility

(1) Antisperm antibodies

ASA are one of the main causes of immunological infertility by binding to the sperm membrane and impairing sperm functions and can be present in males and females

(554). They occur in seminal plasma, bound to the sperm surface, in blood sera of men and women, in cervical mucus and follicular fluid of women.

The incidence of ASA in infertile couples ranges between 9 and 36% in the literature (126). Ten percent of infertile men show ASA in the seminal plasma or attached to the surface of spermatozoa, while in approximately 5% of female infertile partners ASA occur in cervical mucus or oviductal fluid (270). However, these antibodies are also present in approximately 1% to 2.5% of fertile men (533) and in 4% of fertile women (1049), which indicates that not all ASA cause sterility. Serum ASA are mainly of the IgG isotype while ASA in cervical mucus or seminal plasma belong to the IgA isotype (747).
It has recently demonstrated that there is a diversity of ASA bound to the sperm surface, including different Ig classes of ASA, differing localization of the corresponding antigens for ASA, and different biological activities of ASA, in males (1297). Moreover, a relatively high incidence of asthenozoospermia was demonstrated in immunologically infertile males, and a significant effect of sperm-immobilizing antibodies bound to the surface of ejaculated sperm on sperm motility was confirmed (1295).

Aetiology of ASA formation

There are several hypotheses for ASA formation in men. Theoretically, the blood-testis barrier may be breached by a variety of mechanisms resulting in exposure of immunogenic sperm antigens to the immune system which could initiate an immune response, resulting in an inflammatory reaction and ASA formation. Mechanical obstruction of the genital tract may occur as a result of congenital abnormalities, surgical interventions, inflammation and trauma to the epididymis and vas deferens (128). Several reports suggest that between 50% and 70% of men after vasectomy subsequently have sera positive for ASA (533). Frequently, ASA also appear to be of idiopathic origin. ASA in the male may fulfill the criteria of an autoimmune disease (127).

Production of ASA in women may occur in a variety of ways. Mechanical or chemical disruption of the mucosal layer of the female genital tract may permit exposure to foreign sperm antigens and, ultimately, ASA formation. It is known that after pelvic infection or several uterine or peritoneal inseminations with capacitated spermatozoa, some patients can develop antisperm antibodies (872).

Cunningham et al. confirmed in a study of the ASA prevalence in nulligravid women with various gynecologic infectious processes (283). Forty-six percent of women diagnosed with PID had sera and cervical mucus positive for ASA compared with an ASA prevalence of 20% in women who had lower genital tract infection. ASA also were detected in 69% of women with laparoscopically confirmed pelvic adhesive disease or hydrosalpinx but with no prior history of PID.

ASA and fertilization

Several studies have shown an inverse relation between the proportion of sperm bound by ASA and the fertilizing capability of sperm. One such study showed that only 14% of oocytes were fertilized if 70% or more of sperm were bound by both IgG and IgA antibodies; whereas a fertilization rate of 60% was observed when less than 70% of sperm was bound with ASA (305).

Results also imply that different ASA isotypes have different effects on fertilization and some ASA can act synergistically to reduce fertility. ASA of isotypes IgG, IgA, and IgM are all capable of binding to sperm (305). However, a study showed that IgG ASA on sperm correlated with a reduction in fertilization rate, whereas IgA and IgM ASA in female serum correlated with a decrease in fertilization rate (199).

Analysis of cognate antigens of ASA

In order to understand the relevance of ASA it is necessary to characterize the cognate antigens of ASA. There are direct tests to detect the presence of ASA on sperm such as the mixed agglutination assay, agglutination tests using donor sperm and tray agglutination test (538). These tests only detect the gross binding of antibodies to various locations on sperm and do not examine the antigenic specificities of the ASA.

Moreover, ASA may have heterogeneous effects on sperm functions, obviously depending on their binding sites (128). They were found to affect sperm motility, penetration of cervical mucus by spermatozoa, acrosome reaction, interaction between spermatozoa and zona pellucida as well as the sperm-egg-fusion. However, no proper methods exist which indicate which ASA of an individual man are functionally relevant and which sperm antigens are associated with immunological infertility (128).

The possibility exists that fertilization-related antigens may be the targets of ASA with an inhibitory effect on fertilization (1298). Such antigens consisted of sperm surface antigens including PH-20 (995), PH-30 (994), fertilization antigen-1 (FA-1) (662), sperm agglutination antigen-1 (SAGA-1) (327), and lactate dehydrogenase-C4

(LDH-C4) (463), and acrosomal antigens such as SP-10 (499), all found by immunological methods.

Koide et al. investigated the cognate antigens of agglutinating ASA, which were obtained from the blood serum of infertile women or were mAb raised in the mouse against human sperm proteins. Six sperm proteins have been recognized as target antigens for ASA sourcing from fluids of the reproductive tract including YWK-II, BE-20, rSMP-B, BS-63 (nucleoporin-related), BS-17 (calpastatin) and HED-2 (zyxin) (735). In a study by **Bohring et al**. 18 proteins of the sperm membrane were detected as cognate antigens of ASA. Six of these proteins were identified as HSP70 and HSP70-2, disulfide-isomerase-ER60, caspase-3 and two subunits of the proteasome (component-C2 and zeta-chain) (126).

Effect of ASA on sperm motility and transport

Since it is likely that ASA only bind to antigens of sperm membranes it may be speculated that functions of proteins with intra- and extramembranaceous parts may be altered by ASA to interfere with sperm motility (128).

There are several hints that show the ability of ASA of IgG and IgA to inhibit sperm penetration into cervical mucus. The proportion of motile sperm with ASA correlated with the inhibition of sperm penetration in cervical mucus (917). Moreover, ASA were detected in the cervical mucus from up to 29.6% of infertile women immobilizing sperm and preventing passage through the cervical mucus (917). ASA can also impair sperm migration from the uterine cavity through the fallopian tubes (1296).

A study examining migration of sperm through cervical mucus indicated that ASA, mainly IgA, directed against the sperm head, along with IgA and IgG ASA directed against the sperm principal piece, severely impaired the ability of sperm to penetrate the cervical mucus (1522). In contrast, the binding of IgG and IgA to the tail tip of sperm did not appear to affect the ability of sperm to penetrate the cervical mucus providing evidence that cervical mucus aids in the selection of the most fertile sperm of an ejaculate by acting as an immunological filter, preventing the passage of sperm coated with ASA (223).

Impairment of sperm penetration into cervical mucus appears to be a consequence of the activation of the complement cascade by Ig attached to sperm surface resulting in cell lysis and phagocytic processes (128). This impaired penetrating ability, however, seems to be mediated by the effector region (Fc) portion of the Ig

molecules (611). This is also proposed by the study of **Hirano et al**., as a 15-kD protein with an amino terminus identical to that of SPLI is found in human cervical mucus, and this inhibits sperm transport (551). The Ig possess both an antigen-recognition region (Fab) and an Fc that binds to various leukocytes through specific surface receptors (FcR). Sperm exposed to the Fab portion of IgG of ASA can swim through the cervical mucus, whereas those exposed to intact IgG of ASA can not (611).

Effect of ASA on capacitation and acrosome reaction

Evidence has emerged that ASA might prevent membrane fluidity changes needed for capacitation before fertilization (109). In addition, sperm incubated with serum containing immobilizing ASA were found to have lower rates of spontaneous and induced acrosome reactions than sperm that were not incubated with the serum (996). Furthermore, ASA can inhibit the ability of sperm to undergo spontaneous capacitation as an antibody raised against a human sperm protein, BS-17, prevented capacitation of human sperm (1486).

The effects of ASA on acrosome reaction have been contradictory. ASA may have a variable effect on the acrosome reaction and capacitation with some ASA adversely altering the ability of sperm to undergo capacitation or acrosome reaction, whereas other ASA do not (129). A study showed that human follicular fluid contains IgG antibodies capable of inducing the acrosome reaction and inhibiting sperm–ZP binding (872).

Effect of ASA on sperm-oocyte binding

There are conflicting results about the ability of ASA to affect sperm-oocyte binding and it is likely that the antigenic specificity of ASA is important in their effects on fertilization (223). Some studies reported that binding of head-directed IgG or IgA ASA to sperm reduced sperm binding to human ZP (860) while other investigators found that ASA did not affect sperm-oocyte binding (241) or that ASA were capable of both stimulating and suppressing sperm-oocyte fusion (146).

ASA treatment

Several strategies are used in an effort to improve the potentially deleterious effects of ASA-mediated infertility resulting in improved gamete function.

First, methods used to reduce ASA production include condoms and systemic corticosteroid treatment. As repeated or multiple sperm exposure to the female

reproductive tract results in ASA formation, condom use would decrease sperm exposure, resulting in a concomitant decline in ASA production (893).
Mild inflammatory and immune system suppression with corticosteroids may also provide some couples with a limited benefit. In a study of 43 men with ASA-bound sperm, there was a statistically significant decrease in sperm-associated IgG but only little effect on sperm-bound IgA in the steroid versus the placebo group. Furthermore, in spite of a decrease in the antibody titer there was no statistically significant difference in pregnancy outcome between the two groups (494). Cyclosporine was also tested in a cohort of ASA-positive men (133). After treatment, a pregnancy rate of 33% was observed but due to the lack of placebo controls, no definite conclusions can be drawn.

Second, there are methods that attempt to remove ASA already bound to sperm include immunodepletion, sperm washing, and IgA protease treatment (893).
The immunomagnetic separation technique has been tried to separate the antibodies bound on the sperm surface but offered only limited success in isolating sufficient number of ASA-free sperm of good motility (418).
There are mixed reports on simple sperm washing on ASA elution from various laboratories. **Adeghe** found that washing decreased IgG bound on the sperm surface (8) whereas another group did not find the similar positive effects (1511).

Moreover, several studies have examined the use of assisted reproduction technology (ART) in treatment of ASA including intrauterine insemination (IUI), intracervical insemination (ICI), IVF, gamete intrafallopian tube transfer (GIFT), subzonal sperm injection (SUZI), and, more recently, intracytoplasmic sperm injection (ICSI) (893).
IUI has been found to be useful for treatment of female and male immunoinfertility by circumventing problems related to sperm transport in the female genital tract especially, sperm passage through the cervical mucus (1013). However, in women having ASA in the cervical mucus, pregnancy rates after IUI were identical to women who did not have ASA if the male partner did not have ASA (207). However, the pregnancy outcome significantly improved after including the ovarian hyperstimulation treatment along with IUI or in some cases of male immunoinfertility (1013).
In GIFT procedure, sperm and eggs are mixed *in vitro* and then transferred to the fallopian tubes for fertilization. In one study, GIFT was performed in 16 immunoinfertile couples that achieved pregnancy rates of 43% per couple (1439).

This study did not include any control group, and the pregnancy rates are comparable with those that are reported after GIFT in patients having other etiologies.

An analysis of data from IVF programs by **Chiu and Chamley** has provided a great amount of evidence regarding the effects of ASA in serum, semen, and follicular fluid as a possible cause of infertility (223). These studies generally indicate that couples with ASA have lower pregnancy rates in IVF trials than couples without ASA.

Acosta et al. (96) retrospectively examined the combined effects of ASA and sperm morphology on fertilization and pregnancy rates in 85 couples with male factor infertility, treated with 38 cycles of IVF and 57 cycles of GIFT (5). The ASA-negative group had greater fertilization and pregnancy rates than the ASA-positive group. Interestingly, there are also studies reporting increased rates of IVF outcome including implantation and pregnancy rates in immunoinfertile women compared with women with tubal factor infertility (292). In IVF procedure, generally albumin instead of female partner`s serum is used as a protein source in the insemination medium that circumvents the antibodies if present in the female partner. Thus, IVF can take care of female but not male immunoinfertility (1013).

ICSI is a method that may allow some couples to avoid fertilization failure secondary to an autoimmune mechanism. **Lahteenmaki et al**. treated 29 infertile ASA-positive couples with ICSI after 22 of them demonstrated a poor fertilization rate (6%) during IVF (775). After ICSI, the fertilization and cleavage rates for the ASA-positive group were similar to the ASA-negative group. It is notable that 46% of the pregnancies occurring in the ASA-positive group ended in spontaneous pregnancy loss compared with none in the ASA-negative group.

A retrospective analysis of 55 ICSI cycles for 32 different couples with high levels of ASA-bound sperm demonstrated a significantly higher fertilization rate between the ASA-positive group undergoing ICSI and an ASA-negative control group undergoing ICSI (999). Using the ICSI procedure in immunoinfertile men, one can achieve higher fertilization rates than using the IVF procedure; however, the fertilized zygotes show higher degeneration and mortality, and decreased embryonic development (1013). However, compared to other methods, ICSI has been the most successful in treating in ASA-positive infertility (747).

By using hybridoma and recombinant DNA technologies as well as various genomics approaches, several sperm antigens have been defined from various laboratories that may be involved in fertilization and fertility. **Naz et al**. has cloned FA-1 and YLP12 among at least nine sperm-specific cDNAs (1014). Antibodies to FA-1 antigen inhibit human sperm-ZP interaction, and also block human sperm capacitation/acrosome reaction by inhibiting tyrosine phosphorylation.

A study was conducted if immunoadsorption with the human sperm FA-1 antigen would remove autoantibodies from the surface of sperm cells of immunoinfertile men and thus increasing their fertilizing capacity (919). An increased immunobead-free swimming sperm on an average of 50 and 76% for IgA and IgG ASA, respectively, was found. The acrosome reaction rates increased significantly and showed improvement in 78% of the sperm samples after FA-1 adsorption. The IUI of FA-1-treated antibody-free sperm resulted in normal pregnancies and healthy babies, indicating that the antigen treatment does not have a deleterious effect on implantation, and embryonic and fetal development (919).

(2) Anti-ovarian antibodies

AOAs form a heterogeneous group of antibodies recognizing several different antigenic targets such as granulosa and thecal cells, zona pellucida, oocyte cytoplasm, corpus luteum, as well as gonadotropins and their receptors (960). Their involvement in patients with patent premature ovarian failure (POF), a disorder of multicausal etiology leading to infertility in women, is highly likely.

POF is defined as secondary amenorrhoea with a hypestrogenic hypergonadotropic serum profile in the menopausal range which finally culminates into partial or total destruction of the primordial follicle pool thereby leading to infertility (261).

In POF patients, a variety of autoantibodies and possible target antigens have been reported so far, including antibodies binding to mature follicles and corpus luteum, termed as steroid cell antibody (SCA) (113). In the ovary, SCA immunostaining was preferentially localized in theca interna cells of antral follicles, but in some cases the granulosa layer and corpus luteum were also stained. Whereas the prevalence of SCAs in POF is 6.5-10% of patients, it was much higher (87–100%) in patients with Addison`s disease with associated POF (294).

None of these studies showed SCAs to be present in healthy controls, and it is not known whether they could be detected in other ovarian pathologies. Thus, these antibodies seem to be highly specific to anti-adrenal autoimmunity with associated

ovarian failure (960). Moreover, in normally cycling patients with autoimmune endocrine disorders, the appearance of SCAs may be predictive of forthcoming ovarian failure (113).

The localization of AOAs on cells of the granulosa layer and in oocytes, as well as the expression of FSH and LH receptors in granulosa cells and FSH receptors in the oocyte, is consistent with the hypothesis of gonadotropin receptors being antigenic targets for AOAs (836). However, different studies on anti-gonadotropin receptor antibodies suggest that the role of this type of autoantibody in human ovarian diseases still remains to be demonstrated (960).

Only a few studies have demonstrated the existence of antibodies directed against gonadotropins themselves. **Meyer et al**. detected anti-FSH antibodies in 92% and anti-LH antibodies in 65% of low-responder IVF patients, but not in good responders (928). POF patients did not present with anti-FSH antibodies, and only 6% of them had anti-LH antibodies (837). **Haller et al**. showed that patients with endometriosis and polycystic ovary syndrome presented with higher levels of anti-FSH IgA and proposed anti-FSH IgA as a marker for ovarian disorders causing infertility (498). Most of the research on anti-gonadotrophin antibodies has actually been done to develop contraceptive vaccines in humans, which is discussed later on.

Whether the prevalence of anti-ZP antibodies is higher in infertile than fertile women has been examined in numerous studies, but the results are still controversial. In the largest series of infertile women tested, **Kamada et al.** found a significantly higher prevalence of serum anti-ZP antibodies in patients with unexplained infertility than in infertility with known causes, thus indicating a possible aetiological significance of anti-ZP antibodies in some cases of unexplained infertility (655). Results of a study by **Kelkar et al**. indicate that 66.6% women with POF belonged to the autoimmune group and that ZP is an important antigenic determinant of autoimmune POF and that ovarian auto-antibodies circulating in POF patients are specific to ZP (678).

Only a few studies have been devoted to antioocyte antibodies. Studies on human ovarian sections revealed the presence of anti-oocyte antibodies in the serum of 33% of POF patients (295), and in another group of patients with ovarian failure 46% had such antibodies detected by ELISA with extracts from unfertilized IVF oocytes (837).

(3) Non-organ-specific autoantibodies

Presence of organ-specific auto-antibodies to testicular antigens and sperm in men, or antibodies to zona pellucida and endometrial antigens in women, are considered possible causes of infertility in a fraction of patients with unexplained infertility (1442).
However, the presence of other autoantibodies, which are not particularly organ-specific, such as APA, ATA, ANA or anti-smooth muscle antibodies (SMA) have recently been increasingly implicated (1442). Some investigations showed a greater incidence of these antibodies compared to organ-specific antibodies in infertile women and the association between their presence and the period of infertility (460).

In a preliminary study by **Putowski et al**., 82.3% of infertile patients after repeated IVF failure had at least one abnormal result on autoimmune testing but no symptoms of autoimmune disorders (1148). Results of another study on 108 females with reproductive failure versus a control group of 392 women showed that 40.7% of patients' sera and 14.8% of control sera contained one or more common autoantibodies with ANA and SMA most frequently detected (1184).

As mentioned in the chapter about immunologic aspects in early pregnancy loss, APA as a heterogenous group of autoantibodies are highly associated with reproductive failure. Clinical criteria contain venous or arterial thrombosis, thrombocytopenia and recurrent fertility failure as well as the presence of serum IgG or IgM anticardiolipin antibodies (aCL) and lupus anticoagulant (1434). Women with systemic rheumatic diseases and accompanying reproductive failure have a high prevalence of APA, antibodies against cardiolipin and β2-glycoprotein I (765). The latter antibodies have been shown to have a direct inhibitory effect on the endothelial cells of the female reproductive tract, the early embryo and trophoblasts in the placenta (765).
Evidence is also accumulating that antiphosphatidylserine antibodies (aPS) are particularly pathogenic to trophoblast (901). Both animal and *in vitro* experiments have shown monoclonal and polyclonal aPS and aCL to specifically destroy trophoblast, inhibit syncytium formation, halt hCG production and limit trophoblast invasion (901).

Mettler and colleagues demonstrated in their study that 66.7% of 123 women undergoing IVF had at least one pathological marker in autoimmune testing; a combination of pathological assays was found in 25.2% patients (927). Overall, 32.5% of all patients had a positive titer of APA which is significantly higher than the estimated 2% prevalence in a healthy obstetric population.
Würfel compared several studies on the prevalence of APA in IVF-patients (1540). Percentage numbers of patients tested positive for APA range between 15% and 48%; compared to percentage of controls between 0% and 6%; however, in most studies there were no control groups measured.

The presence of autoantibodies that are not specific to components of reproductive system in infertile women are viewed by some authors as an epiphenomenon rather than a factor playing a pathogenetic role. In this context, autoantibodies in infertile patients are seen as a part of generalized activation of the immune system (461), because in these patients, in addition to infertility-associated antibodies, some other classic autoimmune conditions were observed including monoclonal or polyclonal gammopathies, selective IgA deficiencies, and abnormalities of IgG subclasses (460). In addition, there is a plausible possibility that reproductive failure, in any of its forms, is the first clinical sign of impending autoimmune disease (462).

In conclusion, women with reproductive failure and patients with repeated reproductive losses in IVF programs are a highly selected patient group with autoantibody abnormalities. There is a need of the immunological diagnostics in the group of patients with unexplained infertility. In the management of reproductive immunology, it is proposed to be sufficient to examine antibodies against seven different phospholipids in IgG, IgM and IgA, sperm and zona pellucida antibodies (1434).

c) Treatment options of immunologic abortion

Heparin plus aspirin, aspirin alone, intravenous immunoglobulin (IVIG) administration and immunization with allogenic lymphocytes are the most common treatments in women with immunological RSA.

Aspirin/heparin and steroids

Among women with the combined problems of APA and elevated NK cells who become pregnant with preconception treatment, live birth rate is about 70%. The initial treatment of choice is usually low dose heparin and aspirin therapy (166). **Sher et al**. reported that the treatment of APA-positive women with a heparin/aspirin combination significantly improved the birth rate (1293).

The other treatment option for auto-antibody syndromes is prednisolone, which suppresses the inflammatory process and stabilizes the cell. Particularly the combination of steroids and heparin is evaluated increasingly positive (1540).

Intravenous immunoglobulin

For women with RSA due to immunological reasons and failure of the heparin/aspirin therapy, therapy with IVIG remains a safe but still controversial alternative of treatment (1071). It has also been suggested for use in RSA cases with various serum autoantibodies which suggest autoimmunity.

Several studies have shown significant benefit of IVIG treatment in women with recurrent miscarriages, while other studies have failed to confirm this beneficial effect (1366, 1540). Studies have previously shown that IVIG treatment does not affect the pregnancy outcome compared with placebo (226, 607). Also the results of the **German RSA/IVIG group** did not show a significant difference between IVIG and placebo therapy of women with RSA (1411).

Stricker et al., however, reported a significant difference in pregnancy success rate between IVIG-treated and untreated groups in a study with older women with RSA (1365). A following study with a larger group of women confirms these positive results (1367).

The basic effect of IVIG is to neutralize the cytotoxic effect of maternal immune response against the fetus (Table 37). Studies demonstrated that IVIG suppress the activity of APA and passively transferred blocking or antiidiotypic antibodies that inhibit the binding of APA to corresponding antigens (201).

Suppression of lymphocyte activation	Inactivation of complement
Blocking of TCR	Downregulation of NK cell activity
Increase of suppressor T cell activity	Shift of Th1/Th2 cytokines to Th2

Table 37: Proposed mechanisms of IVIG

Results of a study by **Graphou et al**. showed at administration of IVIG in women with RSA influenced the Th1/Th2 lymphocyte ration (477). In alloimmune abortions, this effect was reflected by a shift of Th1/Th2 balance to Th2, while in autoimmune abortion IVIG possibly drives the Th1/Th2 ratio to a normal balance. **Morikawa et al**. demonstrated a downreguation of peripheral NK cell activity and subsets by massive IVIG treatment of women with RSA (968). **Rigal et al**. demonstrated that IVIG had no effect on 15 of 17 tested clusters of leukocyte differentiation (CD) markers used but that it downregulated adhesion molecule lymphocyte function-associated antigen-1 (LFA-1) and NK cells (1196).

However, **Jablonowska et al**. showed that there were no statistical differences in the presence of the blocking antibodies prior to pregnancy in RSA women included in the IVIG trial compared with women not attending the IVIG trial and controls (605). Furthermore, they demonstrated that IVIG does not have long-term effect on T- and B cell subsets in women with RSA (606).

Allogeneic lymphocyte immunotherapy

Taylor and Faulk first reported the possibility of paternal lymphocyte immunotherapy for women with unexplained RSA which has become widely performed since then (1399). They originally based their idea of third party lymphocyte immunization for women with RSA on observations that renal allograft rejection could be delayed by third party blood transfusions while **Beer et al**. based paternal lymphocyte immunization on the belief that maternal “blocking antibodies” were necessary for successful pregnancy (95).

The exact mechanisms of immunotherapy with allogenic lymphocytes have yet to be elucidated (Table 38).

Induction of humoral antibodies (APCA, Ab2, MLR-Bf)	Increase of PIBF on lymphocytes
Decrease of peripheral NK cells	Increase of PR on lymphocytes
Shift of Th1/Th2 ratio to Th2	

Table 38: Proposed mechanisms of allogenic lymphocyte immunotherapy

Immunotherapy with leukocytes attempts to block immunological rejection of the fetus by exposing the mother to an overload of self or third party antigen. This is thought to mimic the presentation of fetal antigens during pregnancy presumably

illiciting maternal immunoglobulin effectors that are believed to be necessary for maintenance of pregnancy (1050).

Some investigators suggested that paternal lymphocyte immunotherapy may act as immunogen to enhance the maternal immune response and to induce various humoral antibodies as immunological regulators for maintaining pregnancy (1073).
Various recent studies demonstrated that paternal lymphocyte immunization in women with RSA induce the level of humoral antibodies like APCA (1054), Ab2 (603), and MLR Bf (1069) that were correlated with the success of pregnancy. It was further suggested that the humoral antibodies APCA, Ab2 and MLR-Bf produced as a result of immunotherapy (1072) would mask the fetal HLA antigens and prevent them from being attacked by the maternal T cells.

Initial reports on paternal lymphocyte immunization used the sharing of HLA antigens between spouses as a criterium for immunization (95). This excess sharing was considered to be increased in couples with RSA and responsible for the hyporesponsiveness which was considered to be shown by a lower incidence of APCA, Ab2 and MLR-Bf in RSA couples.
The anti TCR-idiotypic antibody that has been reported to be present in the sera of normal pregnant women (603) would provide another mechanism for the immunotherapy. After immunization with paternal lymphocytes, maternal T cells recognizing paternal HLA antigens would expand and serve as immunogens to produce anti TCR-idiotypic antibodies. The anti TCR-idiotypic antibody would then bind specifically to the TCR and suppress the maternal immune response against the fetus, allowing the fetus to escape the maternal immunological attack. Thus the beneficial effect of this procedure has been attributed to the induction of various humoral antibodies that may block the mechanism of immune-rejection of the fetus and help in the implantation and fetal growth (603).
In addition, this therapeutic approach also includes a specific and non-specific T cell suppression (882), a shift to Th2-type immunity and suppression of NK cell activity (767).
Recently, **Gafter et al**. demonstrated that monocyte functions such as secretion of IL-1α, TNF-α, IL-6 and cytotoxic activity decreased whereas IL-10 and TGF-β increased after paternal lymphocyte immunotherapy in women with RSA (433). *In vitro* studies showed that low dose of IL-6 stimulated the asymmetric antibodies synthesis and a high dose decreased it suggesting that IL-6 regulates the synthesis of asymmetric antibodies in the trophoblast (1576).

Check et al. reported that lymphocyte immunization causes an increase in PIBF in women with RSA, which may play a significant role in the maintenance of pregnancy by regulating the Th2 shift (206).

Of interest was the observation that immunization with third party leukocytes produced equally good results (1050). Thus some women would abort following immunization with paternal cells but successfully carry pregnancies to term and deliver live babies after immunization with third party lymphocytes. Such an observation could be interpreted to mean that the husband did not provide adequate antigenic repertoire for the wife to raise sufficient immunological response to sustain the pregnancy, a condition that was then fulfilled by the third party leukocyte donor.

LeukoNorm CytoChemia® (LNCC)

The leukocytic ultrafiltrate LNCC has been approved for the treatment of immunologically-based RSA and repetitive implantation failure (1540).

A study by **Würfel et al**. investigated the treatment outcome of LNCC in patients with multiple failed IVF or ICSI cycles (1541). Administration of LNCC on five consecutive days from the day of oocyte retrieval significantly improved treatment results with a pregnancy rate of 55% (40% clinical pregnancies) as opposed to a rate of 21% in the non-treatment group. A consecutive multicenter study in Germany with LNCC has shown similar positive results (1540).

1a, 25–dihydroxy-vitamin-D3 (VD3)

As VD3 and its analogs were already known to be effective in the treatment of Th1-immunity-mediated disease, **Bubanovic** proposed VD3 as new immunomodulatory agent for the treatment of women with RSA another Th1-immunity disease (151). The mechanism of VD3 activity, however, is not yet fully understood but it is thought to downregulate the production of Th1 cytokines, such as IL-2, IFN-γ, TNF-α, IL-1, IL-6 and IL-8 as well as increased Th2 associated cytokines in T cells from adults. VD3 also inhibits not only IL-12-generated IFN-γ production, but also suppresses IL-4 and IL-13 expression induced by IL-4 (1120). As the effects of VD3 are very similar with immunomodulatory effects of IL-10, it was believed that VD3 could be used as local immunomodulatory drug for the treatment of RSA (151).

Dehydrogesterone

Progesterone was recognised early on to be one of the most important steroids required for the maintenance of pregnancy, and so its stereoisomer dydrogesterone became a candidate for the treatment of threatened and habitual abortion (485).

Already in the 1980s, the pregnancy-maintaining effects of dydrogesterone were demonstrated in ovariectomised rats (850). In a study by **Raghupathy et al**. on PBMCs of women with RSA, dehydrogesterone significantly inhibited the production of the Th1 cytokines IFN-γ and TNF-α and induced an increase in the levels of the Th2 cytokines IL-4 and IL-6 resulting in a substantial shift in the ratio of Th1/Th2 cytokines (1161). The effect of dydrogesterone was blocked by the addition of the progesterone-receptor antagonist mifepristone, indicating that dydrogesterone was acting via the progesterone receptor. Dydrogesterone also induced the production of PIBF.

Another recent study compared serum cytokine levels in women with threatened abortion to those of healthy pregnant controls and evaluated the impact of dehydrogesterone in the former group (647). Peripheral cytokine production did not significantly differ between the two groups before and after treatment with dehydrogesterone. The protective effect may therefore be manifested via PIBF rather than controlling cytokine production (647).

In a controlled, randomised clinical trial, 180 women with a history of habitual abortions received either dydrogesterone, hCG, or no treatment until the 12th week of gestation (366). Dydrogesterone significantly reduced the abortion rate as compared with untreated controls with no increase in obstetric complications.

3. Immunocontraceptive approaches

Vaccines are currently being explored as a tool for for contraception. This involves generating a cellular or humoral immune response against antigens that are critical in the reproductive process and therefore interference leads to a block of fertility. This is achieved by linking an appropriate antigen to a foreign carrier molecule and when coadministered with an adjuvant causes the immune system to raise neutralizing antibodies. In reversible methods, fertility is regained subsequent to a decline in antibody response whereas an irreversible block in fertility is evident by a failure to regain fertility in spite of undetectable circulatory antibodies (492).

There are several possible targets for immunological intervention in the female reproductive system (492). These can be divided into three main categories:

contraceptive vaccines targeting gamete production, gamete function and gamete outcome.

For example, neutralization of the gonadotropin-releasing hormone (GnRH) activity in the GnRH/LH/FSH feedback control system by generating a specific antibody response will interfere in the production and maturation of of oocytes. At the level of the sperm-egg interaction, possible targets could be surface determinants on the gametes against which an immune response can be drawn. After fertilization, immunological neutralization of hCG by antibodies would result in a failure of blastocyst implantation. An overview gives Figure 26.

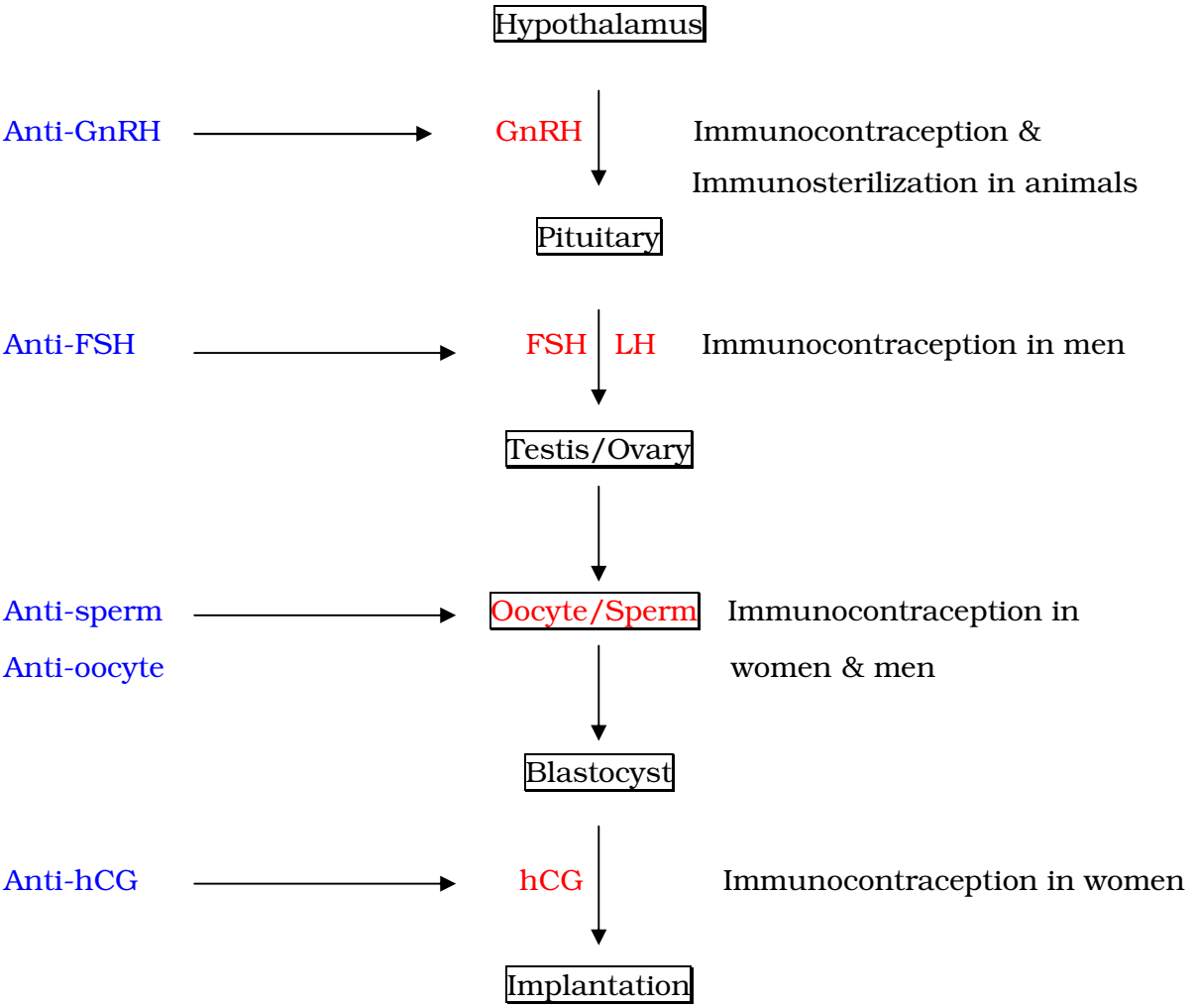


Figure 26: Stages of the reproductive cycle with possible targets for immunological intervention

a) Anti-GnRH vaccines

GnRH is present in males as well as females; hence a vaccine against GnRH is usable in both sexes. GnRH by itself is not immunogenic and has to be conjugated to a carrier to mobilize Th cells (1009).

Vaccines incorporating GnRH have been developed primarily for use as immunocontraception and immmunocastration agents in animals whereas, in humans, their use is primarily for the treatment of prostate cancer in man and various sex hormone-dependent disorders (1392). For the use in human contraception, however, androgen supplementation is required to maintain libido and secondary sex characteristics (394).

Previous studies have already shown that passive or active immunization in order to neutralize GnRH leads to a block of fertility in animals (1388). **Miller et al**. tested active immunization with a GnRH-based vaccine on white-tailed deer (941). Treatment led to reduced fawning rates, altered estrus behavior, reduced concentrations of progesterone, and failure to maintain pregnancy following conception. Infertility lasted up to two years and was reversible, directly related to the antibody titer.

Another application of a GnRH-based vaccine is to perform immunocastration providing an alternative to surgical castration. **Hannesdottir et al**. used a GnRH-analogue conjugated to *Mycobacterium tuberculosis* hsp70 in mice together with two different adjuvants (505). With either adjuvant, all mice made sufficient antibodies to cause atrophy of the urogenital complex and showed significantly reduced serum levels of testosterone. **Zeng et al**. also demonstrated the efficiency of a GnRH vaccine in immunocastration of pigs (1577).

