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DYNAMICS OF THE HIV-2-SPECIFIC IMMUNOGLOBULIN A (IgA) RESPONSE

av

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AKADEMISK AVHANDLING

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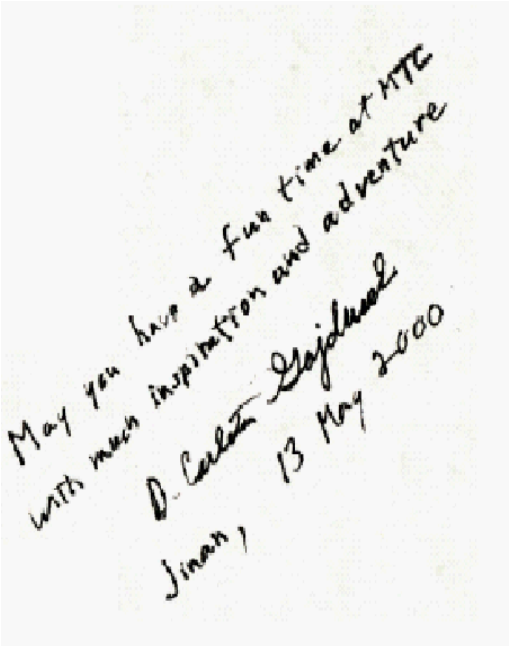

Nobel Laureate:

D. Carleton Gajdusek to Qin Lizeng at Jinan, China in 2000.

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TO MY FAMILY

ABSTRACT

Millions of lives are affected by infection with HIV, which causes AIDS if untreated. HIV transmission occurs primarily through sexual contact across mucosal surfaces. In the mucosal immune response, IgA is considered the first defense line against HIV invasion. Furthermore, a recent study suggests that serum IgA can initiate phagocytosis by Kuppfer cells *in vivo* to avoid bacteria spreading via the blood system, which further suggests that serum IgA can serve as a second defense line.

HIV-2, the second lentivirus known to cause AIDS, appears to be less pathogenic with a lower transmission rate than HIV-1 provides an important opportunity to study immune control and pathogenesis of lentivirus infections in humans.

The overall goal of our investigations was to elucidate the role of IgA in HIV-2 infection and immunity. Four studies were performed.

The first study was designed to investigate the antigenic sites of HIV-2 that are important for the binding of serum IgA. We showed that serum IgA in HIV-2 infected individuals had the capacity to neutralize HIV-2. Additionally, a strong serum IgA binding activity against a peptide (aa 644-658, MYELQKLNSWDVFGN) in the region of the central part of transmembrane envelope of HIV-2 (gp36) was displayed.

In the second study, we found that serum IgA in HIV-2-exposed IgG seronegative (EGSN) individuals had more potent capacity to neutralize HIV-2 than their known HIV-2 positive partners. The ratios of neutralizing titers/mg IgA (T/mg IgA) between EGSN individuals (580) and their known partners (480) was statistically significant ($p<0.05$). Given that IgA had potent neutralizing capacity *in vitro*, it may confer protection in the populations at high risk of HIV-2 exposure. This study may have important implications in understanding HIV-2 pathogenesis and transmission.

In the third study, sera were collected longitudinally from a group of HIV-2 infected individuals during three-to-eight years of follow-up. We demonstrated that HIV-2-specific serum IgA has as broad binding reactivity pattern to the different antigens as IgG. Importantly, our data indicate that HIV-2 specific serum IgA may be induced independently of CD4 T cells or by long lived memory B cells, since loss of CD4 T cells did not give a reduction of serum IgA. Moreover, a sustained serum IgA neutralizing capacity against HIV-2 was displayed during 8 years of follow-up. Thus, serum IgA may be associated with the lower pathogenesis and lower transmisson rates seen in HIV-2 infected individuals.

Based on our prior findings, we were stimulated to explore in depth the serum IgA targeted neutralizing sites on HIV-2. Thus in a fourth study we explored the neutralization capacity of serum IgA against multiple HIV-2 strains and investigated targets for HIV-2 neutralizing IgA within the transmembrane protein (gp36) of HIV-2 in an effort to further understand the mechanism of IgA-mediated immunity. We showed broad HIV-2 serum IgA-mediated neutralization against three primary isolates representing HIV-2 subtype A and B. Furthermore, our results demonstrated that two epitopes in gp36, GCAFR (aa 588-592) and MYELQ (aa 644-648), were important in mediating serum IgA antigenic binding. However, none of the selected six peptides having either the amino acid sequence GCAFR or MYELQ exhibited inhibition of IgA neutralization of HIV-2 in peptide blocking experiments.

In summary, we have shown long-lived virus neutralizing HIV-2-specific serum IgA responses, which may be associated with the lower pathogenesis and lower transmisson rates seen in HIV-2 infected individuals. The importance of serum IgA was further implicated when HIV-2 exposed seronegative individuals were found to have strong serum IgA-mediated HIV-2 neutralizing activity. These studies have provided significant information important for the understanding of immune responses against HIV, particularly HIV-2, and may lay important information for the foundation of the design of an HIV vaccine.

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LIST OF PUBLICATIONS

This thesis is based on the following original papers and manuscripts that will be referred in the text by their Roman numbers.

I. **Lizeng, Q.,** Skott, P., Sourial, S., Nilsson, C., Andersson, S., Ehnlund, M., Taveira, N., Björling, E., 2003. Serum immunoglobulin A (IgA)-mediated immunity in human immunodeficiency virus type 2 (HIV-2) infection. *Virology.* ***308****, 225-32.*

II. **Lizeng, Q.,** Nilsson, C., Sourial, S., Andersson, S., Larsen, O., Aaby, P., Ehnlund, M., Björling, E., 2004. Potent neutralizing serum immunoglobulin A (IgA) in human immunodeficiency virus type 2-exposed IgG-seronegative individuals. *Journal of Virology.* ***78****, 7016-22.*

III. **Lizeng, Q.,** Björling, E., Norrgren, H., Biague, A., Andersson, S., Nilsson., C. Dynamics of the HIV-2-specific IgA response in a longitudinal follow-up study. *Submitted.*

IV. **Lizeng, Q.,** Björling, E., Taveira, N., Andersson, S., Nilsson, C. HIV-2-specific serum IgA mediates broad neutralization of multiple HIV-2 strains. *Submitted*.

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LIST OF ABBREVIATIONS

Aa	Amino acid
Ag	Antigen
AIDS	Acquired immunodeficiency syndrome
APC	Antigen presenting cell
Bp	Base pair
CCR-1,-2,-3,-5	C-C (beta) chemokine receptor 1, 2. 3 or 5
CD4/8	Cluster of differentiation 4/8
CTL	Cytotoxic T lymphocytes
CXCR4	C-X-C(alpha) chemokine receptor 4
DNA	Deoxyribonucleic acid
Ds	Double stranded
Env	Envelope
Gag	Group antigen
Gp120/gp41	Glycoprotein 120kDa/41kDa
Gp125/gp36	Glycoprotein 125kDa/36kDa
HAART	Highly active antiviral treatment
HIV-1, -2	Human immunodeficiency virus type 1 or 2
Ig	Immunoglobulin
IFN	Interferon
LTNP	Long-term nonprogressors
LTR	Long terminal repeat
KDa	Kilo Dalton
MAb	Monoclonal antibody
PBMC	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
PIgA	Polymeric IgA
PIgR	Poly immunoglobulin receptor
RNA	Ribonucleic acid
RT	Reverse transcriptase
R5	CCR5 receptor using virus
R5X4	Dualtropic CCR5 and CXCR4 receptor using virus
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel
SIgA	Secretory immunoglobulin A

SIV	Simian immunodeficiency virus
SU	Surface glycoprotein (gp120/gp125)
Tat	Transcriptional transactivator
TCID50	50% tissue culture infectious dose
TCLA	T-cell line adapted
V1, 2, 3, 4, 5	First to fifth variable region
X4	CXCR4 receptor using virus

Amino Acid	Letter Codes
A	(Ala) Alanine
C	(Cys) Cysteine
D	(Asp) Aspartic acid
E	(Gln) Glutamic acid
F	(Phe) Phenylalanine
G	(Gly) Glycine
H	(His) Histidine
I	(Ile) Isoleucine
K	(Lys) Lysine
L	(Leu) Leucine
M	(Met) Methionine
N	(Asp) Asparagine
P	(Pro) Proline
Q	(Glu) Glutamine
R	(Arg) Arginine
S	(Ser) Serine
T	(Thr) Threonine
V	(Val) Valine
W	(Trp) Tryptophane
Y	(Tyr) Tyrosine

AIMS OF THE THESIS

The general aim of the thesis was to investigate the systemic HIV-2-specific IgA-mediated immunity in HIV-2 infected or highly HIV-2 exposed individuals. The specific objectives were:

1. To determine antigenic sites important for HIV-2-specific serum IgA binding.
To this end, a cross-sectional and a longitudinal study were performed in order to seek the binding sites for serum HIV-2-specific IgA usage.

2. To investigate the role and biological function of serum IgA in HIV-2 infection.
Studies were designed to show whether serum IgA derived from different individuals could neutralize various strains of HIV-2.

GENERAL INTRODUCTION

The worldwide dissemination of human immunodeficiency virus (HIV) over the past four decades is one of the most catastrophic examples of the emergence, transmission, and propagation of a microbial genome (Ho, 2002). Twenty three years ago, the first cases of an acquired immunodeficiency syndrome (AIDS) afflicting young, homosexual American men were reported, heralding what we now know to be the beginning of the HIV epidemic. Since then, billions of US dollars have been invested in HIV research in the hope of gaining a better understanding of this infection and how to prevent and treat it (Rowland-Jones, 2003). Despite this an effective vaccine to protect vulnerable populations has not been obtained. However, many valuable details about the AIDS causative agents, viral transmission, host immune responses and host-pathogen relationship have been resolved. Doubtlessly, all these efforts will be helpful in designing and formulating a promising vaccine candidate.

THE DISCOVERY OF THE CAUSATIVE AGENT OF AIDS

In 1983, experimental data indicating an association between a retrovirus and AIDS was published and the virus was isolated from a homosexual man with lymphadenopathy and designated lymphadenopathy-associated virus (LAV) (Barre-Sinoussi, 1983). One year later, the same virus, called human T cell lymphotropic virus III (HTLV-III), was found in an AIDS patient (Popovic, 1984) and also from individuals at high risk for AIDS in California (Levy, 1984). Soon it became evident that LAV and HTLV-III were identical and the name "human immunodeficiency virus type 1" (HIV-1) was coined and classified as belonging to the lentivirus family (Coffin, 1986). This finding allowed the development of a reliable test for HIV infection-looking for the presence of circulating immunoglobulin G (IgG) antibodies specific for HIV-which provided the basis for screening of donated blood and for large-scale epidemiological studies. It is worth noting that, in contrast to the analysis of many other infections, HIV-specific antibodies have been widely used for two decades as a marker of persistent infection, rather than as an indicator of a past infection that has been cleared.

Soon after the discovery of HIV-1, a group of healthy Senegalese was described, whose sera showed an atypical pattern of HIV-1 in Western Blot analysis, demonstrating a stronger response compared to HIV-1 to the newly discovered simian immunodeficiency virus (SIV) isolated from immunodeficiency macaques in 1985 (Daniel, 1985). This finding indicated

the existence of a human virus more closely related to SIV than to HIV-1. In the following year (1986), the second lentivirus, HIV-2 was isolated (Clavel, 1986). In 1987, another HIV-2 strain, SBL_{6669}, was isolated by Albert and colleagues from a Gambian woman in Sweden (Albert, 1987).

Figure 1 shows the origin of HIV and the close relationship between HIV and SIV (Stebbing, 2004).

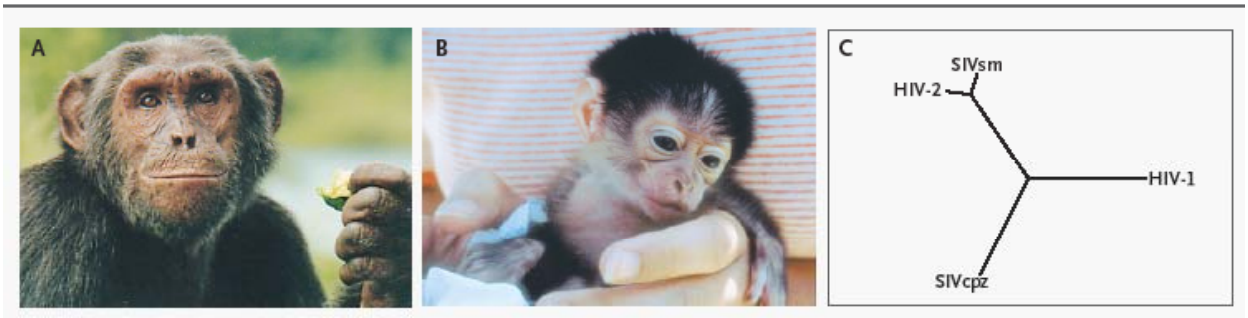


Fig. 1. *Origin of HIV (Panels A and B) and close relationship between HIV and SIV (Panel C). HIV-1 is likely from Chimpanzee (Panel A) and HIV-2 from Sooty mangabey (Panel B).*

TODAYS PANDEMIC OF HIV/AIDS

The HIV epidemic continues to expand at an alarming rate. By 2004, HIV had infected a cumulative total of more than 65 million people, over a third of whom have subsequently died (UN, 2004). Still, almost 40 million people live with HIV/AIDS today. Unfortunately, the catastrophic potential of the AIDS pandemic has not yet been fully realized. HIV and AIDS continue to extract an enormous toll throughout the world, notably in sub-Saharan Africa with 30 million people infected (Jaffar, 2004). The incidence is accelerating in some countries and regions, including China, India and parts of Eastern Europe as well as central Asia (UN, 2004).

While most infections are due to HIV-1, HIV-2 represents a significant minority of all HIV infections. HIV-2 has mainly been reported to be confined to West Africa with the highest prevalence in Guinea-Bissau (Albert, 1987; Andersson, 2001; Clavel, 1986; Kanki, 1987). Fairly high prevalences are reported in Portugal (Schim van der Loeff, 1999), Spain (Mota-Miranda, 2004; Toro, 2004) and India (Bollinger, 1995; Bhanja, 2004; Pfutzner, 1992; Rubsamen-Waigmann, 1991). Additionally, Angola (Bottiger, 1988) and Brazil (Cortes, 1989) have documented HIV-2 infection. Notably, recently Asian countries have also reported sporadic HIV-2 infection, such as Nepal (Chandler, 2004) and East Asia

(Kusagawa, 2003). Further note should be taken to see whether this virus leads to vast epidemics in these areas.

HIV-1 is characterized by a high degree of genetic variation. At least 10 different subtypes (A-J) of HIV-1, representing the major viral groups and M, O representing the minor groups and N are responsible for the AIDS pandemic worldwide (Fauci, 1993; Pantaleo, 1995). In developed countries, such as in North America, Europe and Australia, the HIV-1 epidemic is dominated by subtype B. In India and China by subtype C and in Thailand by circular recombinant forms (CRF_AE) (Carr, 1996; Gao, 1996). Subtype C is thought to be the most prevalent subtype worldwide.

