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Mikko Seppänen

Complement Factor C4 and Immunoglobulins in Recurrent or Chronic Mucosal Infections

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ACADEMIC DISSERTATION
To be publicly discussed, with the permission of the Medical Faculty of the University of Helsinki,
in the Auditorium 3 of Meilahti Hospital, Haartmaninkatu 4,
on Friday, January 12th, 2007, at 12 noon

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© 2007 by Mikko Seppänen
Printed at the Helsinki University Printing House, Helsinki Finland
ISBN 978-952-92-1507-2 (paperback)
ISBN 978-952-10-3658-3 (PDF)
http://ethesis.helsinki.fi

Minds are like parachutes – they only function when open
Thomas Dewar

To Taina, the love of my life

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1. Abstract

Aims: We assessed the frequencies of *C4A* and *C4B* null alleles, and of low IgG subclass levels in the general population (II, III, IV) and in healthy blood donors without sinus problems (II). In a series of case-control studies, we tested whether genetic deficiencies of *C4*, (C4 nulls, in studies I, II, III, IV), low IgG subclass levels (II, III), IgG1 and IgG3 allotypes (*Gm*, III), or *HLA-A**, *-B**, and *DRB1** gene polymorphisms (I, III) are associated with recurrent herpetic gingivostomatitis (I), severe chronic or frequently recurrent rhinosinusitis (II), acute purulent rhinosinusitis (II), genital herpes patients with HSV-2 infection having at least 10 yearly recurrences for longer than 1 year after the first clinical episode (III), or with severe chronic periodontitis (IV). We further aimed to assess whether any immunologic parameters found in the study patients associate with any complications of the diseases studied, or with any associated comorbidities (I, II, III). At the same time, we compared the applicability of a new polymerase chain reaction (PCR)-based quantitative analysis of *C4A* and *C4B* genes with the traditional C4A and C4B protein allotyping (II, III, IV).

Main methods: We recruited, with strict clinical disease criteria, consecutive patients and control subjects from the Division of Infectious Diseases (I, II, III), Vihti Health Care Center (II), from the dental offices of three periodontists (IV), Vita Laboratories Ltd (II, III, IV), and Finnish Red Cross Transfusion Service (II). In the studies, after exclusion, 3 patients with herpetic gingivostomatitis (I), 48 patients with severe chronic or recurrent rhinosinusitis (II), 50 patients with acute purulent rhinosinusitis (II), 52 patients with frequently recurrent genital HSV-2 infection (III), 37 patients with severe adult periodontitis (IV), 100 blood donors (II), and 150 subjects coming for health survey before accepting a new occupational post (II, III, IV) were included. Immunoglobulins (II, III), type-specific anti-HSV-2 IgG (I, III) and IgG subclass antibodies against glycoprotein G (III), IgG1 and IgG3 allotyping (III), levels of C3, C4, and complement classical pathway hemolytic activity (I, II, III, IV), C4 immunophenotyping (I, II, III, IV), *C4* real-time PCR-genotyping (II, III, IV), and *HLA-A*, -B*, -DR**-typing (I, III) were performed.

Results: Patients with the rare chronic recurrent intraoral HSV-1 infection had *HLA-A**, *-B**, *-C**, and *-DRB1** homozygosity, together with total genetic deficiency of complement component *C4A* or *C4B*.

In chronic or recurrent rhinosinusitis patients (n = 48), low IgA, IgG,

IgG1, IgG2, IgG3, and IgG4 were all more common than in the general population (n = 150) and in those without known episodes of purulent rhinosinusitis (n = 48) combined. We searched for clinically relevant differences between patients with chronic or recurrent rhinosinusitis and those with non-recurrent uncomplicated acute purulent rhinosinusitis (n = 50). According to stepwise logistic regression analysis, nasal polyposis (odds ratio, OR 10.64 [95% confidence interval, CI 2.5-45.7] P = 0.001), bronchial asthma (8.87 [2.3-34.9] 0.002), C4A null alleles (5.84 [1.4-24.9] 0.017), and low levels of IgG4 together with either IgG1 or IgG2 (15.25 [1.4-166.8] 0.026) were more common in those with treatment-resistant rhinosinusitis than in acute rhinosinusitis patients.

Compared with herpes simplex seronegative control subjects (n = 70), *G3m*^{g}*,G1m*$^{a/a(x)}$, a haplotype that encodes the heavy chains of IgG3 and IgG1, was more frequent in the patients (P = 0.047). This haplotype has previously been associated with low IgG3 levels and with highly efficient immune evasion from IgG1 by HSV. The *G3m*^{g}*,G1m*$^{a/a(x)}$ haplotype is thus a new genetic polymorphism associated with active genital herpes infection. Compared with the control subjects, low total IgG1 (4.9 [2.0-12.5] 0.001) and IgG3 (3.6 [1.7-7.8] 0.001), unlike anti-HSV-2 antibodies, were associated with frequent recurrences. The strongest statistical significance was reached in the frequencies of low IgG or IgG3 or both between patients and HSV-seronegative control subjects (7.9 [3.3-19.2] < 0.001). Low IgG1 was more common when all infected persons (patients and seropositive control subjects) were compared with seronegative subjects (4.0 [1.1-13.9] 0.030). *C4* nulls were negatively associated with herpetic neuralgias (0.2 [0.06-0.81] 0.022) and prolonged or chronic neuralgias (0.2 [0.05-0.57] 0.004). Certain *HLA* haplotypes and alleles were found more frequently in herpes patients and in control subjects.

In severe chronic adult periodontitis, plasma levels of C3 were higher, and CH50 was lower in patients than in control subjects. Partial *C4* gene deficiencies were more frequent in patients than in control subjects (2.4 [1.1-5.5] 0.032).

Conclusions: Both *C4* nulls and low IgG subclass levels are common in the general population. *C4* null alleles or low immunoglobulin G subclass levels or both were associated with recalcitrant mucosal infections. Changes in complement levels may reflect chronic, recurring inflammation. *C4A* deficiencies are associated with chronic or recurrent rhinosinusitis, potentially through their effect on immune defense and inflammation control. Isolated low IgG subclass levels had limited value in the assessment of patients with rhinosinusitis.

HLA homozygosity, *HLA* alleles, the *G3m*^{g}*,G1m*$^{a/a(x)}$ haplotype, and low plasma levels of ADCC-mediating IgG1 and IgG3 antibodies may predispose to recurrent herpes infections. *C4* nulls are associated with protection from

herpetic neuralgias, potentially through reduced inflammation.

C4 deficiencies are associated with predisposition to chronic periodontitis. The new real time PCR *C4* genotyping test complemented the traditional C4 allotyping method.

Partly, these findings in patients may be explained by the immune evasion mechanisms employed by the causative microbes. *C4* nulls may also regulate the degree of mucosal inflammation. Since low IgG subclass levels are common in the adult population, our findings directly influence the interpretation of subclass levels in the clinic. The found new immunogenetic polymorphisms associated with frequent mucosal infections may also influence, for example, vaccine development.

2. Publications

I Seppänen M, Lokki ML, Timonen T, Lappalainen M, Jarva H, Järvinen A, Sarna S, Valtonen V, Meri S. Complement C4 deficiency and HLA homozygosity in patients with frequent intraoral herpes simplex virus type 1 infections. *Clin Infect Dis*. 2001;33:1604-7.

II Seppänen M, Suvilehto J, Lokki M-L, Notkola I-L, Järvinen A, Jarva H, Seppälä I, Tahkokallio O, Malmberg H, Meri S, Valtonen V. Immunoglobulins and complement factor C4 in adult rhinosinusitis. *Clin Exp Immunol*. 2006;145: 219-27.

III Seppänen M, Meri S, Notkola I-L, Seppälä IJT, Hiltunen-Back E, Sarvas H, Lappalainen M, Välimaa H, Palikhe A, Valtonen VV, Lokki M-L. Subtly impaired humoral immunity predisposes to frequently recurring genital herpes simplex type 2 infection and herpetic neuralgia. *J Infect Dis*. 2006;194:571-8.

IV Seppänen M, Lokki M-L, Notkola I-L, Mattila K, Valtonen V, Nieminen A, Vesanen M, Asikainen S, Meri S. Complement and C4 null alleles in severe chronic adult periodontitis. *Scand J Immunol, accepted.*

3. Abbreviations

For clarity, those abbreviations used only in tables, clarified in their footnotes, have not been included. When the name of a molecule listed below has been written in italics, it refers to the name of the corresponding gene (or gene region) according to the HUGO nomenclature. Some molecules and genes with commonly used abbreviations have not been abbreviated in the text. Then, only for the first time the term is used in the text, its abbreviation may follow in parenthesis.

ADCC	antibody-dependent cellular cytotoxicity
AP	alternative pathway of complement
APC	antigen-presenting cell
ARS	acute/intermittent (presumed bacterial) rhinosinusitis
BCR	B cell receptor
BF	factor B gene
C	complement factor (e.g. C4 and C5)
C4bp	C4b-binding protein
CH50	complement classical pathway hemolytic activity
CDR	complementarity-determining region of an immunoglobulin heavy chain
CP	classical pathway of complement
CRS	chronic rhinosinusitis
CRRS	chronic or recurrent rhinosinusitis
CVID	common variable immunodeficiency
DC	dendritic cell
f	complement factor (e.g. fB)
FcR	Fc receptor
GC	germinal center
Gm	immunoglobulin G heavy chain gene allotype
HIGM	hyper-IgM syndrome
HLA	human leukocyte antigen
HSV	herpes simplex virus
IFN	interferon
Ig	immunoglobulin
IGH	immunoglobulin heavy chain gene
Km	allotypic allele of immunoglobulin light chain gene
LP	lectin pathway of complement
LPS	lipopolysaccharide
LT	lymphotoxin

LTA	lymphotoxin-α gene
MHC	major histocompatibility complex
NK	natural killer
PAMP	pathogen-associated molecular pattern
PCR	polymerase chain reaction
S-IgA	secretory IgA
sIg	surface immunoglobulin
SLE	systemic lupus erythematosus
SCD	immunoglobulin G subclass deficiency
TCR	T cell receptor
TD	thymus-dependent
Th	T helper cell
TI	thymus-independent
TLR	Toll-like receptor
TNF	tumor necrosis factor
TP	terminal pathway of complement
VDJ	recombined variable, diversity, and joining genes
vFcR	viral Fc receptor
V_H	variable immunoglobulin heavy chain gene

4. Introduction

To survive the harsh pressures of the environment, human beings like other organisms must be capable of defending themselves. Physical barriers, such as the skin and mucosal surfaces, tolerate harmless, non-invading, commensal organisms. These aid us by competing with the more virulent microbes, and by keeping our mucosal surfaces alert and intact. Microbes around us act as friends or foes, whereas the immune system has to be able to recognize the difference.

At species level, the changing outside challenges have lead to the evolution of adaptive immunity. A highly developed immune system protects us from intrusion of extrinsic substances and organisms such as bacteria, viruses, fungi, and parasites. The adaptive arm of immunity is organized around T- and B-lymphocytes. Each lymphocyte displays a single type of receptor with unique specificity. Human lymphocyte populations are large and diverse. This greatly heightens the probability of an individual lymphocyte to encounter a matching antigen. After a non-self, offending structure is found, clonal activation, proliferation, selection, and maturation of the adaptive immune response takes place. This will enable a quicker, concerted response during a rechallenge by the same or a closely related organism. Even so, it takes three to five days for sufficient number of clones to be produced and to differentiate into effector cells.

The innate arm of immunity, by scanning for nonself structures and pathogen-associated molecular patterns (PAMP), is able to sense and instantly attack various microbes. Innate immunity also alerts and orchestrates multiple, less rapidly acting effector arms of immunity. Yet, during an individual's life-span, innate immunity is incapable of adjusting to any major changes in the environment.

Gene mutations, duplications, and recombination events in germ line cells aid species to adapt their immunity. Structurally and functionally related genes governing the production of related proteins with specialized functions may emerge. Genomic changes are then handed down to further generations. Some of the best known examples are the immunoglobulin (Ig), major histocompatibility complex (MHC), complement, and immune-type receptor genes.

Within a chromosome, duplicated genes may be tightly linked and form relatively stable combinations called conserved haplotypes. Products of closely related genes within a haplotype, so called isotypes, enable differentiated functions within the immune system. Well known examples of isotypes are Ig subclasses such as IgG1 and IgG3, and complement component C4 isotypes

C4A and C4B. The genes of these isotypes may further go through series of mutations, resulting in polymorphisms. Between individuals, structurally slightly different proteins with highly similar functions may then be encoded by the same gene. These protein allotypes are commonly found in Igs (e.g. $Gm1^a$ and $Gm1^f$) and in complement proteins (e.g. C4A3 and C4A1).

Within the genome, deletions may occur and mutated non-expressed genes may arise. Clinically, this may manifest as an immunodeficiency in an individual with severely impaired immune function. Over 90% of all adult primary immunodeficiencies are caused by humoral immunodeficiencies that lead to missing or very low levels of Igs or complement components. Depending on the genes affected and the types of mutations, changes in the effector protein levels and in the repertoire of available isotypes may ensue. For example, individuals normally have four copies of complement factor C4 (C4) genes in their genome, two of each isotype (*C4A*, *C4B*). Having only one gene copy ("C4 null allele" or "partial C4 deficiency") of either isotype may fail to affect the level of total C4 plasma concentration, but may lead to decreased levels of circulating isotype-specific C4. Activation of the immune system may also be excessive and cause hyper-inflammation, such as seen in autoimmune diseases and in the systemic inflammatory response syndrome during sepsis, sometimes associated with *C4A* null alleles. To avoid deleterious effects to the host, inflammation has to be tightly regulated. Mild genetic defects and regulatory disturbances of the immune system, leading to lower effector protein levels, may thus be either harmful or beneficial to the host.

After the emergence of higher organisms, microbes have acquired means to evade the immunity of potential hosts. Microbes causing chronic or recurrent infections need to evade from the rapidly acting effector arms of the host's immunity. Their ability to evade from different isotypes and allotypes of complement factors and antibodies varies. In the clinical practice of infectious diseases, otherwise immunocompetent patients suffering from a narrow spectrum of recurrent or chronic infections are commonly encountered. Hypothetically, this could be caused by multiple, subtle, and common defects in subjects' immune system. Whether this predisposes the host to specific pathogens or clinical infectious syndromes is poorly known. Although subtly low levels of Igs and partial or total deficiencies of some complement components are very common in the general population, the clinical consequences caused by these have rarely been studied.

The aim of this study was to explore whether common subtle immunologic defects such as *C4* nulls, or low Ig class or subclass levels are associated with recurrent or chronic infections, or their complications. Since mucosal surfaces are exposed to frequent attacks by potentially pathogenic organisms, we decided to recruit patients who suffer from severe and complicated forms of common, usually mild or infrequent mucosal infections.

5. Review of the literature

5.1 Complement system

The effector mechanisms of innate immunity do not require previous contact with an antigen and are activated immediately. Major participants of innate immunity are epithelia, cytokines, chemokines, complement, antimicrobial peptides, phagocytes, natural killer (NK) cells, and Toll-like receptors. Together, they also help the adaptive immunity to differentiate self from non-self structures. This is accomplished by the use of germ line-encoded molecules recognizing PAMPs[363].

In order to sense a wider repertoire of PAMP, complement has evolved into three distinct pathways: the alternative pathway (AP), the lectin pathway (LP), and the classical pathway (CP)[378]. Complement consists of at least 34 circulating factors produced by different cellular sources, and of at least 14 different complement receptors on various cell types (Tables 1.1 and 1.2)[150, 302]. Additional collectins (surfactant protein A [SP-A] and D [SP-D]), pentraxins (serum amyloid protein [SAP]), and galectins interact with complement. All three pathways are activated in a sequential manner, with the activation of one component leading to the activation of the next (Fig. 1.1). Direct B-cell receptor and thrombin activation, as well as bypass-pathways have also been described[155, 324]. During activation, several factors are cleaved. The resulting fragments are designated lowercase suffixes such as C3a and C3b. With the exception of C2, the smaller fragment is designated "a" and the larger "b". Further fragments are designated consecutive lower case letters alphabetically ("C3d"), and inactivated products are marked by i ("iC3b")[378]. The three pathways converge at the level of complement factor C3 (C3), which is the central protein of complement (Fig. 1.1)[378].

Complement has three main, partly overlapping physiologic activities. It readily defends us from invading organisms by opsonization, chemotaxis, activation of leukocytes, lysis of bacteria and cells, and formation of antimicrobial peptides (Table 1.3)[378, 379]. It also interacts with adaptive immunity: it augments antibody responses and enhances immunologic memory. Complement achieves this through CP and C3 activation, which also leads to disposal of immune complexes and apoptotic cells as part of the "scavenger" system (Tables 1.1 and 1.3)[68, 301]. Complement enhances B cell immunity mainly via complement receptors CD21 and CD35 (Table 1.2)[68].

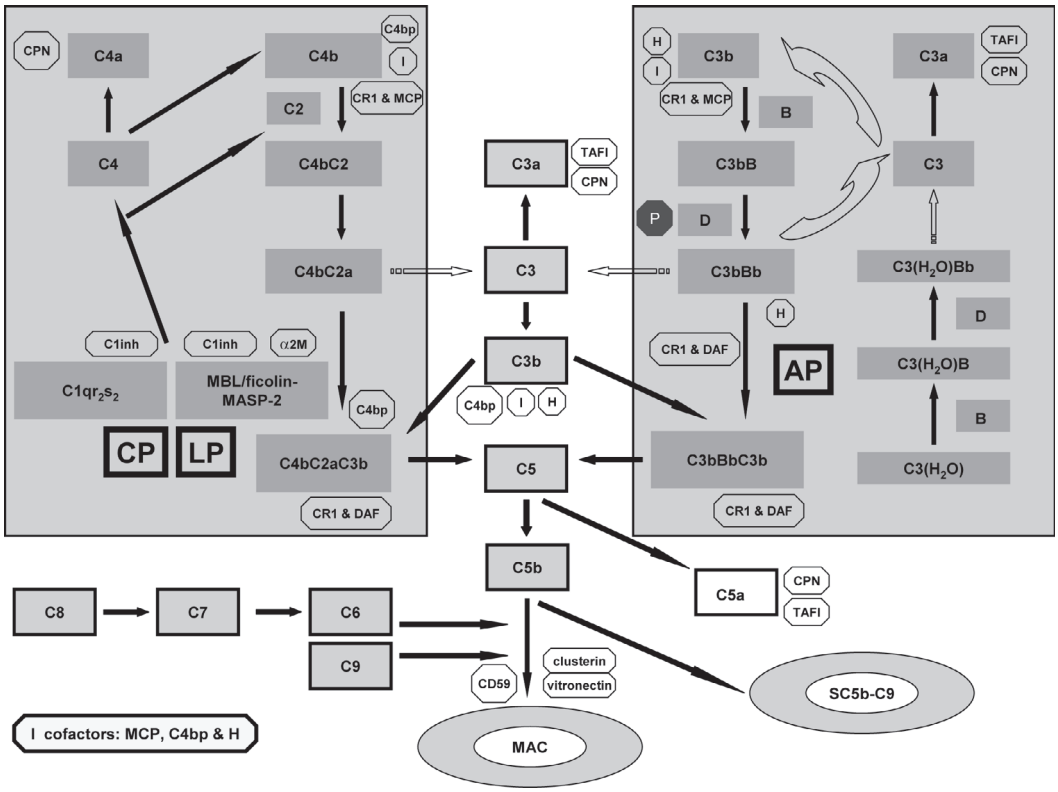

Figure 1.1 Complement pathways.
Some of the smaller fragments released during activation are not shown. For abbreviations, functions, and ligands of individual molecules see Tables 1 and 2.

Table 1.1 Complement proteins

Proteins	Polypeptide chains	Conc. [µg/mL]	Cellular sources[1]	Gene location	Key function
Alternative pathway					
Factor B	1	210	HC, MNP, EpiC, EndoC, AC, FB	6p21.3	Catalytic subunit of AP C3 convertase, part of C5 convertase.
Factor D	1	1-2	MNP, AC	19p13.3	Cleaves factor B bound to C3b or C3(H_20).
Properdin[2]	1-4	25	MNP	Xp11.3-11.23	Binds and stabilizes C3 convertase (C3bBb), positive plasma soluble RCA.
C3	2 (α, β)	1300	HC, MNP, EpiC, EndoC, FB	19p13.3-13.2	Component of AP, CP, and LP. C3b covalently binds activating surfaces, mediates phagocytosis, cytolysis. C3b binds factor B. C3a is part of C3 and C5 convertases, anaphylatoxin. Fragments regulate B cells.
Factor H[2]	1	500	HC, MNP, EpiC, EndoC, AC, FB	1q32	Binds C3b. Accelerates the dissociation of AP C3 convertase C3bBb, Cofactor for factor I. Negative plasma RCA, also regulates CP.
FHL-1[2]	1	10-50	HC, MNP, EpiC, EndoC, AC, FB	1q32	Inactivates the AP convertase C3bBb. Negative plasma RCA.
Factor I[2]	2 (heavy, light)	35	HC, MNP, MB, AC, FB, KC, MB, B cells	4q25	Cleaves C4b and C3b. Negative plasma RCA.
Carboxypeptidase N[2]	2 x 2 (α, β)	30-40	HC	8p22-23, 10	Inactivates C3a/C4a/C5a. Negative plasma RCA. Also called CPN.
Carboxypeptidase R[2]	1	3-60	HC	13q14.5	Inactivates C3a/C5a. Negative plasma RCA. Also called TAFI.
Classical pathway					
C1q	6 x 3 (A, B, C)	80	HC, MNP, FB, GEC	1p34-36	Binds to IgM, IgG, or CRP. Initiates CP.
C1r	1	50	HC, MNP, FB, GEC	12p13	Cleaves C1s.
C1s	1	50	HC, MNP, FB, GEC	12p13	Cleaves C4 and C2.
C4 (C4A, C4B)	3 (α, β, ã)	250	HC, MNP, FB, epithelial GUC, AlvC type II	6p21.3	Part of CP C3 convertase. C4b is acceptor for C2, binds to activating surfaces, and regulates B cells. C4a anaphylatoxin.
C2	1	20	HC, MNP, FB, epithelial GUC, AlvC type II	6p21.3	Catalytic subunit of CP C3 convertase. Part of C5 convertase.
SIGN-R1 (CD209a)			MΦ (described in mice)	n.a.	Binds microbial polysaccharides, C1q. Initiates CP without the involvement of immunoglobulins on the surface of marginal zone MΦ.
C1inh[2]	1	200	HC, MNP	11q11-13	Binds and inactivates C1r and C1s, inhibits MBL-MASP-2 and ficolin-MASP-2 activities. Negative plasma RCA.
C4bp[2]	8 (6-7α, 0-1β)	250	HC, MNP	1q32	Binds C4b. Cofactor for factor I. Accelerates decay of CP C3 convertase C4b2a. Negative plasma RCA.
C-reactive protein	5	0.5-3	HC, MΦ, SMC, K, neurons	1q22	Binds to phospholipids of microbes, apoptotic cells, modified LDL, FcγR I and IIa, C1q, C4, factor H. Activates CP, opsonizes, and inhibits TP (LP, AP).

Lectin pathway					
MBL	2-8 x 3	1-5	HC, AstroC, Kd, SI, T	10q11.2-q21	Binds to mannans of microbes. Initiates activation of LP.
MASP-1	2	6	HC, AstroC, Kd, H, L, SI, Plac	3q27-28	May be involved in direct cleavage of C3, inefficient.
MASP-2	2	6	HC, SI, T	1p36.21	Cleaves C4, C2. Mediates ficolin- and pentraxin-initiated complement activation.
MASP-3	1?		Widely expressed in tissues tested	3q27-28	Unknown. Product of alternative splicing of MASP-1.
sMAP/MAp19	1?		HC, SI, T	1p36	Unknown. Truncated form of MASP-2, associates with MASP-1.
L-ficolin	2-4 x 3	10	HC	9q34.3	Binds microbial N-acetylated groups, associates with MASPs, activates LP.
H-ficolin	4-6 x 3	15	HC, AlvC type II, bronchial EpiC	1p36.11	Binds microbial oligosaccharides associates with MASPs, activates LP.
M-ficolin	4 x 3		MC, NP, AlvC type II. Secreted locally.	9q34	Binds microbial N-acetylated groups, associates with MASPs, activates LP.
α2-macroglobulin[2]	4	200	HC, T, ovaries, uterus	12p13.3-12.3	Inhibits MASPs. Negative plasma RCA.
Terminal pathway					
C5	2 (α, β)	75	HC, MNP, FB, EpiC, AstroC, T and B cells	9q33	Binds to C3b. C5b binds C6, C7 and initiates the assembly of membrane attack complex. C5a anaphylatoxin.
C6	1	45-70	HC, NP, AstroC	5p12-14	Binds to C5, C7.
C7	1	55-60	HC, MNP, FB, AstroC	5p12-14	Binds to C6, C8.
C8	3 (α, β, ã)	80	HC, PC, AstroC	1p32	Binds to C7, C9.
C9	1	60	HC, AstroC, FB, MΦ, MC, TC	5p13	Binds to C8, polymerizes.
Vitronectin[2]	1 or 2 x n	505	HC, male genital tract?	17q11	Inhibits membrane attack complex. Binds C5b-6/7. Negative plasma RCA.
Clusterin (SP40-40)[2]	2 (α, β) x n	50	Produced in most organs	8p21	Inhibits membrane attack complex. Binds C5b-7/8/9. Negative plasma RCA.

[1]Abbreviations of cell types: see Table 1.2 footnotes. [2]RCA = regulator of complement activation. Conc. = concentration, CP = classical pathway, AP = alternative pathway, FHL-1 = factor H-like protein, LP = lectin pathway. Modified from refs.[150, 300].