Enough data have accumulated to conclude that the vaccines against GnRH can be employed in humans and in animals without side effects. Recombinant vaccines would be substantially cheaper to make on an industrial scale than synthetic vaccines. **Talwar et al**. reported the ability of a multimer recombinant anti-GnRH vaccine to cause decline of testosterone to castration level and atrophy of prostate of rats (1389). Some GnRH vaccines for veterinary use have already been commercialised (Vaxstrate™, Improvac™) (349, 569).

b) Anti-FSH vaccine

A promising vaccine for males aims to prevent spermatogenesis by immunization against FSH (394). **Moudgal et al**. have performed successful studies in male bonnet monkeys in which infertility was induced by immunisation with ovine FSH (982).
Therefore, a phase I clinical trial was carried out using either the ovine FSH αβ-heterodimer or isolated ovine β-chain, purified from sheep pituitary, as the immunogen (981). FSH-specific antibodies were elicited in all immunized individuals but, unfortunately, there was no overall reduction in the sperm count in the volunteers due to the antibodies being of fairly low titer. Further trials are intended using recombinant proteins and different doses of the vaccine.

c) Anti-sperm vaccines

As discussed in the last chapter, several clinical studies in women have demonstrated that antibodies against sperm are frequently associated with unexplained infertility. Men and women who have infertility attributed to ASA are involuntarily vaccinated models to show how a sperm vaccine will work in humans. Sperm have both auto- and isoantigens, so they can be used for both the sexes for contraception. Sperm antigen vaccines are designed to cause agglutination of spermatozoa in the female reproductive tract, by IgG and IgA antibodies in cervical mucus (394). These vaccines may also be applied to males but it remains a challenging prospect to produce effective neutralizing antibodies in the relevant areas of the male reproductive tract.
However, the whole spermatozoon cannot be used for vaccine development, due to the presence of several antigens on sperm cell that are shared with other somatic cells (1012). Therefore, a sperm antigen suitable for vaccine development must be sperm-specific and immunogenic, it must participate in the fertilization process and its surface expression should be accessible to antibody binding (332).

Many studies have been performed so far on candidate antigens from male and female gametes in order to demonstrate their ability to induce auto and/or isoimmune response interfering with fertility (1009). Some of them have been further characterized and the complementary DNA (cDNA) encoding these antigens have been cloned and sequenced (1009). Notable among them are the fertilization

antigen (FA)-1 (1015), sperm protein (SP)-17 (796), testis-specific antigen-1 (1254), protein A-kinase anchoring protein (931), and sperm-associated antigen 9 (1283).
Active immunization of female animals with some of these antigens, for example FA-1 and SP-17, has been shown to reduce fertility *in vivo*. To date, the maximum effect so far obtained is up to 75% reduction in fertility of a single antigen in the mouse model (1009).

In humans there are at least four sperm proteins involved in oocyte ZP binding (1007) so that multiple antigens may be involved in the fertilization cascade. Vaccination with a single antigen may also not raise enough antibody titer, especially in the local genital tract, to completely block fertility. At this time, there is no published report examining the contraceptive effect of more than one sperm antigen in a single vaccine formulation in any animal model (1009).
Only a few studies have examined the effect of vaccination based on a sperm antigen on fertility in a non-human primate model. Vaccination with testis-specific lactate dehydrogenase (LDH-C4) reduced fertility in female baboons in a study by **O'Hearn et al** (1036) whereas another study interestingly reported no effect on fertility in female monkeys after vaccination with LDH-C4 (1422). Recently, male monkeys (*Macaca radiata*) were immunized with an epididymal protein, designated as epididymal protein inhibitor (Eppin) (1037). After immunization, 78% monkeys developed high antibody titres to Eppin and became infertile which indicates that anti-sperm vaccine can also be used for men.
Several synthetic sperm peptides have also been investigated for contraception having caused various degrees of contraceptive effects in animal models (510, 796). A vaccine prepared by conjugating the synthetic YLP12 peptide with recombinant cholera toxin B subunit (rCTB) was tested in intranasal and intramuscular vaccination of female mice (1008). All vaccinated animals showed a sperm-specific immune response inducing some degree of inhibition of fertility while the animals with high antibody titres in sera as well as vaginal washings showed a complete block.
Perhaps, monospecific antibodies to sperm antigens may be combined for immunocontraceptive purposes in the form of intravaginal sperm-specific spermicides (332). A first recombinant miniantibody has been already engineered to the tissue-specific carbohydrate epitope located on the sperm glycoform of the CD52 antigen (331) and shown to agglutinate human sperm cells in a tangled pattern (1028).

d) Anti-oocyte zona pellucida (ZP) vaccines

Zona pellucida (ZP) as matrix around the oocyte is mainly composed of glycoproteins ZPA, ZPB and ZPC (492). It serves as docking site for species-specific recognition and binding of spermatozoa to the oocyte and protects the growing blastocysts before implantation. The glycoproteins have become promising candidate antigens for the development of an immunocontraceptive vaccine.

Antibodies generated against ZP glycoproteins from a given species were shown to react to some extent with the ZP from other species and thus permit heterologous immunization (492). After immunization with porcine ZP, infertility together with follicular atresia and an abnormal hormone profile was observed in female rabbits (1530).

Studies employing different animal models demonstrated that immunization with heat-solubilized porcine ZP led to a block in fertility that was likely to be due to ovarian dystrophy rather than a block in fertilization. It was suggested that the observed side effects might be due to the impurities of other ovarian-associated proteins that may be present in the heat-solubilized ZP preparations. The group of **Bagavant et al**. showed that monkeys immunized with highly purified porcine ZPB and ZPC failed to conceive in the presence of high circulating antibody titers but did not show any adverse effects on ovarian functions (70).
Recombinant proteins may diminish the problems of probably contaminated ZP glycoproteins isolated from a native source. In a study by **Govind et al**. immunization of female baboons with recombinant bonnet monkey ZPB led to a reversible block of fertility (472). Ovarian histology of the immunized animals revealed the presence of atretic follicles with degenerating oocytes, which may explain failure to conceive.

Also the development of live recombinant vectors encoding ZP glycoproteins has been started. Immunization of mice with murine cytomegalovirus as live recombinant vector expressing mouse ZPC has led to infertility (825).
Another approach is the use of plasmid DNA encoding ZP glycoproteins. In a study by **Xiang et al**. immunization of mice with the plasmid DNA encoding partial sequence of rabbit ZPC led to inhibited fertility without any disturbances in folliculogenesis (1544).

A series of experiments demonstrated that the "oophoritogenic" T cell epitopes present in the zona proteins may be responsible for ovarian dysfunction often observed after immunization with ZP antigens (832). To circumvent the ovarian pathology, synthetic peptides devoid of "oophoritogenic" T cell epitopes as immunogens have been proposed (492). However, *in vivo* studies did not show consistent reduction in fertility (1096).
Another approach may be to employ chimeric recombinant protein comprising ZP and spermatozoa antigens (797).

e) Anti-hCG vaccine

Two fundamentally different strategies were taken regarding the development of hCG-based contraceptive vaccines. In one approach the main consideration was to produce a vaccine that would provide an hCG-specific immune response that did not cross-react with the structurally-related LH. The alternative approach considered that such cross-reaction, if it were to occur, would not be harmful and therefore the prime consideration should be to produce a vaccine capable of eliciting high titer of antibodies (319).

As probably the most promising candidate for development of an immunocontraceptive vaccine for females, the efficacy of hCG linked to tetanus toxoid (TT) was tested in various animal studies (1392). For this vaccine the intact β-chain was used and there was no great concern about possible cross-reaction with LH.
Talwar et al. conducted a multicenter phase I clinical trial on 63 women which showed the presence of both anti-hCG and anti-TT antibodies (1390). However, antibody titers and the duration of immune response highly varied among immunized women.
A more immunogenic vaccine formulation was tested in extensive phase II clinical trials and led to generation of anti-hCG antibodies levels enough to protect against conception in 80% of immunized women (1391).

The other vaccine against hCG developed by **Stevens et al**. with support from the WHO Task Force on Vaccines for Fertility Regulation is based on a synthetic peptide based on the portion of the β-subunit of the hormone linked to diphtheria toxoid (DT) (1354). Potentially contraceptive antibody titers were induced in a phase I

clinical study which was followed by a phase II clinical trial that was abandoned due to unacceptable local reactions at injection sites (1009).
The immunogenicity of a β-hCG-based DNA vaccine has also been illustrated. The DNA vaccine together with a C3d adjuvant led to a generation of a 9-fold higher antibody response compared to β-hCG alone and increased the expression of antigen-specific Th2 humoral immune response in mice (1477).

f) Other vaccination strategies

Other potential approaches for development of immunocontraceptive vaccines include riboflavin-carrier protein which is a major transporter of vitamin to the embryo across the placental barrier. Active immunization in animals with this agent significantly reduced fertility (9).

A project by the German group of **Frank et al**. is aimed at the immunological inhibition of syncytial trophoblast fusion as a novel approach to contraception (422). Fusion-inhibiting recombinant antibodies were generated and used together with autoantibodies from patients with repetitive IVF failure that were shown to inhibit syncytial fusion and are expected to inhibit implantation, to generate anti-idiotypic peptides. These peptides mimic trophoblast epitopes essential for syncytial fusion and are, therefore, considered specific immunogens for the generation of antibodies that will inhibit implantation. Of 300 anti-idiotypic peptides which were tested for their binding capacity to patient autoantibodies associated with repetitive IVF failure, habitual abortion and preeclampsia only three peptides were found to selectively bind to autoantibodies of patients with repetitive IVF failure and were considered safe and efficient enough for evaluation in preclinical and clinical studies required for the development of immune contraceptives (422).

III. Discussion

A. General immunology of the genital tract

The aim of this review is to summarize and evaluate the latest studies dealing with the subject of the immunology of the female genital tract mucosal tissue. The main focus is thereby first to present results concerning the basic principles of innate and adaptive immune mechanisms in the female genital tract.
This distinct site had long been neglected as a subject of studies but its importance and specialty among the human immune system has now been noticed during the last years. Advances concerning the field of female genital tract immunology may implicate conclusions for other related research fields such as the immunology of genital infections and immunology of pregnancy. Especially the continously rising number of STDs provided the need for further investigations in this field.

It is now established that the mucosal immune system is a distinct and separate component of the host`s immune apparatus and differs from the lymphoid tissues in peripheral sites which contribute to the antibody isotypes found in the blood circulation (762, 1266, 1423). Furthermore, regulation of the mucosal immune system is different than that of the systemic immune system. The mucosal immune system can be divided into discrete inductive sites where antigens or vaccines are encountered and are endocytosed, processed, and presented to B and T cells and to separate effector areas where immune cells actually function (1018).

Protection against potential pathogens in the female reproductive tract is provided by a variety of measures that can be grouped into the two broad categories of innate and adaptive immunity.
The innate immune system differs from the adaptive immune system in the cells involved, the type and specificity of receptors for antigen, the immediacy of response, and the nature of the response to antigenic challenge (615). Recognized as the first line of defense, the innate immune system functions to prevent and control the invasion of pathogens. It has evolved to recognize foreign structures, the so-called PAMPs via pattern recognition receptors of the host expressed on cells of the innate immune system, the TLRs. Signaling through TLRs in response to PAMPs involves a number of adapters and other molecules that lead to recruitment of immune cells as well as the production of intracellular and secreted antimicrobial

factors that both kill invading microbes as well as link innate and acquired immunity. Adaptive immunity encompasses pathogen-specific defense mechanisms.

Of those mucosal surfaces in the body, the female reproductive tract has unique requirements for the regulation of immune protection, because it must deal with sexually transmitted bacterial and viral pathogens, allogeneic spermatozoa, and the immunologically distinct fetus (1514, 924, 779, 763). The immune system of the female genital tract is the least understood part of the mucosal immune system with respect to the origin of its immune cells, the role of tissue CTL responses and the induction of antibody responses, as well as the contribution of serum-derived versus mucosally produced antibodies. Although it is considered as a part of the common mucosal immune system it offers some distinct features which outlines its special role.

A major difference between the genital tract and the intestinal tract is the compartmentalization of the genital mucosa. Vagina and ectocervix present with commensal flora whereas endocervix, uterus and fallopian tubes are sterile, lacking the presence of a microbial flora (630, 1151, 1512). The epithelium of the vagina and ectocervix therefore is required to provide a strong barrier whereas the epithelium of uterus and endocervix is less afflicted by microorganisms. A sophisticated barrier function is also given by the cervical mucus as part of basic innate immune mechanisms that filters bacteria but allows sperm to ascend to the uterus (630).

By analogy to a primary immune response in the MALT, antigen reaching the submucosa of the vagina is taken up by APCs (373), which then migrate to draining lymph nodes. Once in the lymph nodes, the APCs stimulate B and T lymphocytes, including memory subpopulations, that enter the bloodstream via the efferent lymph and thoracic duct. These T and B lymphocytes migrate to the genital tract and, on exposure to the antigen, participate in a secondary immune response.
In contrast to the intestinal tract, the mucosal immune system of the female genital tract is under hormonal control of estrogen and progesterone that regulate the transport of Igs, the levels of cytokines, the distribution of various cell populations, and antigen presentation in the genital tissues during the reproductive cycle (1512).

Instead of M cells in the intestinal tract, it is likely that mononuclear phagocytes such as macrophages and DCs as well as genital ECs present in the vagina are

capable of acting as APCs and of initiating an immune response (630, 1151, 1514). There are lymphoid aggregates in the basal layers of the uterus comparable to the intestinal tract but their presence and distribution strongly varies under the hormonal influence.

Especially the role of ECs in the female genital tract has gained a completely new significance. Acting as the first line of defense, ECs provide host protection in a number of ways that includes providing a mechanical barrier, secreting antimicrobial molecules, transporting IgA, processing and presenting antigen and communicating with underlying immune cells by secreting cytokines and chemokines (1151, 1512, 1514).
Besides their role in providing a physical barrier against microbes by establishing tight junctions and TER (372, 1034) they have to be regarded as a genuine part of the mucosal immune system that recognizes antigen and leads to production of antimicrobials (1093, 1512, 1514, 1514).

It can be concluded from different studies that uterine ECs as well as APCs in the uterine stroma and vagina are capable of presenting antigen which initiates an immune response in the female reproductive tract. Isolated uterine ECs express MHC class II antigen and CD40 or CD1d proteins and are able to process and present tetanus toxoid to T cells (375, 1470).
Moreover, antigen presentation in the female reproductive tract is supposedly regulated by sexual hormones and soluble factors of stromal cells (1515, 1516). More recent studies demonstrated that stromal antigen presentation is regulated by cytokine production by ECs. In response to estrogen, uterine ECs produce TGF-β which suppresses underlying APCs in the stroma (1517).

Various defensins have been detected in the female genital tract secretions (1152, 1436) and seem to be regulated by cycle-associated changes in sexual hormones and influenced by contraceptive use (415, 707). These factors may contribute to an altered susceptibility to infections. In the same way, SLPI and SP-A/D production by uterine ECs varies due to the stage and status of the menstrual cycle (376, 704, 809, 855) and seems to be regulated by sexual hormones as well (214, 709).

However, estradiol and progesterone seem to regulate genital tract EC proliferation, apoptosis, secretions and effects on pathogenic microbes (1034, 1513). Several studies suggested that estrogen regulation of EC proliferation is mediated indirectly

by uterine stroma (250, 595, 1121). In experiments with uterine ECs of mice, estradiol decreased TER and therefore EC layer integrity whereas other sexual hormones had no effect (474). Uterine epithelial monolayer integrity seems to be directly influenced by estradiol and is ER-mediated which suggests a cycle-dependency. Sex hormone regulation of EC function is both direct via ER in ECs and indirect via ER located in the underlying stromal cells (250, 278).
Soluble factors expressed by uterine stromal cells such as HGF act as mediators of estrogen-induced proliferation of uterine ECs, increase of TER (473, 475) and regulation of EC cytokine production.

Cytokines are now recognized as principal components of the complex intercellular communication among cells in the uterus (374, 672, 673, 674), and temporal release patterns during both the menstrual and estrous cycles show many to be regulated by sex hormones. Cytokines secreted by ECs recruit other immune cells such as neutrophils and lymphocytes (268, 374, 674, 1292) and could also account for the influx of leukocytes and lymphocytes that form lymphoid aggregates observed during the secretory phase of endometrium (1560).
Concentrations of cytokines and chemokines vary in the endometrium and show temporal release patterns related to the menstrual cycle (880). Many cytokines and chemokines released by ECs peak during the late proliferative phase and at menses such as LIF, RANTES, MIP-1α, IL-8 (48), IL-6 (1382, 1564), TGF-β, IL-1β, and M-CSF.
The local production and transport of Ig in ECs in the reproductive tract is also influenced by the female sex hormones estradiol and progesterone (1519). Estradiol increases pIgR and SC expression and therefore IgA transport and IgG accumulation are stimulated in the uterine lumen whereas this is inhibited in CVS by estradiol and progesterone (918, 1519, 1520).

TLRs, a family of structurally related receptors that recognize specific products of pathogens referred to as PAMPs as well as endogenous ligands associated with cell damage, have recently received growing attention in the female genital tract. They are present mainly on macrophages, neutrophils and DCs, but low levels of expression have also been observed on fibroblasts, endothelial and epithelial cells (1514).
The expression of TLRs in epithelial cells from the vagina, ectocervix and endocervix by the detection of mRNA was reported by several authors. **Fichorova et al**. found mRNA for TLR-1, -2, -3, -5 and -6, but not -4 (395), which is partly consistent with

other findings of **Young et al**. (1567) and **Pioli et al** (1126). TLR1-6 and TLR9 were expressed by endometrial ECs whereas TLR7, -8, -10 were not detected. Results indicate that ECs that line the uterine lumen are sensitive to viral infection and exposure to viral dsRNA released from killed ECs (1262, 1263). In addition to releasing proinflammatory cytokines and chemokines that mediate the initiation of an inflammatory response and recruitment of immune cells to the site of infection, ECs also express β-defensins, IFN-β, and IFN-β-stimulated genes that can have a direct inhibiting effect on viral replication.

Macrophages as another representative of APCs can be found throughout female reproductive tract tissues and make out about 10% of the leukocytes (459, 584). In endometrium, their number continously rises during menses, implantation and early pregnancy among other leukocyte populations such as uterine NK cells and neutrophils (638). In the ovary, their distribution also differs depending on the stage of menstrual cycle (1538).
As mediators of phagocytosis of foreign pathogens and cellular debris, they play an essential role in the initiation, maintenance, and resolution of host inflammatory responses in the female genital tract, for example by secreting pro- and antiinflammatory cytokines (920). In their function as effector cells of ovarian function with specific localization and their presence in periovulatory human follicular fluid they seem to play diverse roles in the folliculogenesis, tissue restructuring at ovulation and corpus luteum formation and regression.

It is well known that estrogen levels influence macrophage proliferation and function as well as cytokine production (1512) and there is high evidence that steroid hormones regulate the recruitment of uterine macrophages throughout the menstrual cycle due to regulation of cytokine and chemokine expression (318). Human peripheral blood monocytes and macrophages have similarly shown expression of both receptor isoforms, ERα and ERβ (1114, 964) whereas only one study detected PR (1451) Paradoxically, macrophages have also been showing an effect to progesterone, which could be explained by cross binding of progesterone to the glucocorticoid receptor (1495).
However, recent data suggest a predominantly immunosuppressive role of estrogen in modulating immune responses, mediated by nuclear ERα (777). Human studies have demonstrated that macrophages selectively aggregate into premenstrual endometrial stroma, concurrent with depression of estrogen and progesterone levels as the result of luteolysis (639, 656). This is probably due to hormone-regulated

variations in cytokine and chemokine levels as the expression of macrophage chemoattractants MCP-1, MCP-3, FKN and MIP-1β in human endometrium is also increased perimenstrually (638, 926). **Arici et al**. have demonstrated that treatment of endometrial stromal cells with estradiol significantly inhibits expression of MCP-1, which correlates with suppression of macrophage migration (48). This implicates that fluctuations in estrogen and progesterone levels are coincident with changes in the migration of macrophages to the endometrium (318).

To be precise, accumulation of endometrial macrophages together with macrophage chemokines also occurs during the mid-secretory phase of the menstrual cycle with high estrogen and progesterone levels (638). Although these data are inconsistent with estrogen inhibition of MCP-1 expression, it is possible that regulation of MCP-1 is mediated by estrogenic modulation of another factor, such as IL-1 (48). Estrogen has been shown to stimulate IL-1 mRNA and protein expression and IL-1 again has been shown to induce expression of MCP-1 (18).

As sentinels of immune function, DCs take up microbial antigens at sites of infection and then migrate to draining lymph nodes to stimulate antigen-specific T-cell activation which is induced either by pathogen invasion of mucosal surfaces or epithelial damage (73).
Female genital tract LCs can be found in the epithelial layer of the vaginal and ectocervical mucosa of women (119), whereas DCs are predominant in the submucosal layers.

DCs express mRNA for both ER isoforms at all stages in their differentiation. Data suggest that nonsteroidal anti-estrogens such as tamoxifen and also estrogen inhibit the differentiation of immature DCs and therefore the development of inflammatory Th1 responses but not via ER (737). Others showed in a *ex vivo* mouse model that estradiol promoted the differentiation of functional DCs from murine bone marrow precursor cells via ER (1060). This suggests a mechanism by which estradiol levels in peripheral tissues might modulate both the number and functional capabilities of DC *in vivo*, thereby influencing immune responses. These contradictory results could be explained by the cell type and species specific effects of estradiol or by the use of different hormone concentrations.

Estrogen has also been demonstrated to increase the expression of cytokines and chemokines in human monocyte-derived DCs (106). Moreover, mature DCs treated

with estradiol had an increased ability to stimulate naïve CD4+ T cells. Collectively, these data implicate a role for estrogen in the regulation of DC effector function and suggest that estradiol plays a key role in the induction and maintenance of inflammatory responses. Nevertheless, further investigation of the influence of estrogen on DC differentiation and maturation is warranted.

Considered in this context, the identification of DCs in the human reproductive tract has significant implications for the progression of inflammation and disease as well as for the maintenance of pregnancy. DCs are resident in human decidua (439), where they have been proposed to mediate tolerance to the conceptus as well as to mediate maternal immune responses to pathogens. This should be discussed further in the next chapters.

Similarly to T cells, NK cells can utilize a variety of effector mechanisms including cytokine production and perforin-mediated cytotoxicity to help the host to eliminate pathogens and tumor cells (1051). They are able to differentiate between healthy cells and abnormal cells by using a sophisticated repertoire of cell surface receptors that control their activation, proliferation and effector functions (783). They are focused on recognition of MHC molecules by surface receptors KIR, Ly49 and CD94/NKG2 with both activating and inhibitory isoforms which distinguish themselves from phagocytes which rely on conserved pattern-recognition receptors such as TLRs (1177). Downregulation of MHC I due to infection or transformation of cells would alleviate inhibition of NK cell positive signaling and may result in initiation of cytotoxicity and cytokine production (483, 783, 784).
However, blood and uterine NK cells also express various TLRs whose agonists are able to trigger IFN-γ production of NK cells (568, 749, 1512). The hypothesis is therefore that microorganisms may initially activate ECs which produce cytokines and these cytokines in combination with PAMPs will activate NK cell cytokine production, resulting in further activation of innate immune responses.

In the female genital tract tissues, NK cells make out 10-30% of all leukocytes and localize to the endometrium in large numbers following ovulation, then accounting for almost 70% of leukocytes prior to menstruation (459, 584) which suggests the involvement of sex hormones in regulation of this NK cell migration. Pathological conditions such as gynecological malignancies and pregnancy loss are associated with altered NK cell numbers and activity (338, 848, 902).

NK cells in the uterus express CD56, CD94 and KIRs, few express CD16, and none express CD57 or CD8 and, unlike blood NK cells, uterine NK cells express CD9 and CD69 on their cell surface (370). Thus, uterine NK cells have a cell-surface phenotype that is unique. A large molecular study indicated that decidual NK cells were more similar in their gene expression profile to $CD56^{bright}$ NK cells than to $CD56^{dim}$ NK cells (740).

The question if decidual NK cells are derived from blood NK cells which differentiate in the decidua or if they represent a special cell line which is produced only for the decidua is still not resolved. The hypothesis that NK cells in the decidua are derived from uterine NK cells in the endometrium at the time of implantation or from newly recruited blood NK cells has been favoured. NK cells isolated from nonpregnant endometrium and decidua may not represent different phenotypes but a rather continuous process of differentiation due to alterations in hormone levels and changes in stromal cell environment (1512).
Concerning NK cell phenotype in other female genital tract tissues, there have been performed only few studies (902, 1512).

Uterine NK cells have shown similar chemokine receptor expression compared to blood NK cells and it can be proposed that migration of uterine NK cells to the endometrium is induced by high expression of chemokines (598, 715, 858, 1275). **Sentman et al**. suggested that sex hormones induce specific chemokines in nonpregnant human endometrium that can activate NK cell migration (1275).

Also decidua can produce chemokines that can recruit NK cells (504, 1180); however, it is not exactly clear which chemokines are involved in NK cell recruitment. CXCL10 induced by sex hormones in endometrium prior to implantation may recruit uterine NK cells whereas chemokines derived from trophoblast such as CXCL12 may recruit uterine NK cells to reorganize placental arteries and facilitate trophoblast invasion of maternal tissue (1512). NK cell attachment to uterine endometrium needs specific adhesion molecules. L-selectins and α4 integrins are important for the binding of lymphocytes (200, 392). The adhesion of NK cells also involves VCAM-1 which is expressed at the site of trophoblast invasion and might allow NK cells to migrate continuously to these sites (200, 392).

In general, NK cells as part of the innate immune system help to protect against infections, for example by promoting macrophage and CTL activation via Th1 and Th2 cytokine production (370, 634, 1052, 1107).
In pregnancy, high numbers of decidual NK cells also seem to be involved in regulation and restructuring of maternal spiral arteries through production of angiogenic growth factors and LIF as well as in decidualization and trophoblast invasion (156, 815, 1512). The invading trophoblasts express HLA-G and can interact with the KIR receptors on uterine NK cells. Membrane-bound HLA-G has been shown to stimulate uterine NK cells and IFN-γ production and to suppress mononuclear cell effector functions (1438). NK cell-derived IFN-γ seems to be necessary for vascular remodelling of spiral arteries and placental formation so that the recognition of HLA-G on trophoblasts by uterine NK cells may be important for placental development. Uterine NK cell-deficient mice demonstrated failure to sustain decidual integrity and loss of spiral artery modifications (481).

Recruitment, differentiation and activation of NK cells within the uterus seem to dependent from specialized CD8+ Treg cells (1288) and cytokines such as IL-15, IL-18, IFNs, TGF-β and prolaktin (30, 209, 252, 348, 370, 387, 716, 1371).
Uterine NK cell numbers and migration, however, are regulated by sexual hormones as they show cycle-dependent changes with numbers up to 70% of leukocytes in the late secretory phase (459, 702). Two mechanisms have been discussed as reason for the increase of uterine NK cells: on the one hand, in situ proliferation (658, 1383) and on the other hand, selective recruitment from the peripheral NK cell pool. However, an active recruitment of these cells to the uterus is likely to play a major role with data indicating that uterine NK cells are derived from blood or bone marrow cells and not from NK cells within the uterus (488).

However, it is not exactly clear how NK cell functions are regulated by sex hormones. Various studies have been coming to different and partly conflicting results. One reason may be the different kind of tissue conducted for experiments, including nonpregnant endometrium as well as decidua. Estrogen has been shown to suppress human NK cell cytolysis *in vivo* and *in vitro* in a dose-dependent manner (391). Another study illustrated that men showed significantly higher NK cell activity in blood than women with regular menstrual cycles or women using oral contraceptives (1568).
Uterine NK cells of decidual tissue do not express receptors for estradiol or progesterone; therefore the activity of sex hormones on NK cell function is likely

mediated via hormone action on other cells such as fibroblasts or ECs (539). However, NK cells do express glucocorticoid- and estrogen-β1 receptors which may be mediating steroid hormonal effects on NK cells.

Considered as a first line of defense against pathogens, neutrophils follow both endogenous and bacterial chemoattractant signals and migrate to the site of infection (536, 600, 812, 1266). The presence of pathogens are detected by germline-encoded receptors that recognize PAMPs; human neutrophils express TLR 2 to 10 except TLR3 (525). Pathogens are then eliminated by phagocytosis, microbicides or production of toxic compounds via the NADPH oxidase system (1512). Neutrophils themselves produce several cytokines, for example IL-1β, IL-8, TNF-α, TGF-β1 (182), that attract more neutrophils, macrophages and other immune cells helping to initiate the adaptive immune response.

In the female genital tract, the highest number of neutrophils can be found in the fallopian tube with consistently decreasing numbers down through the lower genital tract (459, 1529). This seems striking as the vagina contains the most pathogens and one would expect the highest number of neutrophils there.

As a consensus, the number of neutrophils in different female genital tract tissues is dependent of IL-8, a potent chemoattractant and activator for neutrophils. Moreover, neutrophils in the female reproductive tract seem to be regulated by GM-CSF which can potentiate induction of neutrophil chemotaxis and regulate neutrophil activation (1292, 1512).
However, despite a increased expression of IL-8 in the late proliferative phase around ovulation there seem to be no studies on neutrophil number variation in the fallopian tubes during menstrual cycle (1067, 1512). This difference and the exact role of neutrophils in the fallopian tubes as part of the local immune system remains quite unclear. Fallopian tube neutrophils compared with blood neutrophils expressed higher levels of the adhesion molecule CD31 and CD15. CD31 is increased on transmigrated neutrophils (844), which suggests that fallopian neutrophils have crossed the endothelial barrier and are not only part of the marginated pool associated with the luminal surface of blood vessels. CD15 is involved in the binding of neutrophils to E-selectin and P-selectin. CD15 ligation is reported to induce a release of granule content; the high CD15 expression may be important for innate immune responses in the fallopian tube.

Fallopian tube neutrophils have also shown lower amounts of specific granule-associated molecules which suggests that they have undergone some kind of degranulation. Furthermore, they also demonstrated high levels of intracellular VEGF and IFN-γ (1512). As VGEF plays an important role in vasodilatation and vascular permeability (86) it would make a leukocyte infiltration of tissues possible.

High levels of IL-8 correlate with high levels of neutrophils in the female vagina (192). This corresponds with the findings that neutrophils cannot cross the epithelial barrier unless they are under the influence of a chemokine gradient of IL-8, for example (692). Investigating vaginal neutrophil numbers during the menstrual cycle, **Patton and colleagues** found constant numbers of neutrophils throughout the cycle (1098) whereas in mice neutrophil migration showed a sexual cycle dependency and positively correlated with MIP-2 concentrations, a functional IL-8 homologue (1327).
Studies demonstrated that IL-8 production and therefore neutrophil infiltration into the vagina only takes place in case of vaginal epithelial infection. If infection was asymptomatic or did not take place, there was no neutrophil infiltration in the vagina (400).

Neutrophils represent the highest population with 83% of all leukocytes in human cervical secretions (1414). Both insemination and infection of the cervix with increased local production of IL-8 as chemoattractant increase the number of neutrophils in the cervix significantly (125, 1481).
In the uterus, a decrease in progesterone levels prior to menses is responsible for a rise in IL-8 production and therefore a rise in neutrophil numbers (47, 267, 1249) which serves to guard against infection at this time of lower epithelial defenses.
Endometrial neutrophils also biase immune responses through production of IFN-γ (1559) and may play a role in endometrial breakdown through release of elastase and MT1-MMP (1579).

Studies on ovarian neutrophils proposed that they are involved in tissue remodeling during the ovulatory process with high numbers in the developing ovarian follicle wall (144). This is also consistent with peak IL-8 concentrations in follicular fluid around ovulation which seem to be stimulated by FSH and LH (1225). Through the release of and activation of MMPs and collagenase, ovarian neutrophils seem to be effector cells in the process of ovulation whereby the oocyte is expelled from the interior of the follicle (143). In addition, the corpus luteum that remains after a

rupture or tearing of the ovarian wall represents an injured tissue in need of protection from infection which may also be an explanation for the presence of neutrophils in this region (1512).

Findings of a study on distribution of neutrophil chemoattractants in different female genital tract tissues do not correlate with neutrophil recovery from tissues by enzyme digestion (1512). Cervical tissue should contain the greatest number of neutrophils as they presented with the highest amount of chemokine and IL-8 expression. Moreover, one would expect a higher amount of microorganisms at the cervix than in higher regions of the reproductive tract, resulting in greater need for innate immune protection by neutrophils.

Also with respect to adaptive immunity, the genitourinary immune system displays characteristic features that are distinct from those of other typical mucosal sites or the systemic compartment.

Antigen-specific IgA, IgG and IgM antibodies and SC can be detected in the external secretions of the human genital tracts. The dominance of IgA in the majority of external secretions, such as intestinal and nasal fluids, saliva, tears and milk, has for decades been considered a cardinal characteristic of the humoral arm of the mucosal immune compartment.
Despite highly variable information about the presence of different Ig classes in human female genital secretions (1467), recent findings have revealed that humane urine, seminal plasma and cervical or vaginal washings collected at various stages of the menstrual cycle contain an equal or higher proportion of IgG than IgA (556, 566, 608, 764, 1090, 1155). However, IgG/IgA ratios ranged from 2:1 to 10:1. Such discrepancies seem to reflect both the differences in the applied sampling method and individual variability such as age and stage of menstrual cycle (1090).

IgG and IgA antibodies in female genital tract secretions are in part produced locally by resident plasma cells and also derived from plasma, as reflected in their structural heterogeneity.
IgA1 and IgA2 are found in equal proportions in the female genital tract, therefore resembling secretions of the lower intestinal tract (922, 925). Unique is also that cervical mucus contains about 70% polymeric IgA and vaginal secretions contain almost equal proportions of polymeric and monomeric IgA whereas plasma contains about 90-99% monomeric IgA and saliva 95% polymeric IgA.

The ratios of IgA1 and IgA2 and the predominance of polymeric IgA in cervical secretions indicate that much of the IgA originates from local production, not from plasma (764). However, the high representation of the serum monomeric form of IgA in the vagina clearly demonstrates that, contrary to other mucosal surfaces, the genital tract relies heavily on antibodies derived from serum and systemic immunity. Because of the intrinsic resistance of IgA2 to IgA1 proteases of many pathogenic bacteria, the increased proportions of IgA2 may provide functional advantage to certain specific antibodies (763).
As there is a high percentage of the IgG1 subclass, IgG occurring in genital secretions is not only serum-derived, but also produced locally (556, 1227). However, the mechanisms involved in the transport of IgG into cervical secretions remain unclear (273).

Furthermore, many immunological aspects in the genital tract besides the distribution and properties of immunocompetent cells, namely the proportions of Ig isotypes and their molecular forms are under hormonal influence (423, 1408). Estrogen is said to increase Ig production and drives differentiation of CD4+ helper T cells toward Th2 regulation whereas androgens drive differentiation of CD4+ helper cells toward Th1 regulation. This could partly explain higher Ig levels and a a stronger humoral immunity in females than males (929).

The role of sex steroids in regulating immunity may be especially pronounced in the genital tract. Similar to the rat model (1518), both IgA and IgG peak shortly before ovulation with significantly higher Ig levels in women on contraceptives compared to other women (423, 764), which supports the general agreement that reproductive hormones enhance immunity via increasing Ig and expression of pIgR on uterine ECs (1518, 1519).
This relationship between estrous cycle and specific antibody levels likely reflects the changes that occur in the female reproductive tract during the course of the estrous cycle. Preovulatory until the time of ovulation, or at the time of mating, the female genital tract is subjected to numerous pathogens (1144). In fact, sperm has been shown to be a vector for bacteria, whereby the bacteria attach to the tails of sperm as they move up the reproductive tract. The need for protection of the female reproductive tract against pathogens is therefore especially high during ovulation.

Estrogen is said to increase IgG and IgM production by PBMCs due to inhibition of CD8+ suppressor cells (1058) whereas another study proposed secretion of IL-10 as responsible for the increased Ig production (661).
It is tempting to speculate that the effects of estradiol and progesterone on Ig levels and secretion are mediated indirectly by hormonal effects on CD8+ (and possibly CD4+) T cells, or accessory cells such as macrophages. In this regard it is interesting that the lymphoid aggregates found in human uterus have a central core of B lymphocytes surrounded by large numbers of CD8+ T lymphocytes which, in response to hormonal changes, would be in exactly the right anatomical location to regulate local antibody secretion (91).

Concerning distribution of Ig-producing cells, earlier immunohistochemical studies by **Kutteh et al**. found predominantly IgA-producing cells with highest percentages in ectocervix and vagina (759). Later, studies using ELISPOT found at least four times more IgG- than IgA-producing cells (273).
Brandtzaeg proposed that immunohistochemical studies have underestimated the IgG class because of interstitial staining (141). Moreover, the quantification of Ig-producing cells is difficult to perform due to their uneven distribution. The actual quantities of Ig-producing cells were measured as density by number of cells/10 low-power fields without any accurate definition of the evaluated tissue compartment. The endocervix was found to have the largest number followed by ectocervix, fallopian tubes and vagina (273, 761).