In sub-Saharan Africa, all known HIV-1 groups and subtypes are evident (Gurtler, 1994; Janssens, 1997; Simon, 1998; Takehisa, 1998). Subtypes A, C and D are common in east African countries. Subtype C predominates in the northeast and southern African countries, while, CRF02_AG is found in West and West-Central Africa (Carr, 1998; McCutchan, 1999). However, this subtype geographic distribution is not so strict, with new subtypes entering this area (Hamers, 2003, 2004).

HIV-1 TRANSMISSION

The AIDS pandemic has exacted a terrible toll in terms of loss of life and decreased quality of life worldwide, especially in Africa, where 70% of deaths from HIV-1 infection have occurred (UN, 2002). The known predominant route of HIV-1 infection is heterosexual transmission through mucosal surfaces (or transmission by oral or parenteral routes). Apart from this, mother-to-child transmission, drug injection needles as well as contaminated transfusion blood and blood reagents also play roles in HIV transmission. Almost 60% of individuals infected with HIV-1 are women (Pope, 2003). There is therefore, a great urgency to develop vaccines, microbicides and other preventive strategies to blunt the progress of the epidemic and, in particular, to stop the transmission of HIV-1 to women. The main lesson to be learned from HIV-1 and SIV studies is how quickly the virus establishes persistent infection in a lymphatic tissue reservoir and compromises host defences. The virus becomes well established in a lymphatic tissues reservoir during 2-3 weeks following sexual mucosal transmission (Haase, 1999). This reservoir is the principal site of virus production, storage, persistence (Fox, 1991; Pantaleo, 1993; Schacker, 2000) and pathology (Racz, 1986) with associated $CD4^+$ T cell depletion and destruction of follicles and the lymphatic tissue architecture.

Viral transmission is related to the viral dose. In fact, the probability of transmission for each encounter with HIV-1 is quite small (about 0.001), and is related in part to dose (Gray, 2001). There is a direct relationship between viral load in peripheral blood and transmission, with a cutoff of around 1,500 copies of viral RNA per ml of blood, below which there is no evidence of transmission (Quinn, 2000). The extent to which the concentration of virus in semen mirrors that in peripheral blood is unknown.

HIV-1 strains can be classified according to co-receptor usage in infection of host cells, CCR5 (R5 virus) and CXCR4 (X4 virus). R5 strains are generally transmitted, whereas X4 strains emerge later and are associated with a rapid progression to AIDS (Schuitemaker, 1992).

In HIV transmission, many factors can influence viral penetration of the mucosal barriers, such as inflammation, menstrual cycle and hormones. Generally, in HIV-1 infection, thinning and breaches of epithelia may underlie the increased transmission associated with sexually transmitted diseases, bacterial vaginosis and nonoxynol-9 use (Cohn, 2001; Martin, 1999; Sewankambo, 1997; Van Damme, 2002) as well as the association between cervical ectopy and increased HIV-1 transmission (Moss, 1991). However, β-chemokines such as

RANTES, MIP-1α and MIP-1β can inhibit virus infection by blocking CCR5 (Alkhatib, 1996).

It may be easier for viruses to traverse the single layer of epithelium that lines the endocervix than the multilayer squamous epithelium of the ectocervix and vaginal mucosa. This would account for both the association between cervical ectopy and increased HIV-1 transmission (Moss, 1991), and the higher concentration of SIV-infected cells found in the cervical submucosa after intravaginal inoculation (Zhang, 1999). The fact that the virus crosses the mucosal barrier so rapidly and gains access to dendritic cells (DCs) and CD4+ T cells to facilitate virus production and dissemination limits opportunities for a vaccine-induced anamnestic response to HIV-1 that could prevent or fully control infection.

MECHANISMS FOR CROSSING AN INTACT MUCOSAL BARRIER

HIV-1 can traverse the intact stratified squamous epithelium of the vaginal mucosa and the simple columnar epithelium of the cervix. Transcytosis via a vesicular pathway is one mechanism by which HIV-1 can cross an intact barrier to infect susceptible host cells in the underlying tissues and this event can be mediated by epithelial galactosyl ceramide and the HIV-1 envelope glycoproteins (Bomsel, 1997; Mascola, 2000). It is also a stage at which the virus is vulnerable to mucosal antibody responses because secretory antibodies neutralize the infectivity of transcytosing virus both intracellularly and on the surface of epithelia (Alfsen, 2001; Belec, 2001; Bomsel, 1998) (Fig. 2). In addition, SIV bound by neutralizing antibodies specific for the viral envelope is also significantly less effective at infecting macaques through the vaginal route than is unbound virus (Veazey, 2003).

Epithelial cells selectively capture R5 HIV-1 and then transfer infection to CCR5-expressing target cells underneath the epithelia, which could account for the preferential transmission of HIV-1 strain R5 (Meng, 2002). Dendritic cells (DCs) positioned in vaginal and cervical epithelium are also prime targets for HIV. DCs express CD4, CCR5, DC-SIGN (DC-specific ICAM grabbing non-integrin) (Geijtenbeek, 2000) and other C-type lectin receptors (CLRs) as well as mannose receptor that facilitate capture and infection by HIV-1 and SIV (Frank, 2002; Lee, 2001). After successfully entering the mucosal surface, viral replication and gene products including Nef, Tat and Vpx further help to infect naïve and activated CD4 T cells. This may partly explain the predominance of infection in resting CD4+ T cells during early SIV infection after intravaginal inoculation (Zhang, 1999) and in genital organ cultures *in vitro* (Collins, 2000; Gupta, 2002).

Additionally, a number of mechanisms may be involved in the transmission of mucosal surfaces (Fig. 2):

1. Direct infection of mucosal epithelial cells (Delezay, 1997; Furuta, 1994).
2. Transcytosis of HIV through mucosal epithelial cells to initiate infection of immunocompetent cells in the underlying tissue (Bomsel, 1997; Spira, 1996).
3. Transcytosis through mucosal microfold cells (M cell) (Amerongen, 1991; Kraehenbuhl, 2000; Spira, 1996). M cells are present throughout the intestinal tract and in palatine and sublingual tonsils but have not been identified in the cervical or vaginal mucosa of the genital tract. These specialized cells bind macromolecules or microorganisms, including viruses such as poliovirus and reovirus (Wolf, 1981), to their apical surfaces and transport them by a nondegradative process to the basal surface (Owen, 1977). Once past the epithelial barrier, lymphocytes, macrophages or dendritic cells may be infected with HIV. After local infection, productively infected cells can quickly spread systemically to peripheral lymph nodes and other lymphoid tissues.
4. Transport through the epithelium by, or direct infection of, Langerhans cells that reside in the mucosal epithelium for transmission via a cell-cell route to neighbouring immunocompetent cells (Pope, 2003; Spira, 1996).
5. Disruption of the epithelium in the gastrointestinal and genital tracts by trauma, inflammation or thinning by menstrual cycle or hormones as well infection provides inoculated HIV-1 direct access to the microcirculation and mononuclear cells in the underlying lamina propria (Pope, 2003).

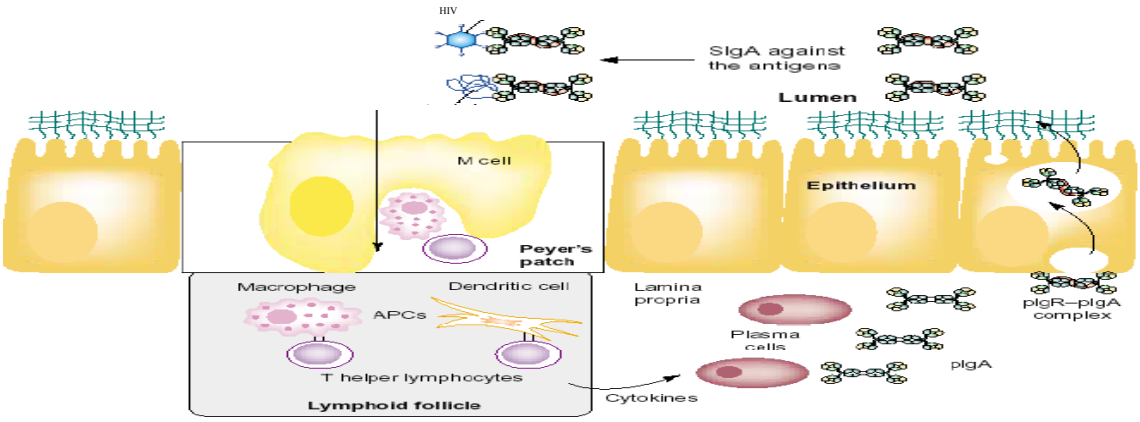


Fig. 2. *Schematic drawing of possible mechanism of HIV transmission through transcytosis and active transport of dimeric IgA-pIgR complex in vesicles. On the one hand released secretory IgA on apical surfaces can neutralize HIV. Non-neutralized HIV can target M cells or other epithelium to penetrate mucosal surface and further infect other cells, such as DCs, macrophages and T cells, (modified from Corthesy, 2002).*

HIV-2

THE ORIGIN OF HIV-2

There is persuasive evidence that HIV-2 arose from the SIV_{SM} of the sooty mangabey (*Cercocebus atys*) monkeys of coastal West Africa, from Senegal to the Ivory Coast, the endemic epicentre of HIV-2 (Hahn, 2000). In these areas, nonhuman primates are kept as pets and butchered for food, suggesting routes of transmission from monkey to human. HIV-1 originated from the chimpanzee (*Pantroglodytes*), which harbors the related SIV_{CPZ} and lives in central Africa (Gao, 1999; Santiago, 2002). The most recent common ancestors of HIV-2 subtypes have been dated to the 1940s (Lemey, 2003). For HIV-1, the earliest documented case of infection in humans was identified in a serum sample from Kinshasa that was stored in 1959 (Zhu, 1998). As has been estimated, the main (M) group of HIV-1 strains diversified in humans in about 1931 (Korber, 2000).

In addition, phylogenetic relatedness demonstrates that SIVs from chimpanzees cluster phylogenetically with HIV-1; in contrast, HIV-2 is a zoonosis from the sooty mangabey (Fig. 3). It appears from phylogenetic analysis of divergent HIV-2 strains that there have been seven independent transmissions from sooty mangabey to humans, resulting in HIV-2 subtypes A-G (Chen, 1997). Only one member each of subtypes C, E, F and G, and two members of subtype D, have been identified and it is thought that these rare subtypes may be primary zoonotic infections (Schim van der Loeff, 1999).

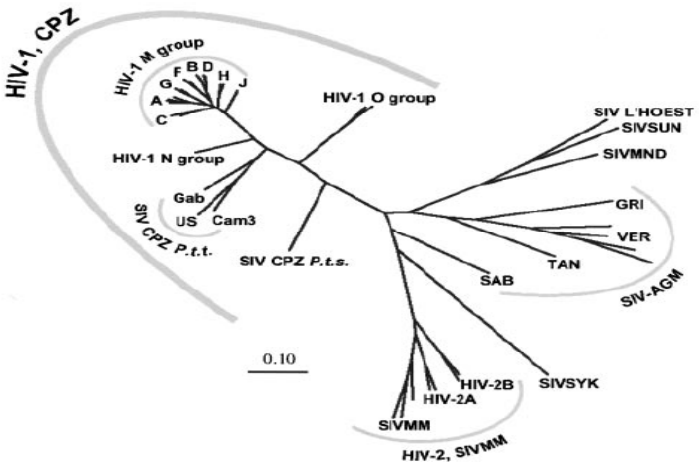


Fig. 3. *Phylogenetic tree illustrating the relationship of primate lentiviruses. Modified from (Reeves, 2002).*

HIV-2 GENOME AND STRUCTURE

HIV-2 is an enveloped virus and belongs to the lentivirus subfamily. The genome information is carried as two identical single strands of RNA which consists of around 9.7

kb nucleotides, a little bit longer than that from HIV-1 because of a longer LTR gene. The genome organizations of HIV-1, HIV-2 and SIV are similar. However, HIV-1 and HIV-2 differ by 40-60% at the nucleotide level (Chakrabarti, 1987; Guyader, 1987). Additionally, HIV-2 and SIV lack the *vpu* gene. They carry *vpx* gene instead, which does not exist in HIV-1 (Fig. 4).

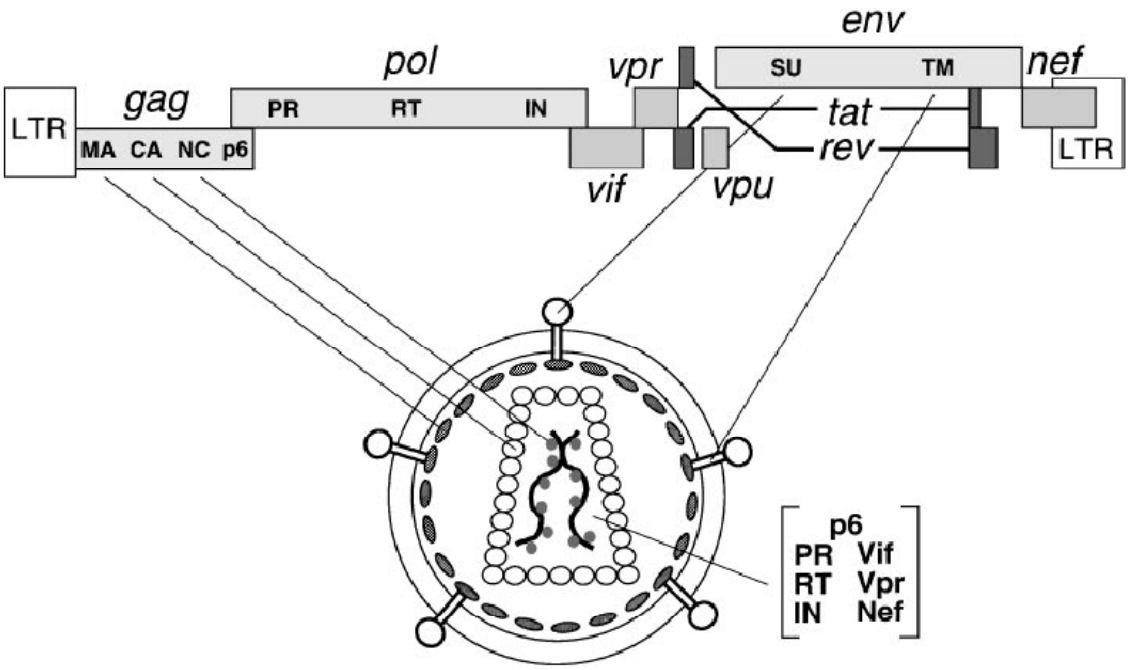


Fig. 4. *HIV genome organization and virus structure. Modified from (Frankel, 1998).*

HIV-2 *gag* gene encodes polyprotein which can be cleaved into internal proteins such as MA (p17), CA (or p26), NC and p6. The *pol* gene overlaps the *gag* gene and encodes a large *gag-pol* fusion protein which is cleaved into protease (PR, p10), reverse transcriptase (RT, p55/p66) and integrase (IN, p32). Primate lentiviruses contain two regulatory genes encoding Tat and Rev and the accessory gene products Vif, Vpx (Vpu in HIV-1) Vpr and Nef. Nef has been reported as a down-regulator of MHC class I molecules (Collins, 1998).