Table 1.2 Complement receptors

Receptors	Polypeptide chains	Cell type[1]	Binds	Gene location	Key features
C1qRp (CD93)	1	EndoC, TC, NP, GC, MC	C1q, MBL, SP-A	20p11.21	Mediates phagocytosis of C1q-opsonized apoptotic cells, immune complexes, and pathogens
Calreticulin (cC1qR)	1	MC, EndoC, B cells, DC	C1q, MBL, SP-A, SP-D	19p13.3-13.2	Mediates phagocytosis of C1q/MBL-opsonized apoptotic cells
gC1qbp (gC1qR)	1 x 3	EndoC, B and T cells, DC, TC, MastC	C1q, MBL, vitronectin	17p13.3	Unknown. Chemotaxis of mast cells?
CR1 (CD35)[2]	1	Ery, Eos, MC, MΦ, NP, GlomP, FDC, Mast, B and T cells	C3b, C4b, iC3b, C3c, C1q, C4b, MBL	1q32	RCA, accelerates decay of CP and AP convertases, cofactor for factor I, helps processing of immune complexes, involved in phagocytosis of C3- and C1q-opsonized particles
CR2 (CD21)	1	FDC, mature B cells, T cell subsets, BP, Mast, KC, EpiC, and CD4-CD8- thymocytes	iC3b, C3d,g, C3d, CD23, IFNα	1q32	Immunoregulation with CD35, part of CD21-CD19-CD81 coreceptor, lowering of B cell activation thresholds
CR3 (CD11b/CD18)	2 (α, β)	PMN, MC, NK, DC, some B and T cells	iC3b, C3dg	16p11-13 21q22.3	Phagocytosis and cytotoxicity of cells with complement activation products, neutrophil adhesion
CR4 (CD11c/CD18)	2 (α, β)	MC, MΦ, NP, NK, ADCC effector lymphocytes, B cells, DC	iC3b	16p11.2 21q22.3	Cell adhesion
C3aR	1	NP, MC, MΦ, BP, Mast, Eos, TC, AstroC, microglia, DC, L	C3a, C4a	12p13.31	Chemotaxis, -kinesis, cell aggregation and adhesion, release of lysosomal contents
C5aR (CD88)	1	NP, MC, MΦ, Mast, BP, Eos, LPC, DC, L vascular SMC, EndoC, EpiC, AstroC, microglia, MesC	C5a, C5adesArg	19q13.3-13.4	Chemotaxis, cell adhesion and aggregation, release of granular enzymes, superoxide anions, and histamine. Augments humoral and cellular responses
C5L2 (gpr77)		Immature DC, PMN, MC, skin FB, AC	C5a, C5adesArg	19q13.33	Unknown. A decoy receptor for C5a?
DAF (CD55)[2]	1	Ery, TC, and all leukocytes	C3b, Bb, C4bp, C2a	1q32	RCA, accelerates decay of AP, CP C3 convertases. Lysosomal enzyme release, leukocytosis.
MCP (CD46)[2]	1	MC, TC, RC, lymphocytes, PMN, mesenchymal, EndoC, EpiC	C3b, iC3b, C4bp	1q32	RCA, cofactor for factor I
Protectin (CD59)[2]	1	Widely expressed, all circulating cells, vascular EndoC, EpiC	C8, C9	11p14-13	RCA, inhibits MAC on host cells
CRIg		Kupffer cells, MC, L, Plac, H, adrenal glands	C3b, iC3b	Xq12	Required for efficient binding and phagocytosis of C3-opsonized particles

[1]HC = hepatocytes, MNP = mononuclear phagocytes, EpiC = epithelial cells, EndoC = endothelial cells, AC = adipocytes, FB = fibroblasts, KC = keratinocytes, MB = myoblasts, GEC = gastrointestinal epithelial cells, GUC = genitourinary cells, SI = small intestine, T = testis, H = heart, L = lung, Plac= placenta, NP = neutrophils, AlvC = alveolar cells, AstroC = astrocytes, Kd = kidney, PMN = polymorphonuclear cells, PC = pneumocytes, MΦ = macrophages, MC = monocytes, TC = platelets, GC = glial cells, Mast = mast cells, Ery = erythrocytes, Eos = eosinophils, GlomP = glomerular podocytes, FDC = follicular dendritic cells, NK = natural killer cells, BP = basophils, LPC = liver parenchymal cells, SMC = smooth muscle cells, DC = dendritic cells, MesC = mesangial cells, RC = reticulocytes. [2]RCA = regulator of complement activation. CP = classical pathway, AP = alternative pathway, MAC = membrane attack complex. Modified from refs.[150, 300].

Table 1.3 Complement functions

FUNCTION	COMPLEMENT FACTORS AND MECHANISMS
Host defense against infection	
Opsonization	Covalently bound fragments of C3 and C4
Chemotaxis and leukocyte activation	Anaphylatoxins C5a, C3a, C4a, and their leukocyte receptors
Generation of antimicrobial peptides	C3a, C3a-desArg
Lysis of bacteria and cells	Membrane-attack complex (C5b-C9)
Interface between adaptive and innate immunity	
Regulation of antibody responses Foreign antigen recognition Self-antigen recognition	1. C3b and C4b bound to immune complexes and to antigen engage CD21-CD19-CD81 coreceptor on B1 cells and enhance positive selection of B1 cells 2. Coligation of BCR and CD21-CD19-CD81 coreceptor by C3- or C4- coated self-antigen induces anergy or apoptosis in bone marrow and at transitional stage 3. Antigen coupled to C3d activates naive mature follicular B cells through BCR and CD21-CD19-CD81 coligation; with T cell help leads to activation and expansion of B cells 4. GC-formation initiated by activated B cells in FDC-organized splenic follicles; engaged CR on GC enhance BCR signaling
Enhancement of B cell memory	Post-GC B cells require complement and antigen selection for the efficient maintenance of long-term memory B cells
Regulation of T cell immunity	Priming of CD4+ and CD8+ T cells by fragments of C3
Disposal of waste	
Clearance of immune complexes from tissues	C1q; covalently bound fragments of C3 and C4
Clearance of apoptotic and necrotic cells	C1q, C4b, (i)C3b, MBL; together with IgM, collectins, and pentraxins

BCR = B cell antigen receptor, FDR = follicular dendritic cells, GC = germinal centers, CR = complement receptors, MBL = mannan-binding lectin, IgM = immunoglobulin M. Modified from refs.[68, 300, 378].

5.1.1 Complement pathways and factors

Alternative pathway

While CP and LP are activated through their PAMP-sensing initiating factors, AP is kept at a low level of steady-state activation ("tick over") through the spontaneous hydrolysis of the thioester group of native C3[191]. C3 is able to interact with over 20 different proteins of complement and non-complement origin[150]. Various bacteria, fungi, viruses, and tumor cells activate AP[378]. Likewise, IgA and IgG2 can activate AP at high antigen concentrations[85]. The activation of C3 through AP requires three additional plasma proteins: factor B (fB), factor D (fD), and properdin (P). During activation, the metastable C3b fragment forms a properdin-stabilized C3bBb convertase with fB cleaved by fD. This leads to the cleavage and activation of additional C3 molecules[363]. Once C3 is activated through any of the pathways, the C3b fragment can bind covalently to the hydroxyl and amino groups of the activating structure. This leads to opsonization of foreign structures. Activation of the terminal pathway (TP) by the AP is achieved through C5 convertase C3bBbC3b. The smaller cleaved fragments C3a and C5a further act as anaphylatoxins. To avoid self-damage, the positive, continuously-activated feedback loop of AP needs to be tightly controlled (Table 1.2). Naturally occurring antibodies (at least of the IgG2 subclass) forming a complex with C3bC3b ($C3b_2$-IgG) may also amplify AP[212].

To be pathogenic, microbes need to produce various immune evasion molecules against AP[161, 206, 299]. Those bearing large amounts of surface sialic acid are usually poor activators of complement. These include successful pathogens such as group A and B streptococci, *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenzae* type B, *Escherichia coli* K1, and some *Salmonellae*. Pathogens further escape the AP by exploiting, for example, host complement regulators and complement receptor analogues on their surfaces[161, 206].

Lectin pathway

The main human PAMP recognition molecule of the LP is a collectin named mannan-binding lectin (MBL). Similar to its counterpart in the CP, C1q, MBL comprises three polypeptide chains forming a trimeric structure[368]. Their globular CRD heads recognize specific carbohydrate groups present on microbial surfaces, for example D-mannose, *N*-acetylglucosamine (GlcNAc), and *N*-acetylmannoseamine (Table 1.1)[338]. MBL recognizes various pathogenic organisms: Gram-positive, Gram-negative, and anaerobic bacteria, *Mycobacterium avium*, viruses like influenza A, herpes simplex virus 2, and human immunodeficiency virus 1 and 2, as well as yeasts, filamentous fungi, and protozoa[125, 159].

In addition, plasma L- and H-ficolin, and locally secreted plasma soluble H- and M-ficolins in the airways are capable of initiating the LP. The basic

structure of ficolins is similar to MBL[151]. H-ficolin binds to GlcNAc and *N*-acetylgalactosamine, whereas L- and M-ficolins bind to *N*-acetylated carbohydrates and noncarbohydrate compounds on microbes and other surfaces (Table 1.1). L-ficolin binds to the capsules of serotype III group B streptococci, *Streptococcus pneumoniae* serotype 11F, and multiple serotypes of *Staphylococcus aureus*. H-ficolin agglutinates human erythrocytes coated with lipopolysaccharide (LPS) derived from *Salmonella typhimurium*, *Salmonella minnesota*, and *Escherichia coli*[201].

MBL and ficolin oligomers form complexes with MBL-associated serine proteases (MASP) (Table 1.1). After binding to an activating structure, MBL/MASP-2 and ficolin/MASP-2 complexes opsonize the target structure and activate the LP. MASP-2 then efficiently cleaves C4 and C2, which leads to the formation of the CP convertase C4b2a[304, 361]. The biologic roles of the other MASPs are unknown[324]. The LP also takes part in the clearance of dying and apoptotic cells. MBL deficiencies are frequent in certain populations[70].

Classical pathway

Classical pathway is activated by the binding of C1q to its target (Table 1.4). C1q has six subunits radiating like a bouquet of tulips, its overall structure thus resembles MBL and ficolins. Each of the subunits comprises three structurally related polypeptide units (A, B, C) with an *N*-terminal domain, a collagen-like sequence, and *C*-terminal globular domain (gC1q) (Table 1.1)[124]. The globular domains of each polypeptide appear to be structurally and functionally independent[178]. In addition to immune complexes, various pathogens, infected cells, molecules, and cellular structures can bind C1q directly and initiate CP (Table 1.4). Since many diverse structures are recognized by C1q, it is likely to function as a charge-pattern recognition molecule[124].

Antibody-dependent complement activation is initiated by C1q that recognizes IgM- or IgG-molecules bound with sufficient density to an antigen[178]. C1q binds to the Fc regions of IgM and IgG, i.e. to the Cμ3 domain (Asp417-Glu418-His420) on IgM and the Cγ2 domain on IgG[178, 291]. The binding sites of IgG1 and IgG3 differ from each other[178]. In addition, the relative efficiency of IgG subclasses and Caucasian allotypes to bind C1q differs in the order: $G3m^g > G3m^b > G1m^f > G1m^a >> IgG2$[85, 291]. Although IgG1 binds fewer C1 molecules, the deposition of C4b on the cell surface is much more efficiently mediated by IgG1 than by IgG3. Thus the efficiency to initiate complement-dependent cell lysis varies in the order: $G1m^f = G1m^a > G3m^b > G3m^g >> IgG2$[85, 291]. The efficiency of classical pathway-mediated complement lysis also varies with antigen concentration. At higher antigen concentrations, IgG1 is more active than IgG3, whereas IgG3 is relatively more efficient at lower concentrations[228]. IgG2 initiates efficient lysis only at very high antigen concentrations. IgG4 does not fix complement at all[228].

C1q circulates in a complex with two C1r and two C1s molecules

($C1qr_2s_2$), forming C1 (Table 1.1)[124, 178]. C1r and C1s have homologous modular architectures that resemble the structures of MASPs: an *N*-terminal, non-catalytic interaction domain that mediates $C1r_2s_2$-tetramer assembly, and a *C*-terminal catalytic serine protease domain[17]. Both domains take part in the interactions between the C1q collagen-like stems and the $C1r_2s_2$-subcomplex[124]. The binding of C1q to a target via the *C*-terminal globular domain induces a conformational change in the collagen region of C1q[124, 178]. This elicits the self-activation of C1r, a serine protease which, in turn, converts proenzyme C1s into a protease[17, 124]. C1s cleaves C4 and C2 in a highly specific manner[126].

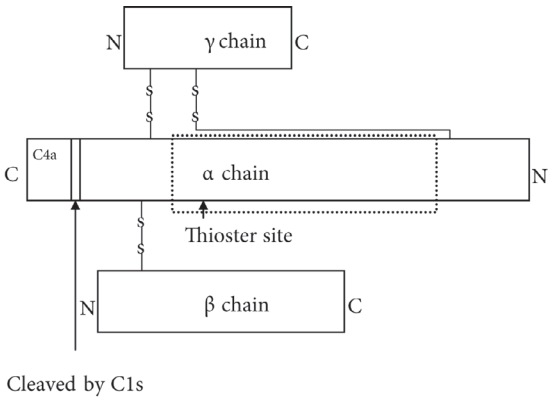

Figure 1.2 Schematic structure of C4.
S-S represent the interchain disulphide bonds, a dashed line marks the C4d region.

When C4 is activated, a single arginyl bond at its *C*-terminus is cleaved, leading to the release of C4a (Fig. 1.2, Table 1.3). In the remaining C4b molecule, an internal thioester bond is exposed. Within a brief time period, and in the immediate vicinity of C1 at the surface of the activator, its acyl group becomes highly reactive with amino or hydroxyl groups. Although the binding of C4b is indiscriminate, complement regulators on normal cells prevent complement deposition and amplification on their surfaces, providing self-nonself discrimination[16]. Attached C4b is then capable of binding C2 (Table 1.1). Binding by C4b renders C2 available for limited proteolysis by C1. After C1s cleaves a single Arg-Lys bond, C2 splits into an *N*-terminal C2b fragment and a *C*-terminal catalytic fragment C2a[126]. C2a remains bound to C4b, and the classical pathway C3 convertase C4bC2a is formed. C4bC2a then cleaves C3, releasing the C3a fragment. C3a and C4a have similar functions (Table 1.3). A thioester on the remaining C3b fragment is exposed, and like C4b, C3b becomes capable of covalently binding to the nearby target surface. When C3b binds to C4bC2a, the classical pathway C5 convertase is formed, and the C2a subunit acquires specificity for the cleavage of C5[16].

Table 1.4 Examples of activators of the classical pathway

Class	Activator
Immune complexes	IgM-, IgG1-, IgG3-, (IgG2-)[1] antigen complexes, CD209a-antigen complex[2]
Bacteria	*Escherichia coli, Salmonellae, Klebsiellae, Mycoplasma pneumoniae*
Viruses	Sindbis, Epstein-Barr, cytomegalovirus, human immunodeficiency virus 1 and 2, human T lymphotropic virus, *Paramyxoviridae*
Parasites	*Schistosoma mansoni, Trypanosoma brucei*
Cellular structures	Cellular and subcellular membranes, apoptotic cells
Proteins	C-reactive protein, myelin, β-amyloid peptide, serum amyloid protein A, prion proteins
Other molecules	Oligosaccharides, polysaccharides, lipid A, cardiolipin, polyanions (DNA, heparin)

Ig = immunoglobulin. [1]IgG2 fixes complement weakly, see text. [2]Described in mice. Modified from refs.[16, 362].

Terminal pathway

The TP is activated by C5 binding to C3b either as part of the AP (C3bBbC3b) or CP (C4bC2aC3b) C5 convertases[336]. C5 is cleaved into C5a and C5b. C5a acts as a chemotactic factor and an anaphylatoxin (Table 1.1). C5b then interacts with the terminal complement proteins in a nonenzymatic sequential association, resulting in the formation of the membrane attack complex (MAC). As each protein incorporates into the complex, a conformational rearrangement exposes a binding site for the next component. C5b-7 becomes cell membrane-bound and binds C8. This allows C8α to become more deeply buried in the target cell membrane. C9 binds C8α, goes through a conformational change, and traverses the membrane[336]. Additional binding sites for C9 are exposed, and 12-18 C9 molecules may become incorporated into the C5b-C9 complex in a circular configuration. A pore, able to function as a protein-walled channel and causing the loss of membrane integrity, is formed[336]. MAC then causes cell activation, or cell death through cell necrosis or apoptosis[94, 336]. Sublytic levels of C5b-9 promote cell survival and protect from apoptosis[94].

Regulators of complement activity

Since complement is capable of attacking host cells as well as nonself structures, its activation must be confined to designated targets. This is accomplished by plasma soluble and membrane-bound regulators of complement activation (RCA) (see Tables 1.1 and 1.2 for details). AP is constantly activated at a low level and has a powerful positive feedback loop. Thus it needs to be tightly regulated. CP, being a linear cascade that is activated mostly by adaptive immunity or tissue destruction, is less tightly controlled.

Regulation takes place at different levels of complement pathways[16]. Properdin is the only known positive regulator of C, and stabilizes the AP C3 convertase. Most of the other RCA: membrane-bound regulators on

autologous cells including complement receptor 1 (CR1, CD35), decay accelerating factor (DAF, CD55), and membrane cofactor protein (MCP, CD46), as well as soluble regulators factor H, factor I, and factor H-like protein 1 (FHL-1) inhibit mainly the C3 convertases by binding C4b and C3b[16, 175, 227]. In addition, carboxypeptidases N and R (TAFI) inactivate anaphylatoxins and opsonins (Table 1.1). Vitronectin, clusterin, and protectin (CD59) inhibit MAC formation[175]. Various RCA proteins also regulate bradykinin and coagulation cascades[175, 227]. RCA are exploited by various pathogens; bacteria use RCA for cell entry and viruses have RCA homologues on their surfaces that prevent complement activation[52, 188, 206, 239].

Only those RCA that regulate CP are discussed in more detail below. In addition, any RCA that inactivates C4 activation products may be viewed as a regulator of CP. These include DAF, fI, its cofactor fH, MCP, and CR1 that regulate the CP C3 convertase (Fig. 1.1)[16, 175].

C1 inhibitor (C1inh), a serine protease inhibitor (serpin), is the only known serum inhibitor of C1 protease, MBL-MASP-2, as well as ficolin-MASP-2 activities (Table 1.1)[16]. It also inactivates factors of the contact (factor XII, kallikrein), coagulation (factor XI, thrombin), and fibrinolytic (tPA, plasmin) systems. When C1inh is cleaved by activated C1s and C1r, its major fragment forms a covalent complex with the active site of the two proteases and renders the complex inactive[83]. C1inh, through non-covalent binding, prevents spontaneous C1 activation[16, 227].

C4b-binding protein (C4bp) is a potent inhibitor of CP and LP at the level of C3 convertase assembly. Its major plasma isoform, which constitutes approximately 80% of plasma C4bp, has seven identical α-chains and one β-chain (Table 1.1). In circulation, all isoforms that contain the β-chain of C4bp circulate in a complex with an anticoagulatory protein, protein S[52]. C4bp prevents the assembly of the CP C3 convertase C4bC2a and accelerates its decay by promoting the dissociation and replacement of C2a from C4bC2a (Table 1.1). It is also a cofactor for factor I in the cleavage of C4b to C4c and C4d (Fig. 1.2). Via its interaction with the membrane-binding protein S, C4bp is able to localize its RCA activity to the surface of apoptotic cells. By interacting with CD40, it may also act as a survival factor for B cells[52].

5.1.2 Complement factor C4

C4 structure and function

C4 is synthesized as a single-chain precursor (Table 1.1). The major polypeptide sequences of the C4 precursor show homology with α2-macroglobulin. In the central part of the molecule is a short anaphylatoxin motif. During proteolytic processing, a basic tetrapeptide is excised at the *N*-terminal end of the anaphylatoxin module, followed by a two-step removal of a 26-residue sequence[16]. The mature circulating protein has three chains (α, β, γ) linked by disulfide bridges (Fig. 1.2). C4 has a metastable thioester site in its α-

chain. When C4 is activated by C1, a single arginyl bond at the *C*-terminus of the anaphylatoxin module is cleaved, leading to the release of C4a, a weak anaphylatoxin. C4b, the activated form of C4, then forms a covalent bond with either hydroxyl or amino groups on cell surface macromolecules or immune complexes. C4b and the cleaved *N*-terminal fragment C2a form the C3 convertase of CP. Bound C4b is also able to bind non-covalently to CR1 of phagocytes and thus acts as an opsonin[16].

C4 genes, isotypes, and allotypes

C4 is the most polymorphic protein of the complement system. Human C4 is encoded by a duplicated gene giving rise to the genes *C4A* and *C4B*, which lie 10 kb apart within the central, class III MHC region in chromosome 6 (Table 1.1). *BF*, *C2*, *C4A*, and *C4B* gene alleles together form a complotype[9]. Complotype SC01, for example, contains the polymorphic alleles *BFS*,*C2C*,*C4AQ0*,*C4B1*. Each *C4* consists of 41 exons coding for a 5.4 kb transcript. In Caucasians, ~ 75% of *C4* genes harbor a 6.4 kb human endogenous retrovirus *HERV-K(C4)* that gives rise to long (L) variants. The long *C4* gene is 20.6kb and the short (S) 14.2 kb. Moreover, 55% of Caucasian MHC haplotypes have a 2-locus, *C4A-C4B* configuration, but 45% have an unequal number of *C4A* and *C4B* genes (Fig. 1.3)[51]. Duplication of a *C4* gene always concurs with the adjacent genes *RP* (a nuclear protein kinase), *CYP21* (steroid 21-hydroxylase), and *TNX* (extracellular matrix protein tenascin X); together they form a genetic unit called RCCX (Fig. 1.3). Monomodular, bimodular, and trimodular RCCX structures with one, two, and three *C4* genes have population frequencies of 17%, 69%, and 14%, respectively[51]. Further, quadrimodular RCCX units with SLSL or LLLL structures and, for example, a trimodular RCCX with the homoexpression of C4A3 protein have been described. In duplicated RCCX units, *RP*, *CYP21*, and *TNX* genes between *C4* genes are mostly nonfunctional. Over 24 different RCCX haplotypes have been described in Caucasians. Consequently, there is great variation in the *C4A* and *C4B* gene dosages between individuals[51]. In a diploid genome, Caucasians have 2-6 *C4* genes and about 52% of them four *C4* genes. Having fewer than two functional *C4A* or *C4B* causes a low level of the corresponding C4 protein (C4Q0, "C4 null") and is considered a deficiency. C4 deficiencies are caused by gene deletions, point mutations, insertions, or conversions[51, 158]. In Caucasians, a complete deficiency of C4A is present in 3% and C4B in 8% of the population. Up to 35-40% of individuals have at least one null allele[51]. In the human genome, somewhat comparable variations in the number of immunologically active genes are only seen in two other complement genes *MBL2* and *C2*, which also take part in LP and CP activation, respectively[170, 361].

In the MHC region, genetic polymorphisms are often inherited as conserved, fixed combinations resulting in linkage disequilibrium. This happens in ~ 50% of Caucasian MHC haplotypes[9]. C4 genes are also

inherited with these extended conserved ("ancestral") MHC haplotypes together with other immunologically active genes such as candidate genes associated with CVID in human leukocyte antigen (HLA) region carrying *HLA-DR* (important in antigen presentation), and with complement and cytokine genes *BF*, *C2*, tumor necrosis factor (*TNF*), lymphotoxin α (*LTA*), and lymphotoxin β (*LTB*)[9, 65]. At least a third of common (frequency > 0.01) European Caucasian haplotypes are fixed from *HLA-B* through the complotype to *HLA-DR/DQ*. This extensive linkage equilibrium makes difficult the determination of single, functionally relevant polymorphisms associated with diseases[9, 67].

The two C4 isotypes C4A and C4B are more than 99% identical in primary amino acid sequence[64]. All mammals have C4B. C4A and C4B express different, serotypically identifiable antigenic determinants. In general, C4A expresses the Rodgers, and C4B the Chido blood group determinants. Only four amino acids between 1101 and 1106 are specific to either C4A or C4B[64]. C4A binds predominantly to free amino groups, and C4B preferentially to hydroxyl groups. Consequently, C4B is hemolytically more active[294]. The different binding specificities of the isotypes can be correlated with a single His (C4B)/ Asp (C4A) substitution at position 1106. This substitution also gives rise to a prolonged half-life of activated C4A (~ 10 s) when compared with the very short half-life (~ 0.5 seconds) of C4B[198]. The biological significance of having the two isotypes remains enigmatic. C4A may be more efficient in binding immune complexes and in maintaining immune complex solubility than C4B[294]. The HLA-independent association between C4A null phenotypes and systemic lupus erythematosus (SLE) might be explained by impaired clearance and processing of necrotic and apoptotic cells, and immune complexes. Even though C4A may bind CR1 (involved in immune complex clearance) more efficiently than C4B, in certain populations only C4B null alleles are associated with SLE[294]. Experimentally, C4A-reconstituted guinea pigs are also able to class switch from IgM to IgG, whereas C4B-reconstituted animals are not[395].

C4A and C4B further exhibit extensive structural polymorphism giving rise to > 41 allotypic forms[51]. These allotypes are designated according to their electrophoretic mobility by increasing Arabic numbers from the cathode to the anode[1]. The most common allotypes are C4A3, C4A2, C4A4, C4A6, C4B1, C4B2, and C4B3, all of which are found in > 1% of Caucasians. C4A6 is hemolytically inactive due to its inability to form a functional C5 convertase. In addition to these, rare structural variants have been recognized[64]. Only minor differences in the three-dimensional structures of the two most common allotypes, C4A3 (in ~ 85% of Caucasians) and C4B1 (in ~ 87%) have been found. However, activated C4A3b and C4B1b differ in secondary and tertiary structure, electrostatic surface charge, and surface hydrophobicity. Hypothetically, this may confer selective advantage for binding to different microbes and different types of immune complexes[294].

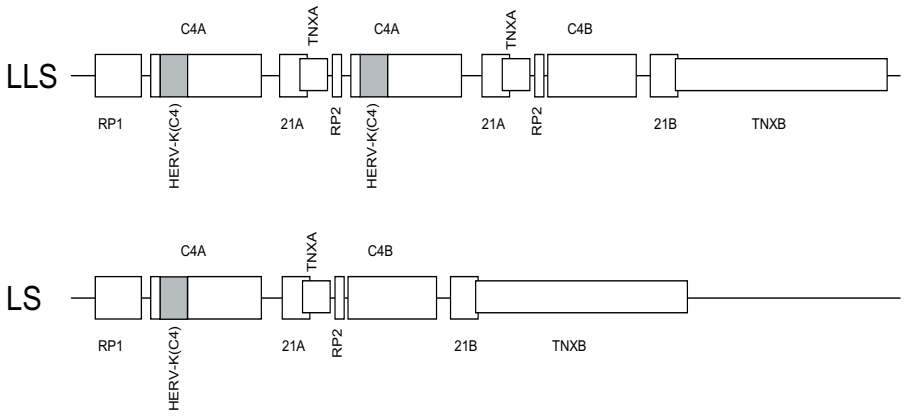

Figure 1.3 Schematic representations of a trimodular and a bimodular RCCX gene element
Mono-, bi-, tri-, and quadrimodular structures of RCCX can be found in the population. Modified from ref.[51].

C4 synthesis

Like other complement components, C4 is mainly produced by the liver. Many extrahepatic tissues are also capable of producing C4 (Table 1.1). Plasma or serum protein levels of total C4 vary between 0.05 and 0.8 g/L among individuals, as do the relative quantities of C4A and C4B[296, 395]. Likewise, the CH50 differs greatly between individuals. Part of this variation is caused by acquired factors: obesity and age between 40 and 65 years increase C4 concentrations[297, 395]. Most of the variation is caused by genetic differences between individuals. C4 concentration and its hemolytic activity are increased by higher C4 gene dosages, the presence of C4B, and by C4(S) genes. C4(S) genes are more common in blacks, who also have ~ 40% higher plasma C4 concentrations[395].

5.1.3 Genetic complement deficiencies

Complement genes have a high degree of polymorphism (Table 1.5). Several complement genes belong to related gene families: the C5 family (C3, C4, C5, and α2-macroglobulin), the C9 family (C6, C7, C8α, C8β, and C9), the complement serine protease family (C1r, C1s, MASP-1, MASP-2, MASP-3, C2, fD, fB, and fI), and the RCA family (C4bp, fH, MCP, DAF, CR1, and CR2). CR3 and CR4 are integrins, whereas C3aR, C5aR, and C5L2 are G-protein coupled receptors[300]. Various complement genes are clustered in the same gene regions and may present as conserved haplotypes: the RCA

region (1q32; MCP, CR1, CR2, DAF, C4bp, fH), the major histocompatibility complex (MHC) class III complement cluster (6p21; C2, fB, C4A, C4B), the MAC I cluster (1p32;C8α and C8β), the MAC II cluster (5p12-14; C6, C7, C9), and C1r and C1s in 12p13 (Tables 1.1 and 1.2)[300, 394]. Some complement proteins are the result of alternatively spliced products (e.g. FHL-1) or consist of multiple separately encoded gene products (e.g. C1q and C8, Tables 1.1 and 1.2)[300].