Almost all of the IgA-producing cells contain J chain, a marker of the synthesis of pIgA. Furthermore, cells in the single-layered epithelium of the fallopian tubes, uterus, endocervix, and ectocervical glands express the pIgR (923), which is essential for the selective transport of locally produced pIgA. Thus, all the structural and cellular components characteristic of active transepithelial transport of pIgA are present.
Among different genital tract tissues, the endocervix does not only show the highest number of Ig-producing cells, but also the highest percentage of J chain, SC and S-IgA (141) which suggest that the endocervix is likely to be a focal point for mucosal immunity in the genital tract.
Endometrial tissue was shown to have only few Ig-producing cells with predominantly IgG-producing cells (141, 759). The detection of IgA in the apical epithelium without presence of IgA immunocytes and the appearance of SC

throughout the endometrial epithelium suggest the selective external transport of serum-derived polymeric IgA, which was supported by staining for J chain (140).

SC-expression and IgA uptake in the endometrium also seems to be dependent on the phase of the menstrual cycle, showing a rise from the proliferative to the mid- and late secretory phase (118). The same study showed that the endometrial glands contained significantly more of all components of the secretory immune system in the mid- and late luteal phase than in the early half of the menstrual cycle.
This again confirms the thesis that steroid hormones also have a significant influence on the adaptive immune system in the female reproductive tract.

Whereas B cells are present in low numbers in the female genital tract compartments, T cells seem to make out up to 50% of all leukocytes, with CD8+ T cells predominating over CD4+ T cells (459, 1146). The highest number of T cells was found in the cervical transformation zone whereas normal vaginal mucosa contained few T cells.
Two studies at least found characteristic cervical and endometrial lymphoid aggregates consisting of a B cell core surrounded by CD8+ T cells and macrophages (626, 1560). Their structures presented similarities to inductive lymphoid tissue in various respect and as expected, they seem to be regulated in size and presence by sex hormones (1560). ER has proven to be present on T cells whereas the presence of PR on T cells remains controversial (91).
Further studies are needed to investigate the effect of sexual hormones on T cell function, especially in the genital tract.

It seems clear, however, that T cells throughout the genital tract are provided with cytolytic activity which is also hormonally regulated (1146, 1497). However, menstrual cycle and menopause had no apparent effect on cellular localization or abundance in any of the lower genital tract tissues which makes clear that the cervix, especially the transformation zone, is the major inductive and effector site for cell-mediated immunity in the lower female genital tract (1146).

To sum up, analysis of the female genital tract indicates that the key cells of the innate and adaptive immune systems are present and functionally responsive to antigens; however, there is a certain degree of compartmentalization. The identification of TLRs in the fallopian tubes, uterus, cervix, and vagina and the presence of ECs, macrophages, DCs, NK cells, and neutrophils throughout the

reproductive tract along with their responsiveness to selected PAMPs indicate that the female reproductive tract has evolved to meet the challenges of STDs, while at the same time supporting an immunologically distinct fetal placental unit. The innate immune system is characterized by a significant contribution of chemokines and cytokines that recruit and activate immune cells as well as produce bactericidal and virucidal agents, which confer protection at times when adaptive immunity is downregulated by sex hormones. Findings suggest that the mucosal immune system throughout the reproductive tract is orchestrated by sex hormones so that immune coverage occurs in a way that confers continuous protection.

The consequent link between endocrine and immune system in the female genital tract can also be observed during menstruation. In opposite to older theories which favoured the theory of vasoconstriction due to hypoxia (873), the concept of menstruation as the result of an inflammatory event has come up and been subject to several studies (413). This is mainly supported by the presence of leukocyte invasion and production of inflammatory markers and MMPs in the endometrium during the secretory phase (1249).

An interesting theory proposes a first phase of endometrial vasoconstriction and cytokine changes induced by progesterone withdrawal (12, 686, 873, 1384). A second phase then involves inevitable activation of lytic and apoptotic mechanisms due to hypoxia which may be due to steroid hormone withdrawal or and probably is progesterone independent (686, 1381, 1384).
Hypoxia, prostaglandins and cytokines such as IL-1 also stimulates local VEGF in endometrial stromal cells which both induces vascular permeability and acts as a chemoattractant for monocytes (1299, 268).

During the praemenstrual and menstrual phase there is a significant influx of activated inflammatory cells, i.e. mainly macrophages, NK cells, eosinophils and neutrophils, into the endometrium comprising up to 40% of total cell number within the functional endometrium, possibly due to progesterone withdrawal affecting PR-expressing endometrial cells (471, 623, 940, 1249, 1250).
However, most studies show a lack of evidence for PR or ER expression on endometrial leukocytes which suggests that effects of steroid hormones on human leukocytes may only be indirect (708, 1356). As glucocorticoid receptors are similar to PRs; it is highly likely that progesterone could act in progesterone-dependent tissues in a similar manner than glucocorticoids in other tissues.

Besides that, leukocyte migration into the endometrium is likely to be mediated by chemokines and cytokines such as IL-1, IL-8 or MCP-1 (105). Data propose that progesterone withdrawal thus can stimulate the premenstrual rise in chemokines IL-8 and MCP-1 (635, 639, 684, 685).
It can be concluded from several studies that chemokines modulate the recruitment of leukocytes into the endometrium and their expression is stimulated by the withdrawal of progesterone at the end of cycle.

Also the regulation of adhesion molecules responsible for leukocyte attachment during the menstrual cycle with a higher expression of ICAM-1 and PECAM in the secretory phase fits in this theory (1397, 1413). Besides entering the endometrium from the blood, some leukocytes, especially granulocytes, have been observed to proliferate in the endometrium, mostly during the secretory phase (1383). The question arises how inflammatory cells differentiate to different phenotypic subsets in the endometrium and how are they activated to release their mediators at appropriate time and place for menstruation.

This leukocyte influx induces production of MMPs and other enzymes with the potential to degrade components of the extracellular matrix (1248), which supports the theory that MMPs play a decisive role in the breakdown of cell-cell and cell-matrix adhesions of endometrial tissue (117).
Most MMPs are produced by endometrial stromal/decidual cells except MMP-7 which is an EC product and MMP-9 which is mainly produced by leukocytes including eosinophils, neutrophils, macrophages and some endometrial T cells (1209, 1248, 1580). Also MT1-MMP, perforin and elastase are produced by neutrophils and endometrial NK cells (1248, 1579) while mast cells with their proteases chymase and tryptase can activate the MMP cascade as well (623, 1249).

Regulated by cytokines and steroid hormones (1178, 1249), the patterns of expression of MMPs during the menstrual cycles are different in normal human endometrium. MMP1, MMP-3, MMP-7, MMP-9 and MT1-MMP mRNA and protein are all substantially increased immediately prior to and during menstruation (1209, 1579) which proposes them as the mediators of tissue breakdown at menstruation.

Withdrawal of progesterone may contribute to the recruitment and activation of leukocytes by inducing the production of cytokines and chemokines by endometrial and ECs of the endometrium. Furthermore, withdrawal of progesterone may directly

promote MMP production by resident endometrial cells (868, 1247). Several *in vitro* studies demonstrate that progesterone is a potentially important player in regulation of MMPs in the endometrium.

The unique immunological characteristics of the female genital tract must be considered in the design of vaccines for the protection against microbial diseases, especially against STDs.

Despite the uniqueness of the female genital tract as a mucosal immune system, the concept of a common mucosal immune system with dissemination of activated immune cells from inductive sites to other distant mucosal effector sites offers great hopes and oppurtunities in the field of vaccine development (287). This is dependent on characteristic homing receptor-ligand interactions. The presence of adhesion molecules like ICAM-1, VCAM-1 and selectins, but not MAdCAM-1, in female genital mucosa was reported by **Johansson et al**. (626).
This indicates that the lymphocytes which are destined to the genital tract do not use the MAdCAM-1-α4β7 integrin interaction used by intestinal lymphocytes, at least in non-inflamed tissue (1364). However, it can be proposed that, in response to infection, cytokines produced by genital tract ECs such as TNF-a, IL-1 and IL-6 can induce the expression of both VCAM-1 and MAdCAM-1.

Investigators have attempted to elicit secretory immunity in the genital tract by using different routes of immunization, i.e. vaginal, nasal, rectal and parenteral routes. Systemic immunization warranted some success but further studies are needed (272, 1227, 1410). Remote inductive site immunizations, especially by intranasal route or combinations of systemic and various mucosal routes, results in induction of sustained IgG- and IgA-mediated humoral immune responses.

Numerous experiments have shown that the local instillation of various antigens, especially together with an adjuvant, into the vagina of experimental animals or of human female volunteers can result in the development of specific antibodies in the local secretions (1041, 1409, 1410).
The study by **Kozlowski et al**. where oral, rectal and vaginal immunization increased levels of specific IgG in serum and specific IgA in saliva similarly but only vaginal immunization significantly increased both specific IgA and specific IgG in female genital mucosa (743, 744).

Generally, the levels of these responses are quite modest and moreover the responses are not disseminated either to remote mucosal sites or to the systemic compartment represented by serum antibodies. Local intravaginal immunization required large and repeated doses of antigen. Also the influence of the menstrual cycle should be taken into consideration. Furthermore, local immunization of the male tract is unlikely to be practicable.
These considerations and findings sustain the notion that the genital tracts represent effector sites of the central mucosal immune system, but as inductive sites they serve only for the generation of local responses.

The potential importance of rectal lymphoid tissues as an inductive site for stimulation of humoral immune responses in the human female genital tract is based on the equal distribution of IgA subclasses at both sites.
In general, the rectal immunization route generated variable results, depending on the type of antigen, frequency of vaccine administration and stage of menstrual cycle (272, 743, 744, 745, 760). To generate long-lasting humoral immune responses, however, the combination of several mucosal immunization routes was more effective, for example oral and rectal immunization in the trial of **Kutteh et al**. (760).

Animal studies also emphasized the importance of the inductive sites in the nasal cavity for the generation of mucosal, including genital, and systemic immune responses that may exceed in magnitude those induced by oral immunization (1227).
Experiments on mice and women convincingly demonstrated that viral or bacterial antigens in combination with CTB instilled into the nasal cavity induced superior immune responses in local secretions as well as in saliva and female genital tract secretions (111, 625, 1222, 1228, 1537). Furthermore, the complicating factor of hormonal influences could be avoided by using the nasal route, which offers the additional advantage of potentially inducing high levels of specific IgG antibodies in the circulation, and requiring lower antigen doses.
It was concluded that a combination of nasal and vaginal vaccination might be the best vaccination strategy for inducing protective antibody responses in both cervical and vaginal secretions, provided that the vaginal vaccination is given on optimal time points in the cycle (627).
Therefore, the nasal immunization route in particular deserves further testing with vaccines and potential adjuvants for prevention of STDs.

B. Immunology of genital tract infections

1. HSV

Genital herpes infection remains one of the most prevalent STDs worldwide. About 25% of sexually active adults are infected in the United States (416) and 20-35% of pregnant women are estimated to be HSV-2 seropositive (880). Its increasing prevalence (1337), the frequency of clinical recurrences and asymptomatic shedding (1465) as well as its link to HIV transmission (1466) emphasize the urgent need to develop new preventive strategies to control HSV.
Genital herpes is more common in women than in men, with approximately one in four women versus one in five men having specific antibodies to HSV-2 (416). This gender difference suggests that the female genital tract provides a more permissive environment for HSV-2 infection and the establishment of latency.

The role of immune factors in the control of HSV infection, especially recurrent lesions after viral reactivation, appears complex.
TLR2 and TLR9 seem to be involved in cell signaling in response to HSV-1 and HSV-2 mediating HSV-induced cytokine secretion (840, 756). The first line of defense against genital HSV infection then consists of mucus, normal bacterial flora, acidic pH and secreted proteins such as complement, defensins or SLPI. The second implies early virally induced responses by ECs and resident DCs, which are characterized predominantly by IFN production. Thirdly, there is the recruitment of cellular effectors including neutrophils, macrophages, and NK cells (347, 1584).

Recent studies by **Mascasullo et al**. demonstrated the irreversible antiviral effect of acidic pH≤4.5 on HSV-1 or HSV-2 (880), which confirms hints of earlier studies (1218). Exposure to acidic pH seems to disrupt the viral envelope and prevents binding and invasion. The significance of these *in vitro* observations was confirmed in a murine model with the vaginal buffer AmpHora (440), which suggests a protective role of vaginal acidic pH against HSV infections.
Findings suggest that CVS might contribute to innate resistance to HSV-2 and identified defensins as contributors to this activity (633). They may provide a prototype for future topical microbicides. This is also implicated in earlier *in vitro* studies on the protective effect of HNP1-4 and HD-5/6 against HSV (1557, 880).

However, due to a relatively low concentration of HNP 1-3 in CVL these findings suggest also the effects of other defensins as contributors to this activity.
Also other genital tract proteins such as SLPI (880), lactoferrin (869) and SP-D (921) may play a role in innate immunity against HSV, but this has to be investigated further.
Studies suggest a potential role for complement as a genital tract host defense against HSV besides other infectious diseases (835, 432); and HSV has evolved several strategies to evade immune attack by the classical and alternative complement pathways in inhibiting C3, for example (429).
Less is known about the role played by MBL and the lectin pathway as a host defense against genital herpes infection or whether HSV also evades this complement pathway.

The precise role played by the different DC populations in the immune response to HSV in the female genital tract is not clear; and, notably, differences have been observed for HSV-1 and HSV-2.
HSV-1 may interfere with immune responses through infection of immature DCs and selective downmodulation of costimulatory molecules (932), which leads to delayed activation of T cells and allows more time for replication of HSV type 1 in epidermal cells. These findings support the notion that immature DCs respond to HSV challenge by impaired maturation. Further results indicate that HSV-1-infected mature DCs are limited in their capacity to migrate to lymph nodes, thus inhibiting an antiviral immune response (1141).
In a study on rhesus macaques, HSV-2 was found to induce apoptotic death, decreased expression of costimulatory molecules and increased release of cytokines by monocyte-derived DCs (1106). This coincided with HSV-2-infected DCs stimulating weak T cell responses. Similar effects were observed in HSV-2-exposed human DCs.

In contrast, results from other studies suggest a previously unanticipated role for submucosal DCs in the generation of protective Th1 immune responses to HSV-2 in the vaginal mucosa (821, 1584). The observation that submucosal DCs, but not LCs, are the primary cells responsible for T cell priming in the draining lymph nodes after intravaginal HSV-2 infection is also supported by work from **King et al.** (711) and **MasCasullo et al.** (880).
Further studies are ongoing to define better the role that DCs play in mucosal response to HSV infection in the genital tract.

The exact role of NK cells and neutrophils in genital HSV infection is elusive.
Studies in mice have shown a potential role of NK and NK T cells in immunity against genital HSV-2 (58) and the role of NK cells in innate immune responses against HSV-2 in humans also seems essential (293). However, because NK cell deficiencies involve other immune functions, it is difficult to determine the precise role played by NK cells in these cases.
Studies on lymphocyte-deficient mice implicated that their impaired innate resistance to HSV-1 is not dependent on NK cells (497) but due to the differences in these study models one probably cannot transfer these results to human HSV-infection.
Murine studies suggest that neutrophils may not be critical for primary HSV infection (946) but somehow help in virus clearance from the genital mucosa after primary infection. The mechanisms by which neutrophils mediate antiviral activity are not well understood but may implicate release of antiviral cytokines or ROI/RNI (880, 182).

Despite the recognition that innate immunity provides the first line of defense during both primary and recurrent HSV infection and plays a critical role in preventing infection and in limiting viral replication, the specific factors such as antimicrobials and TLRs that contribute to the mucosal response in the female genital tract have only recently begun to receive attention.
Further studies are needed to provide a better understanding of the contribution of specific components of mucosal immunity and the mechanisms by which virus may evade these immune strategies. Defining this activity is crucial, as these factors might be exploited in the development of microbicides.
Susceptibility and immune responses to STDs including genital HSV-2 infection are profoundly affected by sex hormones. Studies have already indicated that intake of oral contraceptives influences susceptibility to candidiasis, HSV-2, HIV-1, and chlamydial infections in women (876).

Interestingly, previous studies have shown that mice are susceptible to intravaginal HSV-2 infection primarily during the diestrous phase of the estrous cycle (1086). Thus, treatment of mice with progesterone which maintains the mice at a diestrus-like stage is often required for consistent intravaginal infection with HSV-2.
However, the precise mechanism by which progesterone treatment increases the susceptibility to intravaginal HSV-2 infection is unknown. It is suggested that this

is due to diminishing thickness and increased permeability of the vaginal epithelial layer during diestrus with high progesterone levels (604).
Kaushic et al. demonstrated that progesterone (Depo-Provera) treatment of mice led to a 100-fold increase in susceptibility to genital HSV-2 compared to untreated mice at diestrous phase (671). Further, longterm effects of Depo-Provera treatment included reduction in protective immunity to HSV-2. This study highlights the immunosuppressive role of Depo-Provera and also demonstrates that the thickness and gross morphology of the vaginal epithelium alone cannot account for the susceptibility to HSV-2 infection.

As another possible mechanism for the increased susceptibility to HSV-2 at the diestrous phase it can be hypothesized that APCs are less abundant in the vagina during the susceptible diestrous phase and more abundant during the resistant estrous stage. However, analysis of the distribution of the DCs during the estrous cycle revealed that these cells are abundantly present in the epithelium of mice at diestrus, whereas they are only sparsely present in the basal layer of the vaginal epithelium in estrus (1584), which suggests that the distribution of the APCs per se does not account for the susceptibility to virus infection at different stages of the estrous cycle.

Finally, it is possible that the virus entry receptors are expressed differently during the hormonal cycle and that the relevant receptors are expressed only during the susceptible stages. Interestingly, nectin-1, the major co-receptor for HSV entry in the genital tract, is expressed in the epithelium of the mouse vagina only during the stages of the estrous cycle in which mice are susceptible to vaginal HSV (821).
Limited clinical studies suggest that asymptomatic HSV viral shedding is not related to contraceptive use or menstrual cycle (145). Moreover, nectin-1 is expressed throughout the menstrual cycle in human beings (821).
Further studies are needed to elucidate the relationship between mucosal immunity, the hormonal environment, and response to HSV challenge.

Extensive studies have demonstrated that in HSV-2, as in many other viral infections, both antibodies and cell-mediated immune responses in the systemic and mucosal compartments contribute to protection against infection and to clearance of infected cells. However, the majority of studies have been performed on mice due to ethical and methodologic limitations.

Several basic indications show the greater importance of T cell responses than the persistence of neutralizing antibodies in recurrent infections. AIDS patients have longer prevalences of herpetic lesions (1301) and mice depleted of CD4+ and CD8+ T cells prior to genital HSV-2 infection shed virus for a prolonged period, confirming a role for T cells in virus clearance (1087).

Early murine studies of the immunology of HSV infection suggested that IFNs and macrophages were an important part of the initial immune response and that the most important protective specific T-lymphocyte response was that mediated by CD8+ lymphocytes. However, immunohistology of biopsies of human recurrent herpetic lesions revealed a sequence of immune cell infiltration beginning with CD4+ lymphocytes and macrophages in the first 2 days around the infected epidermal cells which also develop strong HLA-DR expression (281). This was followed by an influx of CD8+ lymphocytes, which normalized the balance between CD4 and CD8 lymphocytes. The CD4/CD8 ratio is not restored to that of the blood until after 2 days, indicating an early CD4+ and a later CD8+ lymphocyte influx (281).

Several studies have demonstrated that Th1 CD4+ T cells are necessary and sufficient to provide protective immunity to HSV-2. Murine studies on the local T cell response indicate that CD4+ and Th1-like cells dominate in number in genital lymph nodes and secretions after intravaginal HSV inoculation and suggest a predominant role for CD4+ T cells in anti-HSV-2 immunity (944, 753).
Further, it was shown that the CD4+ T cell-meditated protection was linked to their ability to secrete IFN-γ (509, 281).

The mechanism of the Th1-mediated protective immunity to HSV-2 likely involves several pathways. It is possible that CD4+ T cells are involved in the direct killing of HSV-infected ECs, as these effector cells are rapidly recruited to the sites of secondary infection (1086). IFN-γ has also been shown to act on vaginal ECs to restore MHC class I expression and up-regulate MHC class II expression (1427), which may contribute to the recognition by CD4+ effector T cells. Alternatively, IFN-γ could exert protection by activating macrophages and neutrophils for enhanced phagocytosis of infected cells, resulting in the secretion of TNF-α and the release of NO. Finally, IFN-γ secreted during the secondary viral challenge may also be important in the recruitment of memory and effector lymphocytes to the infected vaginal epithelium (1091).

Antigen-specific T cells have been detected in the uterine cervix of women with genital HSV-2 infection where CTL activity was both associated with CD4+ and CD8+ T cell populations (732). This is consistent with the finding that clearance of HSV-2 skin lesions correlated with the infiltration of CTLs of both CD4+ and CD8+ phenotypes (731). Recent studies concerning the specificity of CTLs indicate that CD8+ T cells from genital herpes lesions recognize viral tegument and immediate early proteins as their targets (729).
The importance of CTL-mediated clearance of HSV is evidenced by yet another immune evasion mechanism employed by the virus. Its genome encodes the ICP-47 gene product, which can bind and block the TAP complex to prevent assembly f MHC class I peptide and antigen presentation to CD8+ T cells (1424, 544).

Women with symptomatic genital herpes have antibodies to HSV-2 of both IgA and IgG isotypes, as well as IgM in CVS (59, 60, 894) and in serum (519). External secretions including cervical mucus contain both IgG and IgA antibodies, but these differ in their specificity for various HSV-2 antigens (60). Moreover, cervical anti-HSV IgA antibodies either free or with SC as S-IgA also differ with respect to their levels and specificities for various HSV-2 antigens, suggesting origin from circulation as well as by local production.
Remarkably, nearly all specific viral antibody in vaginal mucus in HSV-immune mice was of the IgG isotype whereas S-IgA seems to contribute little to protection (1079, 943, 1082, 1085). However, these observations did not eliminate the possibility of contribution of vaginal IgA in immune protection against HSV-2, since ELISA titers of specific antibodies do not necessarily predict functional virus neutralization (1092).
The importance of IgG in HSV immunity is also evidenced by the immune evasion mechanism employed by the virus. The HSV gE and gI together comprise a high affinity Fc receptor expressed by infected cells for the purpose of absorbing and inactivating anti-HSV IgG (998).

The role of antibodies in protection of the female genital tract against HSV-2 could be seen controversial.
Anti-HSV mAb failed to protect against vaginal challenge infection and specific antibody was not detected in vaginal secretions after parenteral immunization that produced high antibody titres in serum (896). Immunity against vaginal HSV-2 infection was comparable in intact and IgA-deficient mice and only little vaginal specific S-IgA was detected after local immunization in the vagina (1082, 1088).

Kuklin et al. reported that intranasal immunization of mice with recombinant vaccinia virus produced high titers of specific IgG and IgA in the vagina but failed to prevent epithelial infection (753).
These results would suggest a protective immunity in vaginal mucosa which is mainly based on T cells which was underlined by other experiments as well (945, 1310).
Passive transfer of serum IgG from immune mice diminished a vaginal challenge infection even though mean antibody titer in vaginal secretions of mice was only 8% of that measured in actively immunized mice (1082). Purified IgG from vaginal secretions of immunized mice neutralized HSV-2 in vitro. The protective effect of HSV-2 specific antibodies has also been demonstrated by passive intravaginal immunization shortly before HSV-2 challenge given by the same route (1496, 1575).

The importance of B cells and antibody-mediated protection in vaginal HSV-2 infection was shown in B cell-deficient mice. In contrast to intact mice, these animals displayed diminished immunity to intravaginal challenge as well as increased viral shedding (1088). Antibody is supposed to mainly act early during immune resistance to challenge infection, whereas cell-mediated immunity primarily acts later. An early role for antibody is consistent with the presence of neutralizing IgG in vaginal secretions of immunized mice (1082) whereas memory T cells, on the other hand, require several hours to secrete substantial amounts of IFN-γ in the vagina in response to the challenge antigen and may require even longer developing cytolytic activity (1091).

Differences in study designs and experimental procedures may be responsible for different results. The route of immunization also influences antibody-mediated protection. Secreted antibody is relatively more protective in vaginally immunized mice than in parenterally immunized mice as local immunization leads to accumulation of plasma cells and antibody in the vagina (1083).

The conclusion may be that herpetic lesions show all the hallmarks of the predominantly Th1-pattern, with the induction of MHC class II on epidermal cells being particularly prominent. IFN-γ has a critical role within the lesion in up-regulating MHC classes I and II on infected cells, in activating macrophages and in preventing the spread of epidermal infection by the virus in synergy with IFN-α. Destruction of infected cells and the induction of antiviral immunity within adjacent

uninfected cells are complementary activities of early CD4 and late CD8 lymphocyte infiltration within the lesion.
This indicates that recurrent disease is prevented by virus-specific Th1 immunity, most notably by IFN-γ, and its ability to enhance the innate immunity. Treg cells and Th2 cytokines such as IL-10 downregulate this immune profile, thereby allowing for unimpeded replication of reactivated virus and recurrent disease. A Th2 pattern leading to neutralizing antibody production may prevent cell-to-cell transmission of the virus.

Alternative immunologic treatment options have gained attention during the last years.
The IRM Resiquimod is said to initiate immune cells such as DCs and macrophages to produce Th1 immune responses via cytokine production (942) and may therefore be suitable for treatment of genital herpes. Animal models and phase II studies showed quite promising effects in delaying genital herpes recurrency (112, 1333); however, phase III clinical trials were suspended due to the lack of efficacy of resiquimod gel in recurrent genital herpes lesions (1333). It may have a comeback as a vaccine adjuvant for viral infections that require a strong Th1 immune response.
A similar effect with induction of vaginal Th1-type cytokines and increase of B and T cells in genital lymph nodes was demonstrated by vaginal application of CpG ODN in mice and ginea pigs (507, 1149).
The purpose of a study by **Kwant et al**. was to determine whether intravaginal immunization with recombinant glycoprotein B (rgB) of HSV-2 plus CpG ODN can induce specific immunity and protect against genital HSV-2 challenge (769). Mice immunized with rgB+CpG had higher levels of anti-gB IgA and IgG in the vaginal washes and serum compared to mice immunized with rgB alone. Mice immunized with rgB +CpG showed higher survival and lower pathology scores following genital HSV-2 challenge than mice immunized with rgB+non-CpG ODN or rgB alone.

Gallichan et al. showed that intranasal immunization with CpG ODN plus rgB of HSV-1 resulted in significantly elevated levels of specific anti-gB IgA antibodies in vaginal washes that remained high throughout the estrous cycle (434). Additionally, dramatically elevated numbers of specific IgA-secreting cells were present and persisted in the genital tract in response to intravaginal HSV-2 challenge. Strong CTL responses were observed locally in the genital tissues of both CpG and non-CpG ODN-immunized mice following intravaginal HSV-2 challenge. Interestingly,

mice immunized intranasally with rgB plus CpG ODN, but not non-CpG ODN, were significantly protected following intravaginally HSV-2 challenge. In conclusion, these results indicate that intranasal immunization with CpG ODN plus protein mediates immunity in the female genital tract capable of protecting against a sexually transmitted pathogen.

McCluskie et al. recently compared the efficacy of CpG ODN and resiquimod for topical immunotherapy of intravaginal HSV-2 infection in mice and demonstrated efficacy for CpG ODN but less so for resiquimod (895). Intravaginal administration of GpG ODN resulted in a strong local but weak systemic immune response, as determined by the levels of chemokines as IP-10 whereas intravaginal administration of resiquimod resulted in high levels of plasma IP-10 and weaker local immune responses.

These findings provide a basis for further intervention studies of the efficacy of CpG ODN in humans.

Vaccination to prevent HSV infection, or to reduce reacitivation frequency and severity, would reduce morbidity, the risk of dissemination to newborns and the risk of HIV acquisition. Vaccines may be sought for two independent purposes; prophylaxis in uninfected individuals and therapy in already infected.

History of HSV vaccine development goes back for almost 80 years. HSV vaccines may be divided into the two groups of live or inactive vaccines. Live vaccines contain organisms capable of at least limited replication in vivo, whereas inactive vaccines are incapable of replicating. The vaccines may be further subdivided into the categories: attenuated HSV vaccines (912), replication-limited HSV vaccines (904), vaccines consisting of live nonpathogenic replicating vectors engineered to express HSV gene products, inactivated HSV vaccines (689, 1490, 881), subunit vaccines (1336, 956), and nucleic acid (plasmid) vaccines (136, 449).

Many types of vaccines have been evaluated in clinical trials of immunogenicity and prophylactic or therapeutic efficacy (339, 689, 1490, 881, 758, 1315, 1360, 1361, 1339). Some of the early vaccines either showed low immunogenicity, safety flaws or appeared immunogenic but the trial design then precluded interpretation of efficacy.

The only randomised clinical trials of a vaccine against genital herpes showing efficacy so far, are those with the HSV-2 glycoprotein-D-alum-MLP vaccine of GlaxoSmithKline (1339, 1338). These studies demonstrated that the vaccine was

over 70% effective in preventing genital herpes disease, but only in women who were negative for both HSV-1 and HSV-2 before receiving the vaccine. The vaccine was shown to induce both gD-specific neutralizing antibodies and a Th1 cell-mediated immune response.

Another vaccine containing HSV-2 glycoprotein B2 and D2 together with the adjuvant MF59 failed to show long-term efficacy in preventing HSV acquisition; HSV disease was not reported as an endpoint in these trials (254).
Interestingly, the HSV-2 antibody titers did not differ between vaccinated, uninfected and vaccinated, finally infected individuals which suggests that neutralizing antibody alone is not sufficient to protect against genital HSV-2 infection and that the vaccine failed to induce critical cell-mediated immune responses against HSV-2 infection. Possible explanations for the loss of efficacy could be that the protective immune response was only short time or that initial protection was lost with frequent exposure to the virus (1339).
Also the adjuvant used in both vaccines may be critical in facilitating the induction of protective immune responses as the MF59 adjuvant induces a Th2-pattern of response (1313), whereas the MPL adjuvant induces a Th1-pattern of cytokine response.

The difference between herpes disease and herpes infection is crucial in interpreting the studies (495). The primary endpoint of the GSK study was the occurrence of genital herpes disease and no distinctin was made between HSV-1 and HSV-2 as causes. As HSV-1 is responsible for a a high rate of primary genital herpes, recipients may be protected against disease caused by either virus.
An effective HSV-vaccine might mimic the immunological process underlying the phenomenon that previous HSV infection protects against HSV disease by reducing its severity. It appears that in HSV-1 seropositive, HSV-2 seronegative persons, the GSK vaccine provided no additional protection over their presumed HSV-1 induced immunity.
There might also be hints in statistical analysis of the GSK studies that the study was insufficiently powered to detect a difference in infection rates between placebo and vaccine (495). Also the significantly different results in efficacy for men and women rise questions.
To evaluate all this further, a larger phase III study of 7550 females with the GSK vaccine is currently ongoing.

However, the development of an effective vaccine against genital herpes seems to be under way. If it is ideal to protect the public from rising rates of genital herpes remains speculative. Further areas of investigation should include the route of immunization and suitable adjuvants.

2. HIV

Women comprise an alarming number of newly diagnosed cases of HIV infection (1531, 571). Worldwide, 70 to 80% of HIV-1 infections are transmitted through heterosexual contact, and the majority from male to female. Nonetheless, only one in 200 to one in 1000 encounters results in productive infection which emphasize the effectiveness of structural and cellular barriers to virus entry (479).

As the genital tract mucosa is the site of initial contact with HIV-1 for the vast majority of exposed individuals, study of the virus from the genital tract is critical for the development of vaccines and therapeutics. Therefore, a better understanding of the immunological mechanisms in the genital tract mucosa occurring during HIV infection is essential. During the last years, significant progress has been made in understanding anti-HIV immunity in the vagina by using the SIV/rhesus monkey model of heterosexual HIV transmission (937).
Dysregulation of the immune system of the body is seen in association with HIV infection, with a fall in the CD4+ lymphocyte count and a reversal of the CD4/CD8 ratio now being well recognized systemic manifestations of infection. However, alterations in mucosal immunity in the presence of HIV infection have been clearly documented in both the gut (617) and the lungs (10) whereas in contrast, the female lower genital tract has not been as extensively studied.

During sexual intercourse, free HIV virus and virus-infected cells in semen are deposited within the vagina (1101). Understanding the mechanism of HIV-1 transmission within the female genital tract requires definition of the cellular targets for infection, and whether susceptibility to infection varies under different hormonal and inflammatory conditions.

Several reports have identified DC, resting and activated memory CD4+ T cells, and macrophages as the earliest cell populations to become positive for SIV- or HIV RNA following a non-traumatic exposure to the virus (491, 574).

As mentioned, the cervicovaginal mucosa contains a complete set of immune cells including APCs, CD4+ and CD8+ T cells and B cells. Cervical biopsies from HIV-positive women have shown significantly reduced levels of LCs but increased numbers of macrophages as well as an increased proportion of activated macrophages (13) suggesting that local mechanisms of T-cell activation are in place. Although in chronically HIV-1 infected women, T cells, macrophages, and LCs in cervical tissue are infected with HIV-1 (1137), analysis of lymph nodes in HIV-infected men indicates that active virus replication occurs in activated and resting CD4+ T cells (1583).

However, there are no *in vivo* data to indicate the cell types that first become infected in the reproductive tract of women.

In the SIV-rhesus monkey model, there are controversial data on which cells are first infected during sexual transmission. **Zhang et al**. proposed CD4+ T cells as the first cells to become infected after intravaginal inoculation of SIV (1583) whereas **Hu et al**. demonstrated the presence of SIV RNA in DCs shortly after intravaginal exposure of SIV (574).

One problem in studying HIV-1 transmission in humans is the lack of a suitable *in vitro* model. **Gupta et al**. used a cervical tissue-derived organ culture model which seems suitable to provide the natural tissue architecture and identified memory CD4+ T cells as the first cells that became infected during HIV-1 transmission across the cervical mucosa (491). This would imply a higher replication of HIV in T cells which would allow the virus to expand and get transferred to DCs. DCs are considered important target cells in HIV infection and transmission, as the cell surface receptor DC-SIGN is thought to be one of the receptors to bind and internalize virus prior to its transmission to lymph nodes where the infection would be passed to CD4+ T cells (333).

Although HIV can be recovered from the vagina of women who have had a total hysterectomy, most genital virus arises from the cervix and possibly the upper genital tract (378, 251). Studies on leucocyte populations in the human cervix showed leucocytes in the squamous mucosa and submucosal stroma to be predominantly T lymphocytes and DC which both did not vary in numbers or distribution during the menstrual cycle (1138).

Due to the findings of **Pudney et al**. with the cervix and especially the transformation zone as the major inductive and effector site for cell-mediated immunity in the genital tract, it would also speak for the cervix as primary infection site of HIV-1 (1146).

Expression of HIV receptors and coreceptors is likely to correlate with susceptibility to viral infection. In defining if the ectocervix or the uterus serves as first site of HIV infection, the studies by **Yeaman et al**. on expression of HIV receptors CD4 and GalCer and coreceptors CCR5 and CXCR4 in the female genital tract have given important clues.

It has been shown that HIV-1 infects viable tissue sections and isolated cells from both the lower and upper female genital tract suggesting that both ECs and submucosal leukocytes may be targets for initial HIV-1 infection (571).
HIV-1 strains that utilize the CXCR4 chemokine receptor (X4) for infectivity are able to undergo reverse transcription, integration, viral DNA transcription and viral release whereas viral strains that utilize CCR5 (R5) do not undergo these early replicative events and are only released unmodified from these cells (570).
Endometrial ECs were shown to express all four receptor types and altered expression of these receptors as a function of menstrual cycle stage could serve to either enhance or inhibit HIV-1 infection (1561).
It also seems probable that chemokine receptors, by virtue of their selective binding to HIV-strains X4 or R5, play a role in the selective uptake and transmission of virus within the genital tract (61). Results indicated that uterine ECs could preferentially bind R5 strain during the proliferative phase whereas during the second hal of the cycle they may be more susceptible to X4.

In contrast, there was a lack of CCR5-, CXCR4- and CD4-expressing cells accessible to HIV in the lumen of the ectocervix (1561). Results suggest that HIV infection of cells in the ectocervix could most likely occur through GalCer and CCR5 which were both expressed on ectocervical ECs throughout the menstrual cycle.