The *env* gene codes for the glycosylated envelope proteins gp125 (gp120 for HIV-1) and gp36 (gp41 for HIV-1). Gp125, the outer surface protein (SU), has five variable regions V1-V5, which is anchored to the virus membrane via the gp36 transmembrane protein (TM). The HIV-1 envelope shown by crystal structure is a trimer of gp120-gp41 heterodimers. The trimer is held together by interactions involving conserved gp120 surfaces that are not exposed on the virion surface but, on gp120 shedding, act as a decoy to stimulate largely irrelevant antibodies (Wyatt, 1998).

VIRUS TROPISM AND RECEPTOR USE

HIV-2, like HIV-1, uses CD4 as a main receptor to infect its target cells, such as T lymphocytes, monocytes, macrophages and dendritic cells (Clavel, 1986; Dalgleish, 1984; Klatzmann, 1984). Importantly, it is not sufficient for HIV-1 to use CD4 alone to infect cells. A coreceptor, a seven transmembrane G protein coupled protein receptor (7 TM GPCR), is a must for its effective infection. According to coreceptor usage HIV-1 is divided into CCR5 (R5), CXCR4 (X4) and dual usage for CCR5 and CXCR4 (R5X4) subtypes. HIV-2, besides CCR5 and CXCR4, can use a broad range of coreceptors and is more promiscuous than HIV-1 strains in use of coreceptors (Mörner, 1999). HIV-1 usage of alternative coreceptors is usually inefficient.

A quite striking difference between HIV-1 and HIV-2 strains is that, while HIV-1 requires both CD4 and a coreceptor, CD4-independent usage for HIV-2 infection is frequently reported (Endres, 1996; Thomas, 2003). Two strains (ROD/B and vcp) have been analysed in detail (Lin, 2001; Reeves, 1997) and two mutations conferring efficient CD4 independent infection by increasing the fusogenicity of envelope protein as well as increasing the sensitivity of the envelope to undergo conformational changes induced by sCD4 have been reported. This also suggests that CD4-independent HIV-2, like HIV-1 (Edwards, 2001; Hoffman, 1999), is easily neutralized by antibodies (Thomas, 2003). CD4-independence can be acquired by multiple mechanisms and only a few amino acid changes are needed to confer CD4-independence.

Moreover, coreceptor mutations, such as CCR5Δ32, a 32 bp deletion of the CCR5 gene product have been demonstrated to mediate resistance to HIV-1 sexual transmission (Liu, 1996; Samson, 1996). More recently, blocking CCR5 by a RANTES-like chemokine, PSC-RANTES, prevented SHIV transmission in rhesus macaques, indicating the importance of coreceptors in HIV transmission (Lederman, 2004). Additionally, coreceptor usage has long been used to predict the disease progression. Usually, HIV-1 in the late stage of infection uses CXCR4 more frequently than CCR5. An evolution from R5 to X4 is not obvious in HIV-2-infected individuals as many primary isolates use a range of coreceptors including both CCR5 and CXCR4 and only a limited number of X4 viruses have been isolated from symptomatic patients (Guillon, 1998; Morner, 1999). In addition, it is not known if R5 HIV-2 strains are largely responsible for virus transmission, as only a few isolates from asymptomatic individuals predominantly use CCR5 (Guillon, 1998). Data of the study of coreceptor mutation in HIV-2 usage has not been reported.

EPIDEMIOLOGY, TRANSMISSION AND PATHOGENESIS

Epidemiology

HIV-2, as described before, is primarily restricted to West Africa. A growing concern is also the increase of infections in certain parts of Europe and in the southwestern region of India. In Guinea Bissau, a former Portugese colony, there is an HIV-2 prevalence of up to 8-10% (Wilkins, 1993). The highest prevalence, 28%, is evident among commercial sex workers in the Gambia (Ghys, 1997). In addition to West Africa, countries with past socio-economical links to Portugal, including southwest India and Brazil, have significant numbers of HIV-2 infections (Schim van der Loeff, 1999).

Subtype A accounts for the majority of HIV-2 infections and is the predominant genotype in Guinea-Bissau and Europe (Norrgren, 1997; Schim van der Loeff, 1999). The ROD strain, the prototype of HIV-2, is a subtype A virus that was isolated from a Cape Verdian (Clavel, 1986). In 1987, another HIV-2 subtype A strain, SBL_{6669}, was isolated by Albert and colleagues from a Gambian woman (Albert, 1987). Subtype B viruses have also been isolated from individuals in Europe (Schim van der Loeff, 1999). Although Sierra Leone has a low prevalence of HIV-2 (0.02%), it has the highest diversity of HIV-2 subtypes (A, B, E and F), probably resulting from zoonoses from local sooty mangabeys infected with diverse strains of SIVsm (Chen, 1997). A growing number of HIV-2 endemic areas have documented the dual infection of HIV-1 and HIV-2 (Norrgren, 1999), which shows these dual infected individuals resemble single HIV-1 infection more than infection with HIV-2 (Nkengasong, 2000).

Transmission and pathogenesis

The transmission of HIV-2 has been previously described in detail in this thesis. Generally, HIV-2 appears to be transmitted via the same routes as HIV-1, but the frequency of transmission is reduced, probably due to a very low virus load in many asymptomatic individuals (O'Donovan, 2000). Usually, sexual and vertical transmissions of HIV-2 are around 5-9 and 10-20 fold reduced, respectively, compared to HIV-1.

Naturally infected sooty mangabeys, Africa green monkeys and chimpanzees do not develop SIV-related disease, even though high virus loads can sometimes be detected in their plasmas (Rey-Cuille, 1998). However, a more recent publication reported that a sooty mangabey developed typical AIDS symptoms after 18 years' natural infection, which gave

the traditional concept a small knock (Ling, 2004). As described before, primate HIV infections are zoonotic infections with disease only associated with cross-species transmission of the viruses. For example, SIVs from sooty mangabey and chimpanzees can cross-transmit into humans to yield HIV-2 and HIV-1 infections, respectively.

Although the clinical features of HIV-2 infection are similar to those of HIV-1 infection, HIV-2 is generally less pathogenic. This may be due to differences in virulence of these two viruses, the lower plasma virus load that is usually associated with HIV-2 infection or better immune control of HIV-2 replication compared to HIV-1 (Andersson, 2001). In HIV-2 infection, individuals also generally manifest a longer disease progress or longer latent period of 10 years or more, resulting in a mortality rate estimated to be two-thirds lower than that for HIV-1 (Marlink, 1994), and some will not progress to AIDS at all (Poulsen, 1997). A broad antibody neutralization has been suggested to correlae with longer disease progression (Fenyo, 1996). Notably, the burden of proviral DNA is similar in HIV-1 and HIV-2 infections. The lower virus load observed in HIV-2 infected individuals may be accounted for by differences in virus production (Popper, 2000).

Table 1. Summarized comparison of HIV-1 and HIV-2

	HIV-2	HIV-1	Ref.
Epidemiology			
Geographical distribution	Endemic		Schim van der Loeff, 1999
Transmission mode	Sexual, blood, vertical		Schim van der Loeff, 1999
Mother-to-Child transmission	<5%	20-25%	Schim van der Loeff, 1999
Age-related prevalence	Increase with age	Peak 20-40 years	Wilkins, 1993
Clinical signs			
Pathogenic	Less		Jafffar, 2004
CD4 decline	Slower		
Time to AIDS	Longer>20 years	10-12 years	Marlink, 1994
Viral aspects			
Plasma viral load	Lower		Popper, 2000
Proviral load	Same		Popper, 2000
Genetic similarity	40-60%		Chakrabarti, 1987; Guyader, 1987

IMMUNE RESPONSES TO HIV

AIDS is essentially an infection of the immune system. An early effective host immune system is crucial to prevent against HIV infection. Two major defense pathways are described within the host immune system: innate and adaptive immunity.

INNATE IMMUNITY

Innate immunity is the earliest response carried out by a class of immune-sensor molecules termed pattern recognition receptors (PRRs), against pathogen invasion. The most important receptors are Toll-like receptors (TLRs) (Boehme, 2004). From this primitive immune response, the adaptive immune system has time to develop (Levy, 2001).

Indeed, innate immune responses may play a critical role in the early stage of HIV transmission (Lehner, 2003). The large number of Langerhans cells in vaginal, foreskin and oral epithelia, γδT cells in rectal and vaginal epithelia, and macrophages, DCs and NK cells in the sub-epithelial tissues enable these cells to secrete a number of chemokines and cytokines which may block HIV transmission and replication in the mucosal tissues. The CC chemokines (RANTES, MIP-1α and MIP-1β may also block HIV access to the CCR5 co-receptors (Cocchi, 1995), and they may attract the entire immunological repertoire of cells (T and B cells, DC and macrophage) to the mucosal site. Type I IFN, the complement system and lectin-binding proteins may play a crucial part in these processes (Levy, 2001).

Plasmacytoid dendritic cells (pDCs), formally called Interferon-producing cells (IPCs), produce IFNα. IFNα is a very important antiviral factor, and a positive correlation between blood IPC number, IFN-α production, and clinical state of HIV-infected subjects has been demonstrated. High viral load and a progressive disease in HIV individuals have been suggested to have a close correlation with a decreased number of pDCs in peripheral blood (Levy, 2003; Soumelis, 2001).

Among innate factors, secretory leukocyte proteinase inhibitor (SLPI) is the only one previously reported to be capable of HIV-1 inhibition at physiological levels (McNeely, 1995, 1997). Recently, one group confirmed a role of SLPI which was able to neutralize HIV-1 but not HIV-2 *in vitro* (Skott, 2002). Soluble factors, such as cellular antiviral factor (CAF) have been suggested (Walker, 1986) and alfa-defensins may also inhibit HIV-1 replication in LTNP individuals (Zhang, 2002). The concept that the innate immune system may drive the adaptive immune system and modulate T cell polarization has been gaining support. B1 cells, the non-specific antibody secreting cells, may play a role both

systemically and locally through expression of early non-specific antibodies against HIV invasion. More work is therefore needed to do in this area.

ADAPTIVE IMMUNE RESPONSE

The relationship between viral load and adaptive immune response following acute HIV infection is shown in Fig 5.

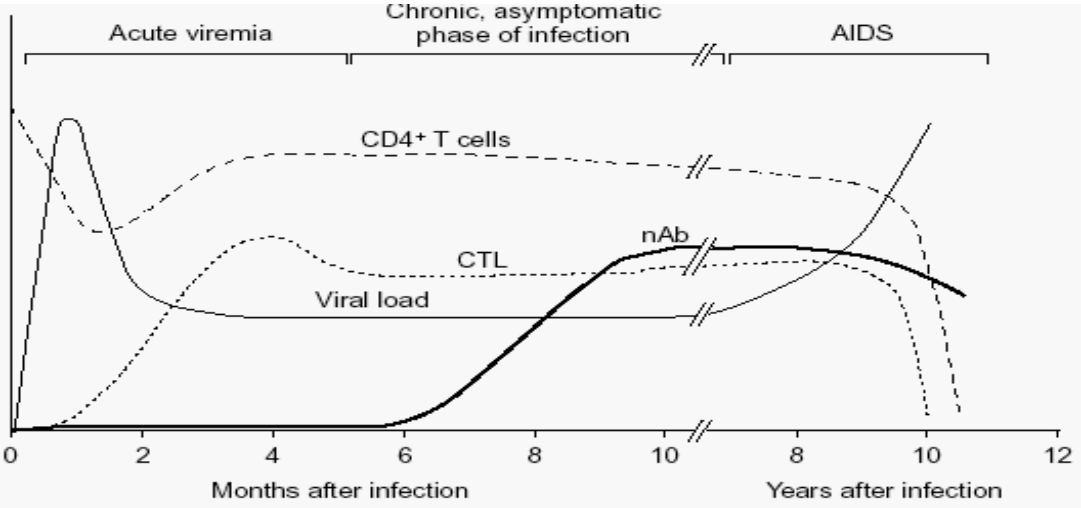


Fig. 5. *Time courses of viremia, CD4+ T cell counts, and CTL and neutralizing Abs responses during HIV infection and disease progression.*

Cellular immune response

Substantial evidence suggests that cellular immune responses have an important role in HIV-1 and HIV-2 as well as SIV infections (McMichael, 2001). In HIV-1 and SIV infections, several observations have implicated the correlation of CTL to protection.

1. Natural experiments. Viral-specific $CD8^+$T cell responses have been detected in individuals who were exposed to HIV-1 but who remain seronegative (Kaul, 2000; Kaul, 2001; Rowland-Jones, 1995).

2. Early stage of HIV-1 infection. A serial study has shown that viral replication is controlled during the early days after infection and correlates temporally with the emergence of an HIV-1-specific $CD8^+$ CTL response, Fig. 6. An association was shown between the appearance of effector cell populations that lyse HIV-1-expressing target cells and the decline in plasma viral RNA during the period of primary HIV infection (Borrow, 1997; Koup, 1994).

3. In LTNP individuals, $CD8^+$ T cells can inhibit HIV-1 replication *in vitro* (Walker, 1986).

4. In animal models. Perhaps the most compelling evidence for the importance of CD8+ CTLs in containing HIV-1 replication comes from studies in SIV-infected rhesus monkeys.

In vivo depletion of $CD8^+$ cells in monkeys, achieved by infusion of monoclonal antibodies to CD8, had profound effects on the replication of SIV (Jin, 1999; Schmitz, 1999). When the duration of depletion was greater than 28 days, primary viremia was never cleared after infection and the monkeys died with a rapidly progressive AIDS-like syndrome. In addition, transient $CD8^+$ lymphocyte depletion of chronically SIV-infected rhesus monkeys was associated with a substantial rise in viral replication that returned to baseline levels coincident with the re-emergence of the CD8+ cell population.

Viral specific-$CD4^+$ T cells also have an important role in controlling HIV-1 infection. $CD4^+$ T cells mainly facilitate a $CD8^+$ CTL response, which documents for priming of a $CD8^+$ T cell response (Ridge, 1998), for maintaining $CD8^+$ T cell memory (Walter, 1995), and for maturing $CD8^+$ T cell function (Zajac, 1998).

In viral infections, EBV and CMV CTL mediated immune responses have been proved to be effective (Riddell, 1995).

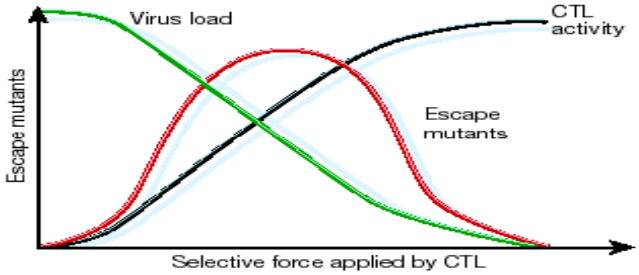


Fig. 6. *Schematic representation of the relationship between virus replication rate, manifested as viral load, the level of CTL response and the selective pressure on epitope escape mutants.*

Strong CTL responses are a feature of HIV-2 infection (Whittle, 1998) with the majority of asymptomatic patients studied demonstrating CTL activity direct from peripheral blood (Gotch, 1993). The extent of specific cytolytic activity against HIV-2 gag, pol and nef in cultured CTLs from asymptomatic Gambians is inversely correlated with proviral load (Ariyoshi, 1995). Strong CTLs, particular gag-specific responses to HIV-2 are detected to and frequently cross-react with the HIV-1 strain (Bertoletti, 1998; Rowland-Jones, 1995). Additionally, HIV-2-specific T lymphocyte proliferative responses have also been reported in HIV-2-exposed uninfected individuals (Andersson, 2005).