Genetic complement deficiencies comprise about 1-6% of primary immunodeficiencies[248]. Genetic deficiencies for nearly all of the complement components have been described. With the exception of C1inh, MBL, C2, and C9, complete or near complete complement deficiencies are rare (Table 1.5). There is great regional variation in the frequencies of TP deficiencies. It is estimated that approximately 1 in 10 000 individuals is deficient in one of the components of the TP, but, for example, 1 in 1000 Japanese are C9-deficient[394].

The importance of MBL deficiencies has been questioned, because in a large population study the incidence of hospitalization or death from infections or other common serious disorders did not differ between MBL-deficient homozygotes and non-carriers[100]. In adults, MBL deficiency has been associated with, for example, infectious morbidity or mortality in patients with cancer, burns, mycoplasma infections, and sepsis[103]. The high frequency of MBL deficiencies in certain populations may imply that limited LP activation is even beneficial to humans in epidemic situations[70]. Some of the suggested consequences of MBL deficiencies are discussed below together with C4 and C2 deficiencies.

Patients with CP component deficiencies commonly suffer from autoimmune phenomena: systemic (SLE) or discoid (DLE) lupus, vasculitides, renal, immune complex -mediated, and other immune diseases[36, 300]. SLE is also found in some MBL deficiencies[361]. The frequency of SLE in partial or complete C1q, C4A and C4B, C2, and MBL deficiencies has marked regional differences[301]. The more proximal the defect, the more probable is the development of SLE (Table 1.5). In population, the most frequently found CP deficiency in SLE is C4A deficiency, found in up to 15% of Caucasian SLE patients[36]. A defect in the clearance of apoptotic cells may be more important than the ability to clear immune complexes or to negatively select self-reactive B cells[36, 300]. Cutaneous and renal manifestations are especially common in early CP deficiencies, which usually manifest in patients aged less than 20 years. Antibodies against nuclear (75%) and extractable nuclear (70%) antigens, and against double-stranded DNA (usually absent) are found less commonly than in the general SLE population. Only 1% of Caucasian SLE patients have C2 deficiency[36].

Alternative pathway (C3, fH, fI), and membrane-bound RCA deficiencies that are caused by GPI anchor deficiency are associated with renal and hematological manifestations (Table 1.5). No firm associations between

autoimmune diseases and TP, properdin, fB, or fD deficiencies have been found[36].

Almost all complement deficiencies, with the exception of those of membrane-bound and TP regulators of complement activity, are associated with an increased risk of infections. The infections are commonly invasive and caused by encapsulated bacteria[119]. Those with AP and early CP component deficiencies often manifest at an early age. Late CP and TP deficiencies may manifest only after the patient has reached adulthood[119]. Mostly neisserial infections are seen in properdin, TP, and fB deficiencies. Isolated strains of meningococci often belong to the less common serotypes Y, W135, B, and C, or cannot be typed. A deficiency of any of the TP components increases the risk of meningococcal disease 1000-fold. Defects leading to CR3 or CR4 deficiency cause leukocyte adhesion deficiency (LAD) type I[300].

C4 deficiencies

C4A null alleles are most often inherited as part of the conserved haplotype 8.1 (*HLA-A*1,B*8,SC01,DRB1*0301*), associated with a wide variety of autoimmune diseases[65]. In Finland, C4B nulls are most often found together with *HLA-B35** or *-B27**[203]. C4 deficiencies have mainly been associated with various autoimmune and infectious diseases[36, 202]. In many of the diseases studied, other MHC-genes may also be involved and the relative risks or odds ratios may be stronger for other MHC antigens (Table 1.6)[332].

C4A, C4B, C2, and MBL are functionally related, being active in CP and LP activation. Interestingly, overlapping associations with human polygenic diseases have been suggested for their genetic deficiencies (Table 1.6). Disease associations with C4 deficiencies have frequently been disputed, based on studies with varying selection criteria from different general or patient populations[48, 74, 112, 117, 305]. Yet there are marked similarities in the described associations with deficiencies in these four complement genes, examples of which are listed in Table 1.6[170, 361]. This may point towards functional significance of complement deficiencies in the etiopathogenesis of these diseases. The most consistent association of CP deficiencies (e.g. C4, C2) is with immune complex diseases, especially with SLE (which is also associated with MBL deficiency)[36]. Repeatedly, C4 deficiencies have been associated with diseases where an association with C2 or MBL deficiency or both has also been described (Table 1.6). Of infectious diseases, invasive or respiratory bacterial infections caused by encapsulated bacteria (*S. pneumoniae*, *H. influenzae*, *N. meningitidis*) and chronic viral infections (hepatitis B, hepatitis C, human immunodeficiency virus) seem to be associated with CP and LP factor deficiencies[48, 74, 112, 117, 145, 170, 230, 269, 305, 361]. Notably, in the available studies partial C4 deficiencies and MBL and C2 deficiencies mainly modulate disease progression, and may thus require other minor deficiencies in immunity to manifest clinically[169].

Table 1.5 Genetic deficiencies and gene polymorphisms of complement proteins

Protein	Coding gene polymorphism[1]	Human deficiency described	Inheritance in deficiency	Published cases / frequency	Notes and disease association
C1q	-	+	AR	≈ 40	A/B/C chain deficiency: SLE (93%), glomerulonephritis, bacterial, viral infections.
C1r	+	+	AR	> 20	Mostly with C1s deficiency. SLE, bacterial infections.
C1s	-	+	AR	3	Selective C1s deficiency rare. For both types SLE, bacterial infections.
C4	+	+	AR	33	SLE (75%), glomerulonephritis, bacterial infections.
C2	+	+	AR	≈ 1/20 000	Invasive infections, SLE (10-25%), collagenosis, AS. ARMD: PP and SP
C3	+	+	AR	27	Pyogenic infections, MPGN, other glomerulonephritis.
C5[2]	+	+	AR	36	*Neisseria* infections.
C6[2]	+	+	AR	> 80	*Neisseria* infections, also with C7 deficiency.
C7[2]	+	+	AR	> 70	*Neisseria* infections, also with C6 deficiency.
C8[2]	αγ / β	+	AR / AR	> 70	*Neisseria* infections.
C9[2]	+	+	AR	regional	*Neisseria* infections, common: Japan (1/1000), Korea. Often asymptomatic
Factor B	+	+	AR	1	*Neisseria* infections. PP, susceptibility polymorphisms in ARMD.
Factor D	-	+	AR	7	*N. meningitidis* infections.
Properdin	-	+	XL	94	Types 1/2/3 exist, with absent/low/nonfunctional protein. *N. meningitidis* infections.
MBL[3]	+	LOF	AR	16%	High regional variability, 7 haplotypes. See table 6 for symptoms.
MASP-2	+	+	AR	1	1 case with SLE and bacterial infections. Estimated frequency 1.5/10000.
sMAP/MAp19	+	-	-	-	-
C1inh	+	+	AD	> 10 000	Hereditary angioedema.
α2-macroglobulin	+	-	-	-	-
C4bp	+	+	AR	3	Unknown.
Factor H	+	+	AR	22	Infections, MPGNII, aHUS, fTTP. SP in ARMD, aHUS > 60 cases
Factor I	+	+	AR	39	Bacterial infections, aHUS, vasculitis, also SP in 3 cases
Vitronectin	+	-	-	-	-
Clusterin	+	-	-	-	-
Carboxypeptidase N	-	partial	AR	1	Recurrent angioedema
Carboxypeptidase R	+	-	-	-	-
DAF (CD55)	+	+	AR	7 / (> 1000)	No symptoms / PNH, thrombosis in GPI anchor defect
MCP (CD46)	+	-	-	0 (12)	(Susceptibility polymorphisms in aHUS patients)
Protectin (CD59)	-	+	AR	1 / (> 1000)	Hemolytic anemia, thrombosis / PNH, thrombosis in GPI anchor defect.
CR1 (CD35)	+	-	-	-	-
CR2 (CD21)	+	-	-	-	-
CR3 (CD11b/CD18)	+	+	AR	1	CR3 deficiency: SLE. CD18 deficiency: LAD type I
CR4 (CD11c/CD18)	+	-	-	-	CD18: LAD type I

Only those with reported gene polymorphisms or deficiencies shown. [1]For gene locus see tables 1 and 2. [2]Every 1 in 10 000 may be deficient in any of these components. [3]Frequency of homozygous and compound heterozygous deficiency in Finland shown. Promoter polymorphisms leading to decreased levels are also common. AR = autosomal recessive, SLE = systemic lupus, AS = atherosclerosis, PP = protective polymorphism, SP = susceptibility polymorphism, ARMD = age-related macular degeneration, MPGN = membranoproliferative glomerulonephritis (type I or II), XL = X-linked, LOF = loss of function, AD = autosomal dominant, aHUS = atypical hemolytic uremic syndrome, fTTP = familial thrombotic thrombocytopenic purpura, PNH = paroxysmal nocturnal hemoglobinuria, LAD = leukocyte adhesion deficiency. Modified from refs.[300, 383].

Table 1.6 Suggested disease associations of C4 deficiencies

Disease	Type of C4 deficiency	Association with C2 or MBL polymorphism?	Examples of other MHC genes associated with the disease
Inflammatory diseases			
Atherosclerosis[1]	*C4A, C4B*	*C2, MBL2*	*LTA*
Autism[1]	*C4B*	-	*DRB1*0401*
Autoimmune hepatitis[1]	*C4A*	-	*DRB1*0301;DRB1*0401*
Behcet's disease[1]	*C4A*	*MBL2*	*B*5101;MICA*
Capillary leak syndrome during cardiac surgery	*C4A*	-	-
Diabetic nephropathy[1]	*C4A*	*MBL2* (high production)	*MICA*
Glomerulonephritis[1,2]	*C4*	*C2*	*A2,B5,DR5;B65,DR1*
Henoch-Schönlein purpura[1]	*C4B*	*C2*	*B*35*
Inclusion body myositis[1]	*C4A, C4B*	-	*B*8,DRB1*0301;B*35, DRB*0101*
IgA nephropathy	*C4B*	*MBL2* (high production)	*DQB1*0602* (protection)
Liver cirrhosis of varying etiology[1]	*C4B*	*MBL2*	
Multiple sclerosis, relapsing-remitting[1]	*C4A*	*C2*	*DRB1*15;TNF*
Photosensitivity[1]	*C4A*	-	*B*8,DRB1*0301;DRB1*15*
Primary biliary cirrhosis[1]	*C4A*	*MBL2*	*DRB1*08, HLA-DPB1*
Sarcoidosis[1]	*C4B*	-	*HLA-G**
Severe rheumatoid arthritis[1]	*C4B*	*MBL2*	*DRB1*;TNF*
Sjögrens's syndrome[1]	*C4A*	-	*DRB1*15;DRB1*03;TAP2**
Spontaneous abortion[1]	*C4B*	-	*HLA-E*;G**
Systemic lupus erythematosus[1]	*C4A, C4B, C4*	*C2, MBL2*	*B*8,DRB1*0301*
Systemic sclerosis[1]	*C4A,C4B*	-	*B*8,DRB1*0301;DR5;TAP1 ;TAP2*
Infections			
Hepatitis B, serologic response[1]	*C4A*	*MBL2*[3] (severity)	*DRB1*03;DRB1*07; DRB1*14;TNF*
Hepatitis C, severity[1]	*C4B*	*MBL2*	*DRB1*0701;HLA-DRB4*0101*
Human immunodeficiency virus, progression[1]	*C4A, C4B*	*MBL2*	*B*35-Px group; (B* 27 protective)*
Invasive infections by encapsulated bacteria	*C4B*	*C2, MBL2*	*A3* (recovery)
Leprosy, erythema nodosum[1]	*C4B*	*MBL2*[4]	*DRB1*15;TNF*
Paracoccidioidomycosis[1]	*C4B*	-	-
Subacute sclerosing panencephalitis (measles)[1]	*C4A*	-	-

[1]Refers to ≤1 isotype genes, otherwise refers to total isotype / total C4 deficiency. [2]Includes various histological diagnoses, e.g. membranoproliferative and membranous glomerulopathy). [3]Associated with persistence of the disease. [4]Associated with protection from the lepromatous form. MICA = major histocompatibility complex class I chain-related A gene.

5.1.4 Laboratory assessment of complement

If MAC is assembled on the cell membrane of red blood cells (RBC), lysis will occur. This phenomenon is used in the classical methods to assess the functional activity of CP (CH50), AP (AH50), and LP (CH50MBL). CH50, AH50, and CH50MBL refer to the reciprocal serum dilutions required to produce 50% hemolysis of a standard preparation of antibody-sensitized sheep, rabbit, or mannan-coated sheep RBC, respectively. During the measurement of AH50, CP activation is inhibited by chelating calcium ions from the serum. Controls and standard conditions are vitally important, because the CH50 unit obtained depends on the amount and type of the antibody used[247].

Alternatively, serum diluted together with blockers of the other two pathways is added to ELISA plates coated with a CP (e.g. IgM), AP (LPS), or LP activator (mannan). The appearance of C5b-C9 is detected with a labeled antibody to the neoantigen expressed during MAC formation[247]. Measuring all three pathways side by side allows a presumptive diagnosis of the probable complement factor(s) deficient from the patient's serum sample[323].

The extent of complement activation can be measured by assaying complement activation products (e.g. C4d) or stable macromolecular complexes (e.g.C1s-C1inh, C3bBbP, C5b-C9) produced during complement activation[247]. Novel assays measuring cell-bound complement activation products are under development.

Individual plasma components of complement (e.g. C3, C4, C1inh) can be quantified by with immunochemical assays. C1inh-deficient patients may have measurable C1inh without function, and so it is necessary to also perform a functional assay. When complement deficiencies of selected complement factors (e.g. C1q, C1inh) are suspected, it may be necessary to assay complement autoantibodies (e.g. C3 nephritic factor) in order to exclude secondary complement deficiencies. Flow cytometry with monoclonal antibodies against complement receptors can be used to detect their presence in cell suspensions[247].

5. 2 Antibody-mediated immunity

More than 90% of all primary immunodeficiencies are caused by defects in B cell-dependent humoral immunity[248]. These lead to low or absent serum Ig concentrations, or to qualitatively impaired antibody responses (Table 2.1, Fig. 2.1)[54]. Severe primary antibody deficiencies are caused by genetic or acquired defects in B cell maturation and activation[248]. Low IgG subclass levels are mainly caused either by a heterozygous carriership of gene deletions in the corresponding heavy chain gene, or more commonly by a dysfunctional, cytokine-regulated switch recombination that is partly allotype-linked (Fig. 2.2)[140]. To understand these better, it is of paramount importance to acquire insight into B cell development and regulation.

Not all immunodeficiencies associated with decreased Ig levels are classified as antibody deficiencies. Defects affecting early lymphocyte differentiation before commitment to B and T cells, such as adenosine deaminase deficiency, lead to severe combined immunodeficiencies (SCIDs). Of SCID patients, mostly those with a decreased number of CD4+ T helper (Th) cells have hypogammaglobulinemia[248]. In addition, hyper-IgM syndromes (HIGM) types 1 and 2 with early defects in class switching (Fig. 2.2) that disturb the CD40L-CD40 costimulatory activity may also be classified as combined immunodeficiencies[249]. Circulating B cells may be absent or immature, and an impaired B cell function is found in most SCID, HIGM1 and HIGM2 patients[248, 249].

5.2.1 B cells

The human body contains ~ 2 x 10^{12} T and B lymphocytes. Both cell types are morphologically similar with a dominant nucleus and very little cytoplasm, but express different cell surface molecules. In adults, B cells originate from the hematopoietic bone marrow stem cells, migrate to secondary lymphoid organs (spleen, lymph nodes, Peyer's patches, mucosal surfaces, tonsils, appendix) and produce antibodies[53]. Each B lymphocyte carries ~ 10^5 membrane-bound antibody molecules that determine the unique specificity (idiotype) of its B cell receptors (BCRs)[174]. B cells expressing the same BCR idiotype together form a B cell clone that is committed to synthesize antibodies of the same specificity. An individual's serum contains, as an estimate, between 10^5 and 10^7 idiotypes[53].

Table 2.1 Congenital immunodeficiencies manifesting primarily as antibody deficiencies

Deficiency	B cell numbers	Decreased Ig	Associated features	Inheritance	Gene	Chromosome
Severe reduction of Ig and absent B cells, neutropenia before initiation of therapy relatively common. Manifest at an early age (adult-onset disease in XLA possible).						
BTK	Profoundly decreased or absent	Mostly all isotypes	Severe bacterial infections, occasionally malignancies, MPGN	XL	*BTK*	Xq21.33-q22
μ heavy chain	Absent	All isotypes	Severe bacterial infections	AR	*IGHM*	14q32.33
λ5	Profoundly decreased or absent	All isotypes	Severe bacterial infections	AR	*IGLL1*	22q11.23
Igα	Absent	All isotypes	Severe bacterial infections, dermatomyositis	AR	*CD79A*	19q13.2
BLNK	Profoundly decreased or absent	All isotypes	Severe bacterial infections	AR	*BLNK*	10q23.2-q23.33
LRRC8	Absent	All isotypes	Minor facial anomalies	de novo	*LRRC8A*	9q34.11
Severe reduction in ≥ 2 isotypes with normal or low B cells. Adult-onset disease common.						
CVID	Normal or decreased	IgG, IgA +/- IgM	Recurrent bacterial infections +/- autoimmunity +/- malignancy[1] +/- granulomatous disease	Variable	Unknown	Linked: 6p21.3; 4q; 5p
ICOS	Normal or decreased	IgG, IgA +/- IgM	Recurrent bacterial infections +/- autoimmunity +/- malignancy	AR	*ICOS*	2q33
CD19	Normal	IgG, IgA +/- IgM	Recurrent bacterial infections, postinfectious glomerulonephritis	AR	*CD19*	16p11.2
TACI	Normal or slightly reduced	IgG, IgA +/- IgM	Recurrent bacterial infections +/- autoimmunity +/- malignancy[1]	AD / AR	*TNFRSF13B*	17p11.2
BAFF receptor	Normal or decreased	IgG, IgA +/- IgM	Recurrent bacterial infections	AR	*TNFRSF13C*	22q13.2
Hyper-IgM syndromes with severe reduction in IgG and IgA levels with increased IgM levels and normal numbers of B cells, classified as primary antibody deficiencies						
AID	Normal	IgG, IgA	Severe bacterial infections, enlarged lymph nodes and germinal centers	AR	*AICDA*	12p13
UNG	Normal	IgG, IgA	Severe bacterial infections, enlarged lymph nodes and germinal centers	AR	*UNG*	12q23-q24.1

Isotype or light chain deficiencies with normal numbers of B cells						
Ig heavy chain	Normal	IgG1/IgG2/IgG4 +/- IgA1/IgE	Homozygous deficiency causes absent serum (sub)class(es), heterozygous deletion a 50% reduction. Might be asymptomatic.	AR	respective *IGHG*	14q32.33
κ chain	Normal	All Igs have λ chain	Might be asymptomatic or have recurrent viral and bacterial infections	AR	*IGK@*	2p12
Isolated IgG subclass	Normal	≥1 IgG subclass	Often asymptomatic, may have recurrent bacterial (IgG2, IgG1) or viral (IgG1, IgG3) infections	Variable	Unknown	Linked: 6p21, 14q32.22, 2p12
IgA with IgG subclass	Normal	IgA + ≥1 Ig subclass	Might be asymptomatic, have recurrent infections +/- poor antibody response to carbohydrate antigens +/- allergies, autoimmune diseases. Some cases progress to CVID, some exist with CVID in the family	Variable	Unknown / *TNFRSF13B* mutations	linked: 6p21, 14q32.22, 2p12
Selective IgA	Normal	IgA				
Specific antibody deficiency	Normal	Normal	Inability to make antibodies to specific antigens. Commonly asymptomatic. Severe cases have similar complications to those seen in CVID.	Variable	Unknown	Linked: 14q32.22, 2p12
Transient	Normal	IgG, IgA	Recurrent moderate bacterial infections, only in infants below 36 months of age. Normal vaccination responses.	Variable	Unknown	Linked: 14q32.22

[1]Most commonly lymphoproliferative diseases. For abbreviations, see Fig. 2.1. Modified from refs.[55, 73, 248, 249].

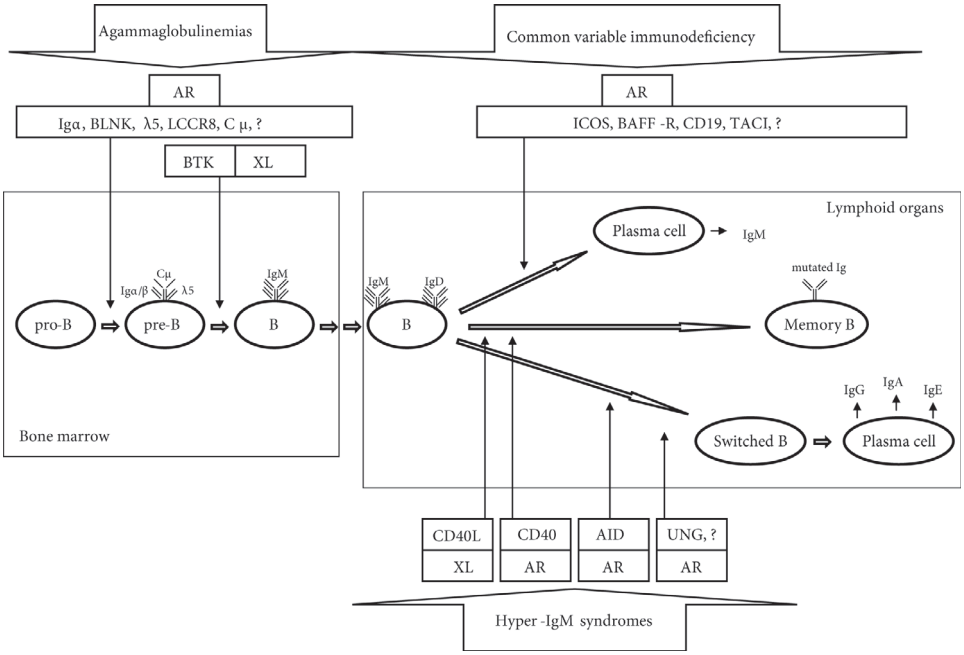

Figure 2.1 Abnormalities of B cell maturation leading to severe immunoglobulin deficiencies

AR = autosomal recessive, XL = X-linked, Cμ = IgM heavy chain, Igα/β = B cell receptor-associated Igα/β heterodimer (CD79a/CD79b) responsible for intracellular signaling, λ5 = V pre-B pseudo-light chain, BLNK = B cell linker, BTK = Bruton's tyrosine kinase, ICOS = inducible T-cell costimulator, CD19 = BCR-associated co-receptor, BAFF-R = B-cell activating factor belonging to the TNF receptor family, TACI = transmembrane activator and calcium-modulator and cyclophilin ligand interactor, CD40 = signaling molecule of the TNF receptor family on B cells, CD40L = CD154, CD 40 ligand on activated T cells, AID = activation-induced cytidine deaminase, UNG = uracil-N-glycosylase. Modified from ref.[107].

B cell development and immunoglobulin gene recombination

The earliest B cell precursors (pre-pro-B cells) express the B cell-specific form (B220) of the leukocyte common antigen CD45, and leucosialin (CD43)[63, 141]. Pre-pro-B-cells have little or no Ig gene rearrangement[141, 214]. During maturation, through targeted and regulated alterations of genomic structure and sequence, variable (*V*) regions of antigen receptors are constructed by gene element recombination (Fig. 2.2)[343]. This takes place between *V*, diversity (*D*), and joining (*J*) elements of Ig heavy chain gene (*VDJ* rearrangement at *IGH@* in chromosome 14q32.33) and between *V* and *J* elements of the Ig light chain genes (*VJ* rearrangement at *IGL@* in 22q11.1-q11.2 and at *IGK@* in 2p12). This creates the necessary repertoire of idiotypes needed for effective defense against pathogens[108, 214, 343]. Superimposed on this, BCRs are further diversified by a factor of 10-100 by insertion of extra *N* (non-templated) or *P* (palindromic) nucleotides at the *V(D)J* junctions[53].

The Ig receptor also has a limited set of conserved effector regions coded by constant region (*C*) gene segments. Altogether *IGH@* encodes more than 100 *V*, 27 *D*, six *J*, and nine *C* genes[253]. *V(D)JC* gene segment combinations are the basis for the generation of heavy chains and light chains, as well as class switching during B cell activation (Fig. 2.2)[53, 214].

IgG1, IgA1, IgG2, IgG4, IgA2, and IgE (sub)class deficiencies may be caused by homo- and heterozygous *C* gene deletions in *IGH@* (Table 2.1). These may cover one or more genes neighboring each other. Multiple types of deletions, created either by non-equal homologous recombination or by looping-out excision, have been described in the Caucasian population[262]. The total frequency of these is ~ 0.015. Heterozygous deletions of *Cγ1*, *Cγ2*, and *Cγ4* are associated with a ~ 50% reduction in the serum level of the affected subclass, causing partial IgG subclass deficiency (SCD). Homozygous deletions that result in complete absence of the corresponding subclass are rare (one per 5000-10000 subjects in the general population)[262]. Subjects with a complete deficiency of any of these IgG subclasses are – paradoxically – usually asymptomatic[192].

Gene rearrangements first take place at *IGH@* during pro-B cell-stage and, starting from the pre-B cell-stage, further at the Ig light chain loci[141, 214]. Pre-B cells express the pre-BCR formed by Igα/β, heavy chain of IgM (Cμ) and by a pseudo-light chain (V pre-B/λ5)[53, 174]. This maturation step is antigen- and T cell-independent and takes place in the bone marrow. Pre-B cells further mature into immature B cells expressing a BCR of IgM isotype. Immature B cells then emigrate to the secondary lymphoid organs, where maturation of the antibody repertoire takes place. In severe antibody deficiencies, the genetic blocks in normal B cell maturation take place from the pro-B stage on (Table 2.1, Fig. 2.1)[73, 86, 107, 249].

To eliminate precursors that fail to produce a complete heavy or light chain, and to avoid self-reactivity, the production of B cells is tightly controlled[63, 141]. Only 10% of the produced immature B cells are exported to the periphery[53, 63]. B cell tolerance, leading to the inability to respond to antigenic stimuli, is achieved both by central tolerance in the bone marrow and by peripheral tolerance in the secondary lymphoid organs. When immature B cells encounter self-antigens in the bone marrow, they undergo negative selection. If a self-reactive V gene is encountered, B cells first try to replace it with a new V gene (receptor editing). Those with high affinity interactions between BCR and self-antigen are clonally deleted, those with low avidity to self-antigen can become unresponsive (anergy)[53]. Autoimmune phenomena are commonly found in humoral immunodeficiencies (Table 2.1)[96, 337].

Immature B cells that leave the bone marrow and enter the spleen can be divided (based on differential surface expression of B220, AA4, IgM, and CD23) into type 1 (T1), T2, and T3 transitional cells that further mature into follicular (B-2), marginal zone (MZ, a subset of B-2), and B-1 B cells[53].

Mature, unswitched B cells co-express IgM and IgD (Fig. 2.1)[53, 141, 214]. In the spleen, T1 B cells with high-affinity BCR for blood-borne self-antigens are negatively selected. Thereafter, T2 B cells moving into follicles proliferate (positive selection). Negative selection is an antigen-dependent event, whereas positive selection requires antigenic and non-antigenic signals such as B-cell factor belonging to TNF family (BAFF) signaling[53].