Thus, on the basis of findings of HIV-1 infection and HIV-1 receptor and coreceptor expression on uterine ECs, it appears that in comparison to the ectocervix, the uterus is a more likely first site for infection and transmission of virus after vaginal intercourse.
In the uterus, HIV-1 transmission could involve secretion of newly synthesized infectious virus from the uterine ECs, or viral transmission by cell-to-cell contact between the infected EC and a susceptible leucocyte. In contrast, the lack of CD4, CCR5 and CXCR4 expression on ectocervical ECs and the inability of these cells to develop a productive infection by HIV-1 (324) suggest that HIV-1 transmission in the ectocervix it is more likely to occur through the gradual release of infectious

unmodified virus that is then able to infect susceptible submucosal leucocytes. An alternative mechanism of HIV infection of the lower female genital tract involves the transcytosis of endosome-internalized HIV through the epithelium barrier, without EC infection (558).
It is also known that mutations in chemokine receptors markedly reduce the likelihood of acquiring HIV infection following exposure to virus. The well-described deletion in CCR5 has been shown to protect against HIV transmission among discordant couples (115).

One of the most important barrier functions to HIV transmission seems to be an intact and healthy vaginal and cervical epithelium with a normal microbial flora and low levels of inflammation. Disruptions to the genital epithelium, which can be induced either by agents in contraceptives or microbicides or infectious agents, enhance HIV transmission. Growing evidence suggest that not only the presence of STDs but also BV supports HIV transmission in the genital tract (28). Enhanced recruitment of activated immune cells, disruption of the epithelium barrier, reduction of Th and CTL function, which are all associated with STDs, may contribute to this STD-mediated enhancement of HIV detection in the female genital tract (27).

Concerning other innate immune responses to HIV infection in the genital tract there have not been enough studies to concretely evaluate these mechanisms at this site.
Newer studies may implicate a role for antimicrobials such as defensins and SLPI in controlling HIV infection within the genital tract. Higher SLPI concentrations in vaginal fluid samples correlate with a reduced rate of perinatal HIV transmission (1123) and SLPI blocks HIV infection of macrophages via annexin II (907, 849). Hormonal regulation of SLPI together with its anti-HIV activity may also be important factors in the susceptibility of women to HIV infection.
HBD1-4 also seems to be involved in innate immune responses to HIV infection in the genital tract. Promising findings underline their possible role (1476, 1539, 1157, 138); however, more studies have to further elucidate the role of antimicrobials in HIV immunity in the female genital tract.

Immature DCs express CCR5 and CXCR4 as well as DC-SIGN and have been demonstrated to be one of the immune cell species which are infected with HIV-1 (1317, 442). However, they are infected with lower efficiency than CD4+ T cells and

macrophages. In contrast, mature DCs do not efficiently replicate the virus and may therefore represent an important reservoir of latent virus infection (1317).

One can speculate that several functional impairments of DCs and other APCs including inability to stimulate T cell proliferation (420) and a reduction of DCs and NK cells may contribute to impaired elimination of infected cells (420). However, further studies need to be done to evaluate these findings and if they can also be transferred to the genital tract mucosal immune system.
It has been proposed that HIV binds to DC-SIGN on DCs in the genital tract and is internalized into nonlysosomal compartments where it retains infectivity, then transported to lymph nodes and presented to T cells. However, DC-SIGN is not expressed on LCs, the most superficially located DCs. It seems likely that there are other types of HIV receptors expressed by DCs (1432).

It might be expected that humoral immune responses to mucosally encountered HV-1 would result in the predominance of S-IgA anti-HIV-1 antibodies. Instead, it is known from several studies that anti-HIV-1 antibodies are present in the vaginal secretions of infected individuals and anti-SIV antibodies in vaginal secretions of infected rhesus macaques with IgG as the major isotype (939, 833, 101). IgA antibodies are either absent or present at low levels. This is also the case in urine, intestinal fluid, saliva, and tears.
The relative contributions of serum-derived and locally produced antibodies needs further study. However, there is evidence that at least some of the anti-HIV-1 IgG antibodies are locally produced (938).
Recently, it has been demonstrated that plasma-derived IgG antibodies were functional in the prevention of vaginally induced SIV infections in monkeys (67). Thus, there is hope that intensive systemic active immunization with relevant HIV-1 antigens may provide long-term protection against genitally encountered HIV-1 infection.
There is also clear evidence of cellular antiviral effector function in the female genital tract. HIV-specific CD8+ CTL are present in the cervicovaginal mucosa of infected women as well as anti-SIV CTL in vaginal and cervical epithelium of rhesus macaques (993, 938, 1281, 827). Elevated numbers of CD8+ cells seen in the ectocervix of HIV-1 women demonstrates the active recruitment of nonresident CD8+ lymphocytes rather than an expansion of the resident intraepithelial population.

Increased proportions of these CD8+ cells in the ectocervix are activated; however, as elsewhere, these cells do not appear to be capable of mounting an adequate immune response to the virus. Mucosal CD8+ T cells may be functionally impaired relative to those in the blood, both in terms of lytic activity and IFN-γ production (13, 906).

In the uterus, CD8+ T cells were found to be noncytolytic during the secretory phase and CTL activity is higher in postmenopausal women than in premenopausal women (571). One could postulate that this would lead to a higher viral susceptibility at the uterine level during the secretory phase.
Furthermore, the decrease in percentage of CD4+ T cells typically seen in HIV-infected persons is also detectable in in the lamina propria of the genital tract in monkeys (1352) and humans (13).
The relative importance of local HIV-specific antibodies and CD8+ CTL immune responses is presently not exactly clear. Animal models suggest that virus-specific CD8+ T cells together with adequate help of CD4+ T cells can efficiently suppress replication of SIV and HIV-1 and slow down disease progression (807). Antibodies play an impotant role in controlling most viral infections, but HIV envelope proteins are variable and evade a neutralizing antibody response.

Studies also show a significant effect of proinflammatory cytokines on replication and spread of HIV. IL-2, TNF-α, IL-1, and IL-6 can upregulate HIV replication, whereas INF-α, TGF-β, IL-10, MIP-1α, MIP-1β, and RANTES can downregulate HIV (380). The balance between HIV-inducing and HIV-inhibiting cytokines may impact the viral load in the mucosa and subsequently, the sexual transmission of the virus.

Levels of the cytokines TNF-α, IL-1β, MIP-1α, IL-6 and IL-10 were found to be significantly elevated in genital secretions of HIV-infected women compared to healthy controls (102, 35, 271). HIV-1 therefore leads to an induction of proinflammatory cytokines in the genital tract mucosa.
These results parallel reports demonstrating increased concentrations of Th2-type cytokines, IL-6 and IL-10, in the systemic circulation and at mucosal surfaces of HIV-positive adults (239, 1045). It also corresponds to reports of increased IL-10 mRNA expression in cervical biopsy specimens from HIV-positive patients compared with those from HIV-negative patients (1045). Moreover, this parallels findings of

increased levels of these cytokines in serum and digestive tract mucosa in HIV infected persons (103, 1189).

As these cytokines are present in CVS in healthy women as well, they could enhance HIV replication as soon as the virus enters the vagina. This hypothesis is in keeping with the higher risk of male-to-female transmission in case of female genital tract infection or inflammation, a situation likely associated with increased local production of proinflammatory cytokines.
Data indicate that concomitant infection of the genital tract with HIV and other viral, bacterial, or protozoan pathogens influences the local concentrations of some immunoregulatory cytokines, as for example IL-10 (271).
In adults, systemic IL-10 concentrations increase with HIV disease progression (326). Most of the patients in the **Crowley-Norwick-study** had CD4+ T cell counts >400/µL and the increased IL-10 concentrations were detected in female adolescents who had been infected relatively recently, suggesting that the local immunoregulatory mechanisms of the cervix are altered early in the course of infection, long before systemic CD4+ T cell counts decline (271).

The impact of the menstrual cycle on genital tract shedding of HIV is controversial. Studies reported that there are correlations between cervicovaginal HIV-1 RNA levels and phase of menstrual cycle or serum levels of progesterone and estrogen (1183, 108). However, other studies were unable to detect a menstrual cycle pattern to HIV genital tract shedding (978). Variation in assay methods, other influencing factors and different number of women sampled in studies may account for this discrepancy.
Recent studies using the SIV-macaque model of vaginal infection indicated that progesterone implants enhanced HIV transmission, presumably by thinning of the vaginal wall (878), whereas estrogen inhibited HIV infection, inversely, by thickening of the vaginal wall (1320).

Expression of HIV-1 receptors and coreceptors in the female genital tract varies as a function of menstrual-cycle stage, suggesting that sex hormone levels may influence a women`s susceptibility to HIV-1 infection (1561). This variation in receptor expression suggests that receptors are regulated by estradiol and progesterone and that a woman`s susceptibility to HIV infection may vary due to this hormonal regulation of HIV receptor expression.

Increased vaginal cytokine levels during menses of HIV-positive women (26) may be correlated with the increased numbers of granulocytes and macrophages in the genital mucosa at menses (102) which in the case of HIV may be hyperactivated, leading to enhanced cytokine production.

In summary, HIV-1 infection leads to a dysregulation of the immune system of genital tract mucosa (Table 39). As in the periphery, HIV-1 infection causes the disruption of CD4/CD8 ratio in the cervical mucosa (13). The increased CD8+ T cells are predominately primed/memory T cells, however, these CD8+ T cells are functionally impaired, as indicated by decreased or lack of perforin and TIA-1 (cytolytic granule-associated protein) expression (13). In the systemic compartment, HIV infection leads to an alteration in the cytokine pattern, most prominent in advanced stage of HIV disease. Likewise, in the CVL of HIV-positive women, proinflammatory cytokines are enhanced and at least one study, using mRNA in situ hybridization techniques, pointed to a shift towards Th2 cytokines (IL-4, IL-5, IL-10), which were significantly higher among HIV-positive women than HIV-seronegative women (1045). LCs are lost or decreased in the cervical epithelium of HIV-positive women (1045), which may be caused by direct HIV infection of LCs or redistribution of these primed LCs to lymphoid tissues. Plasma cells are also reduced from the submucosa of HIV-positive women (1045), which may account for the impaired local production of IgA (101).
HIV infection in women is often associated with chronic viral infection, candidiasis, syphilis, pelvic inflammatory disease, and bacterial infections. These conditions are seen in the genital tract before any manifestation in the periphery, which suggests that breakdown in the genital mucosal immune system may occur before immune system dysregulation (13).

Dysregulation of the immune system in the HIV-infected female genital mucosa	
T lymphocytes	Increase in CD8+ T cells Decreased perforin/TIA-1 production in CD8+ T cells Shift in CD4+/CD8+ ratio
DCs	Reduced levels
Igs	Decrease in plasma cells Decrease in S-IgA production, predominance of IgG
Cytokines	Increased production of proinflammatory cytokines (e.g. IL-6, IL-10)

Table 39: Features of immune system dysregulation in the female genital tract caused by HIV infection

That some individuals become HIV positive after a single virus exposure whereas others remain resistant to repeated challenge indicates that there are factors beyond simple viral contact with particular mucosal cells. Sex hormones and cytokine levels in the environment of the virus and target cells may influence infectivity.

There is the hypothesis that in frequently HIV-exposed but uninfected individuals, HIV-specific mucosal antibody responses may exist and play a role in resistance to HIV. It has been published that HIV-1-resistant sex workers show the presence of HIV-specific IgA in their genital secretions (670) whereas other studies described the absence of HIV-specific antibodies in genital secretions of HIV-resistant sex workers (337). So far, a condition that mimics the reports of HIV infections that produce genital anti-HIV antibodies without exposures of a systemic immune response could not be reproduced in monkeys (938). Seronegative sex workers also have higher frequencies of CD8+ T cells in the cervical mucosa than in blood whereas HIV-infected individuals have higher CTL responses in blood than in mucosal tissues (668) which would again suggest a more important role for cell-mediated responses in the immunity against HIV.

A vaccine that can elicit strong antiviral immunity may provide protection for heterosexual HIV-1 transmission. Further trials of anti-HIV vaccines should include an analysis of potential genital mucosal immune responses induced by the vaccine candidates.

3. HPV

Worldwide, cervical carcinoma is the second leading cause of death from cancer in women after breast cancer. The precursor of squamous cervical carcinoma known as CIN is generally diagnosed in younger women, reflecting that it requires a median of 15 years before invasion occurs (867).

HPV can be considered the most prevalent STD, but infections remain asymptomatic in about 75% (1417) and will regress spontaneously in most cases (549). It is now generally accepted that persistent HPV infection of the cervix is responsible for the development of cervical dysplasia and cancer.

Specifically, the high-risk HPV types 16, 18, 31 and 45 among others account for more than 80% of the cases of HPV-associated HSIL and cervical carcinoma (1340, 1359) while low-risk HPV types such as HPV 6 and 11 cause benign genital warts.

The differences in oncogenicity between high and low-risk HPV result from their different ability to integrate the host DNA and to cause genetic instability. After penetrating epithelium through microabrasions virions infect the stem cells at the basal ECs layers (1059). They replicate their DNA episomally using their own non-structural proteins E1 and E2 and the cellular machinery.
In benign and low-grade cervical lesions, the HPV DNA is maintained in an episomal, non-integrated state (572). The expression of E6 and E7 delays cell cycle arrest and differentiation, which favours the thickening of the epithelium. The integration process observed during the progression of CIN associated with high-risk HPV usually disrupts the E2 region, resulting in an enhanced expression of E6 and E7 after integration of the episomal DNA into the host`s DNA. Binding of E6 to the host`s p53 and E7 to Rb, respectively causes uncontrolled cell proliferation (210, 691) and finally, oncogenic transformation of the cell follows (1417).

Although a very high percentage of women are positive for HPV-16 (555), most HPV infections are subclinical in immunocompetent patients or manifest intermittently as self-limiting warty lesions. A small but medically important fraction of the lesions will progress to HSIL, cervical carcinomas-in-situ, and if left untreated, to invasive and metastatic carcinomas (899). Thus, infection with oncogenic HPV types is a necessary but not a sufficient cause of cervical cancer, other risk factors probably include smoking, genetic factors, cervical inflammation and immune-system dysfunction (1418).
Cervical carcinogenesis includes HPV infection, viral persistence, progression to precancer lesions and invasion. HPV persistence and progression involve a complex interaction between viral and host factors, such as viral variants, viral load, susceptibility genes, immune response against HPV and molecular events associated with progression.
The host immune response is essential for restraining both HPV infections and HPV-related cervical cancer. In women with pre-invasive cervical neoplasia the spontaneous regression of lesions occurs in 30% up to 65% of cases (867, 1260), indicating a role for a successful immune response to HPV infection.

However, HPV does not provoke strong immune responses (328) and especially the cancer-associated HPV types employ distinct immune-evasion mechanisms (427).
There is no cytolysis or cytopathic death as a consequence of HPV replication and therefore no release of the proinflammatory cytokines and little local or systemic presentation of HPV antigens to the immune system by professional APCs (755,

1351). The viral proteins E6 and E7 halt apoptosis until viral replication in the differentiating KC is completed and the KC proceeds to its natural death far from the sites of immune activity. The low levels of HLA I and II of KCs further cause a resistance to CTL-mediated lysis (427) whereas HPV-infected KCs might resist CTL-killing as well by producing proteins that interfere with CTL-lytic mechanisms (238, 1319) or by inducing apoptotic cell death in the CTLs themselves (1570). As a consequence, HPV infection is not accompanied by inflammation resulting in persistent infectons as the host remains ignorant of the pathogen for long periods.
Other immunsuppressive mechanisms include the subversion of IFN responses by interfering with IFN signaling pathways (78, 814, 1016) and downregulation of other immune factors by E6/E7 proteins such as IL-18 (224), TGF-β2 (1017) and MCP-1 (722).
Thus, activity of E6 and E7, and partly E5 (171, 1578) provides the molecular basis for promoting viral persistence and avoiding innate immunity and the consequential activation of adaptive immunity.

Invasive cervical carcinoma cells further evade immune response and CTL-mediated killing by HLA downregulation (248, 1199), IL-10 production (980), predominant Th2 polarity instead of a Th1 response (1294) and therefore induction of anergic or Treg cells (379) and downregulation of signaling components of the TCR such as CD3-zeta (739). Studies also propose a role for FasL expression in cervical carcinoma which allows the tumor cells to evade host immune surveillance (663)
Moreover, the number of LCs in CIN lesions is reduced (1454) and they are not activated by HPV-VLP (384) or uptake of HPV capsids as DCs (385).
In addition, changes in tissue architecture by downregulating E-cadherin expression through E6 modulate LC contact with HPV not allowing optimal antigen capture or activation necessary for the initiation of anti-viral T cell responses (884).
This could probably result in an inefficient primary immune response which also delays the activation of the adaptive immune response in HPV infection (1342, 1418). The host APCs are exposed to low levels of viral proteins in a non-inflammatory milieu for a prolonged time period and, as a result, local immune non-responsiveness may be established in the infected mucosa.
In this HPV antigen-tolerant milieu, host defenses become irrevocably compromised, and HPV antigen-specific effector cells are either not recruited to the infected area and/or their activity is downregulated. Thus, if during a persistent HPV infection there is deregulation of high-risk HPV E6 and E7 with increased protein expression, and this does not result in an armed effector cell-mediated

immune response, HPV-mediated progression to HSIL and invasive carcinoma are unobstructed.

The investigation of the role of natural immunity to HPV infection is problematic as HPV infections can be transient and often asymptomatic. Studies have been carried out largely on patients with established HPV-associated disease and have focused on the oncogenic HPV types, in particular responses against the viral transforming proteins, E6 and E7, the only viral antigens constitutively expressed in transformed cells. Also studies have focused on systemic rather than mucosal immunity, so a substantial component of the host response against HPVs could be missed (867).

Circumstantial evidence suggests a role for both the humoral, e.g. antibodies to capsid proteins, and cellular immune system, e.g. CTLs versus oncogenes, in the clearance of HPV infections. Neutralizing antibodies may be an effective way of preventing early viral infection and spread, but cell-mediated immune response virally may mainly be important in the resolution of infection and associated disease.
HPV infections induce both systemic and local humoral immune responses manifested by the presence of IgG and IgA antibodies to HPV-associated antigens. Although low levels and frequencies of anti-HPV antibodies are present in serum and CVS of apparently healthy women, women with cervical cancer present these antibodies at higher levels and frequencies (131, 316, 342, 714, 1020, 1257, 1452, 1456, 1478).
Increased local levels of HPV-specific IgG in vaginal washes of women with cervical cancer indicate a local inflammation, perhaps due to invasive tumor growth that results in transudation of IgG from plasma to the vaginal secretion. Observations indicate that infection of the genital tract mucosa by HPV preferably induces immune responses at the mucosal site of infection rather than in the systemic compartment.
It is not clear whether these antibodies in CVS were locally produced or a result of leakage from the blood (1418). Also the role of anti-HPV S-IgA which has been detected in CVS of healthy controls and HPV-infected patients remains unclear (76, 496).

There are also some observations relevant to development and persistence of humoral responses to HPV.

HPV-specific local antibodies are apparently induced several months after the initial HPV infection (496) and the persistence of these antibodies is dependent on the concomitant HPV presence (1302). Konization of CIN lesions was shown to be followed by a gradual decline in serum and cervical mucus antibodies (362).
Mucosal IgG and IgA also display different kinetics of appearance and persistence in cervical mucus. IgA responses are more frequent and reflect current HPV infection whereas IgG appears to parallel the development of cervical lesions (1257). Moreover, local IgA responses are elicited earlier than IgG responses with rare detection of IgG in preclinical HPV infection.
Measurement of serum antibodies to HPV 16/18 antigens E6 and E7 does not appear to be useful for the prognosis of cervical cancer (800, 1303). Similarly, local HPV-16 VLP-specific IgA and IgG responses do not correlate with viral clearance in patients with CIN and healthy women (131, 1452). Rather, the mucosal HPV-16 VLP-specific IgA and IgG responses reflect current HPV infection and development of cervical lesions (1257).
Results suggest a selective downregulation of local HPV-specific IgA responses in women with cervical cancer (1020). Gene expression profiling also indicated downregulation of genes associated with Ig synthesis including gene encoding the heavy chain of IgA2 in cervical tumor tissue as compared to that in the normal tissue of the same patient.
Previously, it was also reported that the number of IgA plasma cells infiltrating invasive cervical carcinoma was altered when compared to normal cervix specimens (356).
This was supported by data obtained from cytokine analysis. Th2 type cytokines, including IL-4, IL-5, and IL-10, that play important roles in B-cell differentiation and subsequent Ab production, were not detected in the vaginal washes.

Increased incidence of HPV persistence and HPV-asociated malignancies in immunosuppressed individuals strongly suggests a decisive role for cell-mediated immune mechanisms (32, 390). Furthermore, the regression of HPV-induced warts is associated with lymphocytic infiltration of both CD4+ and CD8+ T cells in both patients with genital warts (245) and animal papillomavirus models (1273) whereas non-regressing warts are characterized by a lack of immune cells. Moreover, there are cytokines such as IL-12, TNF-α, and IFN-γ which are characteristic for a Th1-based immune response in regressing warts (245, 1343).

Cell-mediated responses comprise both CD4+ and CD8+ T cells which take part directly as CTL or indirectly as Th cells for CTL or antibody production in HPV immunity. In almost all studies of cell-mediated immunity against HPV, peripheral blood rather than local T cells have been used (307, 314, 643, 644, 1001, 1000, 1022, 1343, 1565) due to the low yield of T cells from surgical removed cervical cancer tissue and the low availability of relevant cervix tissue.

Studies have shown a correlation of *in vivo* immune responses and the regression of CIN lesions and clearance of HPV (565). In humans, regression of cervical neoplastic lesions was associated with cell-mediated responses to specific HPV 16 E6 and E7 (643, 644), and persistene of HPV infection was reported to be correlated with a lack of CTL responses to HPV 16 E6 (307, 1001).
This is also consistent with trials on vaccination against HPV E6 and E7 in experimental animals which induced cell-mediated immunity. A regression of skin papillomas in rabbits (792) and tumors formed by HPV16 E7-transformed cells in mice (778) has been observed.

Studies suggest a relationship between T cell responses against HPV and cervical disease, with a decreased response in cancer patients and an increased response in women with high-grade CIN and viral persistence (314, 643, 644).
Other studies also showed serological T cell responses against HPV in healthy controls (846, 845, 1441). It is not clear if these results represent *in vitro* artefacts or memory responses based on a prior exposure to HPV.

However, only a limited number of prospective studies have been carried out. Obviously, failure to develop effective cell-mediated immunity to clear or control infection results in persistent infection and, in the case of the high-risk HPVs, an increased probability of progression to HSIL or invasive carcinoma.
Therefore, there is the need of further studies on T cell responses in longitudinal studies and at the sites of disease.

Latest studies indicated that a majority of both patients with cervical lesions and healthy persons display HPV16 L1 peptide-specific type 1 T-cell responses with a similar magnitude (1441). These responses were covering a broad range of peptides within L1 which suggets that during persistent or repeated exposure to HPV16 L1 the immune system maximizes its efforts to counter the viral challenge. Unlike type 1 T cell responses against HPV 16 E2 and E6, type 1 T cell immunity against L1

does not correlate with health or disease. This is in contrast to the previous finding that such patients lack such type 1 T-cell immunity against HPV16 early antigens E2 and E6 (307).
Lately, **Steele et al**. investigated T cell responses to the HPV16 proteins E6, E7, E4, L1 and L2 in women with CIN or cervical carcinoma directly *ex vivo* (1347). The frequency of CD4+ responders was far lower among those with progressive disease, indicating that the CD4+ T-cell response might be important in HPV clearance. CD8+ reactivity to E6 peptides was dominant across all disease grades, inferring that E6-specific CD8+ T cells are not vitally involved in disease clearance.

Concerning the production of cytokines by CD4+ T helper cells, a study found decreased level of IL-2 production but elevated levels of IL-4 and IL-10 in patients who had extensive HPV disease compared with those suffering from localized HPV disease or healthy controls (238).
Production of cytokines that mainly enhance potentially protective cell-mediated immunity is defective in women with extended HPV disease. Moreover, a pronounced shift from type 1 to type 2 cytokine production is associated with more extensive HPV infection. This suggests a shift towards the T-cell production of cytokines associated with the suppression of cell-mediated immunity.
Another study showed a significant IL-2 production against HPV peptides in patients with CIN and persistent HPV infection, as well as to a significantly lesser extent in patients with cervical cancer which suggests a correlation between IL-2 responses and disease (315).

It is widely expected that a vaccine that would prevent HPV infection, which is mediated by antibodies at the site of infection, and restrict the spread of HPV from infected KCs, which depends on the induction of CTLs, would greatly reduce the incidence of cervical cancer and associated mortality. This is especially important in developing countries where screening, early diagnosis, and treatment of precancerous lesions is not available (641, 1417).

Two main types of HPV vaccines are currently being developed: prophylactic vaccines to prevent HPV infection and associated diseases, and therapeutic vaccines to induce regression of precancerous lesions or remission of advanced cervical cancer. The prophylactic vaccines are focused on the generation of neutralizing antibodies mainly against L1 whereas in therapeutic chimeric vaccines, the targets are E6 and E7, which have no share epitopes with human cellular

proteins; therefore, the risk of inducing an autoimmune response is theoretically eliminated (1349).

DNA-free VLP synthesized by selfassembly of fusion proteins of the major capsid antigen L1, or of both L1 and L2, induce a strong humoral response with neutralizing antibodies (713). VLPs are thus the best candidate immunogen for HPV vaccine trials.

At present, several prophylactic vaccines have been developed and tested in clinical trials with proming results (63, 371, 512, 513, 742, 1456). Two pharmaceutical companies, GlaxoSmithKline and Merck, have been the major forces in research and development in prophylactic HPV vaccines to date with two randomized controlled trials published with highly promising proof-of-principle results (512, 742).

The present trial results show a good safety profile and an almost universal induction of high titers of virus-specific antibody by VLP-based vaccines. Further follow-up is needed to see whether the antibody levels remain after decades or whether booster vaccination is needed but encouraging data on HPV VLP L1 vaccines suggests only a slight decline in the high serum antibody titers over a 6-to-8-month evaluation period (1417).

Vaccine efficacy was 100% in preventing acquisition of persistent HPV infection in both studies. Both also showed encouraging results concerning prevention of CIN but these trials had not been designed with sufficient power to detect reductions in CIN incidence. Demonstration of efficacy in preventing high grade CIN is the objective of much larger, phase III trials, which are currently ongoing in different populations. Moreover, evidence from randomized controlled trials has been obtained only for monovalent (HPV 16) and bivalent (HPVs 16 and 18) vaccine candidates.

Based on the successful HPV16/18 L1 VLP vaccine pilot efficacy trial, which established 100% efficacy against persistent HPV16/18 infection, GlaxoSmithKline Biologicals is currently accomplishing phase III trials on 28,000 persons of this prophylactic HPV vaccine (1417). These studies aim to extend the findings in the prevention of high-grade CIN associated with HPV16/18 infection. The efficacy trial is conducted over 48 months as a double-blind, multicenter, controlled study in adolescent and young adult females vaccinated according to a 3-dose schedule. The company also has plans to bring a tetravalent vaccine (with VLPs of cancer-

associated HPV types) into the development phase once it completes the clinical development for its bivalent HPV 16/18 vaccine candidate.
Another multicenter phase III study by Merck & Co with several thousand women is currently carried out to prove efficacy of a quadrivalent HPV vaccine (HPV types 6, 11, 16, and 18) (640, 1342).

However, there also have to be mentioned some important aspects which have to be regarded developing a HPV vaccine.
Levels of specific antibodies in the human female genital tract which are likely to be an important determinant of vaccine efficacy might be influenced over the course of the menstrual cycle. Experiments raise the possibility that the HPV16 VLP vaccine might be less effective during the peri-ovulatory phase (1004).
One has to consider the preferred delivery route for vaccine administration in order to induce effective mucosal immune responses (1349). Obviously, a single dose with mucosal delivery would be the preferred route for vaccine delivery; if nasal or oral delivery can induce antibodies still has to be further investigated but also showed encouraging results so far (1003). An alternative route of administration that avoids parenteral injection while inducing mucosal immunity might also facilitate vaccine implementation in some settings, and partially overcome the substantial variation in HPV16 antibodies at the cervix seen in ovulating women.
The choice and the number of HPV types included in the vaccine is an important issue as there are geographic differences in the prevalence of HPV types involved in CIN and cancer. In order to be effective for at least 80% of the population, the vaccines should theoretically consist of VLPs of the four or five most common types of HPV of that country or region but combining multiple types in one vaccine may be problematic (989, 1417). A pentavalent vaccine with VLPs of HPV types 16, 18, 45, 31, and 33 would potentially prevent 83% of all cervical carcinomas whereas a heptavalent vaccine that also included types 52 and 58 could potentially prevent 87% of the overall cervical cancer burden internationally (989).
Whether such a polyvalent vaccine would result in immunological equivalence such that each component virus-like particle induced an antibody response that correlated with protection is unclear.
Finally, all trials to date of HPV vaccines have enrolled women, but genital HPV infections are mainly sexually transmitted and men will also need to be vaccinated if the whole population is to develop immunity.

In infected patients, targeting already infected basal cells that do not express capsid antigens remains a major challenge. Therefore, vaccine chimeric VLP vaccines are being developed, which contain not only structural viral L1 or L2 proteins but also functional E-proteins (1059). In preclinical data, these chimeric VLP vaccines elicited both neutralizing antibodies to the VLP and T-cell responses to L1 and E7, and could therefore be prophylactic and therapeutic (28).

Therapeutic vaccines consisting of E6 and/or E7 have been tested in patients and have proven to be safe and effective against benign warts, however have had limited therapeutic effect so far in cases of cervical cancer (425).
In general, there are several broad categories of therapeutic vaccine strategies: chimeric VLPs, peptides, proteins, nucleic acid–based, and cell-based.
The major challenge in infected patients remains the targeting of already infected basal cells that do not express capsid antigens. Therefore, **chimeric VLP** vaccines with structural viral L1 or L2 proteins and functional E-poteins are being developed (986). Therapeutic vaccination strategies based on using chimeric VLP (288, 1341), chimeric capsomeres (458) or chimeric HPV VLP immune complexes (383) have been investigated in animal models or clinical trials and may be effective in generating an immune response against HPV-induced diseases such as cervical cancer.
There are only few animal studies and preliminary clinical trials performed with either peptide-based (25, 389, 984) or protein HPV vaccines (426) so that there cannot be concluded if they are an alternative yet.
Finally, various attempts to develop **DNA vaccines** are currently in progress, which tend to be more effective in the generation of both humoral and cellular protective immunity responses. Polynucleotide and recombinant viral vaccines encoding non-structural viral proteins show therapeutic efficacy in animal models and are candidate immunotherapies for established low-grade benign genital infections (299, 502, 822, 962, 1277).

Despite the current enthusiasm about future prospects of preventive HPV vaccines, limitations concerning restricted coverage to only a few HPV types, expenses, potential barriers to wide availability and acceptability in both developed and developing countries, and the potential epidemiological shift of HPV disease to currently less frequent types and variants, have to be taken into consideration.

4. Gonorrhea

Gonococcal infection caused by *Neisseria gonorrheae* remains a major global health problem with more than 60 million cases reported annually worldwide. Teenagers and young adults are at high risk for infection, which is unsettling in view of the increased risk associated with gonorrhea for infection with HIV-1.
In contrast to the inflammatory response generated predominately with gonococcal infection of the male urethra, 50-80% of women with lower genital tract *N. gonorrhoeae* infection are asymptomatic (1330). Infection spreads to the upper female genital tract in up to 45% of patients, possibly causing PID with serious complications including tubal scarring (1330).

Gonococcal pilus binds to CR3 present on the ectocervix and endocervix, positioning the bacterium at the cervical cell surface where complement concentrations would be expected to allow efficient opsonization for the subsequent intimate adherence of iC3b and gonococcal porin to the I-domain (353). Activation of the alternative complement pathway results in C3b deposition upon gonococci; C3b is inactivated to form iC3b.
Ascension to the upper female genital tract is then mediated by both host and gonococcal factors that are subject to cyclic environmental changes occurring within the female genital tract. The LHr present on the endometrial and fallopian tube epithelia mediates the interaction of gonococci with the upper female genital tract (1331).

Gonococci possess numerous surface antigens including LOS, porin, Opa, Rmp and pili, which enable a high grade of antigenic variation.
Adherence to mucosal ECs, the initial step in pathogenesis, is mainly determined by pili and Opa which bind CD46 and CEACAM, respectively (1229). The expression of Opa proteins and the penetration of EC membrane by porin facilitates invasion of nonciliated mucosal ECs through endocytosis.
These virulence factors together with the high heterogeneity and adaptability with repeated phase and antigenic change may be a way to downregulate the functional immune response of the host.

Other mechanisms of *N. gonorrheae* to evade the host`s immune response are the resistance to complement-mediated bacteriolysis and the production of IgA1 protease. However, the relevance of data concerning complement-mediated

bacteriolysis for the reproductive tract with its relative complement deficient setting is not known.

IgA1 protease was detected in vaginal washes from patients infected with *N. gonorrhoeae* by its ability to cleave IgA1 (120) although **Hedges et al.** did not identify characteristic cleavage fragments of IgA1 protease in cervical mucus or vaginal wash samples (530).
These results suggest that IgA1 protease does not play a significant role in the pathogenesis of uncomplicated gonococcal infections within the lumen of the lower female genital tract. As all of the clinical isolates of *N. gonorrhoeae* infecting the patients produced IgA1 protease *in vitro* one could propose that *N. gonorrhoeae* may not be present in the lumen or on the mucosal surface in sufficiently high numbers (530). This hypothesis is supported by the lack of significant local immune or cytokine responses in women infected with *N. gonorrhoeae*. Therefore, the lack of detectable IgA1 protease activity in cervical mucus, in addition to the lack of local host responses, may simply be due to small numbers of bacteria at that site.
In addition, LAMP-1 on ECs is cleaved by neisserial IgA1 protease (820) which may contribute to intracellular gonococcal survival and facilitate escape from antibodies and complement.

In addition to its potential ability to avoid the effects of an immune response, *N. gonorrhoeae* apparently does not elicit strong humoral immune responses during uncomplicated genital infections. Yet, the immunology of gonococcal infections in the genital tract has not been extensively studied so far.
Analysis of cervical secretions obtained from uninfected women and from women infected with the gonococcus revealed that a significant antibody response is not generated with uncomplicated infection (531). Further support for this idea is found in the work of **Hedges et al**. who found that women with gonococcal cervicitis did not exhibit elevated local levels of IL-1, IL-6, and IL-8 (532). In contrast, **Fichorova et al.** have reported increased IL-1, IL-6, and IL-8 expression in similar studies performed with immortalized vaginal and cervical epithelia (396). The release of inflammatory cytokines by the cervical epithelium in response to *N. gonorrhoeae* infection therefore remains under question.

Results indicate that while there are some minor systemic and local antibody responses associated with uncomplicated gonococcal infection in females, the

antibody levels are lower than with other mucosal infections and concomitant infection neither alters total Ig nor antigonococcal antibody levels.
There has not been observed any clear indications of immune memory toward gonococci as evidenced by the failure of previous exposure to this organism to generate more pronounced antibody responses (531). It is unlikely that the poor antibody responses in both male and female patients to *N. gonorrheae* is simply due to an absence of known inductive sites in the genital tract, since female patients with rectal infections did not show any marked enhancement of the antigonococcal responses.
Latest studies have shown that expression of Opa protein results in downregulation of proliferative T cell responses to gonococcal antigens, and eiher downregulation or increased apoptosis of CEACAM1-expressing B cells (134, 1075).
The production of an effective vaccine against *N. gonorrheae* should be an important goal, also due to increasing antibiotic resistance but there is still minimal success despite a long list of potential candidate proteins. Latest approaches with intranasal immunization in mice with gonococcal transferring binding receptor proteins have shown promise due to induction of local and systemic antigen-specific IgA and IgG antibodies (1142), which is also consisting with earlier trials with gonococcal outer membrane preparations or other bacterial antigens in the mouse model (1127, 1226).

5. Chlamydia

Chlamydia trachomatis, an obligate intracellular bacterial pathogen, is a major cause of STDs worldwide with 90 million new cases per year and a prevalence reaching 25% (1543, 683). Women are disproportionally affected because of their increased risk to develop postinfection complications. Chlamydial infections of the lower genital tract of women are frequently asymptomatic in their acute form (187), but can progress to the upper genital tract causing PID, postinfection scarring and finally tubal factor infertility (1527).

To picture the immune mechanisms contributing to the resolution of chlamydial infection and protection of reinfection, it is important to consider the unique developmental cycle of the pathogen in ECs (90, 1527). Protective immunity might include responses directed at the infectious EB, such as neutralizing antibody, or at infected cells, such as CTLs or ADCC.

Despite progress in the past decade, the current understanding of mucosal immunity to this pathogen in the human host remains limited. Most of current knowledge comes from studies in women or female animal models.