The overproduction of β chemokines by PBMCs from HIV-2-infected donors can prevent infection of R5 tropic (see below) HIV-1 strains *in vitro* (Kokkotou, 2000). Furthermore, HIV-2 (but not HIV-1) Env protein can interact with CD8 on T cells that are non-permissive to infection, triggering the production of β chemokines (Akimoto, 1998; Ichiyama, 1999). Higher proportions of $CD8^+$ T cells from HIV-2 infected individuals retain the ability to simultaneously produce the cytokines IL-2 and IFN-gamma. Likewise, more $CD4^+$ T cells

are capable of producing IL-2 than those from HIV-1-infected individuals with equivalent $CD4^+$ T cell counts (Sousa, 2001). These factors may also account in part for the better immune control of HIV-2 infection compared to that of HIV-1 infection. It is also interesting to note that deletions within the *nef* gene of HIV-2 are quite common (Switzer, 1998), while *nef* deletions are infrequently found in HIV-1, where they significantly reduce replication *in vitro* and *in vivo* (Piguet, 1999). Nef downregulates surface expression of MHC class I molecules (Kerkau, 1997; Le Gall, 1998), thus a functional Nef protein may aid evasion of host immune responses. Also notable is the fact that Nef can control Fas ligand expression on infected cells which can trigger apoptosis in neighbouring Fas-expressing cells. A small deletion in *nef* can abolish the apoptosis effect (Xu, 1997, 1999).

Suppression of HIV-2 replication by soluble antiviral factors (CAF) from $CD8^+$ T cells has been described both in monkeys and in people. Ahmed and coworkers have reported that three HIV-2-repeatedly exposed but seronegative monkeys were detected to have higher production of RANTES and MIP-1α. In these monkeys, suppressed SIV infection from $CD8^+$ cell cultured supernatants was available. Further study showed that antibodies to chemokines only partially neutralized $CD8^+$ cell-mediated SIV supression indicating that the anti-viral activity observed in these monkeys was the result of the combined action of several inhibitory factors (Ahmed, 2001). In baboons infected with HIV-2 anti-viral activity appears to be related to the ability to resist superinfection with heterologous HIV-2 strain (Locher, 1999). Recently strong $CD8^+$T cell-mediated noncytotoxic antiviral activity was demonstrated in HIV-2-infected individuals (Ahmed, 2005).

A potent antigen-specific T cell response to HIV infection can contribute to the control of viral replication and is therefore beneficial to the host. However, HIV-mediated increases in generalized T cell activation also appear to accelerate both viral replication and CD4 T cell depletion (Garber, 2004). An investigation from SIV-infected sooty mangabeys (a natural host of SIVsm) demonstrated that limited $CD8^+$ T cell responses in these monkeys enabled SIV-infected mangabeys to avoid the bystander damage seen in pathogenic infections and to protect them from developing AIDS (Silvestri, 2003).

Mucosal cellular response may be a priority for protection

The gastrointestinal and vaginal mucosae play fundamental roles in early HIV disease by serving as a site for viral entry (most often rectal and vaginal) (Kozlowski, 2003; Veazey, 2001), and initial and ultimately predominate as sites of virus replication and amplication (small intestine) (Veazey, 2001). Thus AIDS should be considered as a disease of the mucosal immune system. In particular, studies of SIV infection of Rhesus macaques suggest

that, regardless of the route of infection, the gastrointestinal mucosa, not the peripheral lymphoid tissue, is the initial and predominant tissue site of HIV-1/SIV infection, as indicated by the early local accumulation of SIV-infected lymphocytes and the high viral load in the intestinal mucosa (Veazey, 1998). These studies suggest that a vaccine that induces local mucosal immunity may control viral replication within local tissues prior to systemic dissemination. Support for this possibility was provided by a group demonstrating that local mucosal CTL, induced by mucosal immunization in mice, can protect against mucosal challenge with virus, while systemic $CD8^+$ CTL are not sufficient to protect against mucosal transmission (Belyakov, 1998). Another group reported that mucosal immunization of macaques with an HIV/SIV peptide vaccine was more effective against SHIV challenge than a systemic vaccine because it could more effectively clear virus from the major site of SHIV replication in the intestinal mucosa (Belyakov, 2001).

The period of local mucosal viral replication and local viral interaction with mucosal cells without systemic dissemination is crucial for potential generation of preventive local immune response (antibody and $CD8^+$ CTL), which can eliminate much of the viral load after mucosal transmission and reduce disease. Zhang and coworkers studied SIV replication using *in situ* hybridization in the local mucosal site (endocervical tissue) and systemic lymphoid tissues after intravaginal inoculation in macaques. Detection of low levels of viral RNA was observed in the endocervical tissue. The amount of viral RNA in the endocervical region increased between days 3 and 7. However, the first vial RNA in draining and distant lymph nodes and bone marrow were found on day 12 after mucosal inoculation (Zhang, 1999). This study indicates that immunological clearance mechanisms may have a few days to eradicate the virus locally before it disseminates widely. Understanding early events of interaction between HIV and mucosa will help in the development of new mucosal vaccines and prevention of HIV transmission through mucosal surfaces.

As discussed above, HIV-specific $CD8^+$ CTL was found in the cervical mucosa of HIV-exposed seronegative prostitued women in Nairobi (Kaul, 2000). This is also an evidence to underscore that the CTL response may be important in viral protection.

Furthermore, an asymmetry between mucosal and systemic vaccines for induction of mucosal immunity has been confirmed by several studies not only in HIV (Bertoletti, 1998; Kozlowski, 2003) but also in bacteria infection (Shata, 2001). These studies demonstrate that mucosal immunization can induce both local and systemic $CD8^+$ CTL immune responses. Systemic immunization only induces systemic immune responses, but not mucosal immune responses. This phenomenon further proves that mucosal immune

responses are paramount in protection from HIV infection. Systemic CTL responses may have a limited role in contribution to the local immune response.

In recent studies, it has been found that mucosal immunization of non-human primates leads to more effective induction of mucosal CTL and concomitantly more complete clearance of pathogenic SHIV from the gastrointestinal mucosa (Belyakov, 2001), which is a major reservoir of SIV replication seeding the bloodstream (Stebbing, 2004). The underlying mechanism for this asymmetry is unclear. Studies show that different DC phenotypes, such as DC in Peyer's patches (PP), myeloid DC ($CD11b^+$), lymphoid DC ($CD8\alpha$) and double-negative ($CD11b^-$, $CD8\alpha^-$) can produce different cytokine profiles to that of splenic DCs (Campbell, 2002; Iwasaki, 1999, 2000) which lead to T cell priming and homing.

In conclusion, as suggested above, a vaccine-induced CTL response in the mucosal system may be attractive strategy to eliminate virus at an early stage.

Humoral immune responses to HIV

Protective antibodies and other factors

Although antibody responses have a central role in clearing many viral infections, data suggest that this may not be true in HIV infection, since antibodies in the sera of HIV-1-infected individuals have only weak neutralizing activity for primary HIV-1 isolates (Montefiori, 1996; Moog, 1997; Moore, 1995). Even non-neutralizing antibody to virion debris was often detected (Poignard, 1999). Nevertheless, animal studies have demonstrated that neutralizing antibodies play a role in controlling HIV replication, albeit not in the early infectious stage. One study, using the macaque model to deplete B cells by monoclonal antibody to CD20, showed that the development of neutralizing antibody is delayed, but did not alter the kinetics of early viral clearance (Schmitz, 2003). A more detailed analysis of the generation and evolution of neutralizing antibodies in recently infected patients indicated that neutralizing antibody is indeed involved in controlling viral replication during the first month after infection, and that the pressure these antibodies exert on the virus is significant (Richman, 2003). It was also reported that when neutralizing antibody has pressure on a virus it can cause envelope gene mutation and subsequently result in the modification of the glycosylation pattern of this viral protein (Wei, 2003). This is called a "glycan shield", an important mechanism for viral escape from neutralizing antibody.

SIV and SHIV infection in non-human primates have been widely used as suitable models to study immune responses to lentiviruses and to assess the level of protection conferred by various vaccine strategies. Passive immunization transferred by antibodies is direct

evidence to show immune protection from pathogen challenge. One study reported that after depletion of $CD8^+$ T cells prior to challenge of SHIV in macaques, strong neutralizing humoral response were displayed in all of the tested macaques and that CD4 T cell loss was avoided (Rasmussen, 2002).

A series of animal model based trials have given rise to promising results from passively transferred neutralizing antibodies, for example in protecting newborn macaques (Hofmann-Lehmann, 2002), and avoiding infection after intravaginal or intravenous exposure (Parren, 2001).

Most importantly, neutralizing antibody may block infection. A recent study tested the unique neutralizing monoclonal antibody, b12, as a blocking agent at the vaginal surface applied mucosally in a gel (Veazey, 2003). Protection was observed when high concentrations of the monoclonal antibody were applied and when SHIV exposure occurred shortly after treatment. Recently, an analog of the chemokine RANTES was reported to prevent vaginal SHIV transmission in rhesus macaques through inhibition of CCR5 (Lederman, 2004).

It is worthy of mention that neutralizing antibodies including IgA, IgG and IgM have been extensively reported in mucosal surfaces from individuals who have been repeatedly exposed to HIV, including IgA (Mazzoli, 1997), IgG and Ig M (Ghys, 2000). Antibody from HIV-2 infection may also have ADCC properties, which is an important antibody-mediated mechanism for killing infected cells (von Gegerfelt, 1993). However, previous results showed that HIV-2 exposed uninfected Gambian femal sex workers had no vaginal IgA or IgG in their vaginal secretions (Dorrell, 2000). Additionally, mucosal innate immune responses including SLPI, defensins (Trabattoni, 2004) and chemokines play a role in avoiding HIV invasion across mucosal surfaces.

Humoral immune responses in HIV-2 infection

HIV-2 infection usually induces a strong antibody response. These antibodies have been frequently applied in diagnosis. A small proportion of these antibodies have been reported to have neutralizing ability (see review Andersson, 2001). Nevertheless, neutralizing antibody is thought to play a role in prevention or modulating infection with HIV, SIV or SHIV (Burton, 1997; Haigwood, 2003). Data concerning antibody has been documented in HIV-2 infection showing:

a) Stronger autologous neutralizing antibody in HIV-2 infection than that from HIV-1 (Björling, 1993).

b) Cross-neutralization between HIV-2 and HIV-1 has been shown in several studies, albeit with conflicting results. Some groups showed that HIV-2 antisera could cross-neutralize HIV-1, whereas HIV-1 sera were type-specific (Weiss, 1988). Another group showed weak cross-neutralization between HIV-1 and HIV-2 (Robert-Guroff, 1992). Furthermore, Böttiger and coworkers demonstrated bi-directional cross-neutralization (Böttiger, 1990).

Whether HIV-2 infection confers protection from subsequent HIV-1 infection is hotly debated. *In vitro*, numerous studies show that HIV-2 infection can protect from subsequent HIV-1 infection (Arya, 1996; Browning, 1999; Dern, 2001; Kokkotou, 2000), *in vivo* studies also showed protection (Greenberg, 1996; Travers, 1995). However, Controversial results have been reported (Aaby, 1997; Ariyoshi, 1997; Norrgren, 1999; Wiktor, 1999), since HIV-1 and HIV-2 dual infection has increased in latter endemic area. This is the direct evidence to prove how much the possibility is that HIV-2 can protect from HIV-1 infection (Norrgren, 1999).

Increasing evidence show that neutralizing antibodies might play an important role in HIV-2 particularly, as it has been suggested that a broad serum neutralizing antibodies may be related to slow disease progression and low risk of transmisson in primate lentivirus infection (Fenyö, 1996).

Potent human monoclonal antibodies to HIV-1 and their epitopes

The process of how HIV infects cell can be divided into three distinct steps: the attachment of HIV virions to surface CD4 receptors via the gp120 Env protein; the subsequent interplay of the conformationally altered gp120 protein with the CCR5 or CXCR4 chemokine coreceptors for HIV; and finally, gp41 Env-mediated fusion of virions to target cells (Moore, 2003). Certain neutralizing antibodies showed protecting from infection by coating Env because Env is vulnerable to impairment so it can not block these three steps to avoid virus moving into further steps.

Available data indicates that neutralizing antibody (Nab) mainly recognize three distinct neutralizing domains of the HIV-1 envelope: the CD4 binding sites (CD4bs) of the envelope, represented by mAb IgG1b12; the V3 loop represented by mAb 447-52D, which recognizes the GPGQ motif at the V3 loop, and Nab 2G12 that can react with mannose residues of gp120; the transmembrane gp41 protein domain recognized by mAb 2F5 and 4E10 is extremely conserved.

Additionally, the coreceptor site has been documented to be a target. The conserved coreceptor-binding site is overlapped by the so-called CD4-induced epitopes that are typified by mAb 17b (Zolla-Pazner, 2004).

The properties of mAbs b12, 2G12 and 2F5 have been characterized in detail. The b12 crystal structure shows that it has an unusually long protruding CDR3 loop that can plunge into the centre of the cavity of CD4 binding site. The mAb 2G12 has a more special (maybe a "T"-like) but not the usual "Y"-shaped arms which is well suited to recognize the cluster of oligomannose residues formed as part of the protective sugar coat of gp120. mAb 2F5 can recognize a linear peptide (ELDKWA), on the membrane-proximal region of the gp41 ectodomain. Immunogens that mimic the above mAbs epitopes may hopefully be useful vaccine candidates, but how and when such immunogens can be created is a big question (reviewed in Burton, 2004; Zolla-Pazner, 2004).

HIV-2 neutralization and neutralizing epitopes

Viral neutralization is measured *in vitro* and emerging data from animal studies show that there is a generally good correlation between *in vitro* and *in vivo* results (Ferrantelli, 2002; Haigwood, 2003; Mascola, 1999). The neutralizing determinants in HIV-2 are less well characterized than those of HIV-1. Nonetheless, the V3 loop of the HIV-2 envelope protein has been reported to contain epitopes important for neutralizing activity (Bjorling, 1991, 1994). Analogously, V3-specific mAbs with neutralizing capacity have been produced (Bjorling, 1994; Matsushita, 1995; McKnight, 1996) and synthetic peptides and peptides representing the identified neutralizing epitopes have been used to raise guinea pig hyperimmune antisera as well as for blocking the documented neutralizing activity of human anti-HIV-2 sera (Bjorling, 1991, 1994). Fine-mapping experiment revealed two distinct antigenic sites with conserved motifs within the HIV-2 V3 loop: FHSQ (aa 315-318) and WCR (aa 329-331) (Björling, 1994; Mörner, 1999a). Recent reports show that epitopes important for neutralizing antibody binding have been identified in the V2 and V4 regions of HIV-2 gp125 and one conserved region in gp36 (Skott, 1999). Despite this, peptide immunization failed to induce neutralizing antibody (Robert-Guroff, 1992).