Both MZ and B-1 B cell maintenance requires BCR signaling[53, 141]. MZ B cells are located exclusively in the splenic MZ. They are fully formed only after two years of age and are selectively maintained during decreased B cell output from the bone marrow (e.g. aging, bone marrow failure). It is unknown whether MZ B cells arise from transitional or follicular B cells. MZ B cells provide a first line of defense against blood-borne pathogens and may not typically contribute significantly to the germinal center (GC) reaction[8]. The slow development of MZ B cells may contribute to the susceptibility of newborns to encapsulated pathogens[141].

B-1 cells (CD23-B220+IgM^{hi}IgD^{lo}) dominate in the newborn spleen. Found in large numbers in the tonsils and peritoneal cavity, they comprise a minor subset (3-7%) of adult splenic B cells[53]. In aged subjects, B-1 cell numbers increase again. All peritoneal B-1 cells express macrophage-specific surface molecule Mac-1. Almost all B-1 cells in the spleen and the majority of those in the peritoneum express the T cell differentiation antigen CD5[137, 141]. B-1 cells are further divided into B-1a (CD5+Mac-1+IgM+) and B-1b (CD5-Mac+1-IgM+) cells[53, 137]. B-1 (especially B-1a) cell repertoire is more restricted than that of B2 cells due to limited usage of variable heavy chain (V_H) genes and of N-region addition during *VDJ* rearrangement[141]. B-1 cells are involved in responses against T cell-independent antigens, in the production of so-called natural antibodies, and in autoimmune responses[149, 215].

5.2.2 B cell activation and antibody production

The B cell receptor and the respective antibodies produced after B cell activation recognize antigenic determinants (epitopes) formed by any structure of the antigen that is accessible to solvent[389]. When the BCR of a resting B cell recognizes an antigen, clonal expansion by cell division and differentiation into Ig-producing plasma cells or maturation to memory B cells may ensue. The early events of primary B cell activation are initiated by the BCR. It consists of membrane-bound Ig (mIg, IgM and/or IgD) together with a noncovalently associated disulfide-linked heterodimer of Igα (CD79a) and Igβ (CD79b) that mediates the intracellular signaling pathways[174]. The developmental stage and the context, in which BCR is crosslinked by an antigen, determine whether proliferation, differentiation, anergy, or apoptosis follows. Signals from other receptors on B cell surface (e.g. complement receptors, Toll-like receptors, cytokine receptors) profoundly influence B cell activation and antibody

production[149, 190, 262]. After antigen binding, BCR is internalized and targets antigen for processing. The multiprotein signaling machine (signalosome) mediating early signaling events is relatively well characterized and includes, among others, Bruton´s tyrosine kinase (BTK) and B cell linker protein (BLNK). Further downstream, CD19 forms a complex with CD21, CD81, and CD225 in the membrane of mature B cells. This complex, signaling together with BCR, signals the B cell to decrease its threshold for activation by the antigen. Genetic deficiencies of *CD79A*, Bruton´s tyrosine kinase, and B cell linker protein cause agammaglobulinemia, whereas *CD19* deficiency causes a common variable immunodeficiency (CVID) phenotype (Table 2.1, Fig. 2.1)[73, 86, 174].

BCRs and the respective antibodies recognize antigenic epitopes through their antigen-binding sites (paratopes). Paratopes are highly solvent-exposed structures located at the tip of each Fab arm (formed by the variable regions of light and heavy chains, see below). They are formed by six hypervariable polypeptide loops called complementarity-determining regions (CDR). Three CDRs from the variable light chain region (CDR-L1-3) and three from V_H (CDR-H1-3) exist, of which at least four are used to bind an antigen (Fig. 2.3). These generate a spatial surface (e.g. concave or groove-like) that determines the antigen specificity of the antibody[108, 389, 390]. Mainly H3, H2, and L3 participate in the binding. H3 displays the greatest variability and seems to be the most crucial CDR for antigen recognition[108, 389]. Antibody affinity describes the strength of antigen-antibody bond. It is the sum of the different non-covalent attractive and repulsive forces between the paratope and the epitope[389].

Before the production of an antibody, a resting B cell becomes activated. It then expands clonally and differentiates into an antibody-secreting plasma cell. It may also mature into an (initially quiescent) memory B cell that during a subsequent encounter with the antigen becomes involved in antibody production. The secreted Ig is either its IgM receptor, or it shares the same V region but has a different C region (isotype) (Figs. 2.2, 2.3)[53].

Natural antibodies

Human serum contains polyreactive, low-affinity, mainly IgM isotype antibodies with germ line V gene configuration. These are thought to be produced by B-1a cells without previous exposure to an antigen or in response to B cell PAMP receptor signals leading to the production of so-called "natural" or "innate" antibodies (Table 2.2)[137, 215]. For example, mucosal B-1 cell stimulation by bacteria is thought to enhance the production of natural IgA antibodies at mucosal sites, whereas CR2-dependent natural IgM antibodies may be necessary for the self-recognition of ischemic self-tissues[149, 215, 293]. Natural antibodies contribute to innate immunity and offer first-line defense against invading organisms before specific immune responses[149].

Antibody responses to thymus-independent antigens

B-cells respond to three types of antigens: thymus-independent (TI) antigens types 1 and 2, and to those that are thymus-dependent (TD) (Table 2.2.)[11]. Bacterial lipopolysaccharide (LPS) is the prototypic TI-1 antigen. Some TI-1 and most TI-2 antigens are multivalent polysaccharides or other antigens with repetitive structures. These are found on clinically important bacteria causing respiratory, mucosal, and invasive infections such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Neisseria meningitidis*[11]. Polysaccharides are not degraded *in vivo* and are not recognized by the T cell receptor (TCR). They have the ability to bind directly to BCRs, crosslink them, and to activate B-1 and MZ B cells into an antibody response without Th cells[53]. Yet, for example *S. pneumoniae*-specific plasmablast reaction requires antigen capture and antigen-presenting cell (APC)-derived signals for MZ B and B-1a responses[11]. TI antigens generally fail to elicit a secondary response (which requires specific T cell help), but effective long-lasting TI IgM memory by B-1b has been described for *S. pneumoniae* (Table 2.2.)[137]. Natural antibodies and IgM+ memory B cells are required for protection against pneumococci in humans[69]. Young children, elderly subjects, and splenectomized individuals are susceptible to recurrent infections against encapsulated bacteria. Lack of CD21+ MZ B cells (CD21 is required for MZ B TI-2 responses) in the spleens of children under 2 years of age, or a lack of B-1b cells may be the basis for this susceptibility[11, 54, 137, 238]. These patients also have a limited usage of V_H genes, likewise a feature of (only) B-1a responses[3, 141]. Partly supporting this, children with clinically significant immunodeficiency and an impaired production of pathogen-specific antibodies (selective antibody deficiency, SAD) often become asymptomatic and start producing anticapsular antibodies when they become older[254]. On the other hand, asymptomatic impaired production of antibodies against pneumococci, associated with a limited usage of V_H genes and with specific IgG2 and κ light chain allotypes (see below), is common in the population[237, 238].

Table 2.2 Main characteristics of B cell responses to different types of antigens

Feature	T cell-independent antigen type 1	T cell-independent antigen type 2	T cell-dependent antigen	
			primary response	secondary response
B cell type	B-1a, B-1b	B-1, MZ	B-2	B-2
Clonality of response	Poly- or monoclonal[1]	Polyclonal[2]	Oligoclonal	Oligo-monoclonal
Signals required	BCR and PAMP receptor[1]	BCR crosslinking	Mainly APC	B cell importance ↑
Antigen concentration required	Relatively low[1]	High	Relatively high	Relatively low
Examples of microbial antigens	LPS PnP SIII *Salmonella* polymerized flagellin Vesicular stomatitis virus Rotavirus	As TI type 1[1] LP *Mycoplasma* PPD *Staphylococcus aureus* protein A	Polysaccharide Protein	Protein
Response	Rapid, short-lived[2]	Rapid, short-lived[2]	5-10 days, short-lived	2-5 days
Somatic hypermutation	No	No	Low	High
Predominant antibody isotype	IgM (natural antibodies)	IgM	IgM	Site-dependently IgG or IgA
Affinity	Low[2]	Low[2]	Low	High
Location of response	Bone marrow, GALT	Also spleen	Secondary lymphoid organs	

[1]At low antigen concentrations only specific B cells are activated, at high concentrations the response is mediated by direct engagement of multiple BCRs and becomes polyclonal. [2]Certain antigens (e.g. *Streptococcus pneumoniae, Borrelia hermsii*) can elicit a long-lasting, protective T cell-independent IgM memory by specific B-1b cells. Natural antibodies are polyreactive and of low affinity. PAMP = pathogen-associated molecular pattern, BCR = B cell receptor, LPS = lipopolysaccharide from Gram-negative bacteria, PnP = pneumococcal polysaccharide, LP = lipoprotein from Gram-negative bacteria, PPD = purified protein derivative of tuberculin, GALT = gut-associated lymphoid tissue.

Highly organized and repetitive structures on the surfaces of at least some viruses such as vesicular stomatitis, polyoma, and rotaviruses are also able to trigger specific type 1 TI responses producing high affinity IgG antibodies, but without affinity maturation. These responses are biologically important, because these viruses are exclusively controlled by antibodies, which also serve to maintain cytotoxic T lymphocyte activation[30, 31]. In addition, early TI responses facilitate and enhance the ensuing long-lived TD IgG responses[31, 32].

Antibody responses to thymus-dependent antigens

T cell-dependent (usually protein) antigens have a very limited number of epitopes and are thus less able to crosslink the BCRs to initiate direct B-cell

activation. B-cell responses also tend to remain transient and short-lived without the cognate help by CD4+ Th cells specific for the same antigen. On the other hand, most antigens recognized on viruses, bacteria, and parasites are proteins that are able to trigger Th responses[30]. Beneficially, to avoid autoimmunity, TD activation is also more tightly regulated[30, 162]. In antigen presentation, the antigen is engulfed following binding, degraded intracellularly, and the peptide produced is eventually re-expressed on the surface of the cell in association with MHC class I or II molecules. MHC II molecules are found mainly on cells of the immune system that include B cells, activated T cells, dendritic cells (DCs), macrophages and thymic epithelium. Cognate B cell activation and regulation begins with the MHC II glycoprotein on APCs presenting an antigenic peptide to a specific TCR on CD4+ Th cell[223]. Three types of APCs exist: DCs, macrophages, and B lymphocytes. Another signal relayed by the interaction of costimulatory molecules on APCs and their ligand on T cells (most notably B7-1 and B7-2 interaction with CD28) is required for T cell activation, absence of proper costimulation results in T cell inactivation[223].

DCs, as APCs located at the border zones of the body, are uniquely efficient at protein antigen uptake, processing, and presentation. They travel from the blood to the peripheral tissue to capture foreign antigens[250]. Conserved motifs on pathogens are recognized by various PAMP receptors on DCs[68, 223]. Thereafter, DCs travel to the draining lymphoid organs and prime naive CD4+ Th cells into type 1 (Th1), Th2, and other, less well characterized subgroups of Th cells[342]. Differential activation of CD11c+ DC subgroups further regulates the ensuing immune response[223]. Activated DCs upregulate costimulatory molecules (e.g. CD40, CD80, CD86, TRANCE) and chemokine receptors such as CCR7 to relocate from the inflamed sites to T cell areas of draining lymph nodes. There the interaction between the antigen-primed DCs presenting the antigen to specific TCRs on naive Th cells represents the first immunologic synapse in TD B cell regulation. When Th cells become antigen-primed, antigen-specific clonal Th cells expand extensively and differentiate into effector Th cells[223].

The specific BCRs and TCRs usually recognize different epitopes on an antigen. In TD B responses, both T and B cells need to become activated and to cooperate. This, the second immunologic synapse of B cell regulation that leads to the development of effector B cell responses, takes place as the activated B cells pass through the T cell areas of lymphoid tissue where the activated T cells are trapped[223, 389]. There a large number of cell surface molecules regulate the ensuing responses[174, 223]. For example, CD 40 is constitutively expressed on B cells and CD40L only on activated T cells. It is at this stage, and during the ensuing downstream events that deficiencies of costimulatory, regulatory, and signaling molecules that govern the BCR responses lead to severe congenital antibody deficiencies or HIGM syndromes (Fig. 2.1, Table 2.1)[249, 382].

The strength of the antibody response is further positively (e.g. by Toll-like receptors[TLR]) or negatively (e.g. by CD22, FcγRIIB) regulated by contact-mediated and soluble factor-mediated signals[174, 190]. Activated B cells then migrate to splenic medullary cords and differentiate first to plasmablasts, and then into short-lived, terminally-differentiated plasma cells that secrete IgM or IgG during the primary response (Table 2.2). Alternatively, the activated B and Th cells migrate together into the B cell areas of lymphoid tissue and enter the primary follicles[389].

5.2.3 Somatic hypermutation, affinity maturation, and class switching

In primary follicles, B cells begin to form GCs. GC reaction regulates antigen-specific clonal evolution during the development of B cell memory (the third immune synapse of B cell development)[223]. Compared with a primary response, typical features of a secondary TD response are class switching, somatic hypermutation, and affinity maturation (Table 2.2, Fig. 2.2). During GC reaction, activated B cells displace resting follicular B cells while rapidly and clonally expanding to become centroblasts (sIgD-sIgM^{lo})[160]. Somatic hypermutation occurs during this rapid proliferative phase, when a large number of mutations in the V_H genes occur (Fig. 2.2)[214]. Somatic hypermutation is induced by targeted DNA deamination by the B-cell-specific activation-induced cytidine deaminase (AID). Its effects are counterbalanced by a B-cell-specific reparatory enzyme uracil nucleoside glycosylase (UNG). Genetic mutations in their genes result in HIGM syndromes (Fig. 2.2)[214, 249].

During affinity maturation, V_H mutations may result in centroblasts expressing specific antibody of either lower or of higher affinity (Fig. 2.2)[160, 214]. Centroblasts then further differentiate into centrocytes that begin to upregulate their surface Igs (sIg). Thereafter, centrocytes with high affinity sIgs interact with antigen bound by FDCs (via their complement or Fc receptors), and subsequently with Th cells. Those with lower affinity sIgs undergo apoptosis[160, 223, 389]. Repeated exposure to the same antigen thus results in the production of antibodies that bind, and protect from, pathogenic microbes more efficiently[223, 389]. Impaired affinity maturation is found in humoral immunodeficiencies with impaired class switch recombination or somatic hypermutation (HIGM syndromes) and in some subsets of CVID[106].

During class switching, the antibody-secreting plasma cells switch the C region of the secreted antibody whereas the V portion (and specificity) remains unchanged. This is achieved by class switch recombination events, when the Vμ is joined to a Cγ, Cα, or Cε present in the respective isotype IgG, IgA, or IgE (Fig. 2.2). Which isotype is generated is influenced by a number of factors, including the antigen composition, infection site, and the route of immunization. Class switching is strongly influenced by the dose of the

antigen, by Th-derived cytokines provoked by the infecting agent, and requires CD40L signaling[53, 342]. Defects at the stage of CD40L-CD40 signaling cause combined immunodeficiencies. Deficiency of CD40L results in the classical form of X-linked HIGM syndrome (X-HIGM). CD40 deficiency causes an autosomal recessive disease; this has a phenotype that is indistinguishable from X-HIGM (Fig. 2.1)[214, 249]. CVID phenotype is also shared in the deficiencies of two B and T cell surface-signaling molecules: B-cell-activating factor belonging to the TNF receptor family (BAFF-R) and transmembrane activator and calcium-modulator and cyclophilin ligand interactor (TACI). These participate in isotype switching; deficiency of the latter is the most common known genetic cause of primary hypogammaglobulinemia, found in as many as 10-20% of "idiopathic" Caucasian CVID patients[73].

Cytokines are able to regulate germ line transcription at the *IGH@*. In humans, Th1 cytokines interleukin (IL)-2, interferon (IFN) γ, LTα, and IL-12 are generally thought to promote cell-mediated immunity and the switching to opsonizing antibody subclasses IgG1 and IgG3, whereas Th2 cytokines IL-4, IL-13, and IL-10 would suppress cell-mediated immunity and promote the class switch to IgG2, IgG4, and IgE[53, 148, 160, 342]. However, in experimental conditions *in vitro*, the Th2 cytokines IL-4 and IL-10 may in the presence of CD40L stimulation induce IgG1 and IgG3 switching. Likewise, IL-12 and IFNγ (Th1 cytokines) may promote switching to IgG2. Interestingly, the "anti-inflammatory" cytokines transforming growth factor (TGF)-β and IL-10 promote switching to IgA, which in its circulating form may also have anti-inflammatory actions[53, 97, 262]. IgA deficiency is associated not only with susceptibility to infections, but also with autoimmune and inflammatory complications such as celiac disease[97, 109]. A costimulatory T cell molecule, inducible T-cell costimulator (ICOS), strongly induces IL-10 production and also increases the secretion of IL-4, IL-6, and IL-6. Mutations in inducible T-cell costimulator have been found in ~ 1% of CVID patients (Table 2.1)[73].

5.2.4 Immunoglobulin allotypes

Variations in subclass levels are associated with Ig allotypes[326]. Allotypes are genetic variants of Ig subclasses and light chains (Table 2.3). Though the exact mechanism is insufficiently characterized, different allotypes are associated with differing numbers of B cells producing the particular allotype. This has commonly been attributed to variant efficiencies of the class switch regions of allotypic genes (Fig. 2.2)[221, 257]. Altogether at least 28 Mendelian Ig allotypes defined at the protein level occur (Table 2.3)[257]. Two different, alphameric and numeric, nomenclatures for results obtained by serologic testing are commonly being followed in the literature. The precise designation given to an Ig allotype can be highly variable and complex. An allotype of an Ig molecule is recognized by the expression (e.g. *G2m*^{n}) or

nonexpression (e.g. *G2m*$^{n-}$) of a unique epitope (allotope)[134]. The heavy chain class or the light chain type (κ) is designated by a capital letter, with either a small "m" (Km) or a capital "M" (KM) standing for "marker". The appropriate subclass is often, but not always, shown by the inclusion of the appropriate numeral (e.g. G1m, G3m). Yet italics, superscripts, numeric or alphameric nomenclatures or both, and capital letters are used variably. Thus, the common Caucasian allotype *G1m*^{a} can have multiple designations, for example "G1m(1)", "GM a", or "G1ma(1)". Depending on the antibodies used for typing, these may be unable to differentiate two known allotypes from each other, e.g. *G1m*$^{a/a(x)}$ includes allelic markers "a(1)" and "x(2)" of IgG1 that both give a positive reaction with an antibody against *G1m*^{a}. When additional serotyping with anti-x has not been performed, *G1m*$^{a/a(x)}$ is used (Table 2.3)[134, 257, 390]. At the gene level, even further polymorphism can be recognized within an allotype[154].

Table 2.3 Human antibody allotypes

Gene locus	*IGHG3*	*IGHG1*	*IGHG2*	*IGHA2*	*IGK@*
Designation	G3m	G1m	G2m	A2m	Km
Allotypes	g1(21), g5(28), b0(11), b1(5), b3(13), b4(14), b5(10), s(15), t(16), c3(6), c5(24), u(26), v(27)	a(1), x(2), f(3), z(17)	n(23), n-(23-), ny	1, 2, 3	1, 2, 3

Alphameric designation is shown first, followed by numeric designation in brackets. Modified from refs.[134, 154, 390].

In Caucasians, allelic variants of *IGHG3*, *IGHG1*, and *IGHG2* of heavy chain genes form four predominant, tightly-linked genetic haplotypes (frequencies in Finns in parenthesis) : *G3m*^{b},*G1m*^{f},*G2m*^{n} (0.380); *G3m*^{b},*G1m*^{f},*G2m*$^{n-}$ (0.224); *G3m*^{g},*G1m*$^{a/x}$,*G2m*^{n} (0.017); *G3m*^{g},*G1m*$^{a/x}$,*G2m*$^{n-}$ (0.379) (Fig. 2.2)[257, 326]. *G3m*^{g} and low IgG3, as well as *G1m*^{f} and low IgG1 are associated[262, 326]. *G3m*^{g} is also marginally less efficient to activate complement than *G3m*^{b}[85]. If testing includes other *IGHG* allotypic loci (*Am, Em*), a more extended haplotype may be reported[221, 257]. Patients suffering from symptomatic SCDs commonly manifest together with an increased level of some other (sub)class[192]. Accordingly, the carriership of a haplotype may be associated with multiple Ig concentrations and functions that deviate from the mean. For example, in Caucasians homozygosity for the haplotype *G3m*^{g},*G1m*$^{a/x}$,*G2m*$^{n-}$, *A2m*^{1} is, at the population level, associated with lower mean concentrations of IgG3 and IgG2, less efficient complement activation by IgG3, poor anti-polysaccharide responses, and higher concentrations of IgG1 and IgA2. In addition, the increase in IgG2 concentrations is enhanced but those of IgG1 retarded after birth in the homozygotes[27, 221, 238, 259, 326].

In subjects with low subclass levels, it is common to find a simultaneous

low level of another (sub)class coded by the neighboring gene(s) (e.g. IgG1 and IgG3 or IgA1, IgG2, and IgG4)[27, 192]. Yet the effects of the above-mentioned common Caucasian *IGH@* allohaplotypes on IgG1 and IgG3 serum concentrations are invariably reciprocal (Fig. 2.2)[326]. The allohaplotype of an individual thus explains low subclass levels only in a subset of individuals. Subclass switching is clearly further regulated by other activating signals and genetic differences between individuals than those tested thus far. The local cytokine milieu in lymphatic tissue strongly affects the produced antibody (sub)class[128, 262]. Deficiencies in one arm of immunity may also be asymptomatic, because another arm of immunity is able to compensate[169]. To manifest clinically, low subclass levels probably require additional genetic, regulatory, or structural defects[262].

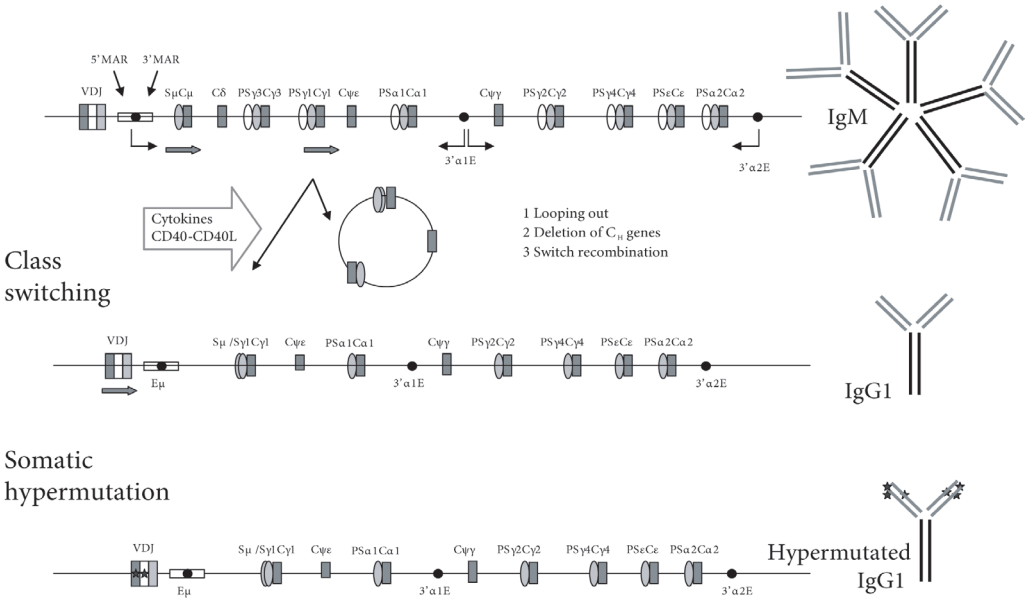

Figure 2.2 Schematic representation of immunoglobulin heavy chain locus in chromosome 14q32.33 during class switching and somatic hypermutation

Promoter regions (P) shown only on the first line. During switch recombination a rearranged and expressed heavy chain variable region (VDJ) is joined to a new downstream constant region (C). Junctions form within switch regions (S). Arrows denote V and S region activation by transcription. Somatic hypermutation alters V sequence (stars). MAR = matrix attachment region, Eμ = intronic enhancer, 3'α1 and 3'α2E = downstream enhancers. ψ = pseudogene. Modified from refs.[214, 262, 389].

5.2.5. B cell memory

During affinity maturation, centrocytes with high affinity sIg move to the edge of the GC and interact with specific Th cells expressing CD40L. Provided that B cells receive the necessary costimulatory signals, they are positively selected and may move back to the dark zone of GC. If again positively selected, they proliferate further. High affinity centrocytes may undergo several rounds before they terminally differentiate into either antibody secreting plasma cells or enter the long-lived memory B cell compartment[223]. Maintenance of serum antibody levels and long-lived B cell memory is tightly controlled, mainly by regulating the number of antibody-secreting cells[215]. The half-time of circulating IgG is exceptionally long. Like other plasma proteins, Igs constantly enter the endocytic compartment of endothelial cells via pinocytosis. Unlike other plasma proteins, IgG is bound in the acidic environment to an atypical Fc receptor (FcR), FcRn, and salvaged from proteolysis back to plasma[2]. Multiple subtypes of affinity-matured B memory cells are defined, for example, by their antibody isotype and by whether they secrete antibody or produce a rapid cellular response to antigen recall[223]. A low percentage of switched memory B cells (CD19+CD27+IgD-) in the blood seems to predict, more accurately than Ig levels, whether patients with missing specific antibody responses to polysaccharide antigens or to CVID develop bronchiectasis, splenomegaly, and autoimmunity (Table 2.1)[6, 69].

5.2.6 Antibody structure, antibody classes, and Fc receptors

Each milliliter of normal human serum contains ~ 10^{16} Ig molecules. Antibodies are glycoproteins that belong to the Ig superfamily. They serve as BCRs, circulate in the plasma and lymph, and can be found in mucosal and lymphoid tissues[207]. Their main function is to protect from extracellular pathogens[85, 207]. The main effector functions of antibodies are achieved by direct effects mediated by their Fab portions and by effector cells that become activated upon FcR binding (Fig. 2.3, Table 2.4)[85, 207]. Cross-linking of multiple FcRs on effector cells is a prerequisite to their activation[207].

The basic structure of an Ig molecule is a four-chain unit that consists of two identical, heavy polypeptide chains (HC) having four tight globular domains and two identical, light polypeptide chains that have two globular domains. IgG, the model of the basic structure, adapts a distorted, somewhat asymmetrical Y shape where chains are linked by disulfide bonds (Fig. 2.3)[310]. Two types of light chains exist, kappa (κ) and lambda (λ), shared by all Ig isotypes. In a given Ig molecule both light chains are of the same type[390]. In humans, 60% of Igs use κ and 40% use λ chains., No functional differences are known between them[390].