Several studies show that innate immune responses are evoked following chlamydial infection which is evidenced by the accumulation of phagocytic cells including neutrophils at the site of infection (1171).
Experiments with neutrophil-depleted mice suggest that these cells may play a role in reducing the initial amplification of *C. trachomatis* and possibly limit local spreading within the genital tract (82) but neutrophils appear not to be critical for eradication of *Chlamydiae*.
NK cells also seem to play an important role in the initial control of chlamydial infection as they are not only responsible for the production of IFN-γ early in the course of chlamydial genital tract infection but are also, via IFN-γ, a significant factor in the development of the Th1 CD4+ response and in the control of the infection (1431).
Clearance of chlamydial infection and protection against inflammatory disease in a mouse model appeared to be associated with recruitment of MHC class II APCs with a DC phenotype into uterine tissue early in infection (1334).

As a result, innate immunity is likely to be essential in shaping the adaptive immune response but alone is unable to resolve genital chlamydial infection.
Humoral and adaptive immune responses are also mobilized during chlamydial infection and are thought to contribute substantially to protective immunity. Study of the infection in both animal models and naturally acquired infection in humans suggest that protective immunity is provoked (50, 665, 695). Moreover, individuals with a previous chlamydial infection show reduced risk of acquiring a chlamydial STD, and immunity to reinfection is partial and of limited duration (665). A proportion of women who had not received antimicrobial therapy were shown to resolve infection (1078). **Brunham et al**. also reported a short-term and serovar-specific immunity against chlamydial infection in female sex workers (148).

However, a number of studies have documented the high rate of recurrences of *Chlamydia* infections in humans (87, 163, 665, 683) and animals (680, 682, 1169). Studies all conclude that some form of short-term protective immunity does develop against chlamydial genital infection in humans but natural production of solid, long-term immunity appears to occur only after multiple infections.

The contribution of the humoral arm of the specific immune response appears controversial but one could propose a minor role for anti-chlamydial antibodies in protective immunity.

Murine studies provided evidence that delayed appearance of anti-chlamydial IgA antibodies in CVS correlated with a delay in the resolution of infection (971). Women with anti-chlamydial IgA antibodies in CVS shed significantly fewer infectious chlamydiae than women without IgA in CVS; this effect was not observed with high serum antibodies (149). Moreover, the presence of these IgA in CVS seems to accelerate the clearance of infection with antibiotic therapy (282).

Equally convincing results from murine models put forward the hypothesis that antibodies are not required for protective immunity against *Chlamydia*. B-cell deficient mice have been shown to resolve chlamydial genital tract infection equally effective than normal mice (632). The presence of anti-chlamydial IgA in mouse CVS alone seems to be insufficient to resolve infection (631) which clearly points to a synergistic role of antibody and cell-mediated immunity in protection (629).

However, a recent study by **Morrison et al**. shows that immune serum and *Chlamydia*-specific mAbs have a profound impact on immunity to chlamydial genital tract reinfection (973). Although the mechanisms by which antibody protects is not understood, the protective efficacy of antibody is highly dependent upon CD4+ T cell-mediated adaptive changes that occur within the genital tract tissues after primary infection.

Extensive studies of the immune mechanisms the control chlamydial infections in the mouse female genital tract have provided evidence that cell-mediated immunity is essential in resolving infection and protective immunity (1172, 1165). Th1-type CD4+ cells are the main effectors in the resolution of genital infection and clearance of challenge infections whereas CD8+ lymphocytes are neither sufficient nor necessary to confer optimal levels of protective immunity (523, 629, 594, 680, 683, 1165, 1506). However, immune CD8+ T cells can confer a limited level of immunity to naïve mice and display measurable cytotoxicity for *Chlamydia*-infected cells *in vitro* (92, 1345).

Results of **Belay et al**. demonstrated that genital chlamydial infection is associated with a significant induction of chemokines and chemokine receptors that are involved in the recruitment of Th1 cells into the site of infection (100). Expression of RANTES, MCP-1, MIP-1α, IP-10 and ICAM-1 as well as of CCR5 and CXCR3, known to be preferentially expressed on Th1 and DCs was upregulated during chlamydial

infection of mice and decreased after clearance of infection, suggesting an infection-driven regulation of expression.
Further data not only demonstrated the necessity of CD4+ cells in the resolution of chlamydial infection but also showed that fewer numbers of CD4 lymphocytes delayed clearance of organisms from the local genital mucosa (971). Consequently, a critical number of antichlamydial CD4+ cells appeared necessary to eradicate infection.

This is also consistent with the postulated interactions between *Chlamydia* and genital tract DCs. Although recognition of chlamydial antigens on genital ECs by CD4+ and CD8+ T cells has not been demonstrated (1084), recognition of antigens that have been processed by DCs is very likely. Following phagocytosis of *Chlamydia* by DCs in the genital tract of immune animals and processing of chlamydial antigen in the MHC II pathway, CD4+ Th1 memory cells are probably located in the mucosa where they rapidly begin to secrete IFN-γ. Several authors had originally suggested that mainly genital ECs present chlamydial antigen to T cells (417, 1345).

The key for understanding cell-mediated immunity to chlamydial genital infection lies in defining the mechanisms by which lymphocytes traffic to the genital tract. The recruitment of leukocytes into tissue sites depends on adhesive interactions between adhesion molecules on ECs and integrin receptors on the leukocytes. However, the role of homing molecules in the recruitment and retention of lymphocytes to genital tract mucosa, which lacks the organized lymphoid structures present in the gut, has not been defined.
Kelly et al. showed that in the genital tract of mice, recruitment of CD4+ cells is mediated through the interactions between homing receptors on CD4+ T cells such as α4β7 and adhesion molecules ICAM-1, VCAM-1 and MadCAM-1 (524, 680). They also reported that during *Chlamydia* infection in mice, cellular recruitment differed with CD4+ cells mainly recruited to the upper genital tract, whereas neutrophils and monocytes were recruited to all regions of the genital tract (682). This correlated with an upregulation of all three adhesion molecules in the upper genital tract during the course of infection.
Remarkably, the mucosal addressin MAdCAM-1 is not expressed by human female genital tract tissue (626).
Some investigators tried to identify the antigen specificity of the B cells that traffic to the sites of human mucosal chlamydial infection. IgG- and IgA-secreting B cells

with specificity for chlamydial MOMP, hsp-60, and a chlamydial plasmid encoded protein accumulate at sites of chlamydial genital tract infection (448).

Moreover, there is strong evidence indicating that IFN-γ is a major effector mechanism of the cell-mediated immune response.
The majority of the antichlamydial activity for both CD4+ and CD8+ lymphocytes is associated with the production of high levels of IFN-γ (167, 593, 683, 1167). Direct evidence for rapid IFN-γ secretion in Th1-immune mice after vaginal challenge with *Chlamydia* has been reported (629).
Besides some minor mechanisms of cytotoxicity such as perforin-mediated lysis (1443), it is proposed that IFN-γ is a primary mechanism for eradication of *Chlamydia* by CTL and CD4+ T cells. However, IFN-γ has also bacteriostatic effects on *Chlamydia* via NO (892, 1112), at least *in vitro* (592).

It was demonstrated that *C. trachomatis* can enter host ECs without inducing the immediate production of inflammatory cytokines such as IL-1, IL-6, IL-8, TNF-α, and GM-CSF, probably due to the small size of infectious EB, a weak stimulatory ability of chlamydial LPS (297, 532, 596, 1174, 1369).
Therefore, these data show that *Chlamydia* can enter host ECs without inducing a vigorous proinflammatory immune response until the developmental cycle is completed. At that time, cell lysis causes the release of IL-1α and the induction of other proinflammatory cytokines. It also appears that *Chlamydia* can further manipulate cells to enhance their survival by inhibiting apoptosis of infected cells through the caspase-3 pathway, which also occurs after initiation of the developmental cycle (377).
Recently **Zhong et al**. reported that *C. trachomatis* infection of APCs reduced the ability of these cells to stimulate T cell proliferation through defects in antigen processing but not presentation (1586). This has been shown to be mediated via a chlamydial protein that induces degradation of transcription factors that control IFNγ-induced expression of both MHC class I and II antigens.
While the ability of *Chlamydia* to manipulate the host cell response to infection obviously is a survival advantage, it may also contribute to observations that immunity to *Chlamydia* is weak, resulting in persistent or recurrent infection.

Therefore, *C. trachomatis* appears to initiate its developmental cycle *in vivo* without immediately alerting the immune system. Possibly, the delayed induction of early

inflammatory mediators may be a survival mechanism that evolved to protect the developmental cycle from immune-mediated disruption.

One serious problem with repeated chlamydial genital infections is tissue damage followed by tubal scarring, chronic salpingitis and distal tubal obstruction.
Recurrent or chronic chlamydial infections, which are associated with damage to the reproductive tract, may result from an insufficient number of antichlamydial Th1 CD4+ cells (1112) or possibly factors that interfere with the effector mechanisms of Th1 cells, for example IL-10 (683, 1555) or TGF-β [207].
Also neutrophils or their products might play a significant role in tubal pathology and infertility since they have the potential to cause damage through the release of proteinases and hydrolases. Furthermore, neutrophils primed by exposure to cytokines such as GM-CSF, IL-8, and TNF-α can release large quantities of ROI and granule enzymes upon activation with soluble immune complexes (357).
In addition, a shift from a Th1-dominated response to a mixed Th1/Th2 response may further dampen antichlamydial responses (974). Unfortunately, cytokines characteristic of Th2 responses are associated with increased collagen deposition, a measure of fibrosis (559), which may contribute to reproductive tract dysfunction. In contrast, IFN-γ inhibited this process (559).
Immunity to chlamydial protein hsp60 which is upregulated during persistent infection (93) seems to contribute to the development of tubal occlusion and adverse pregnancy outcome (352, 150, 1562).
There is a human hsp60 (h-hsp60) homologue to c-hsp60 which is expressed by ECs of the decidua and embryo during early pregnancy (1527). Therefore, a chlamydial fallopian tube infection can induce the development of autoantibodies to h-hsp60 and in women already sensitized to c-hsp60, the exposure to h-hsp60 will reactivate the c-hsp60 lymphocytes. This may lead to immune rejection of the embryo (1527).
The immunologic components that contribute to the production of tubal pathology may also be associated to many factors including the genetic background of the individual. Another question is whether individuals become reinfected with the same serovar or perhaps develop persistent infections.
Although the large majority of women infected with *Chlamydia* are effectively treated and reinfection commonly occurs, it is difficult to document persistence in vivo. However, some less prevalent serovars, such as serovar I, J, K, and H, show increased rates among those with repeat infections (309). More studies are needed

to understand whether these serovars have an increased ability to escape recall responses against *Chlamydia* or these organisms can persist in a latent state.

A major challenge in designing anti-chlamydial vaccines is to develop an immunization regimen capable of inducing and retaining a mucosal Th1 CD4+ response in order to foster long-term protective benefits. This type of immunity would help to resolve infections rather than prevent them and could therefore influence the consequences of asymptomatic infections ascending from the cervix.

Vaccine research is still ongoing and several vaccination methods have been described that target antigens into the endocytic MHC II pathway of DCs and elicit a Th1-type CD4+ immunity. These include immune stimulating complexes (ISCOMS) (591), alphavirus vectors (344), opsonized liposomes (1276), and conjugation of antigen to lysosome-associated membrane protein-1 (Sig/LAMP-1) (212). The antigens that would be the most effective targets for a Th1 cellular immune response have not been identified, but chlamydial MOMP is a suitable candidate (591).
Concerning the route of immunization, data from murine models show that immunization via mucosal routes, i.e. oral, intranasal, and vaginal, induced greater levels of IFN-γ, induced a dominant Th1 response and limited the course and magnitude of infection when compared to subcutaneous immunization (681, 594).
Moreover, both specific IgA and IgG could be found in genital secretions following immunization via the nasal, oral, rectal, and vaginal routes. However, both the nasal and vaginal routes consistently produced the greatest increase in IgA and IgG compared to other routes in humans (594, 743).
The induction of a protective cell-mediated immune response also appears to depend on whether a live infection initiates the immunizing response (681, 1168).

6. BV

BV is the most common adverse alteration of the vaginal flora, with a prevalence of about 10-60% in different populations (1229). It is characterized by a depletion of vaginal lactobacilli accompanied by an overgrowth of a mixed vaginal flora of anaerobic and microaerophilic species in large number, mainly *G. vaginalis*, *Prevotella* ssp., and *Mobiluncus* spp. among others (419, 874).
BV is associated with several adverse pregnancy outcomes such as preterm birth, low birth weight, and spontaneous abortion (548, 1163, 1305, 1215), and increased

susceptibility to HIV and HSV-2 (1265, 285, 220). Studies also raised suspicion that there is a correlation between BV and CIN or cervical carcinoma (329).

It seems clear that there is an increased risk of incurring infections such as HIV and HSV-2 in the presence of an abnormal vaginal microbiota (1265, 285, 220, 1278) and it may be speculated that it is due in part to the fact that vaginal acidity falls as lactobacillus levels decline and this circumstance benefits the survival of other microorganisms (1278).

A further factor arising from the decrease of the lactobacilli is a corresponding decline in production of H_2O_2, which has an antimicrobial effect (547, 1278).

Since SLPI has been shown to inhibit HIV infection *in vitro*, it has been suggested that low levels of SLPI in conjunction with a BV-positive status might contribute to the risk of contracting HIV.

Furthermore, some anaerobes associated with BV augment expression of HIV in T cells *in vitro*, which might enhance the amount of HIV shedding or activate these T cells to further support viral transmission (518).

Studies showed that genital mucosal fluids from women with BV are potent stimulators of leukocytes, eliciting secretion of TNF-α and expression of mRNA for both TLR4 and TLR2 (1573). Thus, findings suggest that TNF-α levels in genital mucosal fluids from women with BV would be higher than those in women with normal flora. Stimulation of cells through TLR 2 and TLR4 with microbial products stimulates HIV expression through stimulation of TNF-α secretion or direct activation of NF-κB and subsequent binding of NF-κB to the HIV promoter (369). Therefore the secretion of TNF-α and mRNA expression of TLR2 and TLR4 by genital mucosal fluid of women with BV could play a role in mediating adverse effects of BV in HIV transmission.

This is also consistent with the studies by **Sturm-Ramirez et al**. (1368). Proinflammatory cytokines such as IL-1 and TNF-α were found to be elevated in BV patients. As these cytokines could upregulate local HIV replication by activating NF-κB in the LTR-promoter region of HIV, this could partly explain the mechanism by which this risk factor enhances HIV transmisssion.

A characteristic of BV is the absence of strong inflammatory signs that accompany other vaginal conditions caused by pathogens. The vaginal mucosa appears neither red nor inflamed and characteristic symptoms for a vaginitis including vaginal pruritus, burning or dyspareunia, however, are rare (969).

The number of vaginal neutrophils is not statistically higher in BV-positive women than in women with normal vaginal flora, which is astounding due to the massive microbial colonization (192, 195). A subgroup of BV-positive patients actually shows high numbers of neutrophils together with a 10-fold increase in proinflammatory cytokine concentrations when compared to the subgroup with low numbers of neutrophils. This implies that some women with BV do experience a strong activation of the innate response.
In addition, other innate immune factors, such as the antimicrobial SLPI, are decreased in BV-positive women (341). The concentration of neutrophil defensins is also not increased in BV (72).

Proinflammatory cytokines IL-1α and IL-1β are released by phagocytes which are stimulated by bacterial surface antigens and trigger an inflammatory reaction by activating T helper cells.
The cytokine level of IL-1α and IL-1β in CVS is higher in BV-positive patients whether pregnant or not (37, 192, 890, 1494). Furthermore, there is a correlation between a gradual disruption of the lactobacillus dominance and a gradual increase of IL-1α and IL-1β (192). This shows that the innate immune system is reacting strongly and trying to fight abnormal microbial colonization, although most BV positive women do not show any inflammatory signs.
However, increased levels of IL-1α seem to be associated with pregnant BV patients (1494, 1569) whereas studies in non-pregnant BV patients have not shown elevated IL-1α levels (37). Thus, there may be a difference in the response of the local immune system during pregnancy.

IL-1β is a master cytokine inducing several chemokines, especially IL-8. IL-8 is a potent chemotactic and activating factor for neutrophils that has been detected in vaginal fluid of women with other vaginal infections such as trichomoniasis (1287). It also has been demonstrated that the numbers of neutrophils in BV-positive patients and in healthy women are associated with levels of IL-8 (192).
IL-8 does not, however, seem to be correlated with the presence of BV in the same distinct manner as IL-1β (192). The BV microbial flora seems to produce virulence factors that specifically inhibit IL-8 more than IL-1β. The resulting low IL-8 levels may be responsible for the low counts of vaginal leukocytes and for the clinically observed absence of inflammatory symptoms in most women with BV.

Specific bacterial proteases could be responsible for the IL-8 degradation in BV (191). Another possibility is that neutrophils as IL-8-producing cells could be impaired by microbial virulence factors, such as *G. vaginalis* cytolysin.

There is no significant difference in IL-6 levels in the cervical mucus sampled from healthy vaginas and that from BV-positive vaginas, also in pregnant women (890, 1471, 1494). Analyses of cervical mucus from pregnant women tend to show a rise of IL-8, though not as high as for IL-1α (1494). In pregnant women, BV can probably result in the induction of a local immune response mediated by IL-1α and IL-8.
As IL-6 is expressed in endometrial ECs, the absence of an IL-6 response in BV may be because the mucosal ECs are not triggered by BV-associated bacteria or their components to secrete IL-6 above the constitutive levels (889).
Nonetheless, oral and vaginal metronidazole treatment of BV-positive pregnant patients has been shown to reduce cervical mucus levels of IL-6 as well as IL-1β and IL-8 (1569). In the group of patients with persistent BV, however, the metronidazole treatment did not result in a cytokine reduction, possibly due to a persistence of bacterial endotoxin.

Anti-inflammatory IL-10 inhibits activation of Th1 cells thereby suppressing cell-mediated immunity. Concerning this cytokine, there are inconsistent results.
The **Munich study** showed no significant difference in vaginal IL-10 levels of BV-patients compared to healthy women (1471) whereas **Cohen et al**. showed higher IL-10 levels in CVS of BV patients compared to controls (243). They suggested that higher IL-10 levels in CVS of BV patients may be another mechanism to increase susceptibility to HIV infection. In another study, vaginal levels of IL-10 were significantly higher in healthy controls as compared to BV patients (37) probably speaking for a suppression of cell-mediated immunity in healthy subjects. This is also consistent with results of a further study where high vaginal IL-10 levels were supposed to be associated with healthy women compared to patients with vaginal discharge (**G. Anton, personal communication**).
These differences may be caused by recruitment of different patients into the studies.

Some evidence points to microbial hydrolytic enzymes, sialidases and prolidases, and toxins as factors that degrade human Igs and thus impair the immune response. So far, the only characterized specific adaptive immune response in BV is

IgA antibody against the hemolysin produced by *G.vaginalis*. Levels of these vaginal IgA antibodies are positively associated with IL-8 and IL-1β concentrations and are inversely related to microbial enzyme activity (191). **Cauci et al**. also proposed an association between levels of IL-1β and anti-*G.vaginalis* hemolysin IgA in vaginal fluids (190) suggesting a parallel induction of innate and specific immune responses. High levels of anti-*G.vaginalis* hemotoxin IgA in BV-positive pregnant women appear to be protective against adverse pregnancy outcomes whereas BV with high prolidase and sialidase activity was associated with a higher risk for low birth weight (194).

All bacterial species associated with BV, with the exception of *Mobiluncus* spp., appear in small quantities in the flora of healthy vaginas, but they increase considerably in the vaginas of women with BV. BV might well be an endogenous infection since microorganisms associated with BV are shown to have their natural habitat in the gastrointestinal tract (562).
The pathogenetic mechanisms that lead to BV among a subpopulation of women are still unknown. The consequences of any interaction between different species of bacteria are not clear. All in all, however, it seems that an increased level of proinflammatory cytokines in conjunction with a disruption of the vaginal flora does not evoke an inflammatory effect in the usual clinical sense, but that it nonetheless is a host response to a growing potential threat.
One could propose that the condition of BV can be considered as a mucosal immunity disorder or genetically determined miscolonization of the vagina resulting in the lack of an adequate immune response aginst the intruding microorganisms. Another option would be that the prevalence of certain bacteria would inhibit the local host response.

It seems to be important to clearly differentiate between the prevalence of BV with its typical clinical picture on the one hand (34), and the state of bacterial dysbiosis on the other hand which is more precisely characterized as a dysbalance of the vaginal flora.
Studies have investigated an abnormal vaginal flora, but concentrated on BV instead of taking also in account other foms of a dysbalaned vaginal flora. These forms have been classified either as “intermediate” flora or assigned to the group of BV or normal vaginal flora, respectively (334, 335).

However, there exist forms of an abnormal vaginal flora, which neither can be classified as normal or correspond with the clinical picture of BV, but could then be classified as bacterial dysbiosis. **Donders et al**. have termed those forms as aerobic vaginitis to clearly seperate them from BV (334, 335).

Women with abnormal vaginal flora showed signs of a vaginitis including a yellow discharge, redness, pruritus and burning pain. This state was characterized by a decrease in lactobacilli, high vaginal pH, absent clue cells and the presence of increased aerobic bacteria and leukocytes (334). This should be clearly separated from BV which shows another clinical picture and is characterized by a different immunologic response.

Women with vaginal dysbiosis were reported to have decreased levels of lactic acids and a local inflammatory response with high concentrations of vaginal IL-1β, IL-6 and LIF (334), which is also consistent with the results of the Munich study group (955). The increase of IL-1β is indirect proportional to the decrease in lactobacilli. Vaginal IL-1β also increases in BV, but significantly more (8-fold) in aerobic vaginitis.

Studies by **Spandorfer et al**. have also found an association of an abnormal vaginal flora with elevated cervical levels of IL-1β (1329). Concentrations of proinflammatory cytokines in an abnormal vaginal flora could be higher due to bacterial release of LPS and endotoxins (890).

Elevated IL-1β was also demonstrated in patients with cervical dysplasia which was more often in women with an abnormal vaginal flora compared to women with normal vaginal flora (98). IL-1β could be responsible for stimulation of HPV replication, similar to HIV.

Also the concentration of neutrophil defensins is increased in patients with "intermediate" flora whereas in BV they are not (72).

The immunoregulatory cytokines IL-2 and IL-4 were found to be significantly lower in the group with a symptomatic vaginal dysbiosis compared to healthy controls (955). This could also be due to bacterial proteins and proteases which have been shown to inhibit cytokine release (1508). The production of prostaglandin E2 by monocytes also inhibits IL-2 production, which was proposed as one important factor in recurrent vaginitis by **Witkin** (1525). An inihibition of the cytokine response results in incompetent effector functions of the immune response stimulating further bacterial growth and the prevalence of symptoms. Possible therapeutic interventions in vaginal dysbiosis such as an immunostimulation through IL-2 are implicated but need further study.

However, if bacterial dysbiosis can be considered a separate entity or a state of transition to BV, remains unclear. There has to be reached a consensus about the diagnostics and classifications of vaginal flora to better define different states of abnormal vaginal flora which also has important implications for therapy.
Further studies to differentiate the effects of BV and aerobic vaginitis on the outcome of pregnancy are needed as well. They might solve the problem of inconsistent results concerning the fact that some studies have found no association between BV and pregnancy outcome whereas others did.

7. Candida

Candidal vaginitis is a common mucosal infection principally caused by the opportunistic yeast-like fungus Candida albicans (915, 1323, 1324). While an estimated 75% of women will experience VVC in their lifetime, another 5-10% have RVVC (585). Whereas acute VVC has several known exogenous predisposing factors (1324), RVVC is usually defined as idiopathic.
Antifungal therapy is highly effective in acute VVC and individual attacks of RVVC (915), but it does not prevent recurrence. The question is therefore, if susceptibility to RVVC results from an immune dysfunction or deficiency.

Results of studies on women with RVVC during the last decades have been conflicting as to whether any deficiency in cell-mediated immunity was present but the consensus was that no deficiency in either cell-mediated immunity or antibody response was observed (408). This is consistent with the fact that HIV-infected women do not acquire VVC more often than HIV-negative women and the systemic T cell responsiveness was similar in HIV-positive women with or without symptomatic VVC (801)
It is obvious that innate and adaptive humoral and cellular immune mechanisms are involved in the host defense against *C.albicans* infections, but they exert their functions site-specific. Innate immunity by PMNs and macrophages seem to dominate protection against systemic candidiasis which results from the high incidence of systemic candidiasis in neutropenic patients (1224).
There is now evidence that human and animal vaginal ECs may represent an important innate anti-*Candida* host defense mechanism (1025). Interestingly, a recent study in humans showed that while the vaginal EC anti-Candida activity was not different at the various stages of the menstrual cycle, it was significantly reduced in women with a history of RVVC (81). Thus, reduced EC anti-Candida

activity may represent, in part, a local immune deficiency associated with RVVC. Other innate immune cells such as DCs and macrophages have not been studied during vaginal *Candida* infections.

The efficacy of humoral immune mechanisms and the role of antibodies against *C. albicans* have been controversial and speak for a subordinate role of antibodies in VCC (1134). Especially *Candida*-IgA has been said to have a protective role.
Candida-specific antibodies also induced by the commensal presence of *Candida* (859) have not been associated with protection against infection (408) while other reports indicated that antibodies against *C. albicans* might be protective against experimental disseminated candidiasis (310, 983).
Similar to other studies (402), a recent study found higher levels of *Candida*-specific IgA, IgG1, and IgG4 in those with symptomatic VVC (306). The authors suggested that the increase in antibodies may indicate a role in fungal clearance. However, similar to previous studies showing similar findings, the increase in antibodies correlated with increased rather than decreased fungal burden, and the study was not designed to examine an endpoint of clearance. Thus, it would appear that the antibodies are induced in response to the organism, but there is no evidence of any role in clearance or protection.Instead, it is equally possible that the antibodies actually contribute to the pathogenesis.

Actually, the role of *Candida*-IgE is considered to be an important one.
Earlier studies already proposed allergic reactions to *Candida* in the sense of a local vaginal hypersensitive immune response and suggested an allergic cause for chronic and recurrent symptoms (1197, 1217, 1525). This was confirmed by the study of **Weissenbacher et al**. who showed significantly higher prostaglandin E2 and Candida-IgE levels in vaginal secretions of women with CRVVC compared to healthy controls. As epitopes of *C. albicans* enolase were already detected as binding sites for IgE antibodies (602), this results in a further proposal of a local allergic immune response in CRVVC. Current diagnostic and therapy should be reconsidered.

A further study concentrated on the comparison of 150 healthy women and 74 women with vaginal *Candida*-infection with respect to IL-8 and glucose in vaginal secretions (486). Healthy women not only showed higher concentrations of physiological lactobacilli, lower levels of neutrophils and a significantly lower presence of gram-positive cocci but also higher titers of IL-8. With respect to

glucose levels, there was no difference between these two groups. An explanation could be that higher vaginal levels of the proinflammatory IL-8 in healthy persons serve as expression of an activated immune state, or that IL-8 may be inhibited in women with vaginal infection or downregulated in a kind of feedback-mechanism.

Candida-specific cell-mediated immunity, acquired by exposure to *Candida* as a commensal early in life, has been considered the predominant host defense mechanisms against mucosal Candidiasis. Specifically, resistance to infection has been considered associated with a Th1 response whereas susceptibility to infection is associated with Th2 responses.
Several observations have led to this conclusion. Individuals with impaired T cell function, including those with HIV infections, are particularly prone to *Candida* infections (397, 725). Clinical studies on RVVC as well as several investigations with estrogen-dependent murine models of VVC led to the suggestion that Th1-type CMI plays a critical role in anti-candidal protection at the vaginal level (310, 402, 406, 408, 1214, 1398).

However, studies from mouse models of vaginal candidiasis and clinical studies on women with RVVC have revealed a general lack of protection by local or systemic adaptive immunity (404, 405, 407, 1534).
The general lack of a role for systemic or local adaptive immunity against vaginal candidiasis is postulated to be due to multiple putative immunoregulatory mechanisms that prohibit more profound adaptive immune responses in the vagina rather than a simple lack of immune reactivity or protective effects.
There are experimental and clinical evidences that support immunoregulation or tolerance against *Candida* at the vaginal mucosa.
Mice deficient of δ-chain TCR are more resistant to experimental vaginitis than WT-mice, suggesting a tolerance role for vaginal γ/δ T cells that are in higher numbers compared with the circulation (1533). Vaginal tissue homogenates in mice showed high constitutive concentrations of the immunoregulatory cytokine TGF-β, which were increased further in vaginally infected mice together with low levels of other Th cytokines (1398). Third, *Candida*-specific T cells with appropriate homing receptors for infiltation into the vaginal mucosa are reduced during a vaginal *Candida* infection in mice despite the upregulation of reciprocal adhesion molecules on the vaginal epithelium (1535). Moreover, PMNs, which have significant killing activity against *C. albicans* in vitro and are often observed in vaginal lavage fluid of infected animals, are not effective against *Candida* in the vaginal environment (403).

One report using a live challenge model in humans revealed a possible paradigm shift for factors associated with susceptibility and resistance to candidal vaginitis (400). Of women with no history of vaginitis only few became symptomatic with vaginitis or even colonized. In contrast, inclusion of women with documented infrequent episodes of VVC resulted in significantly higher rates of symptomatic infection, with the remaining women becoming asymptomatically colonized. Interestingly, protection occurred in the absence of any evidence of inflammation or an inflammatory response, whereas those with symptomatic infection had a heavy vaginal cellular infiltrate consisting entirely of neutrophils. Furthermore, a high neutrophil infiltration score correlated with higher fungal burden.
Therefore, symptomatic infection appears associated with a hyperresponse by neutrophils rather than a deficiency in adaptive immunity, whereas resistance to infection appears to be associated with a noninflammatory innate presence rather than an adaptive cell-mediated response. This would change the paradigm for host defense against VVC away from adaptive immune dysfunction and toward dichotomous roles for innate immunity in both resistance and susceptibility to VVC (399).

Although there are significant efforts to develop a vaccine for *Candida* infections (503, 183, 1136, 360), no effective prophylactic or therapeutic vaccine has been developed against candidiasis so far. One should pose the question if a vaccine against vaginal Candidiasis is useful as women with VVC or RVVC are not generally immunosuppressed. *Candida*-specific antibodies and cell-mediated immunity are present locally and systemically and if immunoregulation acts to prevent more profound adaptive immunity, a vaccine would not be suitable to overcome this (399). It may be better to seek other immunotherapeutic strategies to overcome immunoregulation.

8. Trichomonas

Trichomonas vaginalis, a parasitic protozoan, is the cause of trichomoniasis, a STD of worldwide importance. It is estimated that 170 million cases occur each year, making trichomoniasis the most frequent nonviral STD (186).
It is not only associated with classical symptoms of vaginitis or cervicitis but also with other STDs such as BV or gonorrheae (322, 613), adverse pregnancy outcome (259, 950), and an increased susceptibility to HIV (806, 774, 165). However, about

half of all women infected with *T. vaginalis* are asymptomatic (1113) and in males, the infection appears usually to be asymptomatic.
The existing literature provides little information on the host and parasite factors leading to symptomatic or asymptomatic *T. vaginalis* infection in women. The existence of two types of *T. vaginalis* strains, each differing in its morphological characteristics and intrinsic virulence has been suggested (20).

Observations may indicate that innate immunity involving chemotaxis and subsequent influx of neutrophils is much more important than acquired immunity in controlling infections with *T. vaginalis*, since neutrophils are the predominant inflammatory cells found in vaginal discharges in response to infection. Higher vaginal leukocyte concentrations were directly correlated with both the severity of mucosal inflammation and the number of trichomonads in vaginal exsudates (152, 1186).

Little is known about the exact mechanism of how neutrophils accumulate or mediate the initial inflammatory response after acute *T. vaginalis* infection.
It may be hypothesized that trichomonads in the vagina after acute infection secrete proteins like ESP which have a chemoattractant effect on neutrophils (1077). These neutrophils can be further stimulated by *T. vaginalis* to produce chemokines such as IL-8 and GRO-α, mediated through the NF-κB and MAP-kinase signaling pathways (1232).
IL-8 is found in high levels in vaginal discharges of symptomatic trichomoniasis patients. *T.vaginalis*-membrane components have been shown to induce blood monocytes and neutrophils to produce IL-8, probably with the help of TNF-α (1287). IL-8 has been shown to enhance antimicrobial activities of neutrophils by inducing neutrophil degranulation and respiratory burst.
However, a study by **Chang et al**. found that during prolonged *T. vaginalis* adhesion, later proinflammatory production of TNF-α and IL-12 was suppressed, accompanied by inhibition of NF-κB activity suggesting that *T. vaginalis* may inhibit the NF-κB activity of macrophages (198).

In vitro experiments showed that *T. vaginalis* selectively adhered to human vaginal ECs and exerted their cytopathogenic effects (452). It is possible that IL-8 production is induced in vaginal ECs early in infection when the vaginal ECs are activated with *T. vaginalis* - *Candida* and *Neisseria gonorrhea*e have been reported to induce IL-8 production by vaginal ECs early in infection as well (1346, 396).

These may subsequently induce more infiltration and recruitment of neutrophils by chemotaxis at the reaction site. Finally, the neutrophils are thought to cause continued inflammation in vaginal tissue.

Another leukocyte-derived chemoattractant, which may be generated during the infection either by release from *T. vaginalis* or from neutrophils induced by interaction of trichomonads and humoral immunity, is leukotriene B4.
Leukotriene B4 can be found in high levels in vaginal discharges of symptomatic patients (1286) and appeared be further augmented by IgG antibodies promoting the interaction of neutrophils with *T. vaginalis* and augment the inflammatory response through the amplification of leukotriene B4 production (1285).

Complement components have been documented to play a role in the activation of neutrophils and therefore in the inflammatory response to *T. vaginalis* infection as well (1186). **Shaio et al**. were able to show that both a IgG-enhanced classical component pathway activation and an antibody-independent alternative pathway activation provide C3 to facilitate neutrophil killing of *T. vaginalis* (1284). Earlier studies have already suggested an essential role for the alternative pathway activation of complement (185, 1186, 320).
T. vaginalis seems to have taken advantage of a niche in which little complement is present as cervical mucus is surprisingly deficient in complement (321). Menstrual blood represents the only source of complement available to the vagina and has appreciable complement-mediated cytotoxicity toward *T. vaginalis*, and although a reduction in parasite concentration is seen during menses, trichomonal infection persists even after menses (320).
It was also found that iron was a contributing factor in complement resistance. **Demes et al.** found that fresh isolates of *T. vaginalis* differ in their susceptibility or resistance to complement-mediated lysis in serum (321). It appears that complement-resistant fresh isolates become susceptible to complement after prolonged *in vitro* cultivation, which is consistent with the hypothesis that phenotypic variation allows the trichomonad to avoid lysis by complement. Resistance to complement is dependent upon a high concentration of iron (23), a nutrient which is indeed abundant during menses. It seems that iron upregulates the expression of proteases, which have been found to degrade the C3 portion of complement on the surface of the organism; this allows the organism to evade complement-mediated destruction (23).

Nitric oxide and other ROS/ROI produced by immune effector cells such as macrophages are important cytotoxic mediators in microbial infections. The synthesis of nitric oxide by macrophages also results in antimicrobial activity against another protozoan, *Leishmania major*, which was regulated by the cytokines TNF and TGF controlling parasite survival or killing via IFN-γ (480).
In a study by **Malla et al**., the mean concentration of RNI in vaginal tissue of mice infected with *T. vaginalis* isolates from symptomatic women was significantly higher than that of vaginal tissue of mice infected with isolates from asymptomatic women (865). However, it was less in the vaginal washes and plasma of mice infected with isolates from symptomatic women compared to those infected with isolates from asymptomatic women which may be due to different macrophage populations with different functional capabilities (865). This would suggest a possible role for RNI production in establishing the infection.
A recent publication by **Yadav et al**. strongly supports this hypothesis (1547). Mean RNI concentration was significantly higher in leukoctes and vaginal washes of asymptomatic women as compared to symptomatic women, and was also higher in samples of infected as compared to healthy women. This suggests that RNI may have a role in limiting *T.vaginalis* infection in asymptomatic women.

Human infection with *T. vaginalis* does not appear to induce a state of protective immunity, as repeated infections occur without less intensity or duration (1021).
Serum from infected subjects show high concentrations of IgG, IgM and IgA antibody to trichomonads, and specific IgG and IgA are present in cervicovaginal washes from women with acute trichomoniasis (242, 1536, 1363, 21, 22). A significant increase in the specific IgA antibody response was detected in serum samples and vaginal washes of *T. vaginalis*-infected women (1290).
However, similar to their local counterparts, circulating antibody levels also differ and appear to have no function in helping the host to get rid of the infection. Although an association between the presence of local antibody and low parasite counts has been postulated, there is no conclusive evidence to suggest that the presence of IgA antibodies is specifically related to the immune response to *T. vaginalis*.