Mechanisms of neutralization

A number of mechanisms have been proposed for the neutralization of HIV. The simplest way would be to classify these mechanisms according to the event in the virus replication cycle that they block. Blockade of virus attachment to target cells via receptors or ancillary molecules is a necessary first step not only for HIV, but for all viruses. This mechanism is important in the neutralization of many naked and enveloped viruses. MAbs b12 and b17 block receptor and coreceptor binding respectively (reviewed by Burton, 2001, 2004).

Several antibodies to gp120, the outer Env protein of HIV-1, block attachment of the virus to $CD4^+$ T cell lines and are directed to epitopes directly involved in interactions with CD4, the primary receptor for the virus (Moore, 1995, 2001; Parren, 1998). The potent and broad capacity of sera from HIV-1-infected people to block HIV-1 infection of peripheral blood mononuclear cells has also been attributed to inhibition of virus attachment to the cell surface (Beirnaert, 2001). Although many Abs interfere both with virus-receptor interactions and with attachment, particular Abs that block receptor interactions may neutralize without blocking virus attachment to certain cell types, whereas others that do not directly interfere with receptor binding of viral proteins may block attachment of virions to cells (Klasse, 2001). Interference with receptors subsequent to virus attachment can also effect neutralization (Armstrong, 1996). As long as blockage of attachment does occur, whether through direct occlusion of sites involved in virus binding to cells or indirectly through steric interference by Ab coating of the virions, then infection is by necessity prevented. Theoretical and empirical support is strong for inhibition of virus attachment and early entry functions as being dominant mechanisms of neutralization (Klasse, 2002). Aggregation can be a mechanism of neutralization in that it reduces the number of cells that virions can attach to, although this is unlikely to play major role in HIV-1 infection (see review by Parren, 1999). A second step which antibodies can interfere with is provided by post-attachment interaction of the virus with its receptors and co-receptors. Antibodies inhibition of the interactions between the viral envelope protein and the cell-surface receptors may occur after the virion has attached by binding via the receptors. For example, one receptor may serve as an attachment point as well as a trigger of conformational changes that allow interaction with a coreceptor, which in turn mediate later events such as membrane fusion. Antibodies interference with any of these necessary links in a chain of events that lead to entry would constitute a neutralization mechanism.

A third possibility is that antibodies on the virion may reduce its internalization by endocytosis. This would constitute neutralization when endocytosis is an obligate replicative step. Through Ab-Fc receptor interactions, this step could cause neutralizations. A fourth stage of blocking is the fusion at the cell surface or in an endosomal vesicle of enveloped viruses. HIV also uses this kind of mechanism to infect cell. The only commercially available fusion inhibitor of HIV, T-20, is a mimic of the peptide on gp41 that inhibits the forming of leucine zipper during the fusion process of HIV entry (Kilby, 1998). A fifth stage of interference is the uncoating or appropriate intracellular localization of the core or capsid which may be hypothetically affected by antibodies although only IgA has such possibility (Bomsel, 1998).

A potential sixth step, if antibodies that have bound to the virion surface could inhibit the first metabolic events catalysed by viral enzymes, such as transcription, this would count as neutralization.

In HIV infection, several steps combined could be applied for Ab neutralization. In principal and central is the antibody coating of the virus and the high-occupancy theory, in which neutralizing antibodies (nAbs) obstruct the close approach of virion and target cell, thereby effectively inhibiting virus-cell attachment. This theory is widely accepted (Burton, 2000; Moore, 2001; Parren, 1998; Zolla-Pazner, 2004), although some debate remains (Dimmock, 1993).

Nevertheless, a recent report demonstrated that polyclonal IgG from patients neutralize HIV-1 primary isolates by binding free virions, without interfering with an initial CD4-independent attachment of the virus to PBMC (Burrer, 2003). Two recent studies (Herrera, 2003; Poignard, 2003) reported that the binding of non-neutralizing antibodies to the virion surfaces may hinder the binding of neutralizing antibodies, but do not decrease their neutralizing activity. The hypothesis proposed to interpret this observation is that although neutralizing Abs and non-neutralizing Abs bind to non-functional epitopes on envelopes of virion surface, neutralizing Abs alone bind to functional envelopes.

The process of viral neutralization is complicated and many components could influence the whole procedure of antibody neutralization. Increasing data suggest that initial attachment between viruses and their targeted cells could be mediated by additional molecules to those the targeted cells express. Both ICAM-1 (Fortin, 1997) and LFA-1 (Liao, 2000) have been reported to be incorporated into the viral membrane to initate the attachment between viruses and target cells. HLA-DR (Cantin, 1997) and CD86 (Bounou, 2001) are incorporated into the viral membrane to retain their functional ligand properties. Attachment is also reported to be mediated by cell surface nucleolin (Nisole, 2002), and the cellular proteoglycan heparan sulfate (HS) was shown to be responsible for the attachment of HIV-1 TCLA strains on lymphocytic (Roderiquez, 1995) and HeLa cell lines (Mondor, 1998) as well as to mediate attachment of cloned HIV-1 primary isolate to macrophages (Saphire, 2001) although this had no effect on primary blood lymphocytes or unstimulated PBMC (Blanco, 2002). The attachment of HIV to cellular HS was shown to be mediated either by the V3 loop of gp120 (Zhang, 2002) or by cyclophilin A (CypA) incorporated into the viral membrane (Saphire, 1999). Virion-incorporated cellular components have also been reported to mediate HIV-1 attachment to PBMC through an interaction with celluar CD147 (Pushkarsky, 2001). Taken all of these into consideration, Abs to any of those factors

described above would be effective to block the attachment of virus to cell, and thereby avoiding viral infection.

Additionally, further mechanisms of viral neutralization, of HIV in particular, merit further exploration. More recent reports demonstrate that deliberate removal of T cell help improves virus-neutralizing antibody production (Recher, 2004). Another report from an animal study demonstrated that passive immunotherapy in SIV-infected macaques may accelerate the development of neutralizing antibodies (Haigwood, 2004). The underlying mechanism for the production of neutralizing antibody as well as the factor influencing neutralizing antibody remain to be further investigated.

IMMUNE EVASION DURING HIV INFECTION

HIV has evolved numerous strategies to escape from the host's vigorous immune responses, by which the immune system ultimately fails to contain HIV replication. The high rate of antigenic escape may be the most common reason documented for HIV immune evasion. When effective selection pressure is applied, the error-prone reverse transcriptase (RT) and high replication rate of HIV-1 allow for rapid replacement of circulating virus by those carrying resistance mutations.

Other factors, nAbs and CTL responses for instance, also contribute to immune escape. Selection pressure from neutralizing antibody has been reported both *in vitro* and *in vivo*. Studies *in vitro* demonstrated that neutralizing antibodies could change the amino acid sequence of the antigenic sites in the envelope complex, leading to the change of the antibody binding and neutralizing sensitivity (Park, 1998; Reitz, 1988; Watkins, 1996). *In vivo*, Albert and coworkers have shown that autologous virus could escape from neutralizing antibody even though it remains sensitive to neutralization by control sera (Albert, 1990).

Animal experiments demonstrate that the sequential plasma samples recovered from infected animals could neutralize virus from the starting inoculum but were ineffective against variants that emerged during infection (Burns, 1993). A typical example for HIV-1IIIB, a TCLA strain, is that after several propagations in a nAb-free environment, primary isolates can be changed from neutralization resistant to high sensitivity (Beaumont, 2004). Apart from the change of amino acid sequence in mutated HIV strains, recent studies indicate that HIV resistance to neutralizing antibody is developed quite faster than that caused by antiviral drug therapy (Richman, 2003). The mechanism of HIV escape from antibody neutralization remains unknown. An Env glycan study demonstrated

that the virus faces a great selective pressure undertaken by nAbs and the glycan density on viral envelope remains stable without change in order to protect the virus from nAb attack (Wei, 2003).

Viral escape from CTL responses is another mechanism of immune escape that has been documented. Epitope change from MHC class I prevents $CD8^+$ T cell recognition, whereby the virus escapes from CTL responses. This mechanism was very well demonstrated in an animal experiment, in which animals were challenged with SIV and early infecting virus was well controlled by CTL directed against a Tat epitope. New viruses with mutations in this Tat epitope emerged, and the variant viruses continued to establish chronic uncontrolled infection (Allen, 2000). More recently, two studies reported that HIV escape from CTL responses could revert back into wild type after transmission to a CTL-free environment (Friedrich, 2004; Leslie, 2004).

HIV also faces selective pressure from the HLA alleles that cause specific mutations. This theory has been established based on the population level showing evidence that CTL can drive HIV evolution (Moore, 2002; Yusim, 2002). But exceptions also exist (Goulder, 2004). The different sensitivities to CTL responses from the same HLA alleles remain unclear. Nef, one of the HIV accessory gene product, has been reported to interfere with the CTL response by downregulation expression of MHC class I on the cell surface, and infected cells expressing high levels of Nef become resistant to destruction by MHC-restricted CTLs (Collins, 1998; Schwartz, 1996).

In general, the information concerning immune escape of HIV-2 is limited compared to HIV-1. HIV-1 has evolved many strategies-hide, shield and strike back and infect immune cells, to avoid infected cell immune eradication. A better understanding of these strategies might lead to new approaches in the fight against AIDS.

THE REQUIREMENTS AND CHALLENGES FOR HIV VACCINES

Vaccines have been enormously effective in preventing human diseases caused by viruses. The most successful example known is the eradication since 1978 of smallpox by mass vaccination. However, despite their efficacy, we do not have a clear understanding of how vaccines work (Berzofsky, 2001; Burton, 2002). Until recently, experimental approaches to vaccine development have been mostly empirical. Most vaccine candidates have entered clinical trails in the context of limited knowledge about their ability to stimulate immune responses and a poor understanding of the types of immune responses that they might elicit to confer protection (Pantaleo, 2004). For HIV, even after 20 years of research, a promising HIV vaccine candidate is still sought.

THE REQUIREMENTS FOR AN ALL-EFFECTIVE HIV VACCINE

Through HIV and other vaccine studies, we have now established a greater knowledge of immunology which allows us to better understand the correlations between immune responses and their role in vaccine-induced protection. Vaccine-induced immune responses can vary substantially depending on the nature of the immunogen (Pantaleo, 2004). Table 2 shows the correlates of immune protection in different vaccines.

Table 2. Correlates of immune protection in different vaccines

Type of vaccine	Vaccine-induced protective immunity	Mechanism of immune control during virus infection
Live-attenuated	Nab, some CTL	Nab, some CD4 or/and CTL
Killed virus	Nab	Nab, some CD4 T
Proteins (purified and synthetic)	Nab	Nab, some CD4 T
DNA and virus vector	CTL, less Nab	CD4 or/and CTL

Nab; neutralizing antibody. CTL; Cytotoxic T-cell response. CD4 T; CD4 T cell.

From table 2 we can see clearly that all of the vaccines able to prevent chronicity during natural infection are associated with the generation of virus-specific neutralizing antibodies. Indeed, there is a widespread acceptance that the induction of virus-neutralizing antibodies (Nabs) will be crucial to any truly effective vaccine against HIV-1 (Burton, 2004; Letvin, 2003; McMichael, 2003; Moore, 2004; Pantaleo, 2004). However, traditional strategies or purely empirical approaches, as most recently demonstrated in two VaxGen efficacy trials of monomeric gp120 (Burton, 2004; Cohen, 2003; McMichael, 2003) to create an antiviral vaccine have thus far failed to provide protection against HIV infection. Recent studies have

demonstrated that HIV continually accrues mutations that enable it to escape recognition by neutralizing antibodies in chronically infected individuals (Richman, 2003; Wei, 2003). Additionally, the emergence of an HIV-neutralizing antibody response following an HIV infection can only be detected several weeks after the partial containment of virus replication. The absence of a temporal correlation between early viral control and the development of a neutralizing antibody response has called into question the importance of antibody in early HIV control. Nevertheless, making the reasonable assumption that neutralizing antibody will be needed to block the establishment of infection by cell-free virus, investigators have attempted to create subunit immunogens that elicit neutralizing antibody responses. However, they have been unsuccessful in creating vaccines that elicit antibodies that bind to the neutralization-sensitive domains of the virus. The creation of an immunogen that can induce an antibody response capable of neutralizing a variety of HIV isolates remains a major challenge in the development of an HIV vaccine (Burton, 2004; Letvin, 2004).

Diverse evidence supports the importance of the cellular immune response in HIV containment. $CD8^+$ T lymphocytes can inhibit the replication of HIV in $CD4^+$ T lymphocytes *in vitro*, probably through direct cytotoxicity and the production of soluble factors including beta chemokines and cellular antiviral factors (CAF) (Cocchi, 1995; Walker, 1986). The clinical status of infected individuals is associated with the level of virus-specific $CD8^+$ cytotoxic T lymphocytes (CTL) in their peripheral blood; high levels are predictive of good clinical status (Ogg, 1998). The early containment of HIV replication in acutely infected individuals coincides with the emergence of an HIV-specific CTL response (Koup, 1994). The most direct evidence for the importance of $CD8^+$ lymphocytes in controlling HIV replication comes from studies in monkeys (Schmitz, 1999). Monkeys depleted of $CD8^+$ lymphocytes by administration of a monoclonal anti-CD8 antibody and then infected with SIV never controlled viral replication and died with an accelerated disease course. These accumulating studies make a compelling argument for the importance of $CD8^+$ CTL in controlling HIV replication and suggest that an effective HIV vaccine should elicit high-frequency CTL responses.

However, as HIV is spread by both sexual contact and through contaminated blood products, it is reasonable to assume that an effective HIV vaccine must elicit mucosal immune responses to contain venereal spread and systemic immune responses to control hematogenous spread (Belyakov, 2004; Berzofsky, 2004). Additionally, an HIV vaccine should elicit strong innate immune responses that can give the possibility for adaptive immune responses since the innate immune system is the first line of defense against

invading pathogens (Lehner, 2003; Levy, 2001). Other factors such as CAF (Levy, 2003) may also be important for warding off an HIV infection. Therefore, everything described above should be considered for development of an urgent HIV vaccine (Berzofsky, 2004; Burton, 1998; Peters, 2001).

CHALLENGES FOR AN EFFECTIVE HIV VACCINE

HIV infection has presented several unique challenges for vaccine development (Burton, 2004; Johnson, 2002; Robinson, 2002). The natural immune response is not effective (Desrosiers, 2004). Therefore, the first task is to find a vaccine that can stimulate neutralizing antibodies in high titers in most or all individuals who are immunized. This may require conceptual breakthroughs in protein engineering and an understanding of how predetermined B cells can be preferentially stimulated and selected (McMichael, 2003). This challenge seems to be extremely difficult (Burton, 2004; Zolla-Pazner, 2004).

The second challenge is to find a way to optimize T cell-inducing vaccines. We do not know whether any of the vaccines being currently developed will elicit cellular immune responses that will correlate with protection from infection or disease progression. Also, we should know the breadth of the vaccine-induced CD4 and CD8 T cell response (Pantaleo, 2004). The vaccine will have to stimulate a long-term memory T cell response that is broad enough to cope with variability within clades (Wu, 2002), and this enormous sequence heterogeneity of HIV suggests poor ability of CTL-based vaccines to provide protection (Desrosiers, 2004).