Table 2.4 Main human Fc receptors

Fc receptor	Affinity	Polypeptide chains	Gene location	Preference	Immune cell types that present the receptor	Effect of engagement
FcγRI (CD64)	High	3 ($\alpha\gamma_2$)	1q21.2-21.3	IgG monomers IgG1 = IgG3 >IgG4 > IgG2	MC, Mϕ, NP, myDC	ADCC, uptake, phagocytosis, DC maturation
FcγRIIa (CD32)	Low	1 (α)	1q23	IgG1 = IgG3 > > IgG4; IgG2 only by FcγRIIa-H131 allele	MC, Mϕ, NP, platelets, LC, myDC, pDC, moDC	Uptake, phagocytosis, platelet aggregation, DC maturation
FcγRIIb (CD32)	Low	1 (α)	1q23	IgG1 = IgG3 > > IgG4; IgG2	B cells, LC, myDC, pDC, moDC, mast, NP, Mϕ	Inhibition of activation and DC maturation
FcγRIIc (CD32)	Low	1 (α)	1q23	IgG1 = IgG3 > > IgG4; IgG2	MC, Mϕ, NP, platelets, NK[2]	ADCC[2], uptake, phagocytosis, platelet aggregation
FcγRIIIa (CD16)	Medium	3 ($\alpha\gamma_2$)	1q23	IgG monomers, IgG1 = IgG3	NK, Mϕ	ADCC, uptake, phagocytosis, DC maturation
FcγRIIIb (CD16)	Low	1 (α)	1q23	IgG polymers IgG1 = IgG3	NP	Regulation of phagocytosis ?[3]
FcεRI	High	$\alpha\beta\gamma_2/\alpha\gamma_2$[1]	1q23 (αγ), 11q13 (β)	IgE monomers	Mast cells, BP, LC, myDC, pDC, moDC	Secretion of granules
FcεRIIa (CD23)	Low	1	19q13.3	IgE	B cells, LC, myDC, moDC	Ig isotype modulation, antigen presentation
FcεRIIb (CD23)	Low	1	19q13.3	IgE	Eos, platelets, MC, some B and T cells, myDC, moDC	Secretion of granules, uptake, phagocytosis
FcαRI (CD89)	Medium	γ_2	19q13.2-13.4	IgA	MC, some Mϕ, NP, Eos, moDC, Kupffer cells	ADCC, uptake, phagocytosis, secretion of granules, antigen presentation

[1]Tetramer on mast cells and basophils, trimer on antigen presenting cells. [2]Only in individuals with certain allelic polymorphisms. [3]A decoy receptor, which has no signaling component, but may use the signaling machinery of other FcRs and regulate phagocytosis. FcR = Fc receptor, Ig = immunoglobulin, MC = monocyte, Mϕ = macrophage, NP = neutrophil, myDC = blood myeloid dendritic cell (DC), ADCC = antibody-dependent cytotoxicity, LC = Langerhans cell, pDC = blood plasmacytoid DC, moDC = monocyte-derived DC, NK = natural killer cell, BP = basophil, Eos = eosinophils. Secretion of granules results in the release of inflammatory mediators and cytokines. Modified from refs.[34, 47, 244, 256, 286].

The amino(*N*)-terminal globular domains are encoded by *V* genes and are responsible for antigen recognition. Their structure is identical with the respective BCR (see above)[208, 390]. The functional bivalency created by the two identical recognition sites enables cross-linking of antigens. This, for example, is necessary to inhibit virus replication inside infected neurons, possibly through transmembrane signaling.[131].

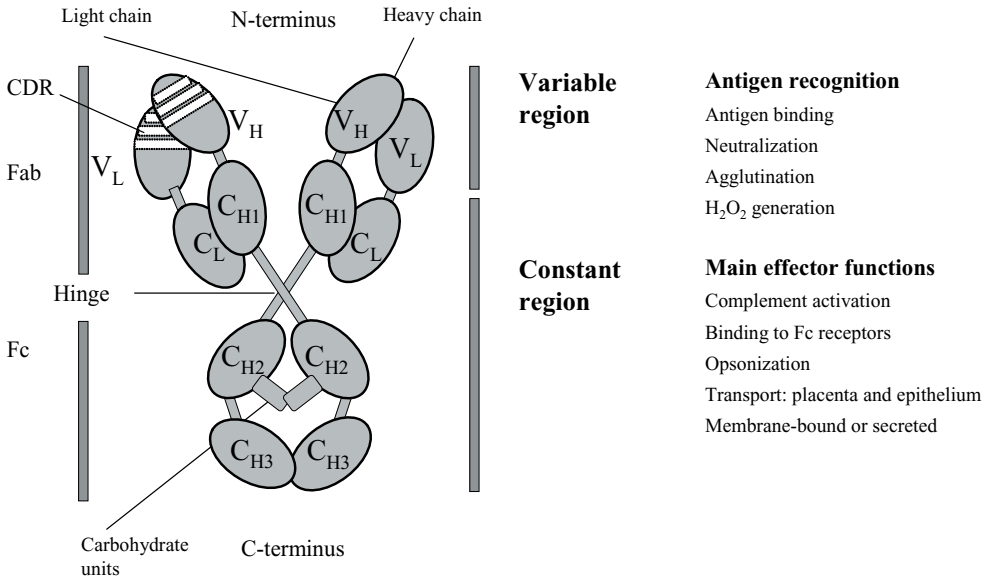

Figure 2.3 Schematic structure of the basic four-chain unit of a human antibody molecule
CDR= complementarity-determining (hypervariable) regions, shown only in one Fab region. Modified from refs.[207, 390].

In humans, five classes (isotypes) of Igs (IgM, IgG, IgA, IgD, and IgE) exist. They differ in their physicochemical, antigenic, and functional properties, and in the amino acid sequence of their constant regions (Table 2.5). IgG, IgD, and IgE are monomers, whereas IgM and IgA predominantly form pentamers and dimers, respectively. Formation of these polymers requires the additional polypeptide-joining (J) chain. In heavy chain structures, the hinge region (Fig. 2.3) shows the greatest variation between (sub)classes. It varies in sequence, length, number of disulfide bonds, and in the presence of carbohydrates[390]. The degree of sialylation of these polysaccharides may regulate the switch from anti- to pro-inflammatory action[172]. In IgE and IgM, the hinge is replaced by an extra constant domain (C_H4)[390]. The hinge allows segmental flexibility of the Fab arm relative to the Fc region. This flexibility is important for antigen binding and effector functions, because

it allows the Igs to cope efficiently with different spacings and orientations of antigenic epitopes[310, 390]. Though the Fc parts of Ig classes share the same general structure, their sequence homology is only about 30%, and their carbohydrate moieties may confer additional heterogeneity[85, 390].

Within the IgG and IgA classes, there are four IgG subclasses and two IgA subclasses having further genetic (Fig. 2.2), antigenic, metabolic, and structural variation (Table 2.5)[85, 97]. Further genetic polymorphisms (allotypes) in the heavy chain (C) genes of IgG1, IgG2, IgG4, IgA2, IgE, and in κ light chain genes are discussed above (Table 2.3)[134, 154, 208, 390].

Different antigens evoke specific antibody responses that have variable (sub)class profiles and, accordingly, variable effector functions conferred by the Fc portion of the antibody (sub)class (Fig. 2.3, Table 2.4). The ability to Fc-FcR interaction is an important determinant of effector functions that a given antibody (sub)class is able to mediate (Tables 2.4 and 2.5)[47, 244]. The only FcR that can commonly be found on B cells, FcRγIIb, is an inhibitory FcR. FcRγIIb downregulates immune activation and serves as a distant checkpoint in B cell maturation against autoimmunity[244]. Other common FcγRs are activating receptors. If several activating receptors of the same isotype specificity are present on the same cell, only those FcγRs that have the optimal affinity are engaged[244]. FcRγII and FcRγIII only bind IgG that is aggregated in an immune complex[207, 244]. The relative binding efficiencies to different FcRs are thus determined by the isotype produced during an antigenic challenge[286]. Since different FcRs are expressed on different effector cells, the isotype produced further modulates the immune response (Table 2.4). This may, for example, regulate the release of Th1 and Th2 type cytokines by activated cells[286]. As an example, activated myeloid DCs induce Th1 responses mediated by IL-12 secretion, whereas plasmacytoid DCs mediate Th2 responses and the release of IL-4, but little or no IFNγ by CD4+ T cells[34]. Genetic polymorphisms of FcRs may modulate infectious and autoimmune diseases (Table 2.4)[244]. For example, the H131 allele (with histidine in amino acid position 131) of FcRγIIA is the only FcγR that binds IgG2. The R131 allele (with arginine) fails to bind IgG2, its frequency is ~ 0.23. Homozygous carriage of this R131 allele leads to an impaired removal and phagocytosis of encapsulated bacteria and *Plasmodium spp.* from the bloodstream. It is associated with an increased severity of invasive disease caused by these pathogens, whereas homozygosity for H131 protects from these[207]. In persons carrying FcγRIIA-H131, IgG2 may mediate opsonophagocytosis[47].

IgM

IgM is mostly found in the intravascular pool. Compared with the basic Ig structure, it has a high content of carbohydrates. These are N-linked to the CHμ1, CHμ2, and CHμ3 domains. IgM exists mainly as a star-shaped pentamer (Table 2.5). Two of its monomers are linked by an extra J chain. The μ chain has an extra CHμ4 domain with a "tail" polypeptide attached

to it, and IgM has no hinge. Still, IgM can adopt a crab-like structure when bound to its antigen, making it a potent binder of antigens even when its affinity is low[207]. Adopting the crab-like orientation leads to the exposure of C1q binding site in each subunit of the pentamer. Accordingly, IgM is a strong complement activator[207]. Natural antibodies belong to IgM class, as well as those produced in responses against TI antigens (Table 2.2). It is the first antibody produced in the primary response (Table 2.2). IgM also acts as an opsonin, as well as aids in the clearance of apoptotic cells[251]. It reacts with ABO blood group antigens and fails to cross the placenta. This protects the fetus against the consequences of blood group incompatibility with the mother[207]. A poorly-characterized IgM/IgA Fc receptor named Fcα/μR may mediate B-cell endocytosis of IgM-opsonized bacterial targets[393].

IgG and its subclasses

IgG is the major antibody class of the secondary response, and most anti-protein antibodies belong to IgG class (Table 2.2)[389, 390]. It is evenly distributed between the intra- and extravascular pools, and accounts for 70-75% of the total serum Ig population (Table 2.5). Structurally, the overall shape of IgG follows the basic Ig structure depicted in Fig. 2.3[85, 207, 390]. IgG subclasses display a 95% sequence homology, but differ in their structural and functional properties (Table 2.5)[85, 208]. IgG1 is the least glycosylated subclass[310]. IgG3, like IgD, has an extended, heavier hinge with additional disulfide bonds compared with the other IgG subclasses. This offers it greater flexibility and thus more adaptable antigen binding. The relative flexibility of IgG subclasses is in the order: IgG3 > IgG1 > IgG4 > IgG2[390]. Half-lives of antibody classes vary greatly between individuals, IgG3 and IgG4 having the shortest[390].

IgG1 and IgG3 production is thought to be enhanced by Th1 responses[148, 342]. Somewhat paradoxically, in *in vitro* models IgG1 and IgG3 production is enhanced by IL-10 and CD40L[128, 262]. IgG1 and IgG3 engage all common human Fcγ receptors present on cells of the immune system (Table 2.4). Thus they are capable of all antibody-mediated effector functions[47, 85]. In general, the IgG1- and IgG3-mediated neutralization, complement activation, complement-dependent lysis, opsonization, and antibody-dependent cell-mediated cytotoxicity (ADCC) defend us from intracellular pathogens such as viruses[90, 184, 219]. In particular, ADCC is primarily mediated by IgG1 and IgG3[85]. The main effector cell of ADCC is the NK lymphocyte. Expression of foreign antigens on the surface of infected cells leads to their recognition by specific antibodies. Antibody-coated target cells are then recognized by the NK cell FcγRIII receptor (or FcRs on monocytes and macrophages). This leads to FcRγRIII crosslinking and NK cell activation, granule exocytosis, and the release of cytotoxic substances such as perforin and granzymes causing cell lysis[207]. The differing hinge-region lengths of IgG1 and IgG3 affect their capacity to mediate ADCC and the affinity to different FcγRs[292].

IgG1 is more active than IgG3 in ADCC and complement activation. Its plasma concentrations are 10-20 times higher than those of IgG3[85]. IgG1 also defends us against pathogens having polysaccharide antigens. In humans, IgM, IgG1 and IgG3 are the main complement-fixing (sub)classes[85].

IgG2 and IgG4 appear during the secondary response. Since IgG2 and IgG4 generally bind FcγRs only weakly, their main function is in the neutralization of extracellular pathogens (Tables 2.4 and 2.5)[85]. The production of IgG2 is enhanced by Th2-dominant responses[128, 148, 342]. IgG2 is the most abundant subclass in response to carbohydrate antigens[390]. IgG2 activates complement CP only at very high concentrations, but has the ability to activate AP (Table 2.5)[85]. Genetic inter-individual variation in the efficiency and development of IgG2- and IgG4-mediated protection from pathogens exists. *G2m*$^{n-}$ is, clinically significantly, associated with diminished or missing specific antibody responses to polysaccharide antigens[54, 254, 259]. In addition to the effect of the above-mentioned FcγRIIa-R/H 131 polymorphism in IgG2-mediated immunity, FcγRIIIa has F/V158 polymorphism; FcγRIIIa-V158 is able to engage IgG4[47]. FcγRIIIA -V158 is a putative risk factor of chronic periodontitis in Northern European Caucasians[204]. IgG4 does not fix complement[85, 390]. It is produced in response to a prolonged exposure to mucosal antigens causing Th2-biased responses, such as those during helminth infections and during simultaneous exposure to multiple allergens[2, 128]. Unlike other antibody classes, IgG4 is often functionally monovalent and bispecific. This is achieved, probably during endocytic salvage, by the exchange of half-molecules of IgG4 between two IgG4 molecules having different specificities[2]. IgG4 may defend us against parasites, and bispecific IgG4 may have anti-inflammatory activities[2, 128].

Secretory IgA and serum IgA

IgA has a more T- than Y-like structure, and it exists in monomeric and polymeric (mostly dimeric) forms; monomeric IgA dominates in the serum (Table 2.5)[393]. Approximately 60% of all Igs produced daily belong to the IgA isotype, but more than half of this is selectively transported into external secretions as secretory IgA (S-IgA)[97, 393]. In S-IgA, the tailpieces of monomers are linked by the J chain. S-IgA also contains the secretory component, which is the extracellular proteolytic fragment of the polymeric Ig receptor (pIgR) responsible for the transport of IgA and IgM into secretions (Table 2.5)[97, 393]. S-IgA is produced locally at mucosal surfaces, mainly by the gut-associated lymphoid tissue (GALT). Approximately 25% of S-IgA in mice is produced thymus-independently by B-1 cells, and 75% comes from B-2 lymphocytes in response to TD antigens[393]. S-IgA serves as the first line of humoral defense – also in a breast-fed infant – by neutralizing toxins and viruses, blocking the entry of bacteria across mucosal surfaces, and by opsonizing pathogens[393]. There is one well-characterized FcαR, FcαRI. It is constitutively expressed on many APCs (Table 2.4)[234, 393].

Serum IgA has a much shorter half-life than IgG (Table 2.5). IgA has two subclasses IgA1 and IgA2. Over 90% of B cells with sIgA in the bone marrow are IgA1 positive. Accordingly, serum IgA1:IgA2 subclass ratio is about 9:1. The subclasses are more evenly distributed in secretions. IgA2 allotypes differ in their susceptibility to bacterial proteases, but no allotype-dependent disease associations are known. Binding of antigens to S-IgA fails to initiate inflammatory processes. Even though polymeric IgA is able to activate complement LP upon binding to MBL, IgA is a poor opsonizer and complement activator. As such it may inhibit and protect mucosal surfaces from excessive complement activation by complement-fixing Igs[97, 234, 393]. The function of serum IgA is poorly known, but may have systemic anti-inflammatory functions. Yet in experimental conditions binding of antigen-complexed IgA to FcαRI can initiate inflammation: ADCC, phagocytosis by resident macrophages such as Kupffer cells, release of cytokines, superoxide generation, and antigen presentation (Table 2.4)[393]. IgA deficiency, the most common primary immunodeficiency, is associated with atopy, frequent mucosal infections, and autoimmunity[97, 393]. SCDs and impaired vaccination responses are frequently found in IgA-deficient patients, but fail to differentiate those with more severe symptoms[5, 109, 123].

IgE and IgD

IgE-committed B cells can be found in the circulation, skin, lungs and gut. IgE makes up about 0.002% of the total Ig pool, 50% of it is found intravascularly with a short half-life (1-5 days, Table 2.5.)[286]. Like IgM, IgE has an extra C_H4 domain and no hinge[390]. IgE mainly exists bound to its receptors on various cells, only very low quantities are present in serum (Tables 2.4 and 2.5)[286, 390]. IgE is produced by Th2-dominant responses in response to IL-4 or IL-13 or both together with CD40L[128, 286]. There is a high affinity IgE Fc receptor FcεRI, and two low-affinity receptors FcεRIIa and b[286]. FcεRII engagement controls IgE responses[60]. The density of human basophil FcεRI α-chain expression correlates with serum IgE[286]. IgE does not activate complement. Instead, activation of complement receptors C3aR and C5aR by anaphylatoxins acts synergistically with IgE-mediated responses[286]. The main actions of IgE are mediated by mast cells and basophils carrying FcεRIs. Their activation through FcεRI cross-linking leads to the release of secretory granules that contain inflammatory mediators, proteases, and cytokines. Besides immediate IgE-mediated hypersensitivity reactions, mast cell activation contributes to the delayed hypersensitivity reaction[286]. IgE is involved in parasite- and allergen-specific responses[128, 286, 390]. Evidence of IgE-mediated response, either by skin prick testing or by the measurement of allergen-specific IgE levels in serum, is required for the diagnosis of allergy[286]. In addition to severe allergies, high levels of IgE are seen in parasitic infections, hematological malignancies, autoimmune disorders, hyper-IgE syndromes, and several other forms of combined immunodeficiencies[132,

Table 2.5 Properties of human immunoglobulin classes and subclasses

Property	Antibody class / subclass									
	IgG1	IgG2	IgG3	IgG4	IgA1	IgA2	S-IgA[1]	IgM	IgD	IgE
Structural and physicochemical properties										
Heavy chain	γ1	γ2	γ3	γ4	α1	α2	α1/α2	μ	δ	ε
κ/λ ratio	2.4	1.1	1.4	8.0	1.4	1.6	n.a.	3.2	0.3	n.a.
Molecular weight (kDa)	150	150	165	150	160	160	400	900	180	190
Number of heavy chain constant domains	3	3	3	3	3	3	3	4	3	4
Number of antigen binding sites	2	2	2	2	2	2	4	10	2	2
Number of amino acids in the hinge	15	12	62	12	20	7	20/7	0	64	0
Number of disulfide bonds between heavy chains	2	4	11	2	2	2	2	1	1	2
Tail piece	-	-	-	-	+	+	+	+	+	-
Carbohydrate content (%)	2-3	2-3	2-3	2-3	7-11	7-11	7-11	12-15	18	15-18
Half-life (days)	21 (36)[3]	27-45 (37)[3]	7 (29)[3]	21 (16)[3]	6	6	n.a.	10	3	2
Occurrence										
Mean serum concentration (g/L)	9	3	1	0.5	3	0.5	-	1.5	0.03	0.00005
Extracellular fluids	+++	+++	+++	+++	++	++	-	+/-	-	-
Secretions	-	-	-	-	-	-	+++	+/-	-	-
Transport over placenta	+++	+++	+++	+++	-	-	-	-	-	-
Transport over epithelium	-	-	-	-	+++	+++	-	+	-	-
Functional properties										
Primary response	-	-	-	-	-	-	-	+	-	-
Secondary response	++	++	++	++	+	+	+	-	+	+
Neutralization	++	++	++	++	++	n.a.	+	-	-	-
Opsonization	+++	-	++	+	+	+	n.a.	+	-	-
Antibody-dependent cellular cytotoxicity	++	-	++	-	-	-	n.a.	-	-	-
Binding to macrophages, other phagocytic cells	+	-	-	+	-	-	-	-	-	+
Binding to basophils and mast cells	-	-	-	-	-	-	-	-	-	+++
Activation of classical pathway of complement	++	+	+++	-	-	-	-	+++	-	-

[1]S-IgA = secretory IgA, [2]kDa = kilodaltons, [3]half-lives in parenthesis obtained from subjects with antibody deficiencies receiving intravenous immunoglobulin replacement therapy. Modified from refs.[12, 207, 390].

[286]. Absence of measurable serum IgE may point to disturbances of class switching (Table 2.1)[286].

IgD is mainly found as a membrane-bound BCR, which kinetically is expressed after IgM. IgD makes up only 0.25% of the Ig population, its serum levels are low (Table 2.5). Both sIgM and sIgD of the same specificity are found on unprimed B cells. Upon antigen stimulation, IgD is turned off and IgM secreted. IgD´s precise function is unknown, but it may regulate B cell activation, development of immune tolerance, immunologic memory, and enhance specific IgM, IgA, and IgG responses[390].

5.2.7 Clinical aspects of low IgG subclass levels

Low levels of IgG subclasses are defined as those below two standard deviations from the mean. Still, there is a lack of universally accepted reference values and analysis methods[26, 61, 315]. The clinical significance of any given subclass(es) in patients without any known immunodeficiency but with recurrent infections is unclear and controversial[54, 61]. Individuals with low IgG subclass levels are common and frequently asymptomatic[61]. Thus, the finding of low levels of one or more subclasses is generally considered insufficient for the diagnosis of immunodeficiency[54].

At population level, the frequency of having low levels of more than one subclass is only known among blood donors, but often considered to better differentiate those with more serious infections[61]. The distribution of low (sub)class levels according to age and sex is uneven. In children, there is a 3:1 male: female preponderance[235]. Boys more frequently have low IgG1[192]. Reflecting the allotype-associated delayed maturation of IgG2- and IgG4-mediated humoral immune responses against polysaccharide antigens, low IgG2 (associated with *G2m*^{n}) and abnormal vaccine responses (associated with IgA deficiency and *G2m*$^{n-}$) are relatively more frequent in children[61, 235, 257, 259, 263]. After puberty, low IgG1 and IgG3 levels together with female preponderance become more common[26, 235]. Nearly half of symptomatic SCD patients aged more than 40 have low IgG1[26].

IgG1 subclass is the quantitatively predominant IgG subclass and may lead to low total IgG levels. Genetically, serum IgG1 levels below two standard deviations from the mean ("[partial] IgG1 subclass deficiency") are uncommonly associated with heterozygous *Cγ1* deletions, and more commonly with the *G1m*^{f} allotype, regardless of the full haplotype (see above)[262, 326]. Clinically, selective IgG1 deficiency (without low levels of other [sub]classes) is associated with mostly moderate upper respiratory tract infections and sinusitis caused by pathogens found in patients with humoral immunodeficiencies, e.g. S. *pneumoniae*, *H. infuenzae*. According to the largest available study on 119 symptomatic IgG1-deficient patients, approximately 9% suffer from invasive infections (e.g. severe bronchopulmonary infections with bronchiectasia, gastrointestinal infections, septicemia, or meningitis).

Vaccination responses were not tested in the study[192]. Severely symptomatic patients with low IgG1 levels, especially if an impaired response to carbohydrate antigens is found, are more properly diagnosed as having CVID. These patients fulfill the internationally accepted diagnostic criteria for possible CVID[54, 87]. If prophylactic antibiotics are not sufficient for the treatment, these patients are candidates for intravenous or subcutaneous immunoglobulin treatment[54, 254].

Low IgG3 levels, regardless of the full Gm haplotype, are codominantly associated with the *G3m*^{g} allotype[326]. They are also autosomally dominantly associated with the *HLA-B*8,SC01,DR*3* haplotype[10]. The clinical significance of low IgG3 levels is controversial[61]. In relatively small patient populations, frequent but mild upper respiratory tract infections, bronchitis, bronchopneumonias, bronchial asthma, erysipelas episodes, and herpes simplex recurrences have been noted[26, 235, 260]. Of haplotypes carrying the low producing allotype *G3m*^{g} in Caucasians, the *G3m*^{g},*G1m*^{a},*G2m*$^{n-}$ haplotype with *G2m*$^{n-}$ is more frequent (~ 0.30) than *G3m*^{g},*G1m*^{a},*G2m*^{n} (~ 0.01). Despite this, diminished vaccination responses are rarely seen in patients with IgG3 deficiency[37].

In adults, low IgG2 levels are associated with the *G2m*$^{n-}$ allotype. During childhood, low serum IgG2 may be caused by the retarded maturation of IgG2 production in connection with the *G2m*^{n} allele[259]. IgG2 deficiency alone is commonly asymptomatic. In children, low IgG2 is relatively weakly and diminished vaccination responses relatively strongly associated with recurrent sinopulmonary infections caused by the typical pathogens *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis*[61]. Though low polysaccharide antigen responses are most commonly seen in subjects with IgG2 deficiency, severely symptomatic specific antibody deficiency without low subclass levels is not infrequently encountered in specialized centers[54]. Symptomatic IgG2 or IgA deficiencies in childhood may progress to full-blown CVID[54, 140]. Patients may also become asymptomatic or (sub)class levels and vaccination responses may become normal or both[54, 254]. No consensus on the clinical phenotype of subjects with selective low IgG4 exists, but in studies on sinus infections it is most often seen in control groups[18, 153, 222, 262, 313, 355].

Combined low IgG subclass levels

The genetic causes of a combined partial IgG1 and IgG3 SCD are mostly unknown. It is thought to be associated with Th2-dominant immune responses affecting class switching of neighboring genes (Fig. 2.2)[148, 342]. Associations between low IgG3, IgG1, IgA1, and IgA2 levels and clinical diseases are complex and allotype-dependent. IgA deficiency by itself predisposes to atopic and autoimmune diseases[54, 97]. Persons with IgA deficiency may have more severely decreased lung function if it occurs together with decreased IgG2 or IgG3 levels[5, 49]. Patients with nonatopic or atopic bronchial asthma with sinopulmonary symptoms frequently have low IgG1 or IgG3[192, 257].

Atopic diseases causing sinopulmonary symptoms in patients with associated IgG1, IgG3, and IgA deficiencies frequently cause misguided therapy and diagnostic confusion with recurrent sinopulmonary infections[61, 97, 192]. A higher prevalence of familial bronchial asthma and IgE-mediated atopy is seen in persons with the *G3m*^{b},*G1m*^{f},*G2m*^{n} haplotype associated with high serum IgG3, low IgG1, and high IgG2[192, 257]. Nonatopic, but asthmatic, children tend to have the opposite haplotype *G3m*^{g},*G1m*^{a},*G2m*$^{n-}$ associated with low IgG3, high IgG1, and low IgG2[257]. Likewise, low levels of IgA2 are found together with the *G3m*^{g} allotype[221]. Accordingly, both low IgG1 and low IgG3 levels are associated with chronic obstructive lung disease and lower respiratory tract infections[260]. Combined IgG1 and IgG3 deficiency may be seen in CVID patients, though *G2m*$^{n-}$ homozygotes and combined IgG2, IgG4, and IgA deficiencies are more common[258, 261, 285].

Vaccination responses and low subclass levels in clinical practice

Diminished vaccination responses to polysaccharide antigens are associated with the *G2m*$^{n-}$ allotype and low levels of IgG2, IgA1, IgG4, and IgA2 (encoded by neighboring genes). In the clinic, all these are more commonly found in children than in adults[54, 235, 259, 263]. In pediatric practice, vaccination responses are preferentially used in patient assessment, and subclass levels are rarely measured since poor vaccination responses have been found to better predict recurrent infections[61]. Yet subjects with impaired responses to polysaccharide antigens are commonly asymptomatic. Impaired responses are seen in the studied populations, as a mixed co-dominant trait, in more than 10% of subjects[238, 263]. There is no universally accepted method for the assessment of vaccination responses. Antigens and the valency of the vaccines differ, and different age-specific criteria may be used[54, 238]. The use of subclass-specific responses may be advocated[372]. Clinical centers assess antipneumococcal responses either by using the sum of all responses, or define an impaired response as an inadequate response to ≥ 70% serotypes measured[54, 339]. Threshold values, numbers of pneumococcal serotypes, and pre-adsorption methods used are highly variable[54, 339, 372]. Severely symptomatic patients with impaired vaccination responses are said to suffer from specific antibody deficiency (SAD). Many of these patients are diagnosed only after the age of 40, and ~80% suffer from recurrent sinus infections[78]. Yet impaired responses to carbohydrate antigens in middle-aged patients with highly-symptomatic sinopulmonary infections are surprisingly infrequent, and poorly differentiate between symptomatic and asymptomatic patients[109, 222, 238, 339]. When 245 successive, mostly adult, patients with recalcitrant rhinosinusitis and SCDs were tested, no adults with diminished vaccination responses were found[222]. The highly variable clinical phenotype of subjects with low subclass levels or an inability to mount antipolysaccharide responses or both suggests that additional genetic, regulatory, or structural defects influence, or are the true cause of, infection-proneness[169, 262].