IgA antibodies may otherwise act as a first line of defense for immune exclusion of the parasite at the mucosal surface. Greater serum and vaginal IgA responses and increased IFN-γ, IL-2 and TNF-α have been reported in mice infected with *T.vaginalis* isolates derived from asymptomatic women than those from

symptomatic women suggesting that specific IgA antibodies might help to protect asymptomatic individuals from severe infection (1061). A higher concentration of proinflammatory cytokines may also lead to a greater activation of macrophages which would result in a greater RNI production again.

Several trichomonad antigens appear to be important for these responses, including α-actinin (7), adhesins (916), and the newly detected TV44 via an IgA mAb (987). Multiple signaling pathways seem to be functional for distinct surface proteins of *T. vaginalis*, and *T.vaginalis* responds during infection both to environmental cues like iron and to adherence to vaginal ECs by up- and downregulating expression of genes (752). Also *Candida albicans* undergoes both up- and downregulation of surface proteins after host cell contact (217).
However, despite the first report describing IgA mAb reactivity to a surface protein of *T.vaginalis*, the protective role of IgA in recognition of surface protein immunogens in trichomoniasis is poorly understood. IgA has been associated with with resistance to a number of other mucosal pathogens, such as *Chlamydia trachomatis* or mycobacteria (1064, 1210).

It is proposed that also IgM and the balance between different IgG subclasses influences the clinical outcome of infection. IgM and IgG1 antibody response was found to be predominantly higher in serum and vaginal washes of mice infected with symptomatic isolates as compared with asymptomatic isolates, whereas no significant difference was observed in the other IgG subclasses (1548).

However, *T.vaginalis* possesses several mechanisms for evading host responses. **Provenzano and Alderete** have reported that numerous proteases secreted by *T. vaginalis* degrade IgG, IgM, and IgA, which allows the organism to survive the antibody response (1145).

The presence of parasite-specific IgG and IgA responses also indicates priming of Th cells, although the relevant antigens are largely unknown, as are the exact effects of antibodies on the parasites. **Paintlia et al**. detected a significant increase in the population of total T cells, as well as CD4+ T cells in mice infected with isolates from asymptomatic women compared with mice infected with symptomatic isolates, but no difference in CD8+cells (1061). Also a significant increase in NK cells was observed in animals infected with isolates from asymptomatic women compared with mice infected with isolates from symptomatic women and control uninfected

mice, indicating that NK cells might be playing a role in the pathogenesis of this disease.
An increase in CD4+ T cells in mice infected with isolates from asymptomatic women appears to be beneficial; it suggests that these cells have a role in the elimination of *T. vaginalis* from their mucosal surface. Clearance of *T. vaginalis* in these animals correlated well with development of anti-IgA antibodies. The results on cytokine production in the study of **Paintlia et al**. also suggest that the Th1 type of response might play a role in the elimination of T.vaginalis.

While it is clear that *T. vaginalis* infection can induce an inflammatory response in the female genital tract, the type of receptors involved in host recognition of the pathogen has not yet been established. A recent study shows that *T. vaginalis* infection of women is associated with the presence in the genital tract of a substance either produced by *T.vaginalis* or by genital tract cells as an innate immune response to infection, that interact with TLR4 (1572).
Interestingly, however, ECs from normal human vagina, ectocervix, and endocervix do not appear to express TLR4 and consequently do not respond to LPS, but do express mRNA for TLR1, -2, -3, -5, and -6 (395). Findings therefore suggest that ECs in the genital tract would not be stimulated during infection with *T. vaginalis* and a severe inflammatory response is initially avoided until the migration of leukocytes into the genital tract would establish an inflammatory response since neutrophils, monocytes, and macrophages express TLR4.
It may be relevant that in many cases, *T. vaginalis* infection is relatively asymptomatic until menses although numbers of *T. vaginalis* organisms decrease at this time (320). Thus, the initiation of symptoms correlates with the introduction of leukocytes into the lower genital tract from the upper genital tract and likely come into contact with *T. vaginalis* organism.

Finally, prospects for vaccine development have been enhanced by the finding that estrogen-treated mice coinfected with Lactobacillus acidophilus can be imunized subcutaneously against vaginal challenge with T.vaginalis, whereas infection alone does not confer protective immunity (2).
The Solco Trichovac vaccine against *T. vaginalis,* which was prepared from inactive lactobacilli and was thought to work by inducing antibodies to abnormal lactobacilli and *T. vaginalis* without adversely affecting the growth of normal lactobacilli in the vagina, showed a lack of antigenic relatedness between the Solco Trichovac

lactobacilli and several strains of T.vaginalis (464). Furthermore, antiserum to L.acidophilus failed to inhibit trichomonad cytoadherence or host cell killing (19).

In conclusion, the findings suggest that both the humoral and cellular immune responses might contribute to the varied symptomatology in trichomoniasis-infected patients, thereby reducing symptoms to asymptomatic levels. Periodic changes in immune competence, perhaps induced by parasite-derived products, might be responsible for an increase in parasite numbers leading to increased epithelial damage and inflammatory response.
However, the current understanding of immunity to *T. vaginalis* remains unsatisfactory, and it is not clear whether acquired immune responses are required for protection and, if so, what role is played by acquired immunity in containing or eliminating infections. Although there is some evidence that protection may be achieved by immunization of laboratory animals, strong protective immunity does not seem to follow natural infection in humans.
A recent study of patients infected with *T. vaginalis* and HIV indicated no evidence of increased levels or longevity of parasite infection in these patients compared to those in patients infected with *T. vaginalis* but not HIV (286). These observations may indicate that innate immunity involving chemotaxis and subsequent influx of neutrophils is much more important than acquired immunity in controlling infections with *T. vaginalis*.

C. Immunology in reproductive medicine

1. Immunology of normal pregnancy

Maternal tolerance of her semiallogenic histoincompatible fetus during pregnancy is still a unique physiological phenomenon contradicting many of the generally established rules. From the immunological point of view, pregnancy is a balancing act of tolerance toward the fetus on the one hand and functional immunodefense on the other hand.
Studies have demonstrated that the maternal immune system recognizes the semi-allogeneic fetus, but that it remains in a quiescent state. The potential mechanisms underlying this phenomenon are likely to be complex and may involve several complementary or overlapping pathways to favor reproductive success. Knowledge of the immunoregulatory processes of pregnancy is necessary to understand and

treat a variety of disorders, which may lead to infertility, premature events or preeclampsia.

The constituents of the maternal immune reaction to the allogeneic stimulus are not different from any other immune reaction and the allogeneic conceptus, the trophoblast, is like all other allogeneic tissue grafts.
Anatomically, placental trophoblasts occupy a unique position at the interface between mother and child. These epithelial cells possess several intriguing features that suggest a role for these cells in preventing fetal rejection. The extra-embryonic trophoblast has several distinctive properties such as the expression of endogenous retrovirus products, oncofetal proteins, and imprinted genes and its DNA is relatively unmethylated (953, 1430). Moreover, it eliminates abortogenic maternal B cell and T cell responses through immunmodulation, probably through progesterone (1159).

Indeed, trophoblasts can influence the immune system during pregnancy through their expression of soluble and cell surface-associated immunomodulatory molecules. For example, trophoblasts secrete IDO, which limits the availability of the essential amino acid tryptophan, consequently limiting lymphocyte proliferation and protecting the conceptus from rejection (988, 1270). Pregnant mice exposed to an IDO inhibitor rejected allogeneic but not syngeneic fetuses. IDO may not only inhibit T-cell proliferation but also be bactericidal by this mechanism (1270). The DC-associated signaling molecule CD200 is also expressed on fetal trophoblast and decidua (235). It was already found essential for induction of allograft tolerance in mice (466) by signaling immunosuppression and experiments in mice support the hypothesis that CD200 plays an unknown immunoregulatory role at the maternal-fetal interface in maintaining pregnancy (235, 466).
There is also growing evidence that trophoblast cells are able to recognize and respond to pathogens through the expression of TLRs, especially TLR2 and TLR4 (3, 561, 754). This suggests that the placenta serves as a highly specialized barrier protecting the fetus against infection and that trophoblast cells themselves initiate an immune response characterized by promotion of cytokines such as IL-6 and IL-8 (3, 4, 489, 561). As it has been found that TLR4 ligation promotes cytokine production, while ligation of TLR2 induces apoptosis in first trimester trophoblast cells it was suggested that a pathogen may directly promote the elevated trophoblast cell death via TLR2 observed in a number of pregnancy complications (4). TLR2-mediated trophoblast apoptosis, therefore, may provide a novel

mechanism of pathogenesis by which certain intrauterine infections may contribute to conditions such as preterm labor or preeclampsia.

Moreover, trophoblasts express little or no classical MHC class I (HLA-A, -B, and -C), but do express a number of nonclassical MHC class I molecules. Examples include HLA-E, -F and -G, which can modulate the innate immune response and prevent NK and NKT cell-mediated cytotoxicity (230, 696, 698, 965, 1143, 1158, 1219). Additionally, trophoblasts have been recently shown to express the nonclassical MHC class I molecule, CD1d, on the surface of first-trimester human trophoblasts (137).
Also the apoptosis-inducing molecule FasL is expressed by placental trophoblast (582) and by first trimester and term human placental villi (500) inducing apoptosis in maternal Fas positive immune cells, such as NK and T cells, at the fetomaternal interface (1042). A reduction in FasL expression seems to be closely associated with activation and infiltration of maternal NK cells and destruction of uterine glands, resulting in rejection of the foetus (1042). Thus, expression of FasL in the uterine glands and cytotrophoblasts may play a role in the downregulation of the maternal immune response, thereby maintaining pregnancy at early stage.

During four phases of the fertilization period maternal immune responses are minimized by different mechanisms, for example protection of the egg through the follicular fluid with its immunosuppressive activity (184), the ZP or maternal cumulus cells (79).
The implantation phase with direct embryo/maternal contact in the uterus is probably the most vulnerable time for the embryo which is now exposed to maternal endometrial immune cells and cytokines. The uterus is designed to prevent implantation except during a narrow implantation window in which embryonic signals help promote endometrial priming and maternal immune tolerance.
Post fertilization, immune tolerance is possibly promoted by the immunosuppressive EPF and PAF (976, 1035, 1156). EPF is non-specific and detectable in serum (506), amniotic fluid (1585) and cervical mucus (218) of pregnant women but also in non-pregnant women which makes its use as a marker for evaluation of pregnancy not very helpful.

How this maternal immune tolerance takes exactly place, what embryo-derived elements are involved and where, if these mechanisms are common to all mammals, and how early embryo tolerance can be detected in the maternal organism are still

are still unanswered questions. **Barnea** postulated that unique embryo-derived compounds are involved in creating the unique maternal immunological response to the embryo (79, 1118). His study group favors PIF as modulator of the maternal immune system, allowing the mother to maintain her ability to fight diseases. **Coulam and Barnea** have found that the presence of PIF activity in maternal serum within 4 days after embryo transfer indicates a >70% chance of successful pregnancy outcome whereas the absence of PIF activity indicated that pregnancy would not develop in 97% of cases (**Coulam and Barnea, unpublished data**).

In the following, results from studies concerning immune cells at the feto-maternal interface during normal pregnancy and their actions are summarized and evaluated.

APCs such as DCs and macrophages are often regarded as the responsible inductors of maternal tolerance against fetal antigens (660).
Studies in mice or rats and humans showed that the population of APCs in the decidua of pregnancy comprises classical macrophages, classical mature DCs (CD83+), immature or intermediate macrophage/DC-like cells (CD83-) and myeloid DCs (121, 157, 158, 439, 579, 657, 659, 660, 808, 900, 952, 1047, 1385). However, the exact functions of decidual APCs are unclear. Specialized APCs may present fetal antigens from the invasive trophoblasts to the maternal immune system which leads to a state of tolerance. Decidual APCs are able to either acquire functions of classical mDCs that can activate T cells or rest in an immature state which likely induces tolerance (843). Cytokine studies show that decidual mDCs secrete less IL-12 than blood monocyte-derived DCs and induce Th2 cells when cocultured with naive CD4+ T cells, which suggests an immunosuppressive phenotype of decidual DCs (951). Recent studies found a significantly higher number of mDCs in human decidual tissue from abortions than in normal pregnancies which leads to speculations that mDCs may play a role in recurrent abortions (62).

CD11c+ CD86+ DC-SIGN+ macrophages are with 20-30% of the decidual cells one of the major cell types in both the maternal and fetal compartments at the site of implantation and unlike NK cells, remain high throughout pregnancy (581, 808, 940, 963). They are closely associated with trophoblast (1316) and it is proposed that they contribute to the feto-maternal immunesuppressive adjustment via cytokines and growth factors (467, 963) and prostaglandin E2 production (584).

However, there is limited knowledge about how decidual APC are driven into immune inhibitory profiles.

Concerning macrophage function, they seem to be involved in essential tissue remodeling by clearance of apoptotic trophoblast cells during implantation and embryo development and thus preventing an extensive inflammatory reaction (107, 583, 963, 1175, 1321). **Mor et al**. stated in their hypothesis that the uptake of apoptotic cells suppresses activated macrophages from secreting proinflammatory cytokines such as TNF-α and IFN-γ and promotes the release of Th-2 type, anti-inflammatory and immunosuppressive cytokines with protective effects on trophoblast survival and immunological tolerance (963). Changes in the cytokine milieu, owing to elevated levels of apoptotic bodies and inefficient clearance, will result in a proinflammatory microenvironment that in turn may result in changes in trophoblast resistance to Fas-mediated apoptosis and the maternal immune system.

The number of endometrial leukocytes continously increases with beginning of pregnancy (1119). 46% of the decidual CD56+ leukocytes are uterine NK cells, 19% are macophages and 8% are T cells, mainly CD8+ T cells, whereas B cells are virtually absent (808, 949) which means that cells of the innate immune system seem to dominate this tissue.

Decidual NK cells are the predominant maternal immune cells in early pregnancy (1158), declining again later until they are absent at term (954). This reduction of uterine NK cells in later pregnancy remains unexplained. Death by both apoptosis and necrosis has been proposed for the loss of uterine NK cells in the late stages of mouse pregnancy (757) but no information is available regarding the fate of the cells in human pregnancy (155).

Results of studies which have compared the distribution of uterine NK cells between decidua basalis, which underlies the placenta and is infiltrated by trophoblast, and decidua parietalis, which lines the remainder of the uterine cavity, have been inconsistent. Some have reported increased numbers of uterine NK cells in decidua basalis and considered this to support a role in the control of trophoblast invasion (1312) but others have failed to detect any difference in the different decidual areas (**Scaife, Bulmer, Robson, Searle, Innes, unpublished data).**

However, the possibility of a functional difference between the two sites has not been considered in detail. Since uterine NK cells express receptors for the non-classical HLA antigens expressed by extravillous trophoblast, contact with trophoblast in decidua basalis could lead to altered function such as a differing

cytokine profiles and increased IFN-γ levels have been reported in decidua basalis (1463).

Phenotypically, decidual NK cells ($CD56^{bright}$ CD16- CD160-) differ from NK cells in peripheral blood ($CD56^{dim}$ CD16+ CD160+) which suggests that either decidual NK cells represent a distinct subpopulation of circulating NK cells or they have undergone some tissue-specific differentiation. There are also morphological differences between $CD56^{bright}$ NK cells in decidua and those present in low numbers in peripheral blood. All the peripheral $CD56^{bright}$ NK cells are small and agranular whereas those in decidua are mostly large granular lymphocytes (699). However, decidual NK cells can change into classical CD16-expressing cells after IL-2 stimulation which can result in cytotoxicity and an alloimmune reaction (701). Women with recurrent abortions have high numbers of NK cells of the conventional CD16+ CD56+ type in the uterus (1448).
This underlines the possibility that decidual NK cells exert different functions with the priority of tributing to placental development and pregnancy outcome. In direct contact with the trophoblast, they can produce cytokines involved in the control of trophoblast invasion, trophoblast differentiation, decidual artery remodeling and placental augmentation (57, 155, 156, 233, 275, 1430).

The function of decidual NK cells is regulated by a balance between activating and inhibitory signals provided by their receptor repertoire upon recognition of specific ligands, most of which are HLA molecules (HLA-C, HLA-G, HLA-E) expressed on invading trophoblast (700, 1158, 1444). These are the KIRs for which HLA-C and HLA-G molecules are the ligands, the immunglobulin-like receptor ILT2 which is expressed by 20-25% of decidual NK cells and interacts with HLA-G, the CD160 receptor with its major ligand HLA-C which is expressed on a minor set of decidual NK cells, and the CD94/NKG2 receptor which binds HLA-E. **Varla-Leftherioti** suggested that among the different interactions of NK receptors with their specific couterparts on trophoblast, the inhibitory KIR-HLA-C interactions appear to be those mainly involved in the functions of an NK cell-mediated allorecognition system in pregnancy (1444).

Several mechanisms have been suggested for this regulation of pregnancy including cytotoxicity, local cytokine production and induction of trophoblast apoptosis. There is strong evidence that decidual NK cells are unlikely to play a role in cytotoxicity. The general consensus of different studies is that the cytotoxic activity of uterine

NK cells is reduced compared with peripheral blood NK cells (155, 701, 787, 793, 1158, 1444).

The functional importance of uterine NK cell cytokine and growth factor production in normal pregnancy is currently not known (1195, 1239). The cytokine profile of uterine NK cells does, however, appear to differ dependent on the phenotype of the surrounding cells, such as HLA-G expressing cells. Altered production of IFN-γ has been reported in decidua basalis compared with decidua parietalis (1463) and **van der Meer et al.** have reported increased production of IFN-γ and VEGF by uterine NK cells in response to HLA-G (1438).

Current interest is directed towards the role of uterine NK cell-derived cytokines, particularly IFN-γ, in the control of trophoblast invasion by a non-cytotoxic mechanism and remodelling of uterine spiral arteries in the first half of normal pregnancy (155). These cytokines may influence trophoblast growth since their receptors have been found in human trophoblast cells (634). In the placental bed of uncomplicated pregnancies during early gestation up to 30% of the extravillous trophoblast cells are undergoing apoptosis and many of these apoptosing trophoblast cells are surrounded by uterine NK cells (330). A possible explanation for this may be that the trophoblast cells which undergo apoptosis recruit uterine NK cells after apoptosis has been initiated (155). If uterine NK cells regulate trophoblast invasion then the most likely mechanism by which this occurs is mediated by uterine NK cell cytokine secretion. Also cytokine production by decidual NK cells might play a role in the defense against viral spreading to the fetus in case of uterine infection, for example, secreted IFN-γ might control cytomegalovirus spreading (1158).

Pregnancy presents itself as an inflammatory condition. Study results generally demonstrate an increased number of granulocytes in pregnancy compared to nonpregnant women (841, 948). An increased expression of surface CD11a, CD11b, CD14, CD49d, CD54 and CD64 on monocytes in pregnant women further supports the hypothesis that the innate immune system is activated during pregnancy (841). Available data on granulocyte activation during pregnancy are contradictory with some studies showing increased surface expression of both activation markers and adhesion molecules, for example of CD11b and CD64 (1234), and an increased production of intracellular ROS and enhanced phagocytosis (997). Other studies showed no upregulation of activation markers and reduced microbial killing activity and chemotaxis of granulocytes (269).

T cells comprise about 10-20% of the leukocytes in the uterine mucosa and continously increase during pregnancy until a decline at term (1119) with rising CD8+ and decreasing CD4+ T cells, respectively (398). Recently, the importance of decidual CD4+CD25+ Treg cells with their increasing numbers during pregnancy in mediating maternal tolerance to the fetus has become clear (33, 1243, 1258, 1259, 1325).

However, the factors regulating CD4+CD25+ Treg cells are largely unknown. Data suggest that estrogen promotes maternal tolerance to the fetus by increasing the number of CD4+CD25+ Treg cells (1129). Decidual CD4+CD25^{high} T cells express a high frequency of intracellular CTLA-4 and 5–7% of these cells express surface CTLA-4 (1259), suggesting that decidual CD4+CD25^{high} T cells are stimulated by some antigens such as fetal antigens. Because antigen stimulation by DCs induced the surface expression of CTLA-4, these activated CD4+CD25^{high} Treg cells should mediate maternal tolerance to the fetus (1241). Recently, **Sindram-Trujillo et al**. reported that labor is associated with a decrease of CD4+CD25+ cells in decidua, suggesting that the disappearance of the CD4+CD25+ T cell population may contribute to the induction of labor, although the study did not explicitly investigate CD4+CD25^{high} cells (1311).

Interestingly, CD4+CD25+ Treg cells express TLR4, 5, 7 and 8 (175). Recent data showed that microbial induction of the Toll pathway, partly mediated by inflammatory IL-6, blocks the suppressive effect of CD4+CD25+ Treg, allowing activation of pathogen-specific adaptive immune responses (1094). Recently, **Yang et al.** reported that DC-based tumor vaccines ablate tumor-specific T cell tolerance only after removal of CD4+CD25+ Treg cells (1556). However, to ablate tumor-specific T cell tolerance in the presence of CD4+CD25+ Treg cells by DC-based tumor vaccines, persistent TLR signals are required to reverse CD4+CD25+ Treg-mediated CD8+ T cell tolerance.

These data suggest that chronic inflammation in recurrent spontaneous abortion, preterm labor and preeclampsia might disturb the suppressive effects of CD4+CD25+ Treg cells resulting in induction of fetal rejection responses (1241).

Taken all together, decidua is a highly complex tissue containing unique, highly specialized leukocyte subpopulations for each stage of gestation that may play a role in determining the nature of local immune responses at the fetomaternal interface. The specific leukocyte composition is controlled at the level of cell trafficking with different expression of cellular adhesion molecules involved in

leukocyte recruitment which has been demonstrated in the mouse (751). Switches in vascular specificity or partial loss of microenvironmental specialization during the second half of mouse development result in dramatic changes in leukocyte populations recruited to the fetomaternal interface.
Studies showed a generalized activation of peripheral blood leukocytes in third trimester normal pregnancy, which is not reflected in the leukocyte populations within the decidua (841). Although a Th2 phenotype predominates within the decidua during pregnancy, this is not the case in the peripheral circulation. As the vast majority of maternal-fetal interactions during gestation occur within the uterus it is not surprising to find a discrepancy in the phenotype of leukocytes between the two compartments.

Future studies are needed to get a more integrated understanding of how these different cell types in the decidua interact with each other and with trophoblast to create the correct environment for implantation to be a success from both the maternal and the fetal perspectives.

However, the observation that NK cells and macrophages, and not T and B cells, comprise the major population of leukocytes in the uterus lends support to the idea that implantation is likely to involve an innate immune system that is distinct from that seen in clinical organ transplantation, where rejection is mediated by cells of the specific immune system, T and B cells.
Pregnancy is characterized by the enhancement of the innate immune system and suppression of the adaptive immune response (541, 1233). There are accumulating evidences in support of this view, for example an increased number of granulocytes in maternal blood as well as phenotypic and metabolic changes in granulocytes and monocytes including increased expression of adhesion molecules, baseline intracellular reactive oxygen, and oxidative burst (997). Moreover, an increased concentration of acute phase proteins such as fibrinogen, clotting factors or globulin (246) and a shift of the Th1- to Th2-type cytokine profile (205).

Moreover, placental tissue in physiological pregnancies contains a fully organized complement system with components C1q, C3d, C4, C5, C6, and C9 detectable (153 381, 1314). As the placenta as a newly formed organ undergoes the process of tissue remodelling, the role of the complement system in the clearing of potentially destructive debris products may be essential. Uncontrolled complement activation

is prevented in successful pregnancy by the three regulatory proteins DAF, MCP and CD59 positioned on the surface of trophoblasts (153, 455, 457, 967).

Deposits of the components were also detected on spiral arteries in normal pregnancy with the highest staining for C3d and C9 suggesting that the complement system is likely to be activated through the classical pathway (1491). A humoral immune response leading to complement activation may thus be involved in the physiological changes of spiral arteries in early pregnancy. The terminal C complex as the final end product of all three pathways was also found to localize in the fibrinoid material of basal decidua, chorionic villi stroma and in vessel walls (1405).
However, the exact way of complement activation in placenta has not been clarified. Potential local activators including cellular and tissue debris could be responsible (153). Another study demonstrated that normal pregnancy is associated with activation of the complement system as determined by increased maternal plasma C3a, C4a, and C5a concentrations remaining high from 20 weeks to term gestation (1194).

Th1- and Th2-type cytokines produced by maternal T lymphocytes present at fetomaternal interface also seem to play a decisive role in the development in pregnancy (205, 1309, 1463). Successful pregnancy seems to be associated with the predominance of antiinflammatory Th2-type cytokines over proinflammatory Th1-type cytokines (1463, 1485).
Murine models and human studies showed that pregnancy rejection or complications are mediated by elevated Th1-cytokines and decreased levels of Th2 cytokines (180, 181, 789, 1115, 1357), whereas an increased Th2-cytokine response and decreased levels of Th1 cytokines confers protection (750, 819, 1485). It is currently believed that for the continuous normal development of pregnancy production of proinflammatory cytokines such as, IL-2, TNF-α and IFN-γ is suppressed, whereas production of antiinflammatory cytokines such as IL-4, IL-6 or IL-10 is enhanced. Therefore, the conception of Th2 overbalance during pregnancy has been a paradigm for immunology of reproduction for many years, while Th1 activity has been presented as unwanted component.
However, the broadening knowledge of immunological mechanisms working for successful pregnancy and birth of the viable fetus brings nowadays the necessity to verify the "Th2-phenomenon" (1485) in favour of conception of "Th1–Th2

cooperation" (203) in which Th1 type activity is no longer only destructive component of physiological pregnancy.

This fine-tuned cytokine balance between Th1 and Th2 seems to be regulated on the intracellular signaling level which is regulated again by a complex of cytokines and other factors, for example the JAK/STAT system (1128). Intracellular signals from cytokine receptors are mostly transduced via the Janus kinases (JAK) and signal transducers and activators of a transcription (STAT) system. Only little is known, however, about the specific intracellular signals in decidual lymphocytes during pregnancy that may support fetomaternal tolerance. Important regulators of cytokine signals in lymphocytes are the suppressors of cytokine signaling (SOCS) which are constitutively expressed in naïve T cells (1128). They have also been detected in gestational tissues and their differential regulation is associated with the onset of labor, but there is no information about their role in decidual lymphocytes in pregnancy so far (123).

CD4+ T lymphocytes of Th2-activity are the source of IL-4, IL-5, IL-6, IL-10, IL-13 and GM-CSF, while CD4+ T lymphocytes of Th1 activity produce mainly IL-1, IL-2, IL-12, IL-15, IL-18, IFN-γ and TNF-α (99).
IL-4, known to be the essential antiinflammatory cytokine for Th2 differentiation (365), IL-6 (1348), and IL-10 (1128) are constantly present in high numbers at the fetomaternal interface where they are involved in pregnancy-supporting mechanisms (365).
It was also found that peripheral blood lymphocytes of pregnant women in the first trimester secrete "*in vitro*" more Th2, i.e. IL-4, IL-10 and less Th1, i.e. IL-2, IFN-γ cytokines compared to non-pregnant patients (879). Also the number of IL-4 secreting cells rises progressively in the course of pregnancy (887). Probably alloantigens localized on trophoblast are the signal for peripheral lymphocytes to Th2 activity which is supported by observation that in further trimester peripheral lymphocytes of pregnant women differentiated to Th2 cells secreting IL-4 after recognition of paternal alloantigens in mixed lymphocyte culture (358).

Studies on mice and humans showed that Th1 activity triggers endometrial leucocytes and embryo itself to produce Th2 cytokines, LIF and TGF-β, activates angiogenic factors (1401) and endothelial cells, thus preparing maternal tissues for embryo implantation (674, 1503). Th1 cytokines stimulate cytolytic activity of

decidual NK cells and lymphokine activated killer T lymphocytes which are able to restrict excessive trophoblast proliferation and invasion (89).

IFNs, especially upon GM-CSF stimulation, can be secreted by cyto- and syncytiotrophoblast. All isoforms of IFNs are present in human placenta, mostly in extravillous trophoblast, but also in villous syncytiotrophoblast (1100). They play a role of immunomodulators as they can decrease proliferative activity of maternal T and B lymphocytes (1463) as well as they cause soluble HLA shedding from the surface of trophoblastic cells. Soluble HLA functions as immunosuppressive factor for maternal macrophages and cytotoxic lymphocytes (1231). IFNs can also increase expression of HLA-G molecules on cytotrophoblast (1463), augment Th2 cytokines production, i.e. IL-6, GM-CSF in endometrial stroma and, together with IL-12, IL-15, IL-18 and VEGF, influences local angiogenesis in uterus (275).
These facts lead to the hypothesis that Th1 activity plays important role in promotion of Th2 response, regulation of placentation process, defense against infections and initiation of delivery. Together with Th2 activity it is necessary component of immunological reactions during pregnancy (1503).

Th1 cytokines also play a positive role for initiation of labor at term (1503). In the sera of delivering women, raised concentrations of IFN-γ and IL-1β were observed (159). Th1 cytokines stimulate decidual prostaglandins which are responsible for the onset of uterine contractions (1132). Proinflammatory cytokines together with mechanical stimuli induce IL-8 production, whose concentration in cervical mucus is positively correlated with the progress of cervical ripening and opening (45).

IL-15 and IL-18 are critical cytokines controlling uterine NK cell cytotoxicity by upregulation of perforin and FasL expression on human decidual NK cells and cytokine production such as GM-CSF, IFN-γ, TNF-α, MIP-1α and MIP-1β (124, 122, 178, 370, 587, 1421, 785). Moreover, IL-15 has been implicated in differentiation and proliferation of uterine NK cells and plays a possible role in induction of IFN-γ production as a mediator in vascular remodelling during early pregnancy with its highest expression in perivascular cells surrounding the decidual spiral arteries (56, 716, 785, 1453). Progesterone induces IL-15 expression and therefore, when progesterone levels fall as a result of failing pregnancy, the manifestation of apoptotic NK cells could be the result of a decreasing level of IL-15 (828).

Similarly, decidual IL-18 enhances innate immunity and both Th1- and Th2-driven immune responses via induction of IFN-γ/IL-4/IL-8/IL-13/GM-CSF production from T cells, NK cells, B cells and DCs (785, 1002). It might also be essential for proper vascularization of the implantation site (274).
However, there are not only high serum IL-18 levels in pregnant women but also in women with implantation failure, fetal growth restriction or recurrent abortions (588, 609). An increased level of IL-18 promotes strong NK cell activation and probably excessive IFN-γ production (785). It seems that a tight regulation of IL-18 is important for normal implantation and decidual remodelling in early pregnancy.
A special role in trophoblast invasion (1117, 1355), endometrial decidualization (45), and angiogenesis inside trophoblastic villi (619) is also considered for T cell production of LIF (1289).

It has been demonstrated that Th2 cytokines increase the proportion of asymmetric antibodies, which are unable to activate effector immune mechanisms such as complement fixation, clearance of antigens and phagocytosis (493). It also has been established that their synthesis is increased under different conditions involving Th2 responses such as pregnancy (104, 864).
Experiments showed that IL-6 is the main responsible factor for the glycosylation of asymmetric IgG molecules and therefore regulates the quality of the humoral immune response during pregnancy (172). **Gutierrez et al**. then hypothesized that during pregnancy, in context of a predominant Th2 immune response, the quality of IgG antibodies synthesized is modified by IL-6 of placental origin (493). If the IL-6 levels secreted by placental cell or normal, there is a preferential synthesis of asymmetric glycosylated antibodies which have a blocking activity and participate in the protection of the fetal antigens whereas if IL-6 levels are abnormal there is a predominance of aggressive antibodies (493).

Another factor regulating immune responses during pregnancy in several ways is progesterone. It can induce the production of Th2-type cytokines (386, 1378) and of LIF and M-CSF production by T cells which is mediated by IL-4 (1116, 1118). Moreover, it can decrease Th1 activity of TNF-α in luteal phase endometrial tissue (1117) which is both at least partly regulated by increased levels of PIBF (624). As urine PIBF concentrations are positively related to pregnancy outcome it could be used as one possible marker for predicting pregnancy outcome (1376).
In summary, results suggest a hormone-cytokine-T cell network at the fetomaternal interface with progesterone partly responsible for the T2 switch there. Defects in

this network can result in fetal loss. Numerous studies have also demonstrated that progesterone via PIBF blocks mitogen-stimulated lymphocyte proliferation, improves allograft survival time, reduces NK cell cytotoxicity and modulates antibody production besides affecting other phases of the immune response (386, 677, 786, 1076, 1105, 1376).

What has become clear from investigations is that labor and the post-partum period also involves an inflammatory reaction with induction of proinflammatory cytokines. The process of cervical ripening is characterized by an influx of neutrophils and macrophages, i.e. cells of the innate immune system, and increasing production of IL-8, IL-1β, IL-6 and TNF-α in the human cervix (1056, 1566). An inflammatory response during labor may also help to remove placental fragments and prepare the uterus for the pathogens that it will undoubtedly encounter during the immediate postpartum period (1105).

These cytokines induce cervical dilatation by production of MMP-1, MMP-3, MMP-9 and cathepsin S (1480) as well as by increasing production of COX-2 and prostaglandin E2 (1105). Furthermore, IL-1β downregulates the expression of TIMP-2, an endogenous inhibitor of MMP-2, which can digest the collagen and elastin fibers in the extracellular matrix of the cervix to further increase cervical compliance (1480). Prostaglandin E2 may then further stimulate labor by increasing the production of proteinases or indirectly by increasing the permeability of blood vessels for leukocyte trafficking (687). NO, another proinflammatory mediator that is increased at term, may also contribute to vasodilation in order to facilitate leukocyte trafficking (687).

Increased levels of G-CSF, IL-8 and IL-6 stimulate neutrophils to release more proinflammatory cytokines and neutrophil elastase that can digest the extracellular matrix produced by cervical fibroblasts. (1274, 1105). These cytokines could be used as markers for predicting labor.

The same proinflammatory cytokines and MMP-9 also stimulate the rupture of the membranes above the cervix (44, 1567, 1546). Increased collagenase activity can then weaken the strength of the membranes and lower their threshold for rupture.

Stimulation of amnion and chorion cells with IL-1β and TNF-α also increases the production of prostaglandin E2 via COX-2 (1131).

Prostaglandin E2 may then either cause increased production of MMP-9 (903) or it could cross the membranes to stimulate cervical ripening in the cervix or stimulate contractions by the myometrium. Hormones and cytokines associated with labor

such as cortisol, TNF-α and IL-1β, have been shown to inhibit the production of the enzyme 15-hydroxyprostaglandin dehydrogenase by chorion and trophoblast, which may contribute to increased prostaglandin production during labor (1095).

The initiation of rhythmic contractions in myometrium is also associated with increased number of leukocytes and changes induced by these proinflammatory cytokines (1566, 1567). IL-1β and TNF-α stimulate arachidonic acid release, activate phospholipid metabolism, and increase the production of prostaglandins by the myometrial cells similar to oxytocin effects (957, 958, 1419). IL-1β and TNF-α can also increase the production of MMP-9 by myometrial cells, which may be important for detachment of the placenta (1212).
IL-6 has no effect on prostaglandin production by myometrial cells (1419) and is unable to stimulate myometrial contractions. However, this cytokine may play a role in labor by increasing the expression of oxytocin receptors on myometrial cells (1176) and like IL-1β, it can also increase oxytocin secretion by myometrial cells (428).

The post-partum period of about one year has been considered as a time for immunological recovery from the profound immunological changes of pregnancy. The immunology of the post-partum period is often viewed as a restoration of Th1/Th2 balance, which has been useful, but not the only explanatory model in explaining autoimmune diseases such as rheumatoid arthritis as Th1-driven diseases which remit during pregnancy but exacerbate during the post-partum period (482).
Delivery is associated with increased serum levels of inflammatory cytokines such as IL-6 and IL-1 (256). The early post-partum period is also characterized by upregulatory inflammatory responses. Both Th1 and Th2 *ex vivo* cytokine production rise during the post-partum compared to pregnancy, with increased Th1/Th2 ratios observed through post-partum months 1 to 12 (1300). **Watanabe et al**. found increased levels of Th cells, CTLs and NK cell subsets with weak cytotoxicity (CD16+ CD57+) in the first months post partum (1479). An increase of suppressor T cells and CD5+ B cells followed during month 7 to 10 post partum which may also be related to the postpartum aggravation of autoimmune diseases (1479). The three immune activation markers neopterin, soluble IL-2 receptor, both elevated during allograft rejection or autoimmune disorders, and soluble CD8 antigen were found elevated at delivery and post partum (162). In a recent study by **Groer et al**. every serum cytokine measured and all proinflammatory macrophage

and acute phase proteins (TNF-a, IL-6, CRP, and neopterin) are higher in post-partum mothers along with a higher lymphocyte proliferative response and the secretion of higher levels of S-IgA (482).