HIV-1 has eluded control by vaccines through its ability to form latent proviral DNA. As an essential step in its life cycle, HIV-1 integrates into the genome of its host to form a provirus. Once integrated, the virus can hide from the immune system through its lack of protein expression (Finzi, 1998). Estimates of the half-life of latent proviral DNA range from 6 to 43 months, indicating that it could take up to 60 years to eradicate a reservoir of as few as $1\ 5 10^5$ latently infected cells (Finzi, 1999).

Finally, HIV-1 is highly variable: HIV-1 arose from a single transmission event from chimpanzees to humans (Gao, 1999), but has evolved in humans to form at least 12 genetic subtypes, which, in turn, have further diversified. Even within a subtype, antibodies that are specific for the variable loops of isolates from one patient typically do not recognize the variable loops of isolates from other patients. As the epidemic has spread, recombinants of different subtypes have gained prominence. The high error rate of reverse transcription generates at least one mutation per provirus (Preston, 1997), which, combined with the rapid turnover of plasma virions (Ho, 1995), provides a broad base of variants for selection and

escape from both cellular and humoural immune responses (Allen, 2000; Evans, 1999; Kaul, 2001; Parren, 1999).

A major step forward might be the combination of a T cell vaccine and a good antibody-stimulating vaccine. Another challenge is to increase Phase 3 trials in developing countries as well as a very large-scale international collaboration. There are signs that this will be possible.

IMMUNOGLOBULIN A (Ig A)

THE BIOLOGY

IgA is the most heterogeneous of immunoglobulins on account of its multiple molecular forms as well as subclasses (Russell, 1992) (Fig 7). In humans there are three molecular forms:

1) Monomeric (mIgA), with a molecular mass 160 KDa, makes up 80-99% of serum IgA.

2) Dimeric (dIgA) or polymeric molecules (pIgA, composed of two or more mIgA) connected with a 15 KDa polypeptide designated joining chain (J) expressed by B cells or precursors (McCune, 1981).

3) Secretory IgA (SIgA) as a secreted form when transferring across epithelium to lumen, which is cleaved from polymeric immunoglobulin receptor (pIgR) expressed on mucosal epithelial cells (Apodaca, 1991). This is the predominant immunoglobulin presented by dimers and tetramers in external secretions. pIgR is connected on SIgA by secretory component (SC), with a molecular mass of 70KDa.

There are two subtypes of IgA, IgA1 and IgA2. The majority (85%) of serum IgA is of IgA1 form. A major difference between the two human IgA subtypes is evident in the hinge region. IgA2 lacks a 13-amino acid segment found in the hinge region of IgA1, that contains five carbohydrate moieties O-linked to serine. The presence of this extend hinge region has been postulated to confer greater segmental flexibility on IgA1, but also renders IgA1 susceptible to IgA1-specific proteases produced by bacterial pathogens at mucosal surfaces (Batten, 2003; Kilian, 1981). The absence of the hinge region in IgA2 makes them resistant to IgA1-specific proteases, which presumably is advantageous for IgA2 antibody function at mucosal surfaces (Kilian, 1981). Thus, because IgA2 does not possess the extended hinge region characteristic of IgA1 it generally does not bind the lectin Jacalin (Skea, 1988).

The IgA system can be functionally divided into two compartments, the secretory or mucosal compartment and the plasma or systemic compartment. These two compartments display a certain degree of independence, but cross-talk over the barrier does occur.

B cells can be defined as B1 and B2 subtypes according to the bearing of CD5 surface on B cells. Both B1 and B2 cells contribute to IgA production mucosally or/and systemically. B1 cells bearing the CD5 surface marker constitute 10-25% of circulating and splenic B lymphocytes in adult humans, but they are the source of low-affinity polyactive antibody and contribute more to natural antibody (Hayakawa, 2000; Kasaian, 1993). B2 cells are thought to give rise to a specific immune response after antigen stimulation. Studies in mice

have shown this kind of IgA is T cell-independent (Macpherson, 2000). Investigation of human intestine biopsies demonstrated that rearranged IgA V regions in B2 cells seems to have a high mutational frequency (Dunn-Walters, 1997), indicating that the response of IgA is specific to certain antigens. The subtype response reflected in the functional properties of IgA antibody is not fully understood. There are differences of antibody specificities between IgA1 and IgA2, such that immunization of adults with protein antigens tends to elicit IgA1 and immunization with polysaccharides IgA2 antibodies (Russell, 1992). In addition, SIgA is particularly stable and resistant to proteolytic cleavage structurally. This property makes SIgA especially well suited to function in the enzymatically hostile environment that prevails on mucosal surfaces.

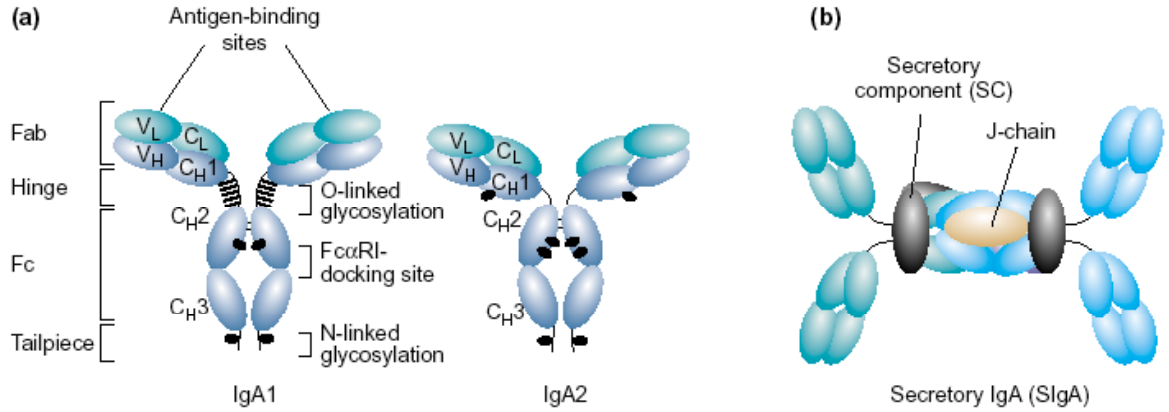


Fig. 7. Structure of IgA. (a) IgA1 and IgA2. (b) Dimeric form of secretory IgA (SIgA). Adapted from (van Egmond, 2001).

QUANTIFICATION OF SERUM IgA AND IgG

SIgA is the predominant immunoglobulin at mucosal surfaces of humans and most other mammals. In addition, IgA is the second most abundant immunoglobulin class in the human circulation. Table 3 compares the serum IgA and IgG levels and their half-lives. The average amount of IgA in serum is 3.5 mg/ml and for IgG 13.5 mg/ml. The half-life for IgA is six days, whereas that of IgG is longer and varies by subtypes from 7-21 days.

Table 3. Comparison of serum IgA and IgG

	IgA		IgG			
	IgA1	IgA2	IgG1	IgG2	IgG3	IgG4
Serum (mg/ml)	3.0	0.5	9.0	3.0	1.0	0.5
Half-life (day)	6	6	21	20	7	21

FUNCTION OF IgA

Numerous studies in animal models and in humans have provided convincing evidence that protection against a variety of bacterial (Fernandez, 2003), parasitic (Robinson, 2001) and viral mucosal pathogens can be achieved by IgA. Administration of influenza virus (Renegar, 2001), RSV (Weltzin, 1996), rotavirus (Ruggeri, 1998) and poliovirus (Buttinelli, 2001) by various routes, such as oral, intravenous, intranasal and vaginal immunization reported to induce IgA production.

In HIV infection, the first two Phase III clinical trials have thus far failed. These trials aimed at inducing neutralizing antibody (Cohen, 2003). A new notion concerning the new generation of an AIDS vaccine is now discussed. HIV is obviously primarily transmitted via mucosal tissues and so in this regard, a mucosal AIDS vaccine may have to be considered (Belyakov, 2004). Of particular importance are studies suggesting that IgA antibodies present in the genital tract secretions of HIV-1-exposed but persistently seronegative individuals may be responsible, at least in part, for their resistance to HIV infection. These studies include female sex workers in African countries (Belec, 2001; Devito, 2000; Kaul, 1999) and Thailand (Beyrer, 1999). This has also been shown for HIV discordant heterosexual couples (Mazzoli, 1997). The common characteristics for these cohorts are that they have neutralizing IgA in mucosal secretions, systemic serum or plasma. Moreover, some studies have demonstrated that purified neutralizing IgA from these exposed uninfected individuals could block HIV-1 transport across epithelial cells (Devito, 2000) or have cross-clade neutralizing capacity (Devito, 2002).

IgA, the only non-inflammational and transcytosing immunoglobulin, might thus play a role in prevention of HIV invasion. A recent study also reported that anti-CCR5 IgA antibodies are displayed in vaginal secretions and saliva in HIV-1 highly exposed uninfected individuals (Barassi, 2004). In conclusion, SIgA may be a useful component induced on mucosal surfaces for protection against HIV infection.

Although the general function of SIgA in protecting mucosal surfaces has been understood for some time, those of systemic, circulating IgA remain poorly understood. In rodents, the mechanism for clearing circulating IgA immune complexes is to transport them via the liver into the bile (Brown, 1982; Russell, 1981). This is because rodent hepatocytes express pIgR on their sinusoidal surfaces. The function of which is to transcytose oligomeric IgA across epithelial cells. However, this mechanism is not available in humans, whose hepatocytes do not express pIgR (Robinson, 2001). Nevertheless, a recent study with human Kuppfer cells showed that serum IgA can initiate phagocytosis of bacteria *in vivo* (van Egmond, 2000).

This suggests a second defense line in the mucosal system, if we assume SIgA in mucosal surfaces as the first defense line.

In human HIV-1 infections, serum IgA neutralization of primary and adapted isolates has been shown *in vitro* (Burnett, 1994; Burrer, 2001). However, the *in vivo* mechanisms of how serum functions to clear HIV remain unresolved.

Apart from immune exclusion of IgA in the mucosal system, the most striking mechanism for IgA is to mediate intracellular pathogen neutralization, which is an unique function for IgA (Bomsel, 1998).

THE INDUCTION OF IgA

IgA switching and secretion in *in vitro* cell culture indicate that TGF-β, IL-4 and IL-5 promoted the switch from IgM to IgA (Coffman, 1989). This finding suggests that TGF-β acts as an isotype-specific switch factor for IgA. In addition, IL-10 also specially promotes IgA isotye switching (Defrance, 1992).

Apart from cytokines, cells may trigger IgA switching. Cells with origins in the mucosa are particularly effective in this regard. Peyer's Patch B (Weinstein, 1991), T and DCs (Schrader, 1993) are more efficient in producing IgA than when these cells are derived from non-mucosal sites. *In vivo* studies have shown that cholera toxin (CT) is a specific adjuvant for induction of IgA (Lycke, 1986). Oral immunization of CT induces CD4 T cell-independent IgA production (Hornquist, 1995).

An important factor has recently been found in that the induction of a mucosal immune response is closely related to the pathway of immunization. Parenteral delivery of a nonreplicating antigen induces mostly systemic immunity and very rarely induces mucosal immune responses. In contrast, mucosal antigen delivery can trigger mucosal immunity at local and distant sites as well as systemic immune responses and is thus an advantageous immunization protocol. This possibility has been proved to be true in more recent mucosal HIV vaccine studies (Kang, 2004; Yao, 2004; Zhang, 2004). The success of mucosal vaccination against polio with live attenuated virus has been one of the great achievements of modern healthcare.

DISEASE RELATED TO IgA

The most common inherited form of immunoglobulin deficiency is selective IgA deficiency, which is seen in about 1 person in every 800. In such patients with IgA deficiency a substitution of IgM or IgG for IgA is usually seen (Fernandes, 1995). Although no obvious

disease susceptibility is associated with selective IgA defects, they are more common in people with chronic lung disease than in the general population. Lack of IgA might thus result in a predisposition to lung infections with various pathogens and is consistent with the role of IgA in defense at the body's surfaces. The genetic basis of this defect is unknown but some data suggest that a functionally unclear MHC class III is involved (Janeway, 2001).

The second common disease related to IgA is IgA nephropathy (IgAN). It is defined as a form of glomerulonephritis in which the IgA isotype predominates or codominates within the glomerular deposits. Usually, an abnormal regulation of immune responses leads to increased IgA production which combined with an increased entry of antigen into systemic circulation gives an impaired clearance of aggregates containing IgA or altered IgA structure. All-in-all, this has a close relationship with an altered mucosal immune system (Ogra, 1999).

MATERIALS AND METHODS

STUDY POPULATIONS (PAPERS I-IV)

The study population in the thesis is listed and described briefly in the table below, and more detailed information is available in the respective papers listed in the appendix.

Table 4. Study populations selected in this thesis

Subjects Positive+controls		Countries (Samples No.)			Notes
	Total	Guinea-Bissau	Portugal	Sweden	
Paper I	29+19	20	9		Positive (P)
		12		7	Negative controls (N)
Paper II	25+15+14	25			HIV-2 Exposed IgG^-
		15			P, HIV-2^+ partners
		14			N
Paper III	8+14	28[a]			P
		14			N
Paper IV	20+10		14 (9)	6 (3)	P (for neutralizing assay)
				10	N

(a) *Longitudinal study, 28 blood samples derived from 8 HIV-2 infected individuals, three to five blood samples were drawn from each subject between 1992-2000.*

PEPTIDE SYNTHESIS (PAPERS I, III AND IV)

In Paper I and Paper III, peptides representing previously known IgG-specific antigenic sites in the envelope proteins of HIV-2 were synthesized as earlier described (Bjorling, 1991; Houghten, 1985). The amino acid sequences were derived from the published sequence of HIV-2_{ISY} or/and HIV-2_{ROD}. All peptides were analyzed using high-performance liquid chromatography (HPLC) to confirm their grade of purity, exceeding 65% before use (Table 5), more detailed information refered to Appendix I, III (papers I and III).

Table 5. Synthetic peptides corresponding to selected linear sites of the envelope glycoproteins of HIV-2 (Papers I and III)

Peptide	Residues	Region
S1-1[a]	110-138/ROD	V1
S1-2	160-186/ROD	V2
S1-19	189-205/ROD	V2/C2
S11-11	283-297/ROD	V3-1[b]
A43-29	311-330/ISY	V3-2[b]
A43-36	318-337/ISY	V3-3[b]
S1-18	399-419/ROD	V4
S1-3[a]	446-470/ROD	V5
S12-37	582-603/ISY	gp36-1[c]
A43-9	615-634/ISY	gp36-2[c]
A43-10	634-648/ISY	gp36-3[c]
A43-12	644-658/ISY	gp36-4[c]

(a) *Peptides not used in paper I. (b) Three peptides were selected to represent the V3 region. (c) Four peptides were selected to represent the transmembrane protein gp36.*

In Paper IV, sixteen overlapping peptides representing the whole gp36 amino acid sequence of HIV-2 were synthesized. The amino acid sequences of the peptides were derived from HIV-2_{ISY} and HIV-2_{ROD}. The condition used for these peptides were the same as for Paper I and III. The detailed information is listed in Appendix IV (paper IV).