Despite being the two most commonly used methods, the relative clinical values of IgG subclass levels and vaccination responses in the assessment of adult patients with recurrent infections are unknown. Subclass concentrations (other than IgG2) and vaccination responses have been simultaneously measured in three pediatric studies. A study on 27 allergic children suffering from recurrent sinusitis reported frequent low subclass levels and no diminished responses[93]. In 61 young children with recalcitrant sinusitis, 21 patients had low response to one serotype and two to two serotypes (4 tested), four hyporesponsive patients also had low subclass values but normal IgG. Seven additional patients had only low IgG2 or IgG3[331]. In a study on 165 symptomatic subjects with unreported symptoms and age, both IgG subclasses and vaccination responses were measured in 59% of the subjects. Total responses to 1-4 vaccines (against tetanus, diphtheria, *H. influenzae*, or pneumococci, in varying combinations) were assessed. With an in-house nephelometric assay, there was a very low frequency of low subclass values (5.2%). According to authors' assessment, but with unreported criteria, only specific antibody measurements influenced the management of the patient[290]. Hypothetically, simultaneous assessment of multiple parameters (e.g. subclass levels, Ig allotypes, vaccination responses, FcR polymorphisms, complement deficiencies, CD27-IgM+IgD+ memory B cells) may more reliably predict differing clinical phenotypes of patients[5, 168, 169, 262]. In the future, enumeration of B cell subsets may even substitute some of the earlier assessment methods[6, 69].

5.2.8 Laboratory assessment of immunoglobulin levels and allotypes

Normal immunoglobulin levels depend on the age and sex of the individual as well as on the ethnic background. Total Ig and Ig subclass levels from plasma, serum, or bodily secretions are usually measured with radial immunodiffusion or nephelometry. In radial immunodiffusion, the antigen (Ig class measured) is allowed to diffuse radially into a gel that contains antibody (anti-Ig antibody). The size of the precipitin ring is directly proportional to the concentration of the antigen, which is determined relative to standards containing known amounts of antigen. Radial immunodiffusion is particularly useful when trace concentrations of Ig are present in the tested sample[50]. Compared with older methods, automated nephelometry is faster, more precise, and less variable. In solution, specific reaction between the measured Ig (sub)class and monospecific high-avidity antiserum creates immune complexes. These are quantified either by end-point or rate nephelometry. Scattered light, which increases with antigen concentration, is measured. In end-point nephelometry, maximum scattered light is measured after antigen-antibody reaction has reached equilibrium or after a fixed reaction time. Ig concentration is calculated relative to calibration curves,

which are obtained with standard serum containing known amounts of antigen (Ig class) tested under similar conditions (including antiserum and equipment). At the same time, a control serum is assayed to check the validity of the calibration curves and the accuracy of the assay. Non-specific light-scatter may be observed in turbid (e.g. lipemic) samples or at low dilutions of serum samples (when trace concentrations are measured). This can be detected by running a parallel assay, in which the anti-Ig antibody is omitted ("blank reagent"). Rate nephelometry, which measures the peak rate of immune complex formation relative to a reference curve, is employed especially in the USA[50]. Mostly polyclonal antisera are used in all methods. No universally accepted antisera or reference values exist, and normally in-house or manufacturers' reference values are used[26, 315].

Serologically, allotypic markers are detected, for example, with double diffusion precipitation in gel or obtained as a byproduct of their quantitation with an inhibition ELISA, usually by employing in-house methods[312, 326]. Allotype-specific monoclonal antibodies are used[134]. Specific serologic reagents against the products of some allelic genes (e.g. $G2m^{n-}$) are not available. Numerous polymorphic sites in the switch regions, Ig genes, and pseudogene regions occur at *IGH@* (Fig. 2.2). Many of them are in positions which do not carry codons for *Gm*, *Am*, or *Em*. These polymorphisms are mostly in linkage disequilibrium with *Gm* and *Am*. Polymorphisms in the *Sμ* and *Cδ* gene regions are an exception (Fig. 2.2)[134]. Consequently, allotyping at the gene level is experimentally performed in highly specialized laboratories, usually in epidemiologic genetic studies[134].

5.3 Human major histocompatibility region and human leukocyte antigens

Human immune system must recognize a high diversity of micro- and macroorganisms. Different effector mechanisms must be applied against these organisms. For example, the elimination of intra- or extracellular microorganisms and intestinal worms requires different effector functions. Unlike B cells and antibodies, MHC molecules recognize both the presence and the location of a pathogen. This is achieved by two classes of MHC molecules. MHC molecules present antigenic peptide structures (derived from self-antigens or pathogens) to TCRs. Class I molecules recognize invading intracellular and class II molecules extracellular organisms[179, 180].

MHC gene region

Major histocompatibility region includes a physical region on the short arm of chromosome six (6p21.3) that spans ~ 4700 kb. *MHC* was first fully sequenced, as a mosaic of different haplotypes, in 1999[179]. With high density, it encodes ~ 240 genes, of which ~ 130 are considered to be functional. At

least 40% of the functional genes are involved in immune response[67]. *MHC* is divided into three distinct non-overlapping regions: class I, class II, and class III. The regions localize, from the telomeric to centromeric end of the region, in the order *MHC I* (1.8 Mb), *MHC III* (0.7 Mb), and *MHC II* (0.7 Mb). Relatively strong associations with *MHC* genes have been found in > 100 diseases[332]. Characteristically, *MHC* contains large numbers of gene duplications and pseudogenes. It has the largest number of polymorphic proteins within the human genome. Some *HLA* genes are known to have > 200 alleles. Of *MHC* genes, ~ 44 are *HLA*-related and ~ 180 are non-*HLA* genes[179, 332]. The extremely high level of polymorphism and heterozygosity is thought to increase the host's fitness against infections by providing the immune system with a selective advantage against the variability of pathogens[67]. Less advantageously, the high level of mutation in *HLA* genes may result in susceptibility to infections, autoimmune and inflammatory diseases[180]. *MHC* genes are inherited as haplotypes. Accordingly, genetic linkage analyses are marred by the large number of functionally related and active loci, and the strong linkage disequilibrium between these loci within the *MHC*[9, 67]. Except for the "classical" class I and II molecules, there is little data to support any functional significance for most of the polymorphisms identified; they may only serve as markers for the neighboring disease loci (e.g. transporters of antigen presentation *TAP1* and *TAP2*)[67].

HLA nomenclature

Replacing older HLA nomenclatures, in the contemporary (gene level) nomenclature the lettered class (and in class II, family and chain) designation is followed by an individual Arabic numeral for the gene, after which the numerous allelic variants are differentiated by a numeric notation preceded by an asterisk[217]. For example, *HLA-DRB1*0401* stands for allelic variant 0401 of gene 1, encoding the β-chain of a class II molecule that belongs to family R[179].

MHC class I genes and function of class I molecules in host defense

Class I genes code for the α-polypeptide chain of the class I molecule; the gene encoding the β-chain (β2-microglobulin, B2M) is located on chromosome 15. Additionally, class I encodes e.g. two coding MHC class I chain-related genes (*MIC*) *A* and *B*. Of the 6 non-pseudogenes in *MHC I*, *HLA-A*, *-B*, and *-C* are called "classical" (class Ia) genes and *HLA-E*, *-F*, and *-G* are called "non-classical" class Ib genes. Ib genes also encode respective α-chains. *MICA* and *MICB* are involved in interactions with γδ T cells, *HLA-E* in interactions with NK cells and together with *HLA-G*, in immune tolerance[179, 332].

Class Ia antigens are highly polymorphic, ubiquitously expressed cellular membrane-bound glycoproteins, which together with B2M form the class Ia molecule. Each assembled class Ia molecule is able to bind a different range of peptides (of 8-11 amino acid residues) that are generated when

an intracellular pathogen infects the cell. After tight intracellular control, class Ia molecules present the intracellularly processed, short, pathogen-derived peptide antigens with the help of their peptide-binding grooves. This presentation to TCRs of CD8+ T cells takes place on the surface of the cell and initiates a cytotoxic response. Class Ia molecules are thus specialized for recognition of intracellular pathogens (such as viruses) that infect any somatic cells of the host[179].

MHC class II genes and function of class II molecules in host defense

Listing from the telomere to the centromere, the classical *MHC II* genes *HLA-DR* (which often contain an additional β-chain gene), *-DQ*, and *-DP* are organized in pairs: one locus encodes the α- and the other the β-polypeptide chain of the class II molecule. Between *HLA-Q* and *-P*, "non-classical" class II genes encode α- and β- chains of molecules that inhibit (HLA-DO; on B cells) or catalyze (HLA-DM; on all professional APCs) peptide-binding to class I molecules. *MHC II* also contains genes that encode other molecules involved in antigen processing and presentation, for example tapasin, *TAP1*, and *TAP2*[179, 332].

Analogous to HLA Ia molecules, the α- and β-chains associate with each other and form a heterodimeric class IIa molecule. It associates with the invariant chain (Ii) in the endoplasmic reticulum. Invariant chain is involved in the maturation of the molecule, and in antigen binding and presentation. Eventually, each class II molecule will present, in its peptide-binding groove, a different range of peptides (of 15-25 amino acid residues in length) at the cell surface. The use of class II molecules is usually restricted to the professional APCs (DCs, B cells and macrophages). Class II molecules primarily present, to CD4+ helper T cells, pathogenic antigens that enter by phagocytosis into the APCs. They thus play an important role in defense against extracellular pathogens such as bacteria and parasites[179].

The extensive allelic polymorphism in HLA molecules is concentrated mainly among amino acid positions that determine their specificity for foreign peptides[67]. Due to protein processing, there are roughly 100 000-300 000 peptide-laden class I or II products of each of the highly expressed *HLA* loci on the cell surface. Uninfected cells display hundreds of thousands of self peptides on their cells. Simultaneous expression of all three classical HLA I and three to four classical HLA II molecules at the surface of cells enables the immune system to simultaneously recognize a wide array of pathogen-produced peptide antigens[179].

MHC, infectious diseases, and HLA homozygosity

For successful protection against a pathogen, an individual's HLA molecules must be able to bind pathogen-derived molecules, and the T and B cell repertoires must include clones that can be activated by the HLA molecule-

peptide complex. If either of these requirements is not fulfilled, this may render a person carrying such inefficient combination of HLA molecules susceptible to the given pathogen. Malaria, HIV, hepatitis B, hepatitis C, and mycobacterial diseases display the strongest recognized associations between pathogens and specific *HLA* alleles[67, 179]. For example, *B*5301* and the haplotype *DRB1*1302,DQB1*0501* protect against severe malaria. In endemic areas, this confers a selection advantage. *B*5301* is present in ~ 25% of Gambians, but only in ~ 1% of Europeans[67, 179]. Other consistently reported associations have been those between slow progression of HIV and *B*27* and *B*57*, rapid progression of HIV and *B*35-Px* alleles, clearance of hepatitis B and *DRB1*1302*, and clearance of hepatitis C and *DQB1*0301*[67]. In herpes simplex type 2 infections, an uneven distribution of *HLA* alleles between patients and control subjects has been reported[200]. In bacterial infections, *HLA* associations have rarely been studied. Recurrent sinopulmonary infections without any apparent primary immunodeficiency and with normal Ig levels have been associated with *HLA-B*44*[168]. Given the large number of *HLA* alleles, conclusive association study on the influence of HLA on an infectious disease requires large samples, accurate clinical information, and proper statistical design and statistical models[67, 179].

Hypothetically, individuals heterozygous at the *HLA* loci present a greater variety of antigenic peptides to effector cells than homozygotes. This may result in more efficient handling of pathogenic challenges. This phenomenon is called heterozygote advantage. Homozygosity of *MHC* genes may allow viral escape from cytotoxic T cell responses. Heterozygote advantage against rapid progression has been reported between HIV and *HLA-A* and *–B* loci, as well as against chronicity between hepatitis B and C and *HLA-DR* and *-DQ* loci[67]. In addition, a heterozygote disadvantage has been reported in succumbing to malaria and in progression of hepatic fibrosis in patients with chronic hepatitis. Thus, a broad immune response may also result in overt injury[270].

5.4 Studied mucosal infections, immune defense, and immune evasion

5.4.1 Herpes simplex viruses type 1 and 2

Herpes simplex viruses type 1 and 2 (HSV-1 and HSV-2) belong to α-herpesviruses. They have diverged approximately 8 million years ago. The virion has an electron-dense core containing the viral DNA, an icosapentahedral capsid, an amorphous layer of proteins surrounding the capsid called tegument, and an envelope. They have a large double-

stranded DNA molecule[386]. The genomic structures of HSV-1 and HSV-2 are identical; their genomic sequences are closely related with over 50% homology[92, 245]. The type-specific genomic regions appear to be important in host immunity.

Both species have a total of 74 genes encoding distinct proteins, with 10 additional genes proposed[245]. Each protein serves multiple, in total several hundred different functions[386]. Most of the produced polypeptides are antigenically related. Their differences can be used in serologic diagnosis and seroepidemiology[273, 375]. The viral envelopes contain at least 12 viral glycoproteins, five of these (gB, gC, gD, gH, and gL) mediate entry and cell-binding to host receptors (e.g. heparan sulphate, nectin-1 and -2, and herpesvirus entry mediator)[340]. gD and the gE/gI heterodimer further mediate cell-to-cell spread[279]. Both viruses have multiple strains[57].

After the fusion of the virion envelope with the host cell membrane, the synthesis of viral gene products (RNA and proteins) commences in three sequential waves. Immediate early (α) gene products shut off host protein synthesis by increasing cellular RNA degradation and block the presentation of antigenic peptides on infected cell surfaces, as well as turn on the transcription of early (β) genes. The β-polypeptides – the main targets for antiherpetic medication – are mainly regulatory proteins and enzymes required for DNA replication or they post-translationally modify viral proteins and their function. The third (γ) class of HSV proteins requires viral DNA replication for expression. They assemble to form the capsid, tegument, and eventually the envelope, before virion transport to extracellular space after approximately 18 hours[92, 387].

HSVs are able to establish latent life-long infections and to reactivate – with or without symptoms – despite pre-existing adaptive immunity. After acquisition, the virus replicates in epidermal and dermal cells and further infects either sensory or autonomic nerve cells[121]. The virus or its nucleocapsid is transported by retrograde intra-axonal flow to the nerve bodies in ganglia, most commonly trigeminal or spinal sensory ganglia in the posterior horn. Experimentally, the virus replicates inside the ganglia and in surrounding neural tissue during the initial phase of infection. The neurons become productively infected, and the virus is transmitted along synapses to more distant sites[181]. The virus then centrifugally migrates to other mucosal sites, causing the more severe clinical manifestations of a primary disease[92]. Though HSV-1 favors trigeminal and HSV-2 sacral ganglia, virtually any site in the central nervous system can be infected, even without readily apparent clinical symptoms[322]. The so-called latency-associated transcript region specific for a given subtype is thought to determine the site specificity of recurrences. After the resolution of primary disease no infectious virus, only viral DNA, can be found in 10-50% of the ganglion cells of the corresponding anatomical region. The viral genome then resides within neuronal cells in

a latent state, without gene expression of the productive cycle[110]. A limited transcription of viral genome to RNA does occur, producing latency-associated transcripts[387]. These may allow more frequent reactivations[110].

Transmission and epidemiology

Humans constitute the sole reservoir for transmission of HSVs[386]. HSV is most commonly transmitted through close contact with a carrier who is asymptomatically shedding the virus at a peripheral site. The host is infected after direct inoculation of the virus onto susceptible (e.g. oropharyngeal, genital, conjuctival) mucosal or fissured skin surfaces exposed to a mucosal site, oral or genital secretions[92]. The first acquired HSV (type 1 or 2) infection is called the primary first episode[59]. When a carrier of either HSV-1 or HSV-2 acquires the remaining HSV type, the infection is called the initial infection, or non-primary first episode[59]. Primary and initial infections are often asymptomatic. Only 37-52% of primary infections are symptomatic[91, 195]. Asymptomatic seroconversion is 2.6 times more likely in patients with initial, non-primary HSV-2 infection[195]. In 15-25% of patients, symptomatic infection occurs after a latent period and is called the first clinical episode[59, 195]. If the two subtypes reside in the same anatomical regions, they both can reactivate and cause clinical symptoms (called recurrence or recrudescence)[353]. Strains with increased virulence have been described[366]. Exogenous reinfections with different strains, called superinfections, are rare. They have occasionally been associated with more aggressive clinical course[295].

Herpes simplex viruses occur worldwide. Seroprevalences vary according to e.g. age, region, socio-economic status, and the number of sex partners[273]. In Finland, the age-standardized seroprevalences are 52.4% for HSV-1 and 13.4% for HSV-2. Females are more commonly seropositive[273]. The mean age of HSV-1 acquisition has shifted to older age groups (over 25 years of age in Finland), and has been associated with a higher rate of HSV-1 genital infections and an altered clinical course of HSV-1 and HSV-2 infections[114, 273, 274]. The rate of transmission of HSV-2 is in women about 9 and in men 1.5 per 10 000 sex contacts[195, 377]. Pregnant women are more susceptible to acquire HSV infections[127]. In serodiscordant couples, the yearly risk is 6-11% for women and 1-3% for men. Therapy with valaciclovir reduces transmission, and condoms offer partial protection for women[91, 377]. Symptomatic genital herpes leads to impaired self-esteem, sexual functioning, personal relationships, and ability to work. It causes a significant decrease in the quality of life, most notably when the yearly recurrence rate is six or more[271].

Reactivations are induced e.g. by mechanical stress, instrumentation, high levels of ultraviolet light, menses, lactation, malnutrition, extensive fatigue, anxiety, and infections with other viruses[387]. Asymptomatic shedding is common and can further be provoked e.g. by hormonal contraception and bacterial vaginosis[80]. The reported rates of shedding vary according to the

immunocompetence of the host and the survey methods. If PCR is used for assessment, approximately 76-100% of immunocompetent HSV-1 carriers shed the virus at least once during 2-11.5 months of follow-up[99]. Similar frequencies have been noted for HSV-2 infection, in which most, if not all, seropositives are likewise considered infectious[307]. HSV is shed from multiple anatomical sites. In genital HSV-1 and orolabial HSV-2, the frequencies of shedding are lower than for the other subtype[307, 376].

Clinical syndromes

Herpes simplex viruses cause multiple clinical syndromes, but infections caused by the two types are clinically indistinguishable (Table 3.1)[92]. The probability to cause a given manifestation and the severity of symptoms vary according to the subtype, strain, anatomical region, age, and immunocompetence of the host. In immunodeficient patients and neonates, atypical and severe manifestations are reported more frequently[92, 387]. In otherwise immunocompetent patients, HSV may rarely cause e.g. cranial neuritis, brainstem encephalitis, transverse myelitis, meningoencephalitis, radiculomyelitis, sacral radiculomyelitis with parasympathetic ganglionitis and urinary retention (Elsberg's syndrome), brachial neuritis, urethritis, and visceral infections (e.g. lungs, liver)[156, 241, 359]. The pathogenesis of some clinical syndromes such as herpetic keratitis and herpes-associated erythema multiforme is considered to have an autoimmune component[28]. Whether the found serious sequelae in immunocompetent individuals are caused by differences in viral virulence or by unidentified narrow immunodeficiencies is unknown.

Oral-facial infections and recurrent gingivostomatitis

A primary oral-facial HSV-1 infection in children is usually asymptomatic[75, 318]. Primary herpetic gingivostomatitis, with fever and oral lesions lasting 10-14 days, is the most common specific syndrome. It is symptomatic in 25-30% of children acquiring the disease, and occurs most commonly before the age of three[13]. HSV-1 viremia has been observed in 34% of cases[142]. In young adults, HSV-1 accounts for 10% of cases of pharyngitis and tonsillitis[345]. Approximately 15-30% of HSV-1 seropositive individuals develop recurrent labial herpes[33, 347]. One quarter of symptomatic adult volunteers experience recurrences more than once per month. The frequency and severity of recurrences decreases with time[33, 347].

Table 3.1 Most frequently reported clinical manifestations of HSV infections in immunocompetent individuals

Common syndromes		**Common agent**
Orofacial infections	Primary gingivostomatitis	HSV-1
	Primary pharyngitis	HSV-1
	Recurrent labial herpes	HSV-1
	Recurrent facial infections	HSV-1
Genital infections	Primary genital herpes	HSV-2 and HSV-1
	Recurrent genital herpes	HSV-2 and HSV-1
	Proctitis and perianal infections	HSV-2 and HSV-1
Common complications		
Extraoral and -genital lesions	Buttock, groin, and thigh	HSV-2
	Perioral, facial and intranasal lesions	HSV-1
	Herpetic whitlow	HSV-1 and HSV-2
Central nervous system	Aseptic meningitis	HSV-2
Uncommon complications		
Extraoral and -genital lesions	Herpes gladiatorum and venatorum	HSV-1 and HSV-2
	Generalized eczema herpeticum	HSV-1
Ocular complications	Blepharitis, conjuctivitis, keratitis	HSV-1
	Chorioretinitis	HSV-1 and HSV-2
	Acute necrotizing retinitis	HSV-1 and HSV-2
Central nervous system	Recurrent aseptic meningitis	HSV-2
	Encephalitis	HSV-1
	Neonatal herpes	HSV-1
Immunologic sequelae	Herpes-associated erythema multiforme	HSV-1
Rare complications		
Intraoral	Recurrent gingivostomatitis	HSV-1
Visceral infections	Esophagitis, pneumonitis, hepatitis	HSV-1 and HSV-2
Autonomic and sensory nervous system	Autonomic dysfunction and neuralgia	HSV-2
Central nervous system	Transverse myelitis	HSV-2
Disseminated infection	Cutaneous and multiple organs	HSV-1 and HSV-2

In immunocompetent children, primary intraoral infection occurs mainly on non-keratinized tissues, most often on inner lip and buccal mucosa. Most recurrent lesions develop within keratinized epithelial tissues at the mucocutaneous junction of the lip. Of symptomatic patients with labial or genital HSV-1, 16-29% have manifestations at a distant site. Intranasal, facial, and hand lesions, erythema multiforme, and keratitis are the most common complications[33, 42, 347]. Recurrent intraoral lesions usually develop within keratinized tissues on the hard palate, gingiva, or tongue[111, 121]. In adults, nonprimary intraoral lesions are mainly seen in immunosuppressed patients with lymphopenia or monocytopenia[320, 392]. Unlike in immunocompetent patients, non-keratinized mucosa represents approximately 50% of all sites[111, 392]. The differential diagnosis of intraoral ulcers is complex. Atypical manifestations such as glossitis have been reported in patients with secondary immunodeficiency[243].

Primary herpetic gingivostomatitis is only occasionally seen in

immunocompetent adults[76, 82, 111, 147]. Recurrent disease is rarely seen. During the last 16 years, approximately 52 adult patients with ≥ 1 clinical recurrence have been described in the Anglo-Saxon literature[82, 111, 147, 308]. Only 28 of these have suffered from multiple recurrences[111, 308]. All the reports come from tertiary referral centers. The clinical course of adult patients follows a more severe course than that seen in young children[147].

Genital HSV-2 infections, reactivations, and neuralgias

Symptomatic primary genital herpes frequently manifests as multiple lesions within non-keratinized epithelial cells (urethra, medial aspects of the labia minora, vagina, and exocervix) and keratinized epithelial cells (penis, lateral aspects of the labia minora, and external genitalia). In immunocompetent individuals, recurrent lesions mainly develop within keratinized tissues[89, 121]. During the first clinical episode of genital herpes, extragenital lesions are observed in 25%, 9%, and 2% of patients with primary HSV-1, primary HSV-2, and initial HSV-2 infection, respectively. Of these patients, 50% will subsequently have extragenital recurrences[42]. After primary HSV-2 with genital lesions only, 6.5% of patients will develop non-genital lesions over time[42]. Extragenital HSV-2 recurrences are mainly seen below the waist, on the buttocks and legs[42, 89]. Dermatomal neuralgias have been reported mainly in those 0.04-0.7% of individuals with concurrent aseptic meningitis, dysfunction of sacral autonomic nervous system, and urinary retention during primary infection (Elsberg's syndrome)[89, 91]. In those with recurrent dermatomal neuralgias, neuralgia is usually the most bothersome symptom[89].

Severe and widespread primary infections are associated with higher recurrence rates[40, 42]. Suppressive antiviral therapy, previous HSV-1 infection, and condom use at the time of the primary infection reduce the frequency of the subsequent clinical disease. Female gender, high number of sexual partners, and previous sexually transmitted diseases increase the likelihood of clinical episodes[89, 91, 195]. Frequent recurrences are less likely in those with HSV-1 genital infection, in a disease manifesting as buttock lesions, and those with remotely acquired infection during the first clinical episode[42, 102, 114].

Data on recurrence rates among seropositive persons are limited. Data mostly come from studies by Benedetti et al., whose patients come from a specialized referral center established for the study of genital herpes. In 15 years, they were able to recruit 457 referred patients with first-episode genital HSV and unknown HIV-status, likely suffering from more severe disease[40-42]. Of HSV-2 patients, 89% experienced a first-year recurrence, the median rate of recurrences was five per year among men, and four per year among women[40]. Of these patients, 38% had ≥ 6 recurrences, and 26% of men, 14% of women had ≥ 10 recurrences during the first year[40]. Those with a longer follow-up (1–8 years) represent the subgroup of patients

with a severe primary and a recurrent disease. In these, with a mean first-year rate of 8.5 recurrences, the annual decrease of recurrence rate was 0.7 recurrences per year[41]. Based on this selected material, approximately 10% of genital herpes patients will have ≥ 10 annual recurrences.

Diagnosis

Diagnostic methods to establish the diagnosis of HSV infection include viral culture, antigen detection, nucleic acid amplification techniques, and serologic tests[306]. Serologic tests accurately distinguish between HSV-1 and HSV-2[375]. When modern enzyme-linked immunoassay methods testing type-specific antibodies against gG are used, seroconversion in primary or initial infection takes place after approximately 3 weeks[23]. Intrathecal antibody detection may be used in central nervous system infections. It has an overall specificity and sensitivity of ~ 80% in herpes encephalitis. Yet intrathecal antibodies take 10-12 days to develop, the response may be delayed or absent, and cross-reactivity with varicella zoster is seen[176]. Therefore, nucleic acid amplification tests are the primary diagnostic methods in central nervous system infections.

Since serum seropositivity is common, the demonstration of anti-HSV-IgG in serum unreliably distinguishes HSV infection from other diseases causing compatible disease forms. Thus in acute disease manifesting with vesicular or ulcerative lesions, viral culture and typing of HSV remains the preferred diagnostic method[306]. Its sensitivity, when used to test early vesicular lesions, is ~ 90% and falls to ~ 70% in ulcerative lesions, and further to ~ 27% in crusted lesions[306]. Cytospin-enhanced direct immunofluorescence assays to detect HSV antigen may be preferred, because they are rapid and do not require living viruses in the sample[193]. Nucleic acid amplification tests to detect HSVs use type-specific primers. These tests are highly specific and sensitive, and they also do not require living virus. Since asymptomatic shedding is common, and also takes place during diseases that mimic HSV infection, care should be exercised in atypical disease forms when laboratory results of samples taken from mucosal sites are interpreted[306]. When isolation of HSV is attempted from otherwise sterile samples (such as cerebrospinal fluid), nucleic acid amplification tests have become the diagnostic gold standard[176].