All these data suggest a broad state of immune activation and an upregulated general and mucosal immunity whose functions are unclear at this time. One can speculate that innate inflammatory activation is both protective and related to the stage of uterine involution. There is increased risk for maternal uterine contamination during birth, and the endometrium is protected from infection by activated macrophages. Uterine involution is associated with myometrial shrinkage, elimination of microorganisms, and repair and restoration of the endometrium which is also associated with both apoptosis and proliferation. This process probably involves inflammatory and immune mediators (482).

2. Disturbances in maternal-fetal interactions

Pathological conditions during pregnancy including preeclampsia, IUGR, PTL and RSA are some of the leading causes of maternal and fetal morbidity and mortality worldwide. Latest studies suppose a common etiology for these pregnancy disorders as they are characterized by similar histological features (885). The endpoint of all disorders may be placental ischemia and inflammation due to an inadequate trophoblast invasion and defect remodelling of spiral arteries.

Studies on the etiology of IUGR are rare compared to the other pregnancy disorders. Results propose an activated state of the immune system with higher serum levels of B lymphocytes and antibodies as well as CD4+ T cells (83, 886, 947, 1272) as compared to healthy pregnancies
Concerning cytokine levels, **Bartha et al**. found in their study that serum TNF-α is increased in women with IUGR and placental insufficiency but normal in those with IUGR and normal placental perfusion (84) suggesting that elevations of TNF-α could be a specific phenomenon of certain subsets of IUGR with placental dysfunction. This was supported by the study of **Holcberg et al**. who reported a correlation between increased placental TNF-α secretion and an abnormal placental perfusion due to vasoconstriction of the fetal placental vascular bed (560). The study of **Walther et al**. detected serum autoantibody against the angiotensin type 1 receptor in a high percentage of pregnant women with preeclampsia and IUGR (1472). This autoantibody may be causative for pathological uteroplacental perfusion and thus

be associated with distinct types of pregnancy disorders rsulting from impaired placental development.
Further studies have to be undertaken if it could serve as an early marker for these disorders.

a) Preeclampsia

Preeclampsia, the occurrence of acute hypertension during pregnancy, is still a leading cause of maternal and fetal death worldwide affecting about 3-7% of all pregnancies (1202). To date, there is no reliable, costeffective screening test for this disease, and there are also no widely accepted or proven measures for primary prevention.
Its histological signs comprise restrained trophoblast invasion, vasculitis, thrombosis and placental ischemia which resembles features of other obstetric complications like IUGR, RSA or abruptio placentae. The question is if these separate clinical entities might have a common immunological etiology (885).

Current hypotheses regarding its etiology focus on maladaptation of immune responses and defective trophoblast invasion. Researchers suppose that an excessive maternal inflammatory response directed against foreign fetal antigens results in shallow trophoblast invasion, defective spiral artery remodelling, and placental infarction which results in the release of proinflammatory cytokines and placental fragments into the systemic circulation and finally in endothelial activation (1203). An abnormal maternal immune response in the sense of an acute allograft rejection was already implicated in earlier studies (717). A recent paper by **Feinberg** presents the thesis of a minimal excess of placental immune complex production versus removal as the cause of a proinflammatory autoamplification cascade of trophoblast apoptosis and oxidative stress, culminating in clinical preeclampsia (388).

For years, there have been discussions on the question of primiparity versus primipaternity in the etiology of preeclampsia (202, 911). For some researchers, preeclampsia is associated with the first pregnancy, with oxidative stress as the main cause of vascular defects. For others, it may be associated with the first pregnancy with a particular father.

The first occurrence of preeclampsia, or its recurrence in primiparous women, seems to be associated with a change of partner (1207, 1208). Furthermore, its incidence is inversely correlated with the duration of sexual cohabitation (1206). In other studies oral sex seemed to be protective; a possible immunological explanation may be that both local and, more important, systemic tolerance can be obtained by oral administration of an antigen.

Although exposure to paternal antigens through a prior pregnancy has a protective effect against development of preeclampsia, exposure to new or different paternal antigens as a result of change in paternity is associated with an altered risk of preeclampsia (813).

Certain reproductive practices that minimize maternal exposure to seminal fluid are associated with increased risk of preeclampsia which include barrier contraception, brief sexual cohabitation, nonpartner donor insemination, oocyte embryo donation, absence of preconceptual oral sex, or intracytoplasmic sperm injection with surgically obtained sperm (1208). Thus, an accumulating body of epidemiological evidence points convincingly toward an immunogenetic basis for preeclampsia.

Perhaps the greatest barrier to enlighten the etiology of preeclampsia is the still incomplete understanding of the immunological basis for normal pregnancy. The success of human reproduction depends on the ability of the mother and fetus to control allogeneic immune responses through multiple, overlapping mechanisms, while maintaining the capacity to mount defense against infectious organisms (726). Evidence suggests that site-specific suppression, whereby maternal immune responses are controlled locally at the maternal-fetal interface, plays a fundamental role (726).

As mentioned above, in normal pregnancy, the ratio of proinflammatory Th1 to suppressor Th2 lymphocytes is shifted toward the suppressor phenotype, which is believed to facilitate maternal immune tolerance of the fetus by suppressing activity of the cytotoxic Th1 cytokines, which are capable of attacking the fetal allograft and impairing trophoblastic invasion (1240, 1485).

Studies revealed that endothelial dysfunction in preeclampsia is found to be associated with excessive release of Th1 cytokines like TNF-α, IFN-γ, IL-1, IL-2 and IL-8 found in PBMC and placenta as well as a deficiency in IL-10 (540, 885, 1242, 1459, 1504).

High levels of IFN-γ, together with TNF-α, can lead to apoptosis of trophoblast (954) which makes them major contributors to many of the local and systemic changes that characterize preeclampsia. Studies of **Mor et al**. and **Pijnenborg et al**. propose

that activated macrophages in the placental bed secrete TNF-α and IFN-γ, inducing trophoblast apoptosis (963, 1122). These macrophages show characteristic changes in distribution compared to normal pregnancies and are located within and around the spiral arteries separating them from the trophoblast cells (1188). Instead of facilitating trophoblast invasion through the placental bed macrophages seem to function as a barrier for trophoblast invasion and differentiation by inducing trophoblast apoptosis and therefore preventing spiral arteries transformation in pathologic conditions (1188).
Increased trophoblast apoptosis would increase the amount of trophoblast debris, syncytical knots, which leak into the maternal circulation and generate a systemic endothelial activation as seen in preeclampsia (1256). Trophoblast debris can activate TNF-α and IL-12 production from monocytes *in vitro*, which further pushes the systemic immune response towards excessive inflammation instead of a normal immune activity.

Consistent with that, **Reimer et al**. reported an upregulated leptin expression in placental tissue of preeclampsia (1185) which could partly explain the production of large amounts of IFN and IL-2, and decrease of IL-4 production (830).
The search for useful markers in preeclampsia have brought up serum granulysin for evaluation of Th1/Th2 balance by **Sakai et al.** (1244) and soluble IL-2 receptor in plasma by **Eneroth et al.** (368), which has to be further evaluated.

Despite the fact that there is a shift to a Th1 response in preeclampsia, accompanied by a systemic inflammatory response, there is also a marked systemic inflammatory response in normal pregnancy and the Th1/Th2 paradigm has been put into question as too simplistic (1485). The state of preeclampsia could be seen as an exacerbation of the inflammation during normal pregnancy as the paper of **Redman and Sargent** does (911, 1181). They demonstrated that the first signs of a systemic inflammatory cytokine response characteristic of pregnancy begin before implantation, and deduce that its origins may not necessarily involve immune recognition of the fetal allograft. Their data show that the predominant changes are in NK and NKT cell populations, whereas T lymphocytes show minimal or no changes. These changes are observed especially for IL-18, which is interesting since there is a Th1 storm in fulminant preeclampsia characterized by excess IL-18 which with IL-12 stimulates IFN-γ secretion and creates a vicious loop (1181).
They then presented data suggesting the systemic stimulus triggering the innate immune system is due to the circulating fetal cells in maternal blood, plus the

release of placental debris from syncytiotrophoblast (1181). This shedding of syncytiotrophoblast microparticles is increased in preeclampsia; and these syncytiotrophoblast microvillous membrane particles increasingly bind to maternal DCs. They likely interact with TLRs, and thus trigger Th1 cytokine production.

Preeclampsia is also characterized by systemic changes in the distribution of lymphocyte populations in peripheral blood. **Sakai et al**. investigated CD4+CD25+ Treg cells, and the ratio of CD4+CD25^{bright}/CD4+ cells is markedly decreased in preeclampsia cases compared to normal pregnancy subjects and even nonpregnant controls (911). Also there is a general consensus that there are increased levels of activated/memory cells (CD4+CD25RO+ and CD4+CD29+) and decreased levels of naïve/suppressor cells (CD4+CD45RA+) (885, 886) whereas there is a switch to naïve/suppressor T cells in normal pregnancy.
A possible explanation might be that antigens have activated these T cells in preeclampsia, indicating inflammatory activity. Reports also characterized lymphocyte subsets in decidua of patients with preeclampsia and reported an increased percentage of CD3-/CD56+CD16+ NK cells and cytotoxic CD8+/CD28+ T cells and a decreased percentage of T CD3+, B CD19+, and suppressor/inducer T CD4+/CD45RA+ lymphocyte subsets (1335, 1504). Systemic immunological deviation towards suppression seen during normal pregnancy is absent not only in peripheral blood but also locally in decidua of preeclamptic women. Moreover, it is consistent with the dominance of Th1 cytokine activation in preeclampsia which stimulates NK cells and CTLs.

Some investigators have proposed that preeclampsia represents a defect in immunological masking that normally allows trophoblast cells to evade maternal immune recognition. **Lim et al**. already described a dysregulation of the expression of HLA-G and MMP-9 on human trophoblast in preeclampsia (818). **Dekker et al**. suggested in their hypothesis that lower expression of HLA-G on trophoblast could result in local IFN-γ- and NK cell upregulation which may be the main factors for pregnancy failure in preeclampsia (317). Defects in the immunosuppressive functions of HLA-G contributing to the control CD4+ and CD8+ T cell activity in preeclampsia may alter the local cytokine profile which is normally controlled by T cells (795).
Kim et al. evaluated the expression pattern of TLR4 and TLR2 at the fetomaternal interface, the placental bed, both in normal and complicated pregnancies (694). TLR4 protein expression was found to be increased in interstitial trophoblasts of

patients with preeclampsia which suggests that dangerous host or microbial signals at the feto-maternal interface, which are recognized by trophoblasts through TLR4, may play a key role in creating a local abnormal cytokine milieu leading to the development of preeclampsia.

To conclude, preeclampsia is based on a cascade of complex immunological events originating from placenta. During normal pregnancy, trophoblast cells interact with uterine NK cells, modifying their cytokine repertoire which results in cellular homeostasis and angiogenesis. Preeclampsia is characterized by the inability of the trophoblast to accomplish this. Trophoblast apoptosis prevents adequate trophoblast invasion into decidua and results in defect remodelling of spiral arteries, hypoxia, placental ischemia and an excessive inflammatory reaction. Leakage of increasing amounts of placental fragments and cytokines into the maternal circulation and a systemic endothelial activation lead to the clinical signs of preeclampsia.
Important for future studies are the further identification of these complex immune factors, perhaps with the help of animal studies, and to discover possible early markers for preeclampsia. Treatment options of preeclampsia which are still focused on signs like hypertension could be widened with respect to modification of immune responses (388).

b) Preterm labor and preterm birth

PTL and preterm birth, defined as labor or birth before 37 weeks gestation, is the major cause of perinatal mortality and morbidity in the developed world. It is preceded in 30% by PPROM, which is defined as membrane rupture prior to 37 weeks gestation (1105).
Infection is considered as the most significant cause of spontaneous PTL and preterm birth in up to 50% of all cases (826, 839). Inflammation at the maternal-fetal interface, mediated by proinflammatory cytokines including TNF-α and IL-1β (345, 346, 1105, 1187), is considered as the main common component and can be caused by intrauterine infections, lower genital infections or distant infections such as periodontitis in pregnancy (781). Animal studies showed that treatment with IL-1β was able to mimic the effects of bacteria and induced PTL, suggesting a causal role for this cytokine in infection-induced PTL (71, 478, 1216).

Recent human studies have demonstrated that fetal carriage of a polymorphism in the IL-1 receptor antagonist gene was associated with elevated second-trimester intraamniotic IL-1β levels and an increased rate of spontaneous preterm birth (1521) as well as a history of spontaneous abortions (1109). The group of **Kalish et al**. also demonstrated that fetal carriage of polymorphisms in genes coding for IL-1 receptor antagonist (649), IL-4 (651), and TNF-α (650, 856), as well as a maternal IL-4 polymorphism (651) was associated with PPROM and preterm birth in multifetal gestations. This is a promising area for future research.

However, recent findings also indicate that midgestation maternal immune hyporesponsiveness, as represented by low cervicovaginal concentrations of various proinflammatory cytokines, constitute an increased risk for subsequent preterm delivery among women with lower genital tract pathological microflora (1308). **Kalinka et al**. reported in their study that the highest risk of preterm delivery was observed among women with low cervicovaginal concentrations of IL-1α and IL-1β; lower but still elevated risk was found for women with genital tract infection and low cervicovaginal levels of IL-6 and IL-8 (648). The increased risk for preterm delivery was found only in the group of women with lower genital tract infection who had low cervicovaginal concentrations of proinflammatory cytokines. The latter condition may also imply a lower reactivity of the maternal immune system that should normally result in diminishing the growth of pathological bacteria in the genitourinary tract (648). Probably, pregnant women with no adequate, i.e. hypo- or hyperimmune responses, are at risk for subsequent infection-related preterm birth.

Intrauterine infections are mostly subclinical (1230) and polymicrobial with *Ureaplasma urealyticum*, *Mycoplasma hominis*, group B streptococci, *G. vaginalis* and gram-negative bacteria such as *Escherichia coli* as the most common pathogens associated with PTL (445, 1030, 1235). Depending on which laboratory technique is performed, prevalences of intraamniotic infection in the setting of PTL range from 0-24% to 30-55% (451, 721). The main mechanism of this infection is ascending microbial invasion by lower genital tract organisms which could produce local inflammation caused by proinflammatory cytokines and prostaglandins (676, 1306), i.e. subclinical chorioamnionitis leading to PPROM, PTL and possibly preterm birth (1306). Important to mention is also the higher probability of respiratory distress, sepsis and cerebral palsy in preterm infants due to fetal inflammatory responses in cases of clinical chorioamnionitis (721).

Once intraamniotic infection is diagnosed, the standard care is administration of intravenous antibiotics and delivery regardless of gestational age (721). However, a recent meta-analysis assessing 11 trials among 7428 patients in PTL with intact membranes showed that overall use of antibiotics did not decrease preterm birth or perinatal morbidity (710). Routine administration of antibiotics to women with preterm labor and intact membranes is therefore not recommended as there are no clear improvements in neonatal outcomes.

Trichomoniasis has recently been associated with a significantly increased risk of PPROM and preterm delivery in several studies (259, 950). Surprisingly, the randomized study of **Klebanoff et al**. reported an even increased risk of preterm birth in patients with asymptomatic trichomoniasis who were treated with metronidazole (718). A possible explanation may be that dying trichomonads could release inflammatory mediators that trigger PTL.

The results of this study may be difficult to extrapolate to the clinical care of the pregnant woman with trichomoniasis as only women with asymptomatic infections were included in the study and much higher doses of metronidazole were used than is the standard of care. It is possible that the latter in some manner had an impact on preterm birth rates. It is also possible that women with symptomatic trichomoniasis may have a different response to treatment than women with asymptomatic infection as a result of organism burden or host factors.
Similar results with increased low birth weight and a trend towards increased preterm birth were reported from a recent randomized trial from Uganda (693). Further studies may be warranted to resolve the conflicting findings. Only symptomatic trichomoniasis should therefore be treated during pregnancy (721).

Also BV is associated with a twofold increased risk of preterm birth among other pregnancy complications, presenting the greatest risk when detected before 16 weeks` gestation (548, 802). This implies a critical period during early gestation where pathogens can ascend the genital tract.

The results of treatment trials for pregnant women with BV have been inconsistent. Two meta-analyses revealed no reduction in overall preterm birth with routine screening and treatment for BV (803, 898). In both studies, however, oral BV treatment led to a decrease in PPROM and low birth weight in a subgroup of patients with a history of preterm birth.

In the study by **Lamont et al**., treatment of low-risk women with BV with a intravaginal clindamycin cream resulted in a decrease of preterm birth (780). Another trial with oral clindamycin showed similar results (1433). Different in those two trials compared to others was that all patients were enrolled and treated before 22 weeks of gestation which suggests that early treatment of BV may be the key to prevention of preterm birth.These results have to be proved in high-risk populations such as women with prior preterm delivery.

Other vaginal colonization including *Candida*, *U. urealyticum* and group B streptococci have not found to be associated with increased risks for preterm birth (176, 258, 719) whereas infection with *C. trachomatis* or *N. gonorrheae* doubled the risk for preterm birth (36, 363).

Future treatment options against PTL and the resulting neonatal morbidity could include the antiinflammatory cytokine IL-10 or progesterone as animal studies showed (552, 1105, 1200, 1407). IL-10 may function by decreasing LPS-stimulated IL-1β that could prevent the induction of COX-2 and ultimately lead to decreased prostaglandin E2 in the gestational tissues (147). Furthermore, IL-10 blocked IL-1β-induced PTL in rhesus monkeys, probably mediated by a decrease in prostaglandin E2 production (1236). In women with a history of preterm birth, the weekly injection of synthetic progestin, 17α-OH progesterone-caproate, significantly reduced the rate of delivery at 30, 32, or 37 weeks gestation (913).

c) Early pregnancy loss

The physiology of human reproduction, including the immune mechanisms that permit pregnancy, is extremely complex and inefficient. Spontaneous abortion is with 15% of all clinically recognised pregnancies the most common adverse reproductive outcome in women. A highly sensitive C-terminal peptide of hCG assay for pregnancy indicates that an additional 20% of conceptions terminate as “occult losses” before pregnancy is detected clinically (1502).
RSA is defined as two consecutive or more than three spontaneous abortions prior to the 20th week of gestation and occurs in approximately 1 to 3% of women with diagnosed pregnancies (457).

Generally, the causes of spontaneous abortion are classified as genetic, endocrinologic, anatomic or microbiologic. After excluding those etiologies, however, still 40-50% of all cases remain unexplained (343). A probable explanation for those cases is that recurrent reproductive wastage had occurred as a result of immunologic failure.

Clinical observations suggest that genetic and immunologic disparity could be a factor in fecundity. This theory is based on earlier clinical observations, as for example a change of partner can sometimes solve the problem (97) or women who have had RSA reject their partner`s skin graft less quickly (77). As the HLA system is the basis of this disparity, the immunologic hypothesis suggests that HLA-sharing induces a weaker tolerance response which leads to rejection of the fetus. Studies have shown that fetuses whose HLA alleles do not differ from maternal alleles are more likely to be aborted than histoincompatible fetuses (738, 1038).

Concerning evaluation of research in this field, there have to be mentioned some general problems. It is likely that there is more than one immune cause of RSA, which may include recognition of paternal antigens on the feto-placental unit by the maternal immune system followed by destruction of the fetus, although actual evidence for this in humans is limited. A population of women with unexplained RSA is likely to comprise subsets with RSA of different etiologies.

As most published studies only involve a small number of women it is possible that some differences are also due to the fact that different populations of women with RSA have been selected. This is inevitable because the recruitment of patients is difficult.

One of the problems in understanding the underlying etiology of this immune failure is that the mechanisms by which the fetus is protected from the maternal immune system during normal pregnancy are not fully understood.

Immunological rejection of the fetus due to recognition of paternal antigens by the maternal immune system, resulting in abnormal immune cells and cytokine production, is postulated to be one cause of unexplained pregnancy loss. To study the role of these immune cells and molecules in the etiology of RSA, it would need placental tissue during the first trimester of human pregnancy which is clearly not possible (776).

Various alternative approaches have been adopted instead including the analysis of immune cell populations and cytokines in the peripheral blood of women with RSA and normal fertile women either before pregnancy or at the time of miscarriage.

Moreover, studies use endometrial tissue obtained from women with RSA and normal fertile women in the periimplantation period in the nonpregnant state, or placental tissue obtained at the time of miscarriage from women with a history of RSA, from women with spontaneous abortions and from women requesting terminations of normal pregnancy (776).

While the study of placental tissue might appear to be the best approach, there are difficulties, particularly with respect to components of the immune system, in determining whether observed differences are due to proinflammatory events as a consequence of the miscarriage.
One must also emphasize the importance of compartment, i.e. either peripheral blood, endometrium or decidua in which the cells and molecules are measured, and the timing of the sampling, both with respect to the menstrual cycle and pregnancy. Both has critical relevance for the interpretation of results. The measurement of factors such as cytokines in peripheral blood may have little significance as this compartment is far removed from where the important interactions are taking place. In addition, the peripheral blood cell population is considerably different to that in the endometrium and decidua. The timing of sampling is also important, both with respect to the point in the menstrual cycle and pregnancy and whether it is at the time or just after miscarriage, as both of these factors will affect the expression of these cells and molecules.

Recurrent miscarriage is normally defined as a loss of three or more miscarriages, but some studies also include women with only two miscarriages. In addition, the timing of the fetal loss may differ between studies and may result in the study of different populations. It must not be neglected as well that immune mechanisms responsible for infertility which is further discussed in an extra chapter and early pregnancy loss might overlap.
Moreover, there is a tendency to extrapolate directly from animal models, particularly those of rodents, to humans, and this has led to assumptions of mechanisms for which the evidence is incomplete.
Lastly, it has recently been suggested that the importance of chromosomal abnormalities in RSA has been vastly underestimated (1154). Karyotyping of the fetus is also important, and should be carried out in future studies so that miscarriages which result from chromosomally abnormal pregnancies can be considered separately from those resulting from chromosomally normal pregnancies.

Concerning NK cells as the predominant leukocyte population in decidua during early pregnancy, there are partly inconsistent results in patients with RSA.
Whereas normal human pregnancy is characterized by a decreasing peripheral NK cell activity during first trimester, several studies have shown increased numbers of CD56+ NK cells in the peripheral blood of women with RSA either prior to or during pregnancy compared with healthy fertile nonpregnant or pregnant controls (38, 766, 1111). A significantly increased number of circulating CD56+ NK cells was also found in RSA women who miscarried compared with RSA women who delivered (1365) and other studies have also shown that levels of peripheral blood CD56+ cells both prior to and during pregnancy can predict pregnancy outcome in women with RSA (262).
However, another study by **Yamamoto et al**. has shown no differences in the levels of peripheral blood CD56+ CD16+ NK cells in normal pregnancies and missed abortions with normal and abnormal chromosomes (1553)

In contrast to the increased numbers of CD56+ cells in peripheral blood, a decreased number of decidual CD56+ NK cells are reported in the placental tissue from spontaneous miscarriages in RSA women compared with tissue from spontaneous miscarriages in women without RSA and women requesting termination (1150). **Vassiliadou and Bulmer** reported a decreased cytotoxic capability of decidual CD56+ NK cells in placental tissue from spontaneous aborters (1447), though women with RSA were not included in this study.
The fact that there appears to be decreased numbers of CD56+ cells in the decidua and increased numbers in the endometrium could be due to the presence of two different populations of CD56+ cells, either CD16+ or CD16- (776). The increased number in the endometrium could be due to CD56+, CD16+ cells as suggested previously while the decreased number reported in decidua could be the CD56+, CD16- population, which is supported by the study showing increased numbers of CD16+ cells in early pregnancy deciduas of women with RSA (367).

Studies have shown increased numbers of CD56+ cells among a general increase of various lymphocyte populations (CD4+, CD8+, CD14+, CD16+/CD56+) in the non-pregnant endometrium of women with RSA, and lower numbers were seen in women with RSA who subsequently had a live birth compared with those who miscarried (773, 1153). This is in contrast to a flow cytometric study which showed similar numbers of CD56+ cells in the endometrium of women with RSA and control

subjects, although the women with RSA did have increased numbers of endometrial $CD56^{dim}$ CD16+ cells compared with control subjects (772).
However, the latter report suggested that CD3+ cells were the major leukocytes within the endometrial leukocyte population, both in women with RSA and fertile controls. This is in disagreement with numerous *in vivo* immunocytochemical studies and suggests that flow cytometry may not be the best means of studying these endometrial leukocyte populations (776).

These results suggest that there are alterations in the CD56+ population of leukocytes in women with RSA but whether these are increased or decreased depends on whether peripheral blood, first-trimester decidua or periimplantation endometrium is analysed (776). There are many reports that support a relationship between elevated peripheral NK cell numbers and/or activity and RSA which means that an abnormal increase in peripheral NK cell parameters prior to conception and during early pregnancy is causally associated with RSA. Recent studies further suggest that a divergence of the specific NK cell repertoire in peripheral blood might be related to the etiology of RSA (1031, 1032, 1552). Therefore, it is proposed that women with alloimmune abortions have a limited inhibitory NK cell receptor repertoire resulting in the recognition of trophoblast HLA class I molecules by decidual NK cells. The relationship between uterine NK cell populations and RSA is still unclear.

Results concerning the numbers of CD3+ T cells as the second most abundant population in endometrium and decidua in RSA patients are also partly inconsistent.
Studies showed no differences in numbers of CD3+ T cells in peripheral blood of RSA and normal fertile women prior to pregnancy (1549); others a significantly decreased number of CD3+ T cells in the peripheral blood of pregnant women with RSA who subsequently miscarried compared with those who had a live birth and normal pregnant controls (766). When investigating the CD56+ subpopulation of CD3+ T cells a decrease in the number of CD56+CD3+ cells in the peripheral blood prior to pregnancy was shown (1549).
Similarly, no differences in the numbers of CD3+ T cells in endometrium and early pregnancy decidua from RSA and control women have been reported (772, 1448, 1150) whereas a decreased number of CD56+CD3+ cells in the decidua of women with RSA compared with control women has been reported (1549).

Thus, although there appear to be no differences in the total T cell numbers in women with RSA, there may be differences in subpopulations of T cells which may be important. This is also suggested by studies investigating the relationship between prevalence of protein components of their TCR and RSA (40, 231, 1374, 1447).

In a study by **Jablonowska et al**. they investigated lymphocyte subsets in peripheral blood of women with history of unexplained RSA during the first trimester of pregnancy and found significant changes in T cell subpopulations in pregnant RSA women (606). Interestingly, the proportions of T helper/memory cells (CD4+CD45RO+), T-killer/effector cells (CD8+ S6F1+), and HLA-DR positive T cells (CD3+ HLA-DR+), all markers of T cell activation, were increased compared with normal pregnant controls. In contrast, the T-suppressor/inducer population (CD4+ CD45RA+) was decreased compared with normal pregnant controls.

Thus, RSA women reveal the opposite phenotype compared to the state of suppression/non-activation during normal pregnancy, indicating that women with RSA have an activated immune system during pregnancy (854). However, it is not known whether this aberration in T cell subsets is pathogenetically involved in RSA, or whether it represents an epiphenomenon (606). Also the proportion and number of B cells (CD19+) were found to be significantly increased in the first trimester of pregnancy in RSA women compared with normal pregnant controls (606).

Studies in rodents, carried out by **Wegmann and colleagues** during the early 1990s, have provided strong evidence that successful pregnancy is associated with a predominant Th2 cytokine profile, and that Th1 cytokines are detrimental to pregnancy (1485) and recent data from human experience confirm that. More recently, it has been suggested that even in mice the Th1/Th2 hypothesis represents an oversimplification of the situation, and the importance of other cytokines has been acknowledged (205).

The evidence for an abnormal Th1 cellular immune response with higher levels of IFN-γ and lower levels of IL-6 and IL-10 to reproductive antigens in women with RSA seems convincing (545, 799, 862, 1159). PBMCs from women with RSA produce TNF-α and IFN-γ in response to stimulation from trophoblast cell extracts, while cells from healthy nonpregnant women and men produced IL-10 (546).

Similar studies have also shown decreased production of IL-4, IL-5, IL-6 and IL-10 and increased production of IFN-γ, IL-2, TNF-α and TNF-β in supernatants of phorbol-12-myristate-13-acetate-stimulated PBMCs obtained from women with RSA

at the time of miscarriage compared with stimulated PBMCs obtained during the first trimester of ongoing pregnancies in women with a normal reproductive history (1160).
However, a recent study of cytokine production by peripheral blood cells of women with RSA taken during early pregnancy before miscarriage, has shown opposite effects with increased IL-4 and IL-10 and decreased IFN-γ in women with RSA (85). In contrast to other studies, the results of this study are not complicated by comparing results from blood samples taken with and without miscarriage and during the first trimester of pregnancy and at birth, both of which are likely to affect cytokine production.

There is also some evidence for differences in endometrial and decidual Th1 and Th2 cytokine production in RSA patients (819, 1115), and in particular decreased production of cytokines such as IL-4, IL-6 and IL-10. However, although the cells in this study originated from the decidua, they underwent considerable *in vitro* manipulations before cytokine measurement (1115). In addition, the T cell population from which these clones were derived comprises only a small percentage of cytokine-producing cells in the decidua.

TNF-α with its elevated levels in RSA patients is proposed to play a central role in triggering immunological pregnancy loss and implantation failure (204, 1420, 1424, 1542). It might have a toxic effect on blastocyst and uterine cells (1068) and is involved in the pathogenesis of stress-induced early embryonic death as experiments with mice showed (41, 470, 1425). Recently, there is also the hypothesis that LIF as another proinflammatory cytokine may be involved in mediating TNF-α-induced stress-induced early embryonic death (1426). LIF knockout mice were shown to produce normal blastocysts but implantation of the embryos did not occur (1355). When transferred to wild-type pseudopregnant recipients, the blastocysts can implant and develop. These results suggest that the implantation failure in LIF-deficient mice was not due to some defects specific to the embryos but to those arising in the uterus which resulted in the total loss of its receptivity.
There is the possibility that LIF signaling may be affected due to alterations in TNF-α expression. Observations showed that LIF must be expressed in the uterus at the right time and at the right level, with activated receptors and signaling pathways, to guarantee a successful implantation (216). Data reported that a sustained

increased TNF-α expression in the reproductive tract of females exposed to stress can alter the temporal pattern of LIF expression (216).

As already mentioned, there has been an increased interest in newly identified cytokines such as IL-12, IL-15, IL-16, IL-17 and IL-18 in relation with RSA. Elevated serum levels of IL-12 and IL-18 were found in RSA patients who miscarried compared to healthy or delivering RSA women (1509, 1510). IL-12 is known to be a Th1-inducer cytokine and IL-18 can synergize with IL-12 in the induction of Th1 immune response resulting in pregnancy loss (991).
The endometrium of women with RSA also expresses elevated levels of IL-13 and IL-15 which might induce a proliferative response in decidual NK cells and augment their cytolytic activity (209). These findings await further investigation.
The role of other cytokines and growth factors have also gained interest. Decreased levels of CSF-1 (468, 1133), GM-CSF (1110, 1133) and TGF-β2 (798) in patients with RSA compared to healthy women seem to be associated with pregnancy failure. However, there are too few studies to evaluate the role of these cytokines exactly.

Similar to the case of unexplained infertility, a high percentage of unexplained RSA cases is found to be associated with certain autoimmune and alloimmune antibodies that may play major role in the immunologic failure of pregnancy and may lead to abortion (1071).
A variety of autoimmune antibodies have been found at increased frequencies in women with recurrent pregnancy failures as for example different types of APA, including Lupus anticoagulant, anticardiolipin β2-glycoprotein IgG/IgM/IgA, anticardiolipin IgG/IgM/IgA, anti-phosphatidylserine prothombin IgG/IgM, and anti-phosphatidylethanolamine IgG/IgM (863, 1462, 1550), as well as a higher incidence of ANA (1365), ATA (164) and anti-endothelial cell antibodies (1221).

Investigators also reported the absence or decreased expression of the protective alloantibodies APCA (11, 517, 1054), Ab2 (603, 1069, 1070) and MLR-Bf (6, 1072, 1394) in patients with RSA which might attribute as another cause to RSA of unknown etiology. However, there are also several studies which reported that MLR-Bf does not play protective role in the maintenance of pregnancy, for example by **Jablonowska et al**. who observed that blocking antibodies have no predictive value for the pregnancy outcome in RSA patients (605).

There are some other interesting approaches concerning further immunological explanations of unexplained RSA which should also be mentioned but still need further evaluation.

A fully active complement system is present in the placenta and protects both the fetus and the mother against infectious and other toxic agents. As fetal tissues are semi-allogeneic and alloantibodies commonly develop in the mother, the placenta is potentially subject to complement-mediated immune attack at the feto–maternal interface with the potential risk of fetal loss. Uncontrolled complement activation is prevented in successful pregnancy by the three regulatory proteins DAF, MCP and CD59 positioned on the surface of trophoblasts (455, 457).

Excessive complement activation in the placenta places the fetus at risk for growth restriction or death. The embryonic letality observed in Crry-deficient mice supports the critical role of the complement regulatory proteins in preventing complement-dependent fetal loss (1545). There is some indication that an altered complement regulation can cause and may perpetuate complications of pregnancy in women, for example in RSA patients (188, 284, 455), although the data need to be confirmed and extended. What is clear is that antibody-induced complement activation can overcome the protective function of the complement regulatory proteins and may be responsible for tissue damage. This situation is encountered in several patients with RSA associated with the presence of APAs (456).

On the other hand, there is the lower ratio of asymmetric/symmetric IgG antibodies in women with RSA compared to healthy women (351). Some investigators consider that the lower levels of the non-precipitating asymmetric-type of antibodies in RSA patients that would allow immunological tolerance are the major determining factor for unsuccessful pregnancy. This still has be investigated further.

The immunmodulation of progesterone and the antiabortive effect of PIBF are also part of the immunological theory behind RSA. In recurrent spontaneous aborters and women showing clinical symptoms of threatened preterm pregnancy termination, PIBF expression and the percentage of PIBF+ lymphocytes and PR+ immune cells was found to be significantly lower than in healthy pregnant women (343, 485, 1374). PIBF which is stimulated by progesterone leads to the inhibition of NK cell activity, enhancement of asymmetric antibody production (1375) and induction of the Th2 response, especially IL-10 (485).

Interestingly, also stress has been shown to influence number of decidual mast cells, CD8+ T cells and TNF-α expression in women with RSA (41, 42, 43). Also the expression of PIBF was decreased by a higher maternal stress perception (624). Psychotherapy has also been reported to result in successful pregnancy outcome in patients with a history of RSA in one study (1362). Furthermore, relaxation therapy, another strategy to improve stress-management, has been shown to decrease peripheral levels of TNF-α (1483). Therefore, women with recurrent pregnancy loss who received psychological counselling may have a better power to manage stress, resulting in decreased levels of abortogenic Th1 cytokines, such as TNF-α (43).

To conclude, improper immune responses and an unbalanced cytokine network may be related to implantation failures, pregnancy loss and obstetric complications. The presence of elevated Th1/Th2 cell ratios, high concentrations of Th1 cytokines secreted by PBMC, elevated NK cell cytotoxicity and levels, and emergence of various autoantibodies are supporting evidence. The underlying immunopathology still need to be investigated but recent data have brought up the induction of procoagulant fg12 by Th1 cytokines as one probable mechanism (232, 234, 235, 723).

3. Immunology of infertility

Approximately 10-20% of couples suffer from infertility, defined as unprotected intercourse for 12 months without conception (1148). In addition, recurrent pregnancy loss, generally defined as three or more consecutive pregnancy losses before 20 weeks gestation, may occur in as many as 2% of child-bearing women. A thorough evaluation of the infertile couple will demonstrate no explanation for infertility in approximately 10% of cases, whereas the evaluation of RSA will demonstrate no cause in as many as 60% of cases.
This “unexplained” reproductive failure can be influenced by some immunological abnormalities, similar to the causes described for RSA (225); therefore, the term immunological infertility has been created (747).
Both reproductive failure and infertility in association with immunological disturbances has been recognized for decades but only recently has it been demonstrated that certain characteristic autoantibodies seem to be involved.

Recent attention has focused on a range of immunological tests for the infertile couple based on the hypothesis that a portion of unexplained infertility and results

from immune-mediated reproductive failure (1434, Table 40). The immunological tests purport to identify autoimmune abnormalities, i.e. APA, ATA, ASA or more generalized immune defects, i.e. a NK cell dysregulation, which are postulated to cause reproductive failure.