WHOLE HIV-2 ANTIGEN, NATIVE GP125 AND RECOMBINANT GLYCOPROTEINS USED IN ELISA (PAPERS I-IV)

Whole killed HIV-2 strain SBL-6669 virions purified from cultured U937: 2 cells were used as whole HIV-2 antigen and were kindly provided by professor G. Biberfeld at the Swedish Institute for Infectious Disease Control. *Galanthus nivalis* agglutinin (Gilljam, 1993) purified native gp125 derived from HIV-$2_{SBL-6669}$ was kindly provided by the Department of Virology at the Swedish Institute for Infectious Disease Control. Recombinant gp105 (rgp105), derived from HIV-2_{ROD}, was produced in a baculovirus expression system. Recombinant gp105 (rgp105) and gp36 (rgp36) were commercially obtained from Bartels (Wicklow, Ireland) and ViroGen Corp. (Watertown, MA, USA), respectively.

VIRAL STOCKS

HIV-$2_{SBL6669}$ (PAPERS I-IV) AND 1653, 1682, 2298 (PAPER IV)

HIV-2 $_{SBL6669}$, isolated from a Gambian woman, has been extensively used in our laboratory. In addition, three primary isolates were used in this study, one originated from the Ivory Coast (1653), the other two originated from Guinea-Bissau (1682 and 2298). These isolates have been previously characterized for biological phenotype and co-receptor usage, as shown in (Paper IV) (Mörner, 1999).

Viral stocks were prepared by infecting 5 $5 10^6$ freshly isolated phytohaemagglutinin (PHA) (Pharmacia, Sweden) stimulated PBMC from two healthy blood donors with 2 ml supernatant from infected PBMC. The PBMC cultures were maintained in RPMI 1640 medium with 3 m M glutamine (Gibco BRL, Paisly, UK) supplemented with 10% fetal calf serum (FCS) (Flow, Costa Mesa, USA), 2 U/ml recombinant IL-2 (Amersham, Buckinghamshire, UK), 2 μg/ml polybrene (Pb) (Sigma, St. Louis, USA) and antibiotics. The culture was maintained for three weeks with the addition of PHA-stimulated PBMC once a week. The 50% tissue culture infective dose ($TCID_{50}$) of the supernatants was determined as previously described (Björling, 1993).

PURIFICATION OF IgA (PAPERS I-IV) AND IgG ANTIBODIES (PAPERS I-III)

IgA1 was purified from serum as described in detail previously (paper I). Briefly, jacalin/agarose beads (Vector, Burlingame, CA, USA) were added to dilute serum (1:10). The mixture was incubated at room temperature for two hours. After binding and washing, the IgA was eluted overnight at 4°C with 200 mM methyl-α-D-galactopyranoside. The following day, the supernatant was harvested, and protein G-sepharose (Pharmacia, Uppsala, Sweden) was used to exclude any remaining IgG from the first purification step. After centrifugation, the supernatant containing IgA was harvested, giving a final volume equivalent to a 1:5 dilution of serum. Similarly, protein G-sepharose was used for IgG purification. IgG was eluted with elution buffer (0.1 M glycine-HCl, pH 2.2) for 10 min and the eluted IgG was immediately adjusted to pH 7 using neutralization buffer (2.5 M Tris-Base, pH 9.0), giving a final volume of 100 μl, equivalent to a 1:5 dilution of serum.

SDS ELECTROPHORESIS GELS (PAPERS I, II)

The purity of affinity purified IgA1 and IgG fractions was assessed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Serum, IgA1 or IgG fractions were mixed with sample

buffer and applied to 4-20 % gradient SDS-polyacrylamide gels. Gels were stained with Commassie-Blue, as detailed in Paper I.

ENZYME-LINKED IMMUNOSORBENT ASSAYS (ELISAS) (PAPERS I-IV)

Whole HIV-2 antigen, native gp125 and recombinant gp105 and gp36 ELISAs were performed in Papers I to IV. Briefly, microtiter plates were coated overnight with whole HIV-2 antigen, gp125, gp105 or rgp36, respectively. Blocking was performed with bovine serum albumin (BSA). Sera and immunoglobulin preparations were added and incubated. To detect IgA, biotinylated goat anti-human IgA and streptavidin-HRP were used. For visualization of HRP conjugates, o-phenylenediamine dihydrochloride (OPD) substrate was added. Optical density (OD) values were read at 490 nm. For IgG detection, alkaline phosphatase (ALP)-conjugated goat anti-human-IgG and p-nitrophenyl phosphate (pNPP) substrate were used. After 30 minutes reaction, OD values were read at 405 nm. Samples were regarded as being ELISA positive when the mean OD values exceeded the mean OD + 3 standard deviations (SD), obtained using a negative control serum.

Peptide ELISAs (Papers I, III, IV)

To determine the reactivity to synthetic peptides corresponding to selected linear sites in the envelope glycoproteins of HIV-2, an indirect ELISA was used. All volumes were 100 µl if not otherwise stated, and each step in the assay was followed by a washing procedure. The peptides were coated onto high–binding microtiter plates. Nonspecific binding was blocked by BSA. Sera or purified immunoglobulins were diluted, added and incubated. To detect IgA, biotinylated goat anti-human IgA and streptavidin-HRP were used. For visualization of HRP conjugates, o-phenylenediamine dihydrochloride (OPD) substrate was added. The optical density (OD) values were read at 490 nm. For IgG detection, alkaline phosphatase (ALP)-conjugated goat anti-human-IgG and p-nitrophenyl phosphate (pNPP) substrate was used. Optical values (OD) were read at 405 nm. Samples were regarded as being ELISA positive when the mean OD values exceeded the mean OD+3standard deviations (SD) obtained using a negative control serum. To verify that the level of IgG reactivity detected were independent of the conjugate system used, biotinylated goat anti-human IgG and streptavidin-HRP were used.

QUANTIFICATION OF SERUM IgA AND IgG (PAPERS I-III)

An ELISA was used for quantification of total IgA in Paper I. Human IgA (Jackson laboratories, WestGrove, PA, USA) was used to generate standards. The OD values were analyzed using DS3-1.46B software (DeltaSoft 3, Biometallic Inc, CA, USA) to calculate the concentrations of serum IgA and IgG.

T- LYMPHOCYTE SUBSET ANALYSIS (PAPER III)

Total CD4 T cells counts in peripheral blood lymphocytes were determined by flow cytometry using FACStrak according to manufacturers instructions (Becton-Dickinson Immunocytometry Systems, San José, CA, USA).

NEUTRALIZATION ASSAY (PAPERS I-IV)

The capacity of purified IgA and IgG and whole serum to neutralize HIV-2 isolates was tested in an assay using phytohemagglutinin stimulated peripheral blood mononuclear cells (PBMC). Briefly, antibodies or sera were incubated for 1 hr at 37°C with diluted tissue culture supernatant of virus-infected peripheral blood mononuclear cells (40-100 $TCID_{50}$, 100 µl). $1x10^5$ PBMC in 50 µl were added to the virus-antibody reaction mixture and incubated overnight. All dilutions were performed in RPMI 1640 medium supplemented with 10 % fetal calf serum, 3 mM glutamine, 20 IU IL-2 and antibiotics. A medium change was performed on days one and four. Seven days after infection supernatants were collected and analyzed for HIV-2 antigen by an antigen capture ELISA. The neutralization titer was defined as the reciprocal of the last dilution step that showed an 80% reduction or more of optical density at 490 nm in the culture supernatant as compared to the HIV antibody negative serum. Titers equal to or above 20 were considered to represent positive neutralizing assays when repeated on at least on two occasions. A previously defined HIV-2 neutralizing serum sample with a known neutralization titer was used as a positive control in the assays. The purified immunoglobulin samples were not concentrated after purification. The purified immunoglobulin samples used in the neutralization assay constituted a 1/5-dilution since 20 µl serum was used initially and the final volume after elution was 100 µl. The neutralization assay was performed in six steps of two-fold dilutions on at least two occasions. Results are reported as reciprocal dilution in the neutralization assay and not the final serum dilution. In Paper II neutralizing titer/mg IgA was compared.

INHIBITION OF HIV-2 NEUTRALIZING ACTIVITY BY PEPTIDES (PAPER IV)

To investigate the target regions of neutralizing IgA in the transmembrane region of the Env, gp36-peptide absorption experiments were performed. Purified IgA fractions diluted two-fold in five steps starting with 1:20 were pre-incubated with 2 µg/ml, 20 µg/ml or 100 µg/ml respectively of peptides for 2 hrs at 37° C. The neutralization assay was then performed as described above. Pre-absorption was also carried out using HIV-2 native gp125 and recombinant gp36 as controls.

STATISTICAL ANALYSIS (PAPERS II AND III)

Unpaired *t* test was used for the comparison of IgA concentration in serum samples.
Wilcoxon signed rank sum test was used for the comparison of the HIV-2 neutralizing capacity of EU and HIV-2 positive IgA purifications when given as titer /mg.
Analysis was performed using StatView (Abacus concepts, INC., Berkeley, CA 1996).
The analysis of correlation between serum IgA and IgG amounts in relation to CD 4 T cell counts were performed using linear regression analysis (Abacus concepts, INC., Berkeley, CA 1996).

RESULTS AND DISCUSSION

BROAD SPECTRUM OF SERUM IgA NEUTRALIZATION TO HIV-2 (PAPERS I-IV)

Serum IgA neutralizing capacity of HIV-2 $_{SBL\ 6669}$ (Papers I-IV)

HIV-$2_{SBL6669}$ was isolated in 1987 from a Gambian HIV-2 infected woman in Sweden (Albert, 1987). Since then, this strain has been extensively used for research *in vitro*. Our studies showed that IgA purified from HIV-2 infected individuals had the capacity to neutralize HIV-$2_{SBL6669}$ (Table 6). Our studies open yet another window for the comparative study of HIV-1 and HIV-2 infections. Thus it is particularly important to further explore the underlying mechanism why HIV-2 infection seems to have less pathogenicity, slower disease progression and lower transmission rates in mother-to-child infection. In our longitudinal study (Paper III), we showed a sustained neutralizing pattern for IgA purified from whole serum, particularly in one patient (P787), over a period of eight years of observation. The IgA neutralizing titres were stable and a sustained neutralization pattern was displayed. This is in agreement with what has previously been suggested, that broad neutralization is associated with a slower disease progression and lower risk of transmission in SIV infection (Fenyo, 1996). Another study also supports this view, in that autologous neutralizing antibodies prevail in HIV-2, but not in HIV-1 infection (Björling, 1993). These findings indicate that HIV-2-specific neutralizing antibodies contribute to the differences between HIV-1 and HIV-2 infection.

Table 6. Serum IgA fractions that neutralized HIV-$2_{SBL66669}$

Paper	HIV-2 positive	HIV-2 negative
I	17/29	0
II[a]	13/15	0, in EGSN group 25/25
III	12/12	0
IV	9/12	0

(a) *In Paper II, HIV-2 negative groups, HIV-2-exposed IgG seronegative (EGSN) individuals were included as a special negative group.*

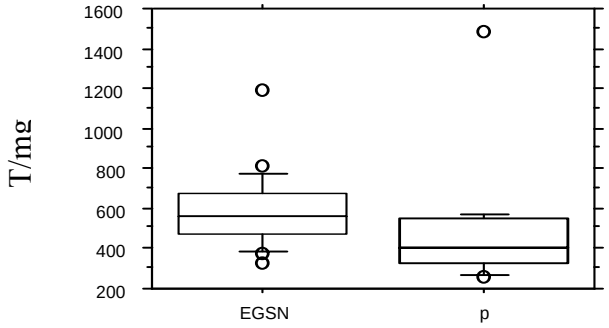
HIV-2-Specific serum IgA in HIV-2-exposed IgG seronegative individuals

Here, we have demonstrated that HIV-2-specific serum IgA from EGSN individuals has the ability to neutralize HIV-2. Importantly, when comparing the potential of IgA-mediated neutralization of an HIV-2 strain, SBL_{6669}, we surprisingly found that IgA from EGSN had a grater ability to neutralize than that of IgA from their HIV-2 known positive partners (Fig.

8). This may have important implications. Given that IgA had neutralizing ability, it may confer protection in a population at high risk of HIV-2 exposure. From this study, we know that exposure to HIV does not inevitably result in productive infection and it is now established that a spectrum of variability exists in susceptibility to HIV (Rowland-Jones, 1995; Shearer, 1996).

Although in the current study (Paper II) we did not investigate the epitope usage for the EGSN group in HIV-2 IgA neutralization, a recent study demonstrated a difference in IgA epitope use between HIV-1 exposed uninfected individuals and their HIV-1 positive partners (Clerici, 2002). In their study, the author showed that a region within HIV-1 gp41 was exclusively bound by serum IgA derived from HIV-1-exposed uninfected individuals. Recent studies have raised the possibility that transiently exposed conformations of proteins that are required for HIV-1 infection can elicit neutralizing antibody immune responses (Chan, 1998).

Fig. 8. Box plot illustrating the anti-HIV-2 IgA neutralizing reactivity expressed as titer / mg sIgA preparations.



The 10th, 25th, 50th, 75th and 90th percentiles of the reactivity titer were displayed after calculating the IgA amount (mg) of purifications from EGSN (n=23, two were not included due to lack of samples) and HIV-2 infected individuals (p) (n=15), respectively (p<0.05).

Broadly neutralizing serum IgA antibodies may exist in HIV-2 infected individuals

Moreover, our results showed that serum IgA from HIV-2 infected individuals could neutralize both subtype A and B HIV-2 strains. When three primary isolates and HIV-2 SBL_{6669} strain were tested, a variable but appreciable neutralization induced by serum IgA derived from HIV-2 infected individuals could be detected. The neutraliation data was as follows: HIV-$2_{SBL6669}$ 75% (9/12), primary isolate 1653 100% (12/12), primary isolate 1682 92% (11/12) and 2298 25% (3/12). This further proved that serum IgA plays a role in HIV-2

infection, which influence needs to be further studied both in *in vivo* and in animal models. Although not investigated in our study, HIV-1 serum IgA has been shown to mediate ADCC reactivity (Black, 1996). This means that IgA may not only play role in adaptive immune responses but it may play a role in innate immune responses. As such, a second defense line role has been proposed through the observation that serum IgA can mediate phagocytosis of bacteria though Kuppfer cells in human liver (van Egmond, 2000). A recent study has shown that serum IgA can clear circulation immune complexes by active transport of IgA from epithelium to mucosal surfaces (Robinson, 2001). Additionally, intracellular neutralization of HIV-1 by IgA has been displayed (Bomsel, 1998). All these studies indicate that IgA (serum and mucosal secretory IgA) can form barriers to protect the human being from pathogen invasions.

It is easy to predict that a new generation of vaccine for mucosal immune protection will be born in the near future.