Immune response to infection

The clinical recurrence rate of HSV-2 is thought to depend on the immune proficiency of an immunocompetent host, but the predisposing genetic factors are largely unknown[181, 385]. During primary infection, the free virion encounters and evades from ever-present, secreted host proteins such as complement and natural IgM antibodies[105, 152]. The free HSV virion is also recognized by TLR2 and TLR9 on stromal cells, NKs, macrophages, and DCs present on infected area[236]. At an early stage, infected epithelial cells

and resident DCs produce type I IFNs (IFNαβ)[105]. During the next 1-2 days, recognition by humoral innate immunity triggers the recruitment of neutrophils, monocytes, and activated NK cells to the area. IFNγ production by recruited NK and T cells commences after 2-4 days[105]. Adaptive responses and resolution of the primary infection is delayed in humans, with CD4+ T cell responses developing approximately 7-10 days, and ADCC 8-10 days after disease onset[183, 387]. A robust DC-driven Th1-dominant cytokine response is considered important[105, 250, 281]. Within infected ganglionic cells and peripheral sites, resolution is dependent on and temporally associated with the infiltration of CD8+ T cells[66, 182]. In ganglia, this is not achieved by CTL-mediated killing of neurons, but rather by cytokine-mediated halt of replication[275].

Cellular immunity is considered more important in recurrent HSV infections, as demonstrated by frequent reactivations in HIV positive and immunosuppressed subjects[282]. The exact role of antibodies is unknown. In recurrent infections, specific antibodies and complement offer immediate protection. Mounting effective cellular immunity against HSV is delayed[281]. Latency in infected sensory ganglia is retained by activated, virus-specific CD8+ memory T cells[284]. HSV recurs in the face of persistent functional HSV-specific CD8+ CTLs[280]. When HSV reactivates and moves from neurons to epithelial cells, complement components, antibodies, CD4+ T cells, macrophages, and DCs are present[105, 281]. During the next 1-2 days neutrophils, CD4+ T cells, and NK cells infiltrate the area[105, 281]. Low IFNγ and Th1 responses in humans are associated with cutaneous recurrences[95, 184, 224, 346]. Yet, IFNγ is mainly secreted by CD4+ T cells and NK cells appearing after 2-4 days[105, 181]. Finally, arriving CD8+ cytotoxic T cells clear the virus[281].

Antibody and complement responses during reactivations

In mouse models, HSV-specific vaginal antibody is mainly IgG[266, 267]. Immunized IgA knock-out mice are not particularly susceptible to HSV-2 in murine vaginal challenge model[181]. Antibody responses reduce neurovirulence of HSV-1 in mice and *in vitro*[138, 229, 380]. ADCC activity is associated with protection from HSV in multiple animal models[184]. Accordingly, in mice intact HSV-specific antibodies confer passive protection against viral challenges, whereas neutralizing F(ab)2 fragments lacking the Fc domain do not[184]. HSV viral Ig Fcγ receptor (vFcR) knock-out viruses are less virulent in mice, and B-cell knock-out mice are more susceptible to HSV infections[239, 267]. The Ig heavy chain locus on murine chromosome 12 (*igh@*)has been identified as a susceptibility factor to corneal HSV-1 infections, suggesting that Ig allotypes differ in their protective capacity[246]. In dermal models with HSV-1 as antigen, the CP of complement is required for efficient formation of specific antibodies[68].

Specific antibodies produced in recurrent HSV infections are mainly

IgG1, IgG3, IgG4, and IgA[144, 219]. Anti-HSV-IgG4 is mainly seen in patients with nonprimary infections. IgG1 and IgG3 generally defend us against intracellular pathogens, and their production is enhanced by Th1 responses[85, 148]. These, in turn, have been associated with protection from HSV recurrences[95, 333, 346]. Serum and local antibody responses are broad, and react with envelope glycoproteins, tegument, and capsid proteins[22]. Symptomatic subjects may have higher levels of antibody than asymptomatic carriers[346]. Vaccine development has largely been unsuccessful[181]. An immunogenic gD-adjuvant vaccine has limited efficacy against genital HSV-2 in women who are seronegative for both HSV-1 and HSV-2[348]. No consistent correlations between levels of antibodies, their subclasses, their functional activities, or their fine specificities and the severity of HSV infection have been demonstrated[181].

Frequent genital recurrences and intraoral HSV lesions are associated with reduced ADCC[90, 130]. Duration of HSV-2 excretion is shortest in patients with pre-existing complement-fixing antibodies[90]. The poor capacity of candidate HSV vaccines to elicit ADCC has been associated with poor efficacy[184]. The relative efficiencies in complement activation and ADCC against HSV-1 vary according to the Ig subclass and allotype[24, 85]. The data above suggest that ADCC and CP are important in defense against HSV[90, 219]. Arguing against the role of antibodies, there is no excess of typical HSV infections in patients with severe hypogammaglobulinemias[385]. Yet in these patients multiple concurrent defects of cellular immunity could even further predispose to HSV[98]. Intravenous immunoglobulin therapy suppresses genital herpes recurrences in humans[218].

Wild-type HSV prevents C5 activation through its immune evasion molecule gC (see below), protecting against complement-mediated neutralization and complement-mediated lysis. This enables HSV to retain full infectivity[340]. Immunologic injury of HSV-infected cells requires both complement and anti-HSV antibodies[58]. The complement-activating innate pattern-recognition receptors recognizing HSV include natural immunoglobulin IgM and MBL. C1q does not recognize and opsonize HSV directly; surfactant protein A may neutralize the virus in the respiratory tract[122, 125, 152, 369]. Complement aids in the selection and maintenance of B1 cells, the main source of natural (IgM) antibody[68]. During the primary response, neutralization of HSV by IgM requires CP[225]. Later, the neutralization of HSV gC-null viruses by human serum also requires CP, but not LP, AP, or MAC , whereas active CP in the absence of natural IgM antibodies is insufficient to neutralize the virus[152]. The extent of HSV neutralization varies up to 10-fold among individuals[152]. Complement fails to aggregate the virus or block its attachment to cells. Inhibition of infection takes place before early viral gene expression[122]. The efficiency and expression of host complement regulators may vary during an infection and between tissues[289]. The inhibition of complement-mediated lysis of infected cells by

complement regulators of the virus may even serve to protect the host[121, 289].

In dermal models with HSV-1 as antigen, CP regulates antibody production at multiple stages. Without an intact early complement CP (C1q, C4, C3, CD21 and CD35), antibody responses against HSV become impaired[68]. Antigens coated by C3 and C4 activation products promote positive selection of specific B cells, their activation and expansion, formation of GCs, antigen retention inside follicular DCs, and effective maintenance of long-term memory B cells[68]. During early lesional stages within keratinized tissue, CP components may be rapidly consumed and not readily available from circulation[121]. To ensure effective coupling of complement CP to HSV locally and in peripheral lymph nodes, local myeloid-derived macrophages and immature DCs produce its early components[72]. Yet, intradermal and intraperitoneal models with C4 knock-out guinea pigs fail to support a critical role of CP in defense against primary HSV-1 infection[352]. Both normal and decreased serum complement levels have been reported in older studies on patients with recurrent genital herpes[173, 374].

Genetic susceptibility

The genetic background of susceptibility to severe or recurrent HSV infections is mostly unknown[181, 385]. Recurrent HSV infections are seen in immunodeficiencies affecting cellular immunity (e.g. SCID, autosomal recessive hyper-IgE syndrome)[132]. Generalized, atypical, or severe infections such as HSV encephalitis may be seen in rare patients having SCID (e.g. signal transducer and transcription activator [*STAT1*], and *UNC93B1* deficiencies), a combined immunodeficiency (e.g. nuclear factor kappa B essential modulator [*IKBKG*] deficiency), or CVID[39, 71, 391]. Encephalitis occurs mostly together with deficient IFNα/β or γ production[71]. In a murine intraperitoneal model, MBL-A and MBL-C double knock-out mice clear HSV-2 ineffectively from the liver. Viral burden of other assessed organs remain unchanged. MBL directly recognizes HSV and may have a role in the hepatic clearance of HSV-2 in humans[56, 125]. MBL may thus protect from HSV infections and their complications, but the mechanisms of action are largely unknown[125]. Studies on genetic deficiencies in humans also point to the importance of MBL in defense against HSV. *MBL2* deficiency and the *MBL2* codon 52 mutation have occasionally been associated with active genital herpes, herpes-associated erythema multiforme, and recurrent aseptic meningitis by HSV-2 in humans[125, 360, 367].

Evasion from host immunity

Delaying and avoiding immune recognition enables HSV to establish a chronic infection. To achieve this, HSV subverts the immune system by using its immune evasion molecules. Most commonly these deflect immune recognition of infected cells by the cellular immunity: they directly inhibit

MHC class I and II expression, attenuate the effects of cytokines, or disarm different effector cells (Table 3.2). HSV mutants lacking immune evasion molecules become largely avirulent. During the first one to two days of a reactivation, the escape from cellular immunity is highly effective[281]. Defense against HSV is then mainly achieved by specific antibodies and rapidly responding arms of innate immunity (see above).

Table 3.2. Immune evasion of herpes simplex viruses from various arms of immunity

Reduced MHC Class I surface expression ICP 47 prevents peptide transport
Reduced MHC Class II antigen presentation Virion-host shutoff protein vhs causes degradation of host mRNA
Reduced MHC Class II antigen presentation $\gamma_1$34.5 protein reverses type I interferon-induced translation block
Disturbed MHC Class II processing pathway Reduction of the expression of invariant chain in infected cells
Inhibition of peptide loading to HLA-DM and -DR Binding of gB to MHC Class II heterodimers
Interference with interferon-dependent antiviral response ICP0 prevents RNase L-independent rRNA cleavage
Interference with interferon-dependent antiviral response Synthesis of 2',5'-oligoadenylate synthetase antagonists
Interference with interferon-dependent antiviral response Reduction of Jak2 and STAT1 levels by SOCS induction
Anti-apoptotic activity of infected cells Actions of US3PK protein kinase, ICP27, gJ, gD and LAT
Apoptosis of activated HSV-infected cytotoxic T cells Expression of US3 protein kinase, upregulated production of fratricide and galectin-1
Functional impairment of cytotoxic T cells Inhibition of T-cell receptor signaling
Apoptosis of activated HSV-infected CD4+ lymphocytes
Disarming of natural killer cells
Disarming of lymphokine-activated killer cells
Inhibition of macrophage differentiation
Apoptosis of immature HSV-infected dendritic cells
Killing and functional impairment of dendritic cells Downregulation of CD1 antigen presentation
Impaired production of interleukin-12 by HSV-infected dendritic cells after LPS stimulation
Loss of CD83 surface expression impairing dendritic cell-mediated T cell stimulation
Evasion from immunoglobulin G and C1q binding Viral Fcγ receptor formed by gE and gI acts through bipolar bridging of specific antibody
Evasion of free virion from complement Viral complement receptor homologs gC-1 and gC-2 bind C3, C3b, iC3b, and C3c
Evasion of HSV-1 infected cell and free HSV-1 virion from complement gC-1 accelerates the decay of the alternative pathway C3 convertase and binds C3b on infected cells

The HSV vFcR, which mediates evasion from antibodies *in vitro* and *in vivo*, is formed by a heterodimer of surface glycoproteins gE/gI[104, 239]. Its affinity to IgG subclasses is in the order IgG4 > IgG1 ≥ IgG2[24]. In Caucasians, vFcR

fails to bind IgG3[164]. The efficiency of IgG1 binding to vFcR also depends on the genetically determined allotype of IgG1[24]. HSV-1 effectively evades IgG1 coded by *G1m*^{a} [21, 24]. At physiologic concentrations, *G1m*^{f} -coded IgG1 is less effectively bound by the HSV vFcγR. Over 80% of vFcγR's are then occupied by non-complement-fixing IgG4[21, 24]. vFcR is able to participate in bipolar bridging, where a single IgG molecule binds by its Fab end to its specific target and by its Fc end to vFcR. Through bipolar bridging the virus effectively resists complement-enhanced antibody neutralization and ADCC[206, 239]. vFcR inhibits the binding of C1q, although vFcR binding site on Fc (on C_H3) does not directly overlap with the C1q binding site near C_H2 domain[344]. gE or gI or both are also indispensable for efficient cell-to-cell spread and axonal localization[279, 380].

HSV has also surface glycoproteins to avoid the AP of complement (gC-1 and gC-2)[105, 206, 340]. gC-1 diminishes the efficiency of complement-mediated neutralization of the free virion and inhibits complement-mediated lysis of infected cells[152]. To achieve this, gC-1 binds C3 and its activation products, C3b, iC3b, and C3c[205]. Furthermore, gC-1 inhibits the binding of C5 and properdin to C3b, blocking activation of both the CP and the AP[187]. It also accelerates the decay of the alternative, but not classical, pathway convertase. gC-1, unlike gC-2, acts as an attachment protein for the virus[340]. HSV-2 glycoprotein gC-2 binds only to C3 and its activation products. Accordingly, gC-2 acts only to reduce complement-mediated neutralization, also that induced by natural IgM, and to facilitate cell-free spread of the virion by binding C3b, iC3b, and C3c[152]. It does not contain a C5- and properdin-interacting domain, and does not accelerate the decay of AP convertase[364]. Mutant viruses unable to express gC are neutralized by the CP, even in the absence of antibodies[340]. In systemic models, gC-2 does not appear to be an important virulence factor[166]. Intradermal models, in which gC-1 of HSV-1 does seem important *in vivo*, have not been studied using gC-2[340].

5.4.2 Acute and chronic rhinosinusitis

Rhinosinusitis is a group of disorders characterized by inflammation of the mucosa of the nose and the paranasal sinuses[226]. Paranasal sinuses, 4-17 in number, constitute a collection of air-filled spaces within the anterior skull. They communicate with the nasal cavity through small meatuses. Goblet cells and nasal glands of the ciliated epithelium that line the nasal cavity and paranasal sinuses produce mucus[115, 341]. This serves to keep the nose moist and to trap particles and pathogens before they enter the lower airways. The mucus contains enzymes that render foreign particles harmless and, with the help of cilia ("mucociliary transport"), transports them down to esophagus[43, 226]. Together, patent maxillary sinus ostia, anterior ethmoidal cells and their ostia, ethmoid infundibulum, hiatus semilunaris, and middle meatus (i.e. "ostiomeatal complex") ensure the maintenance of

optimal sinus ventilation and mucociliary transport. During inflammatory conditions or impaired ciliary function, mucus production may overwhelm the transport capacity of the ostiomeatal complex. This further impairs the ciliary function and export of inflammatory substances and pathogens, causing and exacerbating sinus inflammation. If this condition persists, it can result in chronic rhinosinusitis[115, 341]. The decrease in the quality of life of CRS patients may equal that caused by malignancy[226].

Epidemiology

In US surveys, self-reported chronic "sinus trouble" for more than three months in the previous year affects up to 16% of the adult population, ranking it as the second-most common chronic condition in that country[43, 115]. The prevalence of doctor-diagnosed CRS is much lower, 1-2.4%. CRS is approximately twice more common in women than in men and in the elderly than in young adults[115]. The prevalence of objectively observed nasal polyps is 0.5-2.7%, 30-50% of the subjects are asymptomatic. Polyps are more common in non-atopic (13%) than in atopic asthma (5%). They are approximately twice as common in men and the elderly. Nasal polyps are commonly observed together with bronchial asthma[115]. Women more often have associated comorbidities: the occurrence of polyps and asthma together is twice more prevalent in women than in men. Polyps can be found in 36-96% of subjects sensitive to non-steroidal anti-inflammatory drugs ("aspirin sensitivity"), radiographic findings compatible with mucosal inflammation are found in up to 96% of them. Patients that have aspirin hypersensitivity, asthma, and nasal polyps ("aspirin triad") are frequently non-atopic and over the age of 40[115, 226].

Clinical syndromes

The mildest form of sinus infection is acute viral rhinosinusitis (common cold), with abnormal paranasal sinus CT imaging in ~ 90% of patients[136, 226]. Viral infection and exposure to pathogenic bacteria result in the loss of cilia and ciliated cells. Adults suffer approximately two to five common colds per year[43, 115]. Of common colds, ~ 0.5-2% is complicated by some form of bacterial infection[115]. Factors such as allergic rhinitis, nose blowing, or dental infections may predispose to acute or recurrent (presumed bacterial) rhinosinusitis (ARS)[115, 226].

Numerous subclasses of non-allergic CRS with or without demonstrable inflammatory changes in nasal histology have been proposed, with causative pathogens (e.g. fungal, bacterial) or associated diseases (e.g. polyposis, allergy, diabetes mellitus) as the reason for subclassification[226, 330, 334]. For example, dysregulated activity of autonomic nerve pathways and nociceptive dysfunction may predispose to CRS[226]. In a subgroup of CRS patients, a "ballooning" of the nasal mucosa leads to the development of nasal polyps (Table 3.3). A partly overlapping subgroup of patients have nasal polyposis,

fungal growth from sinuses, and suffer from allergic fungal rhinosinusitis, a disease that immunologically and histopathologically resembles allergic bronchopulmonary aspergillosis (Table 3.3.)[226, 321]. "Non-allergic" or "perennial allergic" rhinitis may be caused by year-round exposure to molds and danders that leads to persistent nasal mucosal edema and eosinophil, mast cell, macrophage, and Th2 lymphocyte infiltration. It has no established definition, but is usually defined as a disease that persists > 9 months each year with symptoms mimicking allergic rhinitis[226, 330, 334]. Its role in the etiopathogenesis of CRS is unclear. The coexistence of CRS and asthma, with upper and lower airway inflammation and lower airway hyperresponsiveness, has been named "chronic inflammatory respiratory syndrome" or "integrated airway syndrome"[226, 255].

Diagnosis

The used diagnostic criteria of rhinosinusitis vary (Table 3.3). According to present definitions, the nasal and sinus symptoms of acute viral rhinosinusitis last < 10 days; allergic symptoms should be included in differential diagnostics[115, 226].

The most important cause of ARS is bacterial superinfection of damaged nasal mucosa. In ARS, there is a sudden onset of two or more of the symptoms: blockage or congestion, anterior discharge or post nasal drip, facial pain or pressure that last > 10 days, or symptoms worsen after initial regress[226]. Objective criteria for ARS include purulent drainage found beyond vestibule or radiographic evidence[226]. The most common bacteria cultured from maxillary sinus aspirates during adult community-acquired ARS are *S. pneumoniae* (~ 40%) and non-typable *H. influenzae* (~ 35%). Other streptococci, anaerobes and *M. catarrhalis* are cultured from 4 to 7 percent of samples. S. *aureus* and *S. pyogenes* are uncommon in ARS[226]. Normal nasal flora in adults includes coagulase-negative staphylococci, *Corynebacterium* species, *S. aureus*, and *S. pneumoniae*[115, 226].

Repeated infections may lead to long-standing impairment of ciliary function and predispose to CRS. The exact definitions for clinical practice and research, of CRS in particular, differ between the three most commonly used criteria (Table 3.3)[115, 196, 226]. Various inflammatory and non-inflammatory conditions can cause symptoms mimicking CRS[226, 330]. The most common cause of diagnostic confusion is allergic rhinosinusitis[334]. Due to the commonness of symptoms mimicking ARS and CRS, radiologic imaging is frequently used. Anterior-posterior and Waters views best visualize the frontal and maxillary, and lateral views the sphenoid sinuses. Ethmoid sinuses are poorly displayed. Radiography cannot differentiate between viral and bacterial sinusitis. In the diagnosis of adult ARS, it has a sensitivity of ~ 0.76 and specificity of ~ 0.79 compared with sinus puncture (the gold standard), but the criteria for its interpretation are controversial[276, 341]. Standard radiographs are inadequate for determination of the need for

and guidance of endoscopic sinus surgery. For evaluation of inflammatory disease secondary to obstruction of the ostiomeatal complex, the coronal plane of computed tomography is the gold standard[341].

In CRS, the most common organisms cultured by various methods (e.g. aspiration, lavage, swab, during endoscopy, cytology) are coagulase-negative staphylococci, *S. aureus*, *S. viridans*, anaerobes (e.g. pigmented *Prevotella*, *Fusobacterium*, *Peptostreptococci*), and gram-negative enteric rods (e.g. *P. aeruginosa*, *K. pneumoniae*, *P. mirabilis*, *Enterobacteriacae*, *E. coli*). Pathogenicity of many of these organisms has been disputed, claimed as colonization, or that isolates are recoverable secondary to a repeatedly damaged mucosal lining having lost its normal sterility[115, 226]. *S. aureus* frequently accompanies nasal polyposis, with a more pronounced multiclonal IgE response to S. aureus enterotoxin (~ 80%) in nasal mucosa compared with other CRS patients (~ 60%)[226]. When bacterial culture and PCR are used, bacteria deemed to be pathogenic are frequently lacking in prominent polypoid tissue[226]. Anaerobe recovery rate varies greatly, but specific IgG antibodies against *F. nucleatum* and *P. intermedia* have been demonstrated in CRS patients and noted to decrease in titer with successful therapy[115, 226]. If there is no invasive growth in histology and the patient has no recognized form of non-invasive fungal rhinosinusitis (i.e. fungal ball, allergic fungal rhinosinusitis), the significance of fungal growth in sinus samples of CRS patients is unclear (Table 3.3)[115, 226]. Since on CT an irregular bony thickening of sinus walls is frequent, a smoldering osteitis has been suggested as a contributing factor to CRS development.

Grading of rhinosinusitis

The most commonly used computed tomography staging system is the Lund-Mackey score. In it, each sinus group is graded between 0 and 2 (0: no abnormality; 1: partial opacification; 2: total opacification) and ostiomeatal complex adds 2 points if obstructed. A total score between zero and 24 is possible[210]. It has been validated in several studies, but the correlation between computed tomography findings and symptoms has consistently been poor, and it is not a good indicator of prognosis[115, 226]. In 2004, a newer classification has been proposed[226]. The role of structural variations of paranasal sinuses (e.g. concha bullosa, nasal septal deviation, displaced uncinate process) in predisposition to CRS is disputable[115, 171, 226]. No consistent difference in the prevalence of anatomical variations between symptomatic and control subjects has been found[115, 226]. The prevalence of both ostiomeatal complex blockade (60%) and low IgG subclass concentrations (81%) has been assessed in one pediatric study on allergic children[93].

The extent of performed operations may be used to grade disease severity. The most accepted surgery score is the Lund-Kennedy score, where seven listed procedures each give one point on each side, up to a total of 14[209]. Accepted absolute and relative indications to surgery have been proposed[14,

[196]. The presence of inflammatory changes should be noted in studies on rhinosinusitis[226]. Sinus fluid of CRS patients undergoing sinus surgery is predominantly neutrophilic. Eosinophil counts per visual field in the mucosal lining of CRS patients without nasal polyposis rarely exceeds 10%, but can be found in ~ 80-90% of patients with polyposis. Tissue eosinophilia may be one of the hallmarks of CRS with nasal polyposis[115].

Immune response to infection

Relatively little is known of local responses during acute rhinosinusitis. Rhinoviruses infect ciliated and non-ciliated epithelial cell[135]. There they activate host immune cells such as monocyte-macrophages through PAMPs such as TLR-3, inducing cytokine and IFN production[165]. Infecting viruses are further transported from the nasal mucosa in the mucus stream to the adenoid region. For example, rhinoviruses reach the lymphoepithelial cells that overlie lymphoid follicles and are rich in rhinovirus receptor intracellular adhesion molecule 1 (ICAM-1)[226]. Plasmacytoid DCs present in perifollicular T cell areas and monocytoid DCs recognize the virus and act as APCs to the cellular immunity[143]. Genetic variations may affect the immune responses to respiratory viruses and lead to the development of asthma and its exacerbations in susceptible individuals[314]. In ARS, a pronounced inflammatory reaction characterized by edema and massive polymorphonuclear and mononuclear cell infiltration, microabscesses, and necrosis ensues locally. Increased numbers of T lymphocytes are diffusely scattered throughout the submucosa, and B cells are organized in aggregates around small veins, adjacent to seromucous glands, and under the epithelium[45].

Histologically, CRS without nasal polyps is characterized with predominantly neutrophilic inflammation with a lesser contribution of eosinophils. In CRS patients with polyps, a more than 10-fold increase in IgE-producing plasma cells and pronounced eosinophilic infiltrate, IL-4, IL-5, and eotaxin expression may be observed locally within the polyps. This happens regardless of whether the patient is atopic or not[226]. Allergic patients with or without nasal polyposis display a more characteristic Th2 prominent response, whereas non-allergic patients have a mixed Th1/Th2 cytokine profile. Th2 profile suggests that delayed hypersensitivity contributes to CRS development in polyposis[226]. Recent – still unproven – hypotheses trying to relate the frequent microbiologic findings and the development of CRS with each other include: 1. biofilm formation that needs debridement, 2. microbial superantigens (*S. aureus*, *Alternaria*, *Aspergillus*) that cross-link MHC II on APC with TCR variable β region and cause extensive T lymphocyte activation (up to 30% of T cells) and generate multiclonal IgE synthesis and anti-superantigen IgE antibodies, and 3. fungal antigen-induced sustained eosinophilic inflammation in most CRS patients[226]. Toxins such as *S. aureus* protein A enhance IL-4 release, expression of CD40L on T cells

and costimulatory molecules on B cells, and cause a V_H bias by interacting with *IGH@* V_H -family gene products[115]. Eosinophilic disease-forms have also been suggested to associate with specific antifungal antibodies of IgG3 subclass, and differentiate these from simple CRS[265].

Bacteria obtained from maxillary effusion aspirates of CRS patients are often, in the order of decreasing frequency, coated with IgG, S-IgA, and C3b[288]. In IgA-deficient patients, low S-IgA levels may be compensated with raised IgM and IgG levels in nasal secretions. In CVID patients on Ig substitution, despite IgG levels in nasal secretions equal to those in healthy control subjects, inflammatory cytokine levels are raised and Ig substitution frequently fails or requires large doses[62, 118, 298, 303]. Thus even in patients with hypogammaglobulinemias, factors other than the lack of sufficient Ig levels may play a role in CRS pathogenesis. An impaired T cell response to recall antigens tetanus and mumps in ~ 55% of patients has been found in a study on 79 CRS patients[77]. Relatively little is known about the significance of complement in protection from CRS. Complement tissue regulators DAF and CD59 are needed to protect nasal epithelium, and both CP and AP are active locally[120, 371]. In ARS, C3b-CR1-mediated phagocytosis takes place, but in CRS C3b binding-capacity of CR1 may be overwhelmed[44, 231]. Genes of complement factors C3, fB, and properdin are similarly expressed in sinonasal tissues of CRS patients with or without nasal polyposis, and in healthy control subjects[194]. Of complement-interacting factors, the gene expression of surfactant protein A is upregulated in CRS, and of serum amyloid A decreased in patients with CRS who suffer from early recurrence of nasal polyps after surgery[194, 199]. Surfactant protein A prevents CP activation and together with C1q enhances the clearance of foreign particles by macrophages[381]. In CRS, low serum C4 and fB levels have been reported[252].

Genetic susceptibility

The prevalence of allergies is higher in CRS patients, but data on it as a predisposing factor to CRS is conflicting[115, 226]. Almost all forms of genetic immunodeficiencies predispose to CRS. In particular, primary antibody deficiencies, primary ciliary dyskinesia, cystic fibrosis (*CFTR*), and hyper-IgE syndromes are frequently complicated by CRS[54]. In a series of 68 CVID patients, recurrent ARS or CRS was present in 76% and preceded the diagnosis of CVID by 15 years[319]. CRS is found in ~ 86% of patients with agammaglobulinemia. The associations between IgG, IgA, and IgG SCDs and bacterial infections discussed in Chapter 2 include CRS. However, there are relatively few studies with even moderate numbers of adult CRS patients recruited, where SCDs would have been assessed. Methodologies of these studies are variable and often poor. Low subclass concentrations, mainly low IgG1[222, 329, 370], IgG2[222], IgG3[18, 153, 211, 313], and IgG4[163] concentrations together with or without low IgA have been described. Frequencies of low subclass

levels may vary depending on the ethnic origin of CRS patients[163]. Despite the reported high frequency of low IgG3 in adult CRS, *Gm3* allotypes are not associated with adult CRS[355]. Yet in adult CRS patients, *G3m*^{g} correlates with low levels of specific anti-*M. catarrhalis* IgG3 antibodies: these, in turn, correlate with adult CRS[129]. The clinical efficacy of pneumococcal polysaccharide and polysaccharide-conjugate vaccines for prevention of mucosal *S. pneumoniae* infections may be poor in homozygous carriers of FcγRIIaR131, which binds IgG2 poorly[388]. Low levels of IgG2 or *G2m*$^{n-}$ of adult blood donors have not been associated with increased susceptibility to recurrent infections[240]. Impaired vaccination responses in adult CRS patients seem extremely rare, with none found in a consecutive series of 245 mainly adult patients with low subclass levels[222].

C2 deficiency predisposes to CRRS[170]. Little is known of genetic factors predisposing to CRS with or without nasal polyposis in patients without overt immunodeficiency. Interestingly, *B8*, *B44*, *B54*, *DR3(17)*, and *DR7* are more frequent in patients suffering from CRRS but having normal antibody levels (not tested in *B54* carriers)[168, 358]. The two most common antibody deficiencies, IgA deficiency and CVID, with susceptibility loci in *DQ/DR*, often occur in association with two extended haplotypes: *A1,B8,SC01,DR3(17)* (with *C4A* deficiency) and/or all or a portion of *A29,B44,SB31,DR7*[189, 319]. *A1,SC01, B8* (and presumably *C4A* deficiency) is overrepresented in patients with IgG4, IgD, or IgG3 deficiency, as well as in patients suffering from nasal polyposis[10, 232]. Further, *DR3* is associated with nasal carriage of *S. aureus*[177]. AFRS and other chronic hypertrophic rhinosinusitis disorders have recently been associated with *HLA-DQB1*03*[321]. Polymorphism of another MHC gene, *LTA*, is associated with higher TNF production and CRS[357]. Recently, IL-1 receptor antagonist gene (*IL1RN*) polymorphism has also been associated with adult CRS[79].

The significance of low IgG subclass values in predicting adult CRS, in patient evaluation, and in the choice of treatment is highly controversial[61]. No studies on the frequency of low subclass values in ARS patients exist, and data on normal population are insufficient (discussed in Chapter 2). Despite the low amount of IgG3 in preparations, intravenous immunoglobulin effectively reduces sinopulmonary infections in severely symptomatic children with low serum IgG3 levels and normal vaccination responses or levels of anti-pneumococcal antibodies[37, 46].

Evasion from host immunity by causative pathogens

IgA proteases that enzymatically destroy IgA1 (distributed predominantly in serum) are produced by *S. pneumoniae* and *H. influenzae*. Such activity results in monomeric Fab bound to microbial antigens and blocks the access of intact antibody molecules and antibody-mediated effector functions[268]. *S. pneumoniae* utilizes attached Fab as a signal to adhere to pharyngeal epithelial cells[213]. *S. pneumoniae* can also interact with secretory component, with the

secretory component portion of polymeric immunoglobulin receptor, and with S-IgA; this may further aid it in immune evasion[101]. *S. pyogenes* is able to degrade both IgG and FcRs[4]. With high affinity, *S. aureus* protein A binds IgG, and *M. catarrhalis* binds human IgD, the proposed benefit is their ability to act as B cell superantigens. This results in an unspecific polyclonal antibody response[309].

Streptococci and *M. catarrhalis* evade from complement by expressing surface molecules that bind fH, FHL-1, or C4bp. Group A and B streptococci also secrete proteins and enzymes that inhibit complement activation and complement-mediated chemotaxis[161, 188]. *H. influenzae* inhibits complement by interacting with vitronectin (Table 1.2)[139]. *S. aureus* staphylokinase degrades cell-bound IgG and C3b, and secretes a chemotaxis inhibitory protein that binds to C5a[157].

Table 3.3 Definitions of chronic rhinosinusitis for research

		Rhinosinusitis Task Force 1997		American Rhinosinusitis Classification 2004		European Rhinologic Society 2005	
Reference		196		226		115	
Symptoms		**Major factors:** Facial pain/pressure Facial congestion/ fullness Nasal obstruction/ blockage Nasal discharge/ purulence/ discolored postnasal drainage Hyposmia/anosmia Purulence in nasal cavity on examination	**Minor factors:** Headache Fever Halitosis Fatigue Dental pain Cough Ear pain/pressure/ fullness	Anterior or posterior mucopurulent drainage or both Nasal obstruction For CRS without nasal polyposis: Facial pain-pressure-fullness For CRS with polyposis: Decreased sense of smell		Nasal congestion/obstruction/blockage and facial pain/pressure or discolored discharge or reduction/loss of smell	
Duration		≥ 12 weeks		> 12 weeks		> 12 weeks	
Criteria	CRS without polyposis	**No subclassification**	≥2 major factors or 1 major and 2 minor factors or nasal purulence on examination	**Subgroups**	≥2 symptoms above and no polyps in middle meatus by endoscopy and presence of inflammation and positive CT imaging with evidence of rhinosinusitis	**Independent groups**[1]	Symptom criteria and no visible polyps in middle meatus, if necessary following decongestant[1]
	CRS with polyposis				≥2 symptoms above and polyps in middle meatus by endoscopy and positive CT imaging with bilateral mucosal disease		Symptom criteria and **No earlier sinus surgery:** endoscopically visualized polyposis in middle meatus[1] **Sinus surgery previously performed:** pedunculated lesions as opposed to cobblestone mucosa > 6 months after surgery on endoscopic examination[1]

[1]Separate criteria for allergic fungal rhinosinusitis, which is not included in the CRS groups and requires demonstrable fungal hyphae from endoscopy samples without invasive growth, positive CT or MRI imaging, eosinophilic inflammation, and evidence of fungal-specific IgE. CRS groups may further be subclassified according to whether there is eosinophilic inflammation or not. CRS = chronic rhinosinusitis, CT = computerized tomography, MRI = magnetic resonance imaging.

5.4.3 Adult periodontitis

Periodontitis is characterized by the gradual loss of deep tooth-supporting connective tissue (periodontium) and alveolar bone as a result of an inflammatory response to subgingival bacteria. This results in the formation of soft tissue pockets or deepened crevices between the gingiva and tooth root. In adult periodontitis, the inflammatory process may progress asymptomatically, cause occasional pain and discomfort and impaired mastication, but eventually results in loosening teeth and tooth loss[277].

Epidemiology

Periodontal diseases are common inherited or acquired disorders. Of US adult population, ~ 22% have mild and ~ 13% moderate to severe periodontitis[277]. Its prevalence varies geographically and according to the used diagnostic criteria[7]. For example, all forms of tobacco use, excessive alcohol consumption, scurvy, osteoporosis, diabetes, and atherosclerosis have been associated with, and may affect the prevalence of adult periodontitis[277].

Diagnosis

The clinical diagnosis of chronic periodontitis is based on visual, spatial, and radiographic assessment of the periodontal tissues and on the space between the tooth and gum. This space deepens from the normal depth of 1-3 mm as the supporting structures are lost. Pocket depths and tissue support are measured at 4-6 locations around each tooth, the amount of supragingival biofilm (plaque), dental calculus, gingival bleeding, and exudate are recorded[19]. In the order of increasing sensitivity, orthopantomography, dental radiographs, or digital subtraction radiography are routinely used to assess the degree of bone support for the teeth[277]. Various diagnostic and classification criteria for inflammatory periodontal diseases have been advocated, but there are no generally accepted, uniform criteria[20].

Bacteria grow as biofilms on tooth surfaces. More than 500 bacterial species can be cultured from dental plaque; supragingival surfaces of one tooth may contain up to 10^9 bacteria. Subgingival load of bacteria is 10^5 times higher in periodontitis patients than in healthy subjects[277]. The number of gram-negative bacteria, anaerobes, and spirochetes that produce leukotoxins, collagenases, fibrinolysins, and other proteinases increases[113]. Most common putative pathogens include *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans* (in young adults), as well as *Prevotella*, *Treponema*, and *Capnocytophaga* species[116]. Epstein-Barr and cytomegalovirus have also been associated with periodontitis[335]. There is insufficient evidence that microbiologic assessment can improve treatment outcomes of chronic periodontitis[277].

Complement in the immune response against associated pathogens

In histologic examination of gingivitis or periodontitis lesions, polymorphonuclear leukocytes line the junctional epithelium in large numbers and appear to attempt to wall off the underlying tissues from the bacterial biofilm[316]. Subgingival dental plaque bacteria are coated with C1q and C4[81]. Gingival fluid C3 is activated and consumed locally during periodontal inflammation. At the same time, the ratio between local and serum C4 decreases[25, 242, 272]. Yet cleavage to C4c has been indemonstrable in gingival fluid[272]. CP activation may thus be halted locally, before or after the cleavage of C4 to C4c. A decreased level of CH50 in periodontitis patients has been suggested by several studies[15, 25, 167, 233].

Genetic susceptibility

It is not fully understood why some adults are prone to overgrowth of oral bacteria, or what triggers the destructive immune response that leads to periodontitis. Data from twin studies indicate that ~ 50% of population variance in periodontitis can be attributed to genetic factors[317]. A number of rare inherited systemic diseases such as Papillon-Lefévre syndrome cause juvenile periodontitis[277]. Of a total of 140 studies on genetic polymorphisms recently reviewed, an association with periodontitis was found in 28 out of 34 studies addressing one or multiple genes in or near MHC. These include 17 out of 20 studies on polymorphisms in class I and II *HLA* loci, 10 out of 14 studies on *TNF*, two out of two on *LTA*, one out of one on *AGER* (advanced glycosylation end product-specific receptor gene), and one out of one study on *EDN1* (endothelin-1 gene)[356]. It is not clear whether these associations are themselves associated with a gene mutation (present in multiple haplotypes in different populations) that causes the susceptibility to periodontitis and is in linkage equilibrium with the candidate genes, or if specific predisposing haplotypes with multiple susceptibility genes exist[349].

Primary and secondary immunodeficiencies predisposing to periodontitis are most commonly associated with impaired opsonization, decreased neutrophil function, neutrophil numbers, production of neutrophil-derived antimicrobial factors such as cathelicidins, or genetic variations in or near cytokine genes[277, 287, 316, 317, 356]. Predisposing primary immunodeficiencies include, for example, cyclic neutropenia, leukocyte adhesion deficiencies, Chédiak-Higashi syndrome, and Morbus Kostmann. Interestingly, patients with Morbus Kostmann are unable to produce cathelicidin LL-37 that is required specifically for defense against *A. actinomycetemconcomitans*[396]. The presence or generation of chemotactic factors is required for polymorphonuclear leukocytes to appear, and effective opsonization of foreign material is required for appropriate function of polymorphonuclear leukocytes. Studies addressing polymorphisms of FcγR genes have found predisposing gene variants, mostly pointing towards the importance of

IgG1- and IgG3-mediated effector functions[204, 316, 317]. CP deficiencies have not been studied in adult severe chronic periodontitis.

Evasion from complement by associated pathogens

The major virulence factors of the best-studied pathogen *P. gingivalis*, proteinases called gingipains, are able to degrade C4, C3, C5, and fB. Gingipains are able to generate local C5a chemotactic activity that allows the prominent accumulation of neutrophils in inflamed subgingival tissues[283]. Whether the assembly of C5a convertase complex, and consequently, the complement-mediated lysis of bacteria is locally impaired, has not been studied (Fig. 1.1).

6. Aims

The aims of the present study were to:

1. assess the frequencies of *C4A* and *C4B* deficiencies, and of low IgG subclass levels in the general population (II, III, IV) and in blood donors without a history of rhinosinusitis (II),

2. assess the frequencies of C4 deficiencies and low subclass levels in patients with rhinosinusitis (II),

3. examine whether genetic deficiencies of *C4* (I, II, III, IV), low IgG subclass levels (II, III), IgG1 and IgG3 allotypes (III), and *HLA-A**, *-B**, and *DRB1** gene polymorphisms (I, III) are associated with recurrent or chronic mucosal infections such as herpetic gingivostomatitis, genital herpes infection, rhinosinusitis and periodontitis,

4. examine, whether immunologic deficiencies or polymorphisms found in the study patients associate with any complications of the diseases studied, or with any associated comorbidities (I, II, III),

5. compare the applicability of a new PCR-based quantitative analysis of *C4A* and *C4B* genes with the traditional C4A and C4B protein allotyping (II, III, IV).

7. Subjects and methods

7.1 Study subjects

Patients were recruited into case-control studies applying strict criteria that were designed to select those with a severe, recurrent or chronic, form of the respective disease. The subjects and their inclusion criteria are listed in Table 4.1. For *HLA** typing, subjects from the Finn90 cohort served as historical control subjects[203]. The local scientific ethics committee approved the study protocols.

7.2 Definitions

The group of 150 consecutive voluntary control subjects (II, III, IV) was named as the "unselected group" (used below). Due to small differences between the unselected and healthy group in Study II, they were analyzed together and named the "combined group". In Study III, those seropositive for HSV-1 or HSV-2 were named "HSV seropositive". In all studies, "low" levels of plasma C3, C4, IgA, IgM, IgG, IgG1, IgG2, IgG3, and IgG4 and serum CH50 were defined as values below two standard deviations from the mean and applying manufacturer's reference values. Activity above 200 IU/ml was coded as 200.

In Study II, nasal polyposis was based on histology, anterior rhinoscopy, or perioperative clinical findings. Septal deviation was diagnosed clinically. Bronchial asthma was diagnosed by a specialist, with the right to reimbursable medication through the Social Insurance Institution of Finland. Hypersensitivity to non-steroidal anti-inflammatory agents (NSAIDs) was based on clinical history of provoked, compatible symptoms. Allergy was diagnosed by a previous positive skin prick test or allergen-specific IgE in serum. Nonallergic rhinitis was defined as recurring or perennial, typical inflammatory symptoms to inciting allergens (e.g. pollens, animals, foods) together with negative allergy test results[330]. In irritant rhinitis, the patient had frequent symptoms to nonspecific irritants (e.g. fumes, solvents). Immunodeficiency or rheumatic disease was based on published criteria[146, 248]. In cases of multiple FESS operations, we used the highest-scoring. In noneosinophilic histology, no surplus of eosinophils was reported by the pathologist.

Table 4.1 Subjects in studies I-IV

Recruited from	Inclusion criteria[1] and study entry	No. studied (excluded)	Study
Division of Infectious Diseases Helsinki University Central Hospital Finland	Consecutive patients with intraoral HSV-1 infection > 10 recurrences yearly Clinically typical recurrent blisters predominantly in the hard palate and gingiva Study entry between March 1999 and June 2000	3 (0)	I
	Consecutive patients with chronic or recurrent rhinosinusitis Rhinosinusitis Task Force 1997 criteria[2] fulfilled No clear response to sinonasal surgery other than septoplasty, to short-course antibiotics, and to maximal topical medical management Study entry between March 1996 and March 2001	48 (7[3])	II
	≥ 10 HSV-2 recurrences yearly for > 12 months after the first clinical episode Clinically typical disease with vesicles Positive HSV-2 culture result from an active genital or extragenital lesion Study entry between April 2000 and June 2004	52 (5[4])	III
Vihti Municipal Health Center Finland	Consecutive voluntary patients with acute rhinosinusitis < 4 yearly episodes of purulent rhinosinusitis, no previous episodes No previous rhinosinusitis episodes lasting > 3 months Symptoms lasting > 7 days Fluid level or opacity in a sinus radiograph or purulent discharge in sinus puncture with lavage Study entry in 4 time-periods between February 2001 and June 2002	50 (0)	II
Dental offices of three periodontists Helsinki, Finland	Consecutive patients attending scheduled post-treatment maintenance care Native Finns treated by the same periodontist for ≥ 5 years Periods of reemerging gingival inflammation and deepening periodontal pockets despite good oral hygiene and regular maintenance care 3-12 months apart Favorable response after the initial series of periodontal treatment 18-55 years old at the time of referral to the specialist clinic Advanced periodontal disease: > 20 affected teeth, alveolar bone loss in > 10 teeth Study entry between March 2002 and November 2003	37 (1[5])	IV
Vita Laboratory Ltd Helsinki, Finland	Consecutive voluntary subjects coming for a health survey before accepting a new occupational post Study entry between February and October 2003	150	II, III, IV
Finnish Blood Transfusion Service Helsinki, Finland	Age- and sex-matched subjects from 100 consecutive voluntary blood donors with no reported history of rhinosinusitis Study entry in 2 time-periods between May and July 2001	48 (52)	II

HSV = herpes simplex virus. [1]In all studies, the subjects had to be > 18 years old. In I, III, and IV, only patients without a known immunodeficiency were recruited. [2]See Table 3.3. [3]2 patients refused, 2 died before inclusion, 1 had small vessel vasculitis, 1 was pregnant, and 1 patient was found to have human immunodeficiency virus infection. [4]3 patients refused, 2 patients were noncompliant. [5]Withdrew from the study.

In Study III, neuralgias were classified as prodromal (ending in ≤ 24 hours after vesicle formation), prolonged (lasting > 24 hours after vesicle formation), or chronic.

7.3 Collection, processing, and storage of blood samples

Blood samples were drawn into tubes containing heparin, citrate, or EDTA (10m*M*), as appropriate. In Studies I and III, all blood samples were taken during an asymptomatic period of > 4 weeks, typically during prophylactic antiviral treatment. Genomic DNA from blood leukocytes was isolated with commercial kits (QIAamp DNA Blood Kit, Qiagen, Austria or Puregene Kit, Gentra Systems, USA). All samples were kept frozen at -70ºC.

7.4 Analytical methods

Microbiologic analyses

In Studies II and III, bacteria and viruses were isolated with standard cultures. In Study I, the combination of positive anti-HSV-gG1 antibodies and negative anti-HSV-gG2 antibodies (HSV-1 ELISA IgG and HSV-2 ELISA IgG; MRL Diagnostics, CA, USA) together with positive in-house HSV-1 PCR, histology with HSV-specific immunostaining, or cytospin-enhanced direct immunofluorescence assay was used[186, 193, 278, 373]. Slides were stained with polyclonal rabbit anti-HSV-IgG followed by fluorescein isothiocyanate-labeled anti-rabbit IgG (Jackson ImmunoResearch Laboratories, PA, USA).

In Study III, type-specific anti-HSV IgG (HerpeSelect 1&2 ELISA IgG, Focus Diagnostics, CA, USA) against gG and IgM (EIAgen HSV IgM, Adaltis, Italy) antibodies were analyzed. For anti-HSV-2 IgG subclass measurements, microtiter plates were coated with HSV gG-2 protein (Aalto Bio Reagents, Ireland). Sera were tested at a dilution of 1:100 (anti-HSV-2-IgG1) or 1:10 (anti-HSV-2-IgG2-4). After incubation with anti-human-IgG, subclass-specific mouse monoclonal antibody, peroxidase-conjugated anti-mouse antibodies (DakoCytomation, Denmark) were used to detect the amount of bound antibody[311, 325]. Results were displayed as enzyme immunoassay units (EIU) with a standard curve as a reference[185].

Immunologic analyses

The principal immunologic studies performed are listed in Table 4.2.

Table 4.2 Laboratory methods used in Studies I-IV

Method	Used in
Allotyping of C4A and C4B proteins by electrophoresis	I, II, III, IV
Serum/plasma C3 and C4 concentrations by nephelometry	I, II, III, IV
Serum classical pathway hemolytic activity by standardized hemolysis-in-gel assay	I, II, III, IV
Serum alternative pathway hemolytic activity by standardized hemolysis-in-gel assay	I
Plasma IgM, IgG, IgA, IgG1, IgG2, IgG3, IgG4 by nephelometry	I, II, III
Allotyping of *G1m*^{a}, *G1m*^{f}, *G3m*^{g}, and *G3m*^{b} proteins and quantitation of allotypic subsets	III
Anti-HSV-1- and anti-HSV-2-IgG by ELISA	I, III
IgG1-, IgG2-, IgG3-, and IgG4-subclass specific anti-HSV-2-antibodies by ELISA	III
HSV isolation	I, III
Non-quantitative isotype-specific PCR amplification of *C4A* and *C4B*	I
Quantitative isotype-specific real-time PCR amplification of *C4A* and *C4B*	II, III, IV
HLA-A*, -B*, -DRB1* genotyping (HLA-C* genotyping in study I)	I, III

HLA = human leukocyte antigen, HSV = herpes simplex virus, PCR = polymerase chain reaction, EIA = enzyme-linked immunoadsorbent assay.

C3 and C4 concentrations in serum (I) or plasma (II, III, IV) were measured by nephelometry (Behringwerke AG, Germany). Serum CH50 was analyzed either by a standardized hemolysis-in-gel assay (I, Binding Site, UK) or by an enzyme-linked immunosorbent assay (ELISA) technique (II, III, IV, CH50, Quidel Corporation, CA, USA). Serum alternative pathway hemolytic activity (I, Binding Site, UK) was analyzed by a standardized hemolysis-in-gel assay.

Immunoglobulins IgG, IgM, and IgA were measured from the sera by nephelometry using the reagents and BN ProSpec Analyzer from Dade Behring (Marburg, Germany). IgG subclasses were measured by nephelometry with Behring BNA Analyzer using PeliClass reagents (Sanquin, Amsterdam, The Netherlands).

Gene analyses

Allotypic markers G1m^{a} and G1m^{f} of IgG1 were detected with a double diffusion precipitation in gel (IsoGel Agarose, FMC BioProducts, Rockland, Maine, USA) containing 3.8 per cent of Polyethylene glycol 6000 (Fluka, Switzerland)[312]. Allotyping for IgG3 proteins was obtained as a byproduct of their quantitation with an inhibition ELISA as referenced[326]. The allotype-specific reagents used were mAb clone 5E7 code 102 for *G1m*^{a}, mAb clone 12D9 code 301 for *G3m*^{b}, mAb clone 8D10 code 303 for *G3m*^{g} (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands) and mAb GG-6 (Bio Makor, Rehovot, Israel) for *G1m*^{f}. Allotyping of C4A and C4B proteins to detect C4 nulls (C4Q0) was performed electrophoretically from carboxypeptidase B (Roche Diagnostics

Gmbh, Mannheim, Germany) and neuraminidase (Sigma-Aldrich Chemie Gmbh, Type IV, Steinheim, Germany) treated serum samples followed by immunofixation with polyclonal anti-C4 antibody (DiaSorin Inc, Stillwater, MN, USA) with the standard procedure[216]. C4A and C4B allotypes were run to specific positions on the gel in relation to the standards.

In Study I, the absence of a C4 isotype was confirmed by isotype-specific PCR amplification of *C4A* and *C4B* genes[35]. For quantitative analysis of *C4A* (II, III, IV) and *C4B* (III, IV) genes we used probe-based Scorpions technology[384]. Both probes and reverse primers were based on published primer sequences[35]. The tests employed 5-carboxyfluorescein (FAM) -labeled Scorpions C4A and 6-carboxyrhodamine (ROX) -labeled C4B probes and an unlabeled reverse primer, according to manufacturer's instructions with minor modifications. The actual probes were designed and manufactured by Eurogentec (Seraing, Belgium). Some of the samples were re-checked with unlabeled primers with SYBR Green QPCR (Stratagene, Cedar Creek, Texas, USA) or Absolute QPCR SYBR GREEN MIX (Abgene, Epsom, UK).

In Study I, DNA samples were genotyped for *HLA-A*, -B*, -C**, and -*DRB1** with commercial kits of Pel-Freez (PF-ABC-SSP, Brown Deer, WI, USA), One Lambda (SSP ABDR, Canoga Park, CA, USA) or INNO-Lipa (LiPA HLA-DRB1, Zwijndrecht, Belgium). In Study III, *HLA-A*, -B**, and -*DRB1** were typed (Biotest HLA-ABDR SSPtray, Biotest, Dreieich, Germany).

7.5 Statistical analyses

In Study I, no formal statistical analysis was performed. In Studies II-IV, differences in proportions between groups were tested by the $\chi 2$ or the Fisher's exact 2-tailed test, as appropriate. Odds ratios (OR) and their confidence intervals (CI) calculated with logistic regression analysis were used to measure the differences between the groups. Forward stepwise logistic regression was used to identify any potential predictors of CRRS, of frequent recurrences of genital herpes, and of severe chronic periodontitis from candidate variables in comparisons of these groups versus their control groups in the respective studies. In continuous variables, comparisons between more than two groups were done by non-parametric analysis of variance (Kruskal-Wallis test, Jonkcheere-Terpstra test for ordinal groups) If the variance analysis showed significant differences between groups, the 2-sample t test with Bonferroni correction was used to locate them. In Study IV, the differences in the means of C3, C4, and classical pathway hemolytic activity of complement (CH50) between groups were evaluated by ANCOVA, and the results were presented with adjustment for age.. Correlations between continuous variables were studied with Spearman's rhos. Stepwise linear regression was used to estimate the effect of C4 gene

numbers, age, and gender on the levels of C4 and CH50.

In Study II, ORs for C4 nulls were calculated with Epi Info version 6. In Study III, patients' MHC haplotypes were constructed, using three markers (*HLA-A*, -B*, -C**), by PHASE V.2.02 software[350, 351]. For all other analyses, we used SPSS package for Windows, versions 12.0.1 (II, III) and 13.0.1 (IV). Missing values were excluded from the analyses.

8. Results

By concurrently surveying multiple arms of immunity, we found subtle disturbances in adaptive or innate immunity or both in all our studied patient groups with exceptionally recurrent or chronic mucosal infections. To the best of our knowledge, our study was the first to suggest the following associations: intraoral herpes and HLA homozygosity or total *C4* isotype deficiency or both; CRRS and *C4A* deficiency or the combination of low plasma levels of IgG4 together with low levels of either IgG1 or IgG2 or both; frequently active genital HSV-2 infection and the $G3m^{g},G1m^{a/a(x)}$ haplotype or low plasma IgG1 or IgG3 concentrations or all; protection from herpetic neuralgias caused by HSV-2 and *C4* deficiencies; and severe chronic periodontitis and *C4* deficiencies. Somewhat surprisingly, this was the first published study to assess the frequency of low Ig concentrations in ARS patients and of low IgG subclass concentrations in a carefully selected general adult population not consisting of blood donors. This was also the very first study to associate a genetic polymorphism or mutation ($G3m^{g},G1m^{a/a(x)}$ haplotype), in a carefully characterized patient population, with frequently symptomatic typical genital herpes[125, 181, 360].

Table 4.3 Percentage of subjects with C4A and C4B gene numbers fewer than two in study groups

Study group	n	< 2 *C4A*	No *C4A*	< 2 *C4B*	No *C4B*	< 2 *C4A* or *C4B*
Frequent intraoral HSV-1 recurrences[1]	3	33	33	67	67	100
Chronic or recurrent rhinosinusitis[2]	48	38	2	46	6	83
Acute uncomplicated rhinosinusitis