Antiphospholipid antibodies (anticardiolipin, antiphosphatidyl antibodies) (APA)
Lupus anticoagulant
Antisperm antibodies (ASA)
Antithyroid antibodies (ATA)
Antinuclear antibodies (ANA)
Anti-smooth muscle antibodies (SMA)
Embryotoxicity assay
NK cells

Table 40: Immunological tests in fertility practice

However, this has to be seen critical due to several reasons.
Precise mechanisms by which these alloimmune or autoimmune disorders contribute to infertility remain to be established. Furthermore, there is considerable variability in the methodologies utilized, the validity, and the standardization for many of the mentioned tests (652). Similarly, the interpretation of test results is inconsistent. Moreover, the rationale for good testing in clinical practice should include the ability to secure a diagnosis and to guide clinical decision-making and treatment plan.

Presence of organ-specific auto-antibodies to testicular antigens and sperm, or antibodies to ZP (655, 678) and endometrial antigens (113, 960), are considered possible causes of infertility in a fraction of patients with unexplained infertility (1442).
Except for some cases of POF, the relationship between anti-ovarian autoimmunity and reproductive failure remains controversial (960). It is even more difficult to ascertain a causal relationship between AOAs and other ovarian pathologies, especially infertility, in patients who have not yet presented with clinical signs of ovarian failure. Currently, several studies suggest a possible role of autoimmunity in these cases of recurrent reproductive failure, but the levels of evidence are low maybe due to the multiplicity of antigenic targets in the ovary.
ASA are present in infertile male and female (126, 270, 1295) as well as in fertile individuals (533, 1049) indicating that there are not necessarily causing infertility.

Causes for ASA formation include trauma, surgery (128, 533) and gynaecologic infections (283, 872) in both sexes.
ASA are considered to be one of the main causes of immunological infertility by binding to the sperm membrane and impairing sperm functions (554). They have been shown to reduce fertilization rates (305), decrease sperm motility (128, 917, 1296, 1522) and may inhibit capacitation and acrosome reaction (109, 996, 1486).

Methods used to reduce ASA production include condoms (893) and systemic corticosteroid treatment whose effects are ambiguous (494). Separation and washing techniques (8, 418, 1511) are not very promising as well. Concerning ART in treatment of ASA-positive infertility, the most capable procedure seems to be ICSI (747, 999, 1013) compared to IUI (207), GIFT (1439) and IVF (1013). However, it is difficult to compare the different techniques as there have not been sufficient well-controlled data.

To conclude, there is not enough evidence to support the use of systemic immunosuppressive methods to treat ASA. However, there are promising animal data that suggest that ASA formation may be prevented by prompt antibiotic treatment in the case of suspected genital tract infection in men (893). The use of ART in many couples with ASA and unexplained infertility is beneficial because it minimizes impaired gamete recognition and fusion.

However, the presence of other autoantibodies, which are not particularly organ-specific, such as APA, ATA, ANA or SMA have recently been increasingly implicated in immunological infertility (460, 1442). Both aPS and antibodies against β2-glycoprotein have been shown to be directly pathogenic to trophoblast (765, 901).
Several studies on infertile patients after repeated reproductive failure showed that 40 to 80% had at least one abnormal result in autoantibody testing (927, 1148, 1184, 1540). However, several studies had no control group measured and if they did, autoantibodies were also observed in control sera but at lower percentages (1184, 1540).
The association of APAs with RPL (1550) has led investigators to explore a role for such antibodies in unexplained infertility. The thrombogenic nature of these antibodies has been postulated to interfere with implantation, placentation, and the normal vascular perfusion of the developing embryo.

Data on the prevalence of APA in infertile patients must be interpreted based on the numbers of autoantibodies tested, the availability of assay controls, the use of non-standardized test modalities, patient inclusion criteria and variable ranges selected to distinguish normal from abnormal test values. The prevalence of serum APAs in the general population has been reported to be 1-3% (364) **Taylor et al**. reported increased ACA levels in 17% of infertile patients versus 6% of the control group (1400). When restricting analysis to fertility patients being treated with IVF, the prevalence of APA was consistently higher (6–38%) than in control populations (when available) consisting of normal parous women (highest prevalence 3.5%) (652).

The consistent observation has been an increased prevalence of APA in the infertile population, especially demonstrated in IVF patients. The essential question has been whether these autoantibodies cause infertility or IVF failure, or simply represent serum markers associated with infertility.

Similar results have also been obtained from studies on the prevalence of ATA, ANA or SMA (446, 447) in infertile patients but it remains difficult to draw conclusions due to small patient numbers and lack of control groups.

Therefore, the use of APA testing in fertility practice is not clear by current data, as well as there is no compelling evidence that testing for ANA, ATA, or SMA in routine clinical practice is relevant to the diagnosis or treatment of otherwise unexplained infertility.

There is also increasing evidence that an abnormal immunophenotype including a high NK cell count may be one of the causes of recurrent abortion and repeated fertilization failures.

A study by **Fukui et al**. showed that an increased blood NK cell cytotoxicity level was associated with recurrent failed implantation after IVF treatment (430).

Beer et al. reported that there were not live births in the group of women with multiple failed in IVF cycles and women with the history of RSA which had the percentage of blood NK cells 18.0% or higher (94). Risk factors for immunologic implantation failure associated with a negative pregnancy test after IVF reported in the literature have included APA, ANA, and ATA, embryotoxic factors detected by an embryotoxicity assay, and elevated levels of circulating NK cells (645). 70% of women with IVF failure had at least one risk factor.

More recent studies have focused on elevated CD69 expression on NK cells as being associated with RSA and infertility of unknown etiology (263). As described by **Thum et al**., CD69 is a triggering molecule on activated NK cells that is capable of

inducing cytotoxicity and stimulating cytokine production (1415). In this study, women with high $CD56^{dim}CD16+CD69+$ in peripheral blood had reduced implantation rates and higher miscarriage rates.

Taken together, the results of the multiple investigations suggest that there are alterations in the NK cell population in women with RSA or infertility. However, whether NK cells are increased or decreased depends on whether peripheral blood, endometrium or first-trimester decidua is analyzed and the timing of sampling with respect to menstrual cycle or pregnancy (776). Data fail to distinguish if the difference in activity and number of NK cells is a cause or effect of reproductive failure.
Further studies, however, are needed to investigate if the composition of NK cells is critical in implantation and what might be the options for intervention aimed at NK cells in reproductive failure as discussed further down.

Different approaches in therapy of immunological infertility and recurrent miscarriage have been used and evaluated. Before effective treatment can be instituted, the cause of pregnancy loss must be determined. Once this has been determined, the most suitable therapy can be recommended and applied.
The most common include heparin plus aspirin, aspirin alone, IVIG administration and immunization with allogenic lymphocytes. There should be no doubt, that immunotherapy seems to be an effective treatment of women with the history of RSA and combined immune abnormalities. Recently, **Jerzak et al**. have used heparin/aspirin, aspirin alone, steroids, IVIG and alloimmunization or combined therapy for the treatment of women with RSA, where he observed 80.5% success rate in immunized women (622).

Without doubt, the most experience exists with a combination therapy of low molecular weight heparin and aspirin in women with repetitive implantation failure. Especially, in women with APA and RSA, it is currently the most recommended treatment form (1416, 1540). Several studies show the effectiveness of this therapy (166, 1293), also compared to aspirin alone (1162). This therapy may not only be effective, but also less costly, and logistically simpler to provide than other treatment options. Possible side effects such as decrease in bone density due to heparin could be neglected (69).

Würfel also reported an increasing role of steroids, above all prednisone, in the treatment of recurrent implantation failure, especially together with heparin (1540). His experience showed a clear benefit in patients with a high autoimmune activity, as presented by high numbers of NK cells or an increased Th1/Th2 ratio. However, there also have been plaebo-controlled studies showing that prednisone and aspirin was not effective in promoting live birth and even increased the risk of prematuity (788). To evaluate this correctly, more data from placebo-controlled, randomized studies seem to be necessary.

The use of IVIG may be restricted to patients with RSA despite conventional treatment with heparin/aspirin (1071, 1416). It has also been suggested for use in RSA cases with various serum autoantibodies which suggest autoimmunity or a high increase in NK cell numbers indicating high autoimmune activity (1540).

However, IVIG therapy still remains controversial. In some trials with IVIG, the lack of a control group made it impossible to evaluate the apparently favourable effect (768, 1365, 1367). So far, there have been published several placebo-controlled trials studies on IVIG therapy with over 300 treated RSA patients. Of those studies, only two showed a possible significant benefit of IVIG treatment in women with recurrent miscarriages (227, 261), while other studies have failed to confirm this beneficial effect compared to placebo treatment (228, 607, 1411, 1350). Results from trials showing a positive effect of IVIG present a live birth rate between 60-85%.

A lack of standardization of IVIG trial design has made their comparison virtually impossible and contributed to the ongoing controversy over IVIG therapy. Major differences were found in patient selection and timing of IVIG regimens. Generally, an evaluation of IVIG therapy may be difficult as different authors propose different indications or no exact indication for IVIG. Patients from different populations with different diagnoses are included in the study groups, making the comparison of results almost impossible. Trials differed in the numbers of previous miscarriages and in the number of patients with RSA after one birth. Concerning treatment protocols, the trials show great diversity with regard to starting time of treatment, number of infusions and amount of IVIG given.
Moreover, additional therapies including aspirin/heparin are often added, and the number of patients included in the studies might be too low to detect any significant effect of IVIG.

Searching for possible reasons, a review showed that factors associated with successful use of IVIG were an older mean patient age, initiation of IVIG therapy prior to conception and repeated intervals of IVIG during pregnancy (1366).

IVIG exhibits a documented effect in many immunological disorders. Immunomodulation by IVIG is thought to result from passively transferred blocking or anti-idiotypic antibodies (201), downregulation of B cell function, inhibition of complement activation (477, 968), reduction of NK cell activity (968, 1196) and shift of Th1/Th2 ratio to Th2 (477). However, studies detected no statistical differences in the presence of the blocking antibodies prior to pregnancy in RSA women treated with IVIG compared to controls (605). The long-term effect of IVIG on T and B cell subsets in women with RSA also seems unsure (606).
It seems obvious that IVIG are capable of reducing high concentrations of NK cells in peripheral blood with a short and long-term efficacy. This was confirmed in a recent study by **Perricone et al**. (1111). The same group also supposed a positive effect of IVIG on GM-CSF levels (1110) which are usually very low in pregnant RSA patients

Appropriate patient selection and valid timing of IVIG administration seem to be crucial factors that determine the success of this treatment. IVIG therapy appears to be safe and effective especially for older women with recurrent failure of natural or IVF-induced pregnancy or recurrent aborters with elevated NK cell activity. Monthly administration of low-dose IVIG initiated prior to conception and continuing through the end of the second trimester of pregnancy appears to be the optimal treatment regimen.
IVIG has been associated with some undesirable side effects (1551); it is costly and the long term effects of its use remain to be confirmed. Further experiments should be carried out with large sample sizes both for experimental and placebo treatments and more exactly defined patient groups and IVIG regime.

Immunotherapy with allogenic leukocytes attempts to block immunological rejection of the fetus by exposing the mother to an overload of self or third party antigen. This is thought to mimic the presentation of fetal antigens during pregnancy presumably illiciting maternal Ig effectors that are believed to be necessary for maintenance of pregnancy (1050).

Various recent studies demonstrated that paternal lymphocyte immunization in women with RSA induce the level of humoral antibodies correlating with the success of pregnancy like APCA, Ab2 and MLR Bf (1054, 603, 1069).

Würfel pointed at the fact that the effect of this therapy seems to be rather inspecific and independent of HLA-sharing (95) as HLA-investigations in several studies indicated. Benefits of this therapeutic approach rather seem to be a short-term suppression of NK cell activity and numbers, a decrease in Th1/Th2 ratio and a increase of PR on lymphocytes (767, 433, 206). Progesterone also decreases NK cells in peripheral blood.

Data on the efficacy of this immunotherapy remain controversial with several of the published results of randomized allogenic lymphocyte immunotherapy for women with RSA showing no significant effect whereas others do. The problem is on the one hand, that most of the trials with positive results have been non-randomized and clinical data were not complete. On the other hand, it was suggested that there are some other factors such as the number of previous miscarriages, presence of prior live births, and time of conception after immunization and patient`s age which may also influence the outcome of pregnancy.

Pandey et al. recently preformed a meta-analysis of various randomized and non-randomized clinical trials for lymphocyte immunization in women with RSA (1073). Women with RSA who received paternal lymphocytes were considered as study group and those who received autologous lymphocytes, third party lymphocytes and normal saline were considered as control group. Comparing the success rate in pooled data of trials, a success rate of 67% in paternal lymphocytes immunized women with RSA under study group as compared to 36% success rate in women with RSA of control group was found.

These data showed the efficacy of paternal lymphocyte immunotherapy as a therapeutic approach for the treatment of women with RSA (1073). However, despite these promising numbers, the point of the small sample size and lack of control groups in several studies has to be made. Moreover, women randomized to immunotherapy tended to be older and reported to be at higher risk of RSA than those randomized to control groups.
The use of allogeneic lymphocyte immunization using partner or third party leukocytes in new meta-analyses of the Cochrane databases was found to be

associated with an odds ratio of 1.23 (12 trials on 641 women) and 1.39 (3 trials on 156 women), respectively (1139). Both thus provided no significant effect over placebo in improving the live birth rate, similar to IVIG with an odds ratio of 0.98. Three authors independently evaluated randomized, placebo-controlled studies with well-defined treatment criteria and patients.
In addition, lymphocyte immunotherapy has been associated with some adverse side effects such as erythrocyte sensitization, thrombocytopenia and IUGR among others (1050).

They have also come up other treatment options for immunological infertility such as dehydrogesterone, VD3 and the leukocytic ultrafiltrate LNCC. Present situation shows that they all have to be evaluated differently.
LNCC has been approved for the treatment of immunologically-based RSA and recently also for repetitive implantation failure (1540) and placebo-controlled, single- and multicenter studies have shown positive results (1541).
VD3 is thought to influence the Th1/Th2 ratio but its mechanisms are not completely understood (1120). The proposal to use it as an effective immunotherapy in women with RSA has to be evaluated in well-controlled studies first.
A similar effect is aligned to dehydrogesterone (1161) which acts through PR and also showed to induce production of PIBF (647). Studies on animals and humans indicate its pregnancy-maintaining effects (850, 366).
However, there is still a need for more controlled, blinded and randomised clinical trials in order to draw firm conclusions as to the usefulness of dydrogesterone in women with a history of recurrent miscarriage.

4. Immunocontraceptive approaches

A continuing population explosion and unintended pregnancies continue to pose major public health issues worldwide. The world population currently increases by 1 billion every 12 years; 95% of this growth takes place in the developing nations.
In the USA, half of all pregnancies are said to be unintended, which results in more than 1 million elective abortions annually (484).
Better education regarding women`s health issues and enhanced contraceptive development are necessary to impact this problem. Contraceptive vaccines may provide viable and valuable alternatives to the presently available methods of contraception.

The molecules that are currently being explored for development of immunocontraceptives either target gamete production, such as anti-GnRH and anti-FSH vaccines, gamete function, such as anti-sperm and anti-oocyte ZP vaccines, and gamete outcome, such as anti-hCG vaccines (1009).

A GnRH-based vaccine would be usable in both sexes and as its primary structure is largely conserved in mammals, rodents can be employed as a homologous model for efficacy and safety. Enough data have accumulated to conclude that immunosterilizing GnRH-based vaccines can be effectively employed in various animal species without side-effects (505, 941, 1389, 1577).
However, generating antibodies against GnRH may have wide-ranging consequences leading to a block in the secretions of pituitary gonadotropins and may therefore not be acceptable for fertility inhibition in humans. For example, the inhibition of testosterone secretion complicates the potential use of GnRH vaccines in man where androgen supplementation would be required in order to maintain secondary sexual characteristics and libido (394).
Also the main research field concerning application of anti-GnRH vaccines in humans is not contraception, but the treatment of prostate cancer and other estrogen-dependent conditions.
Concerning anti-FSH vaccines, early clinical trials on males have not proven to be as successful as expected after quite encouraging results in monkeys (981, 982). One has to remark that it has still not been confirmed that spermatogenesis in humans is dependent on FSH (1238).

The revelation that ASA are frequently associated with unexplained infertility prompted several researchers to identify the immunocontraceptive potential of spermatozoa-specific antigens.
It has to be considered that the selection of a certain sperm antigen for the development of a contraceptive vaccine is limited by its specificity, participation in the fertilization process and its potential to induce a high sperm-specific antibody titer in the genital tract (332).
As no single predominant target of polyclonal ASA but a number of sperm proteins have been identified (796, 931, 1015, 1254, 1283), an effective immunocontraceptive vaccine would be probably consisting of several specific antigenic epitopes included in a single formula.
Active immunization studies on animals with some of these antigens, for example FA-1 and SP-17, has been shown to reduce fertility *in vivo* (1009). To date, no single

antigen has been shown to cause a 100% fertility reduction in the mouse model which may be due to the inherent nature of the model and the involvement of multiple antigens in the fertilization cascade. Another reason may be that vaccination with no single antigen has raised enough antibody titer, especially in the local genital tract, to completely block fertility.

However, it is interesting to note that several studies have observed a complete block of fertility in a few mice after immunization with a single antigen. It is possible that these animals develop a high cell-mediated immune response besides antibody response that also has a deleterious effect on sperm or oocyte function.

Immunization with several antigens does cause induction of cell-mediated immune response and production of cytokines such as TNF-α and IFN-γ (1010) which have deleterious effects on sperm and embryos (1011). Thus, to enhance the efficacy of a vaccine, it may be important to induce both the cell-mediated as well as humoral immune responses.

DNA vaccination may provide a solution but a DNA vaccination approach has not been examined for a sperm antigen (1009). In other systems, it has already been reported that DNA vaccination favours memory and cell-mediated response rather than effector B-cell response (791).

To conduct phase I and II multicenter clinical trials in a quality-controlled manner, the antigens have to be either recombinant or synthetic molecules. Synthetic sperm peptides (510, 796, 1008) were already tested in animals with ambiguous results.

Perhaps monospecific antibodies to sperm antigens may be combined for immunocontraceptive purposes in the form of intravaginal sperm-specific spermicides (332). A first recombinant miniantibody has been already engineered to the tissue-specific carbohydrate epitope located on the sperm glycoform of the CD52 antigen (331) and shown to agglutinate human sperm cells in a tangled pattern (1028). The S19 mAb was shown to immobilize sperm in the presence of complement, agglutinates sperm, and blocks sperm interaction with the zona pellucida.

The functional findings and the demonstration of the male reproductive tract-specificity of the S19 epitope on the CD52 glycoprotein serve as further indications that both the S19 mAb and its unique carbohydrate cognate epitope are strong candidates for vaccine development (1029).

Due to fact that ZP glycoproteins play a critical role in successful fertilization, they have become promising candidate antigens for contraceptive vaccines.

First, porcine ZP glycoproteins became the antigens of choice because of their immunological cross-reactivity with the ZP of various species including humans. However, ZP glycoproteins from a native source may be contaminated with other ovarian-associated proteins as shown in animal studies (1530), which may be prevented by the use of highly-purified porcine ZP (70) or recombinant proteins (472).
Further approaches include the use of live recombinant vectors, DNA vaccines or synthetic peptides (825, 1544, 492)
Despite of quite promising results, there is the need for long-term active immunization studies, before these ZP glycoproteins can be considered for human application.

Finally, the most promising candidate for development of an immunocontraceptive vaccine for females is hCG as it is synthesized pregnancy-specific after fertilization and judged crucial for the establishment and maintenance of pregnancy at east during the first weeks of gestation.
After extensive and promising animal studies with hCG linked to TT, clinical trials have led to variable antibody titers among women (1390). A more immunogenic formula finally resulted in the induction of sufficiently high antibody titers in 60-80% of the immunized women of a phase II trial (1391).
This vaccine against hCG is the first and only birth control vaccine to go through phase II efficacy trials successfully. It is devoid of side-effects showing no bleeding irregularity, and women keep ovulating normally, produce their own sex hormones and have regular menstrual cycles. The fact that luteal phase did not lengthen in vaccinated women provided confirmation that anti-hCG antibodies prevent implantation of the embryo onto the endometrium; interception is therefore before the onset of pregnancy. However, the degree of efficacy is highly satisfactory for vaccines against infectious diseases but a birth control vaccine has to be effective in about 90-95% of recipients in order to be acceptable.
A similar approach employing another anti-hCG vaccine was abandoned due to adverse side effects after promising animal and phase I studies (1009).
Therefore, there is still the need to improve immungenicity of this hCG vaccine by incorporating more adjuvants using other carrier proteins instead of TT. Pilot studies have shown that the conjugation of β-HCG to various peptides not only enhances the quantum of immune response compared to β-hCG conjugated to TT but also assures antibody response in mice of different genetic background (490). Furthermore, cross-reactivity with LH has to be reduced.

The application of contraceptive vaccines in humans still needs further investigation and development. The identification and use of novel target candidates that are crucial for gametogenesis, fertilization and implantation will take more time and effort. Another major challenge will be to increase the immunogenicity of contraceptive vaccines to generate antibody levels in 100% of the recipients. Furthermore, it is unlikely that a clearly defined common length of protection can be obtained.

One could also argue that systemic immunisation is not an appropriate, effective or reliable means of eliciting an optimal immune response in the female reproductive tract. Alternative routes of immunisation, which are likely to promote mucosal immune responses in the reproductive tract, for example nasal immunisation, may offer an effective and acceptable approach to human immunocontraception in the future.

IV. Summary-Zusammenfassung

The objective of this work was to systematically review and discuss recent studies and articles dealing with the subject of the immunology of female genital tract mucosal tissue.
The emphasis hereby lies on the evaluation of studies concerning the basics of female reproductive immunology, research on immunology of the most important genital infections and vaccination strategies, immunologic principles at the fetomaternal interface during normal pregnancy and its complications as well as on immunologic data on infertility and immunocontraception.

It is now established that the mucosal immune system is a distinct and separate component of the host`s immune apparatus and differs from the lymphoid tissues in peripheral sites. Furthermore, despite some common features, the female genital tract mucosal system displays some distinct characteristics which outlines its special role.
Analysis of the female genital tract indicates that the key cells of the innate and adaptive immune systems are present and functionally responsive to antigens; however, there is a certain degree of compartmentalization within the tract. The identification of TLRs in the fallopian tubes, uterus, cervix, and vagina and the presence of ECs, macrophages, DCs, NK cells, and neutrophils throughout the reproductive tract along with their responsiveness to selected PAMPs indicate that the female reproductive tract has evolved to meet the challenges of STDs, while at the same time supporting an immunologically distinct fetal placental unit. To meet these diverse challenges, innate and adaptive immune system in the female genital tract are precisely regulated not only by a network of cytokines and chemokines, but also by the sex hormones estrogen and progesterone.

Understanding the specialty of the genital tract immune system is of critical importance, because STDs are and will be a major worldwide health problem. Despite extensive efforts, only limited success has been achieved in dealing with a growing list of STDs.
The role of immune factors in the control of genital viral and bacterial infections appears complex and needs further studying, also with respect to creating vaccines. Despite the recognition that innate immunity as the first line of defense and adaptive immunity, especially Th1 immune responses, play a critical role in

preventing infection and in limiting viral replication, factors such as antimicrobials and TLRs that contribute to the mucosal response in the female genital tract have only recently begun to receive attention. Further studies are also needed to elucidate the relationship between mucosal immunity, the hormonal environment, and response to pathogen challenge. More data must be collected on the mechanisms of immune evasion by several pathogens such as HSV, *N. gonorrheae* or *Chlamydia*. While considerable information can be obtained from animal experiments, important differences in the physiology of reproduction and the immune sytem result in the need for studies in humans.

Further knowledge on female tract immunology will also impact on immunological approaches to contraception, immunological infertility and the immunological aspects of pregnancy. This does not only involve new options for diagnostics but also for treatment of pregnancy complications such as preeclampsia, preterm birth and early pregnancy loss as well as for infertility.

Pregnancy involves maternal tolerance of the semiallogenic histoincompatible fetus and is characterized by the enhancement of the innate immune system and suppression of the adaptive immune response, probably with progesterone as the important regulator. In opposite to normal pregnancy, improper immune responses and an unbalanced cytokine network may characterize implantation failures, pregnancy loss and obstetric complications. These are the presence of elevated Th1/Th2 cell ratios, high concentrations of Th1 cytokines, elevated NK cell cytotoxicity and levels, and emergence of various autoantibodies.

These immunological approaches needs to be investigated and evaluated further with respect to widening of treatment options by modification of immune responses.

Das Ziel dieser Arbeit war es, eine systematische Literaturübersicht über die aktuelle Studienlage zum Thema „Immunologie im weiblichen Reproduktionstrakt“ zu erstellen.
Die Schwerpunkte liegen dabei zunächst auf der Zusammenfassung und Bewertung von Studien, die die Grundlagen zur Immunologie der Scheide beschreiben. Des weiteren soll ein Überblick über die Forschung hinsichtlich der Immunologie der wichtigsten Genitalinfektionen und neuesten Impfstrategien gegeben werden. Aktuelle Studien zu den immunologischen Grundlagen der normalen Schwangerschaft, Schwangerschaftskomplikationen sowie Infertilität und Immunokontrazeption werden zusammengefasst und diskutiert.

Es gilt heute als gesichert, dass das Immunsystem der Schleimhäute einen eigenständigen und unabhängig funktionierenden Teil der menschlichen Immunabwehr darstellt und sich von der systemischen Immunabwehr vielfach unterscheidet. Das Immunsystem des weiblichen Reproduktionstrakt weist zudem noch einige spezielle Merkmale auf, die seine Sonderstellung unterstreichen.
Untersuchungen des weiblichen Genitaltrakts deuten an, dass die Schlüsselzellen der angeborenen und erworbenen Immunität vorhanden sind und auf Antigene reagieren, auch wenn es gewisse Unterschiede zwischen einzelnen Kompartmenten gibt. Die Identifizierung von TLRs in Eileiter, Uterus, Zervix und Vagina sowie das Vorhandensein von für ausgewählte PAMPs empfängliche Epithelzellen, Makrophagen, dendritische Zellen, natürliche Killerzellen und neutrophile Granulozyten im gesamten Reproduktionstrakt unterstreicht, dass der weibliche Genitaltrakt sich sowohl den immunologischen Herausforderungen durch sexuell übertragbare Krankheiten stellen, als auch gleichzeitig die immunologische Abstoßung des Fetus während einer Schwangerschaft verhindern kann. Um diese vielfältigen Aufgaben zu gewährleisten, wird das angeborene und erworbene Immunsystem im weiblichen Genitaltrakt sowohl durch ein Netzwerk an Zytokinen und Chemokinen als auch durch die Sexualhormone Östrogen und Progesteron genauestens gesteuert.

Die immunologische Sonderstellung des weiblichen Genitaltrakts ist von besonderer Bedeutung im Hinblick auf die stetig wachsende Bedeutung von sexuell übertragbaren Krankheiten weltweit. Trotz intensiver Forschung ist der Erfolg in der Behandlung einer stetig wachsenden Zahl von sexuell übertragbaren Erkrankungen begrenzt.

Die Bedeutung von Immunfaktoren in der Kontrolle von viralen und bakteriellen Genitalinfektionen erweist sich als komplex und bedarf weiterer Erforschung, besonders im Hinblick auf die Entwicklung von Impfstoffen. Trotz der Erkenntnis, daß angeborene Immunität als erste Abwehrlinie und erworbene Immunität, insbesondere eine Th1 Immunantwort, eine entscheidende Rolle in der Infektabwehr im weiblichen Reproduktionstrakt spielen, sind Einflüsse durch antimikrobielle Faktoren oder TLRs erst seit kurzem Gegenstand der Forschung. Weitere Untersuchungen hinsichtlich der Beziehung zwischen Immunität, hormonellen Einflüssen und der Antwort auf Pathogene sind notwendig. Zu Mechanismen von verschiedenen Pathogenen wie HSV, *N. gonorrheae* oder *Chlamydia.*, der Immunabwehr zu entgehen, müssen ebenfalls weitere Daten erhoben werden. Obwohl Tierexperimente beachtliche Informationen liefern, erfordern erhebliche Unterschiede in der Reproduktionsphysiologie und des Immunsystems zwischen Tier und Mensch weiterführende Studien am Menschen.

Erkenntnisse über die Immunologie des weiblichen Genitaltrakts beeinflussen ebenso die Forschung über Immunokontrazeption, immunologische Infertilität und die fetomaternale Immunität während der Schwangerschaft. Dies beinhaltet nicht nur neue Möglichkeiten in der Diagnostik, sondern auch neue Therapieoptionen für Schwangerschaftskomplikationen wie Präeklampsie und Frühgeburtlichkeit oder Infertilität.
Schwangerschaft bedeutet den Schutz des semiallogenen histoinkompatiblen Fetus vor Attacken des mütterlichen Immunsystems und ist charakterisiert durch eine Aktivierung des angeborenen Immunsystems und Supprimierung der adaptiven Immunantwort, was vermutlich durch das Hormon Progesteron mitgesteuert wird.
Im Gegensatz zur normalen Schwangerschaft wird vermutet, dass Infertilität, Schwangerschaftsabbruch und geburtshilfliche Komplikationen durch unangemessene Immunantworten und ein Ungleichgewicht an Zytokinen gekennzeichnet sind. Dazu zählen unter anderem eine erhöhte Th1/Th2 Zellratio, hohe Konzentrationen von Th1 Zytokinen, erhöhte Spiegel und Zytotoxizität der natürlichen Killerzellen, sowie das Auftreten von verschiedenen Autoantikörpern.
Diese immunologischen Erkenntnisse bedürfen weiterer Studien, um neue Therapieansätze durch Modulation der Immunantwort zu entwickeln.

V. Abbreviations

aCL	Anticardiolipin antibodies
aPS	Antiphosphatidylserine antibodies
Ab2	Antiidiotypic antibodies
ADCC	Antibody-dependent cellular cytotoxicity
AIDS	Acquired immunodeficiency syndrome
ANA	Antinuclear antibodies
APA	Antiphospholipid antibodies
APCA	Antipaternal cytotoxic antibodies
APC	Antigen-presenting cell
ART	Assisted reproduction technology
ASA	Antisperm antibodies
ATA	Antithyreoid antibodies
BV	Bacterial vaginosis
CD	Cluster of differentiation
cDNA	Complementary DNA
CEACAM	Carcinoembryonic antigen-related adhesion molecule
CIN	Cervical intraepithelial neoplasia
COPV	Canine oral papillomavirus
COX	Cyclooxygenase
CpG	Cytosine-phosphatidyl-guanine
CRVVC	Chronic recurrent vulvovaginal candidiasis
CTB	Cholera toxin B subunit
CTL	Cytotoxic T lymphocytes
CVL	Cervicovaginal lavage
CVS	Cervicovaginal secretion
DC	Dendritic cell
DHEA	Dehydroepiandosterone
DISC	Disabled infectious single cycle
dMPL	Deacylated monophosphoryl lipid
DNA	Deoxyribonucleic acid
DT	Diphtheria toxoid
EB	Elementary body
EBV	Epstein-Barr Virus
EC	Epithelial cell
ECP	Eosinophil cationic protein
EGF	Epidermal growth factor
EPF	Early pregnancy factor

Eppin	Epididymal protein inhibitor
ER	Estrogen receptor
ESP	Excretory-secretory protein
GALT	Gut-associated lymphoreticular tissue
GalCer	Galactosyl ceramide
G-CSF	Granulocyte colony-stimulating factor
FA	Fertilization antigen
FasL	Fas-ligand
FKN	Fractalkine
FSH	Follicle-stimulating hormone
GIFT	Gamete intrafallopian tube transfer
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GnRH	Gonadotropin-releasing hormone
GRO	Growth-related oncogene
HBD	Human β-defensin
HCC	Hysterectomy for cervical cancer
hCG	Human chorionic gonadotropin
HD	Human defensin
HEV	High endothelial venules
HGF	Hepatocyte growth factor
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HMC	Human mast cell line
HNP	human neutrophil peptide
HSIL	High-grade squamous intraepithelial lesion
hsp	Heat shock protein
ICAM	Intercellular adhesion molecule
ICI	Intracervical insemination
ICP	Infected cell protein
ICSI	Intracytoplasmatic sperm injection
iDC	Immature DC
IDO	Indoleamine-2,3-dioxygenase
IEL	Intraepithelial lymphocyte
IFN	Interferon
Ig	Immunoglobulin
IGF	Insulin-like growth factor
IL	Interleukin
IP	IFN-γ-inducible protein
IRM	Immune response modifier
ISCOM	Immune-stimulating complex

ITAM	Immunoreceptor tyrosine-based activation motif
ITIM	Immunoreceptor tyrosine-based inhibitory motif
IUGR	Intrauterine growth retardation
IUI	Intrauterine semination
IVF	In vitro fertilization
IVIG	Intravenous immunoglobulin
JAK	Janus kinase
KAb	Killer antibody
KC	Keratinocyte
KGF	Keratinocyte growth factor
KIR	Killer cell Ig-like receptor
KTR	Killer toxin receptor
LAMP	Lysosomal-phagosomal protein
LC	Langerhans cell
LCR	Long control region
LDH	Lactat-dehydrogenase
LH	Luteinizing hormone
LHr	Lutropin receptor
LIF	Leukaemia inhibitory factor
LOS	Lipooligosaccharide
LPS	Lipopolysaccaride
LSIL	Low-grade squamous intraepithelial lesion
LTR	Long terminal repeat
M cell	Membranous EC
MAdCAM	Mucosal addressin cell adhesion molecule
MALT	Mucosa-associated lymphoreticular tissue
MAP	Mitogen-activated protein
MBL	Mannose binding lectin
MBP	Major basic protein
MCP	Monocyte chemoattractant protein
mDC	Mature DC
MHC	Major histocompatibility complex
MIF	Macrophage inhibitory factor
MIG	Monokine induced by IFN-γ
MIP	Macrophage inflammatory protein
MLR-Bf	Mixed lymphocyte reaction blocking antibodies
MT-MMP	Membrane-type matrix metalloproteinase
MOMP	Major outer membrane protein
MoPn	Mouse pneumonitis
mRNA	Messenger ribonucleid acid

NADPH	Nicotinamide adenine dinucleotide phosphate
NALT	Nasal-associated lymphoreticular tissue
NF-κB	Nuclear factor-κB
NK cells	Natural killer cells
NO	Nitric oxide
ODN	Oligodeoxynucleotide
Opa	Outer membrane protein
ORF	Open reading frame
PAF	Platelet activating factor
*Pa*KT	*Pichia anomala* killer toxin
PAMP	Pathogen-associated molecular pattern
PBMC	Peripheral blood mononuclear cells
PDGF	Platelet-derived growth factor
PECAM	Platelet cell adhesion molecule
PIBF	Progesterone-induced blocking factor
p.i.d.	Post-infection day
PID	Pelvic inflammatory disease
PIF	Preimplantation factor
pIgR	Polymeric immunoglobulin receptor
PK	Protein kinase
PMN	Polymorphonuclear neutrophil
POF	Premature ovarian failure
PPROM	Preterm premature rupture of membranes
PR	Progesterone receptor
PTL	Preterm labor
RANTES	Regulation upon activation, normal T cell expressed and secreted
rCTB	Recombinant CTB
Rb	Retinoblastoma gene product
RB	Reticulate body
RES	Reticuloendothelial system
Rmp	Reduction modifiable protein
RNA	Ribonucleic acid
RNI	Reactive nitrogen intermediates
ROI	Reactive oxygen intermediates
RSA	Recurrent spontaneous abortion
RVVC	Recurrent vulvovaginal candidiasis
S-IgA	Secretory IgA
SC	Secretory component
SCA	Steroid cell antibodies

SLPI	Secretory leukocyte protease inhibitor
SMA	Anti-smooth muscle antibody
SP	Sperm protein
SP-A	Surfactant protein A
STAT	Signal tranducers and activators of transcribtion
STD	Sexually-transmitted disease
SUZI	Subzonal sperm injection
TAP	Tracheal antimicrobial peptide
	Transporter associated with antigen presentation
TCR	T-cell receptor
TER	Transepithelial resistance
TGF	Tumor growth factor
Th cells	T helper cells
TIMP	Tissue inhibitor of metalloproteinase
TK	Thymidine kinase
TLR	Toll-like receptor
TNF	Tumor necrosis factor
Treg	T regulatory cell
TT	Tetanus toxoid
VAP	Vascular adhesion protein
VCAM	Vascular cellular adhesion molecule
VLP	Virus-like particle
VVC	Vulvovaginal candidiasis
WHO	World Health Organisation
WT	Wild-type
ZP	Zona pellucida

VI. Table index

VII. Figure index

VIII. Bibliography

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