Due to the fact that HIV is a virus of high antigenic variability, we should bear in mind that neutralizing epitopes could be difficult to be identified for the reasons: The neutralization of HIV by antibody to gp120 is determined primarily by occupance of sites on the virion irrespective of epitope specificity. Evidence has indicated that the main mechanism of HIV neutralization is operated through coating the virus by antibody (Burton, 2001; Parren, 1998). Both non-neutralizing and neutralizing antibodies can also recognize the non-neutralizing epitopes on the HIV envelope, but only the neutralizing antibody can recognize the neutralizing epitope on envelope of HIV. Non-neutralizing antibodies to the CD4-binding site on the gp120 subunit of HIV-1 do not interfere with the activity of a neutralizing antibody against the same site (Herrera, 2003; Poignard, 2003). The nature of such non-functional envelope molecules is unclear at the moment, but probably includes various envelope forms that do not support virus-cell fusion. We could not differentiate the non-neutralizing IgA and neutralizing IgA in our IgA fractions. This would also give an unpredictable impact to the results in the peptides inhibition study. Moreover, the peptides here used for blocking IgA neutralization may be too big or IgA binding affinity is too small to compete with the targeted virions used in this study. In general, the underlying mechanism of IgA neutralizing to HIV is unclear as yet, and as known, the process of virus neutralization is much more complex. Further explorations deserve to be done in the future.

BROAD BINDING REACTIVITY PATTERN SHOWN IN SERUM IgA

Serum IgA reactivity to whole viral lysate, native and recombinant envelope proteins

Table 7. Summarizes the serum IgA reactivity to whole HIV-2 antigen, native and recombinant envelope proteins in the different papers.

	HIV-2 whole antigen %	Native gp125 %	rgp105 %	rgp36 %
Paper I	100 (29/29)	96 (28/29)	90 (25/29)	100 (29/29)
Paper II				
EGSN	64 (16/25)	0	12 (3/25)	24 (6/25)
HIV-2^{+}	100 (15/15)	27 (4/15)	33 (5/15)	93 (14/15)
Paper III[a]	100 (8/8)	88 (7/8)	100 (8/8)	50 (4/8)
Paper IV	100 (6/20)	30 (6/20)	35 (7/20)	100 (20/20)

(a) *In paper III, serum IgA reactivity is given as the number of HIV-2 infected individuals with reactivity at any time point divided by the total number of individuals in the study.*

From table 7, it is apparent that serum IgA from HIV-2 infected individuals in different sets of studies recognize HIV-2 whole viral antigen. But for native and recombinant envelope proteins, the reactivity detected differs very largely. This was probably due to the sera arising from different geographic regions and that the patients were in different disease stages. Another possibility that may explain the difference is the envelope conformational change and that antibodies recognize different epitopes or the different outcomes of interactions between antibodies (Cavacini, 2002). As HIV infection can result in an aberrant immune system, B cell function in antibody production may be also affected by HIV infection. Therefore, different phenotypes of antibodies, such as immature and mature antibodies might co-exist within the same individual.

Serum IgA had broad binding ability to peptides representing different regions of variable loops in HIV-2 envelope proteins (Table 8).

Table 8. Show the binding ability by serum IgA to different peptides representing different regions on envelope protein of HIV-2

Peptide	Region	IgA reactivity	
		Paper I	Paper III (longitudinal study)[b]
S1-1	V1	ND[a]	33% (3/8)
S1-2	V2	14%	25% (2/8)
S1-19	V2/C2	0	33% (3/8)
S11-11	V3-1	28%	50% (4/8)
A43-29	V3-2	10%	0
A43-36	V3-3	24%	50% (4/8)
S1-18	V4	7%	33% (3/8)
S1-3	V5	ND	13% (1/8)
S12-37	gp36-1	38%	100% (8/8)
A43-9	gp36-2	45%	25% (2/8)
A43-10	gp36-3	45%	13% (1/8)
A43-12	gp36-4	72%	100% (8/8)

(a) *ND, not done in paper I. (b) In paper III, serum IgA reactivity is given as the number of HIV-2 infected individuals with reactivity at any time point divided by the total number of individuals in the study.*

Two studies were performed to investigate HIV-2-specific serum IgA reactivity to peptides representing different regions of HIV-2 envelope proteins. Although some results differed in these two studies, in general serum IgA had a broad spectrum of reactivity to the different peptides tested. This spectrum was similar to that for serum IgG reactivity (data not shown here, more detailed results see appendix Paper I and Paper III). However, these two studies show that serum IgA had a prominent activity against a peptide aa 644-658 (A43-12) representing the central part of gp36.

These studies may be important in understanding the plasticity of the humoral HIV-specific immune response, antibody maturation process and change in epitope specificity, both cross-sectionally and longitudinally. The breadth of antigenic sites recognized by serum IgA may reflect the flexibility and polyclonality of responses to structural changes due to the genetic drift within the quasi-species of HIV-2. Experiments from SIV infections demonstrated that antibody development from immaturation to maturation is the process of polyclonality to more specificity with the increased specific epitope affinity (Cole, 1997; Haigwood, 1998).

SERUM IgA MAY BE CD4-INDEPENDENT IN HIV-2 INFECTION

Quantification of serum IgA and IgG levels (Papers I-III)

In papers I-III, we quantified serum IgA and serum IgG levels in all samples. The detailed data are displayed in table 9. Overall, the Jacalin purification used gave a good yield. 60%-85% IgA were purified from the whole serum samples (Papers I and II), the average amount of purified IgA being 0.58 mg/ml (Paper II). This is a one-fifth dilution since 20µl serum was used initially and the final volume after elution was 100 µl. The subtypes of IgA fractions were not done. However, 85% of human serum is reported to be IgA1 (Kilian, 1981).

Table 9. Quantification of serum IgA and IgG in samples used in papers I-III

Paper	Serum IgA (mg/ml)		Serum IgG (mg/ml)	
	range	average	range	average
I	0.3-7.1	ND[c]	0.2-7.5	ND
II				
EGSN[a]	2.05-8.46	4.94	ND	ND
HIV-2$^+$	3.61-7.09	4.77	ND	ND
HC[b]	2.33-6.23	3.89	ND	ND
III	0.45-11.21	4.33	4.8-26	10.54

(a) *EGSN; abbreviation of HIV-2-exposed IgG seronegative group. (b) HC; Abbreviation of healthy control group.*

In paper III, we also analysed the correlation between peripheral CD4 T cell numbers and the level of serum IgA in the longitudinal observation, an inverse tendency for serum IgA level and CD4 T cell numbers being suggested. These findings indicated that IgA might be CD4 T cell-independent or produced by long-lived memory B cells, Fig. 9.

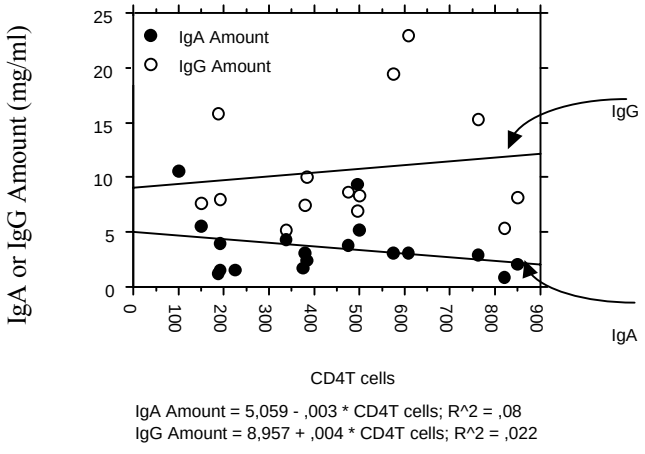


Fig. 9. *Correlation of serum IgA or IgG amounts with CD4 T cell numbers (Paper III)*

In paper II, we found the serum IgA levels in EGSN to be higher than in the healthy group ($p<0.05$). This indicating that a high amount of serum IgA might be related to a strong immune response present in the HIV-2-exposed IgG seronegative individuals, or is present as a compensation for the lack of effective IgG reactivity against HIV-2 repeated exposure. This was also suggested in HIV-1 infection in those patients in the symptomatic phase still in relatively good clinical condition who had high levels of serum IgA antibodies (Matsuda, 1993).

The process of IgA isotype switching and maturation is complex. Many factors can influence such a process, the property of the antigen and the interactions among cells, such as CD4 T cells, dendritic cells (DCs), or macrophages. It is widely accepted that CD4 T and B cell interaction is critical for B cell differentiation and functional responses to foreign antigen. However, the results from our longitudinal study (Paper III) demonstrated a trend towards an inverse relationship between serum IgA and CD4 T cell counts. This implied that after a period of time, in HIV-2 infected individuals, the secretion of serum IgA might be a CD4 T cell independent event. The quality of serum IgA secreted by this manner may be poor, such as lower IgA neutralizing activity. If so, we can partially answer the question why IgA in HIV-2-exposed IgG seronegative individuals had higher neutralizing ability than IgA from their HIV-2 positive partners. Here we hypothesize that, although we are not able to get the CD4 T cell data from the study (Paper II), the serum IgA activity in HIV-2-exposed IgG seronegative individuals was CD4-dependent, with IgA of higher quality, i.e. giving IgA with higher neutralizing activity. In contrast their HIV-2 positive partners, serum IgA reactivity was CD4-independent and as a consequence the IgA was of poor quality. This hypothesis is supported by study of an influenza virus infection mouse model showing that early IgA response to influenza virus infection occurs in the absence of key cognate T cell and B cell interaction (Sangster, 2003). An evolutionary primitive system has been suggested in which immature B cells switch to IgA production at peripheral sites without μ and δ chain expression (Macpherson, 2000). This indicates that B cells have been continuously stimulated and over-activited and overexpression as well, thus producing more immature B cells which secrete a large amount of IgA with lower quality during HIV-2 infection. This could also explain the difference in neutralizing ability between the two groups in paper II (Lizeng, 2004). Alternatively, IgA might also be produced from long-lived memory B cells.

GENERAL CONCLUSION

1. HIV-2 serum IgA is potent, sustained and effective in neutralization.
    - Serum IgA has the capacity to neutralize HIV-2 in a cross-sectional study.
    - In HIV-2-exposed IgG seronegative individuals, serum IgA showed potent HIV-2 neutralizing ability. For the first time the potential for IgA neutralization between the HIV-2 exposed IgG seronegative individuals and their known HIV-2 positive partners was compared. We found that serum IgA has a more potent neutralizing capacity to neutralize a HIV-2 strain SBL_{6669} compared to their HIV-2 positive partners.
    - In a longitudinal study, we have found that serum IgA was stable and sustained titres were maintained in neutralizing HIV-2 during the 3-8 years of observation.
    - Serum IgA from HIV-2 infected individuals could also, have the capacity to neutralize multiple clades, i.e. showed cross-clade neutralizing capacity.

2. HIV-2-specific serum IgA has broad-spectrum reactivity to HIV-2 antigens.
    - HIV-2 specific IgA activities against whole HIV-2 antigen and envelope proteins, both native and recombinant antigens, were shown.
    - Peptide reactivity showed that HIV-2 serum IgA reacted against most of the peptides representing different regions of envelope proteins, both surface protein and transmembrane protein.
    - Serum IgA has high reactivity against the peptide (aa 644-648, A43-12) in the central part of gp36. This was confirmed by samples from three cohorts of HIV-2 infected individuals.

3. Fine mapping of epitopes for serum IgA reactivity. Two epitopes in gp36 may be important in mediating serum IgA binding activity. These are GCAFR (aa. 588-592) and MYELQ (aa. 644-648).

4. Serum IgA levels varied in different individuals but were higher in HIV-2-exposed IgG seronegative individuals as compared to their HIV-2 positive partners.

HARNESSING IgA AS ACTIVE AND PASSIVE IMMUNOMODULATION FOR HIV PREVENTION-A PERSPECTIVE

1. Blocking HIV transmission by IgA. Mucosal surfaces are the first sites for HIV encounter. SIgA in mucosal surfaces and IgA in systemic are both potential immune modulators as they act as the first and the second defense lines. Although the first step of mucosal HIV transmission is not well understood, it is becoming increasingly clear that epithelium, which makes up the target mucosa, is an important determinant in the mechanism of infection. In mucosa, DCs appear to be the first target and vector for HIV viral dissemination into the submucosa. In this regard, IgA should be the first antibody to block HIV-DC contact (Frankel, 1998).

HIV infection will largely impair CD4T cells and gp120 engages CD4 in the first steps of T cell infection. Blocking this interaction is another consideration for how to use IgA (Vincent, 2004).

2. Antigenic sites for IgA targeting merit further studies. Searching for more epitopes on the HIV envelope may be another way for designing an IgA neutralizing antibody vaccine (Clerici, 2002). Additionally, exploration of an effective adjuvant to enhance immune responses may be easier than that for making a new immunogen to induce HIV-specific neutralizing IgA *in vivo*.

3. A variety of immunization pathways elicit effective IgA responses both mucosally and systemically. Alternative ways of administraiton of HIV-vaccines may be needed to induce HIV-specific IgA with efficacy against HIV infection.

The development of a method to make specific monoclonal IgA used in passive immunization may also be helpful in delaying or preventing HIV infection. This research field is in a very early stage, and involves protein engineering and antibody engineering. Recently, recombinant immunoglobulin A (rIgA) studies have been suggested for fundamental and applied research, see a review by (Corthesy, 2002).

4. HIV-specific IgA microbicide in topical administration. The most effective way may be to make an HIV-specific IgA microbicide on topical sites to physically block HIV transmission, such as blocking mucosal CCR5 on T cells (Barassi, 2004). This has been highlighted by two recent studies. The first study used mAb b12 in mucosa gel form, and was demonstrated to be effective in blocking mucosal transmission by vaginal challenge of macaques (Veazey, 2003). In the second study, PSC-RANTES, an amino terminus-modified analog of chemokine RANTES was used to block CCR5 effectively by vaginal

challenge in a monkey model study (Lederman, 2004). In general, to harness IgA, a strategy to prevent against HIV infection requires both active and passive immunomodulation to ultimately eliminate HIV/AIDS.

SUGGESTIONS FOR FUTURE STUDIES

Studies of immune competent cells, DCs, T and B cells in IgA induction

Increasing evidence indicates that the induction of neutralizing IgA is complex. Dendritic cells and other immune competent cells play an important role in the induction and regulation of IgA isotype switching. Extended investigations into the process of IgA induction are needed to further our understanding of how to induce potent neutralizing anti-HIV IgA.

Passive immunization using SIV/HIV-specific IgA

Studies have provided evidence that IgA plays an important role in avoiding infections from influenza virus, poliovirus and rotavirus. The studies described herein provide additional support to further assess the role of IgA in the prevention of infection. Although the consensus indicates that mucosal immune responses are needed in a successful HIV vaccine, to my knowledge there are no studies of HIV-vaccine candidates that specifically employ IgA, especially as a tool in passive immunization for prevention against HIV infection. Passive immunization studies in animal models using neutralizing IgA should prove the benefit of IgA in preventing HIV infection.

Active immunization to induce HIV/SIV-specific IgA mucosally and systemically

Studies have shown that IgA(s) derived from both mucosa and sera in HIV-infected individuals have the capacity to neutralize HIV. Vaccines should be designed to induce IgA production mucosally and systemically. To this end, vaccination routes and adjuvant usage that promotes virus neutralizing IgA induction should be employed.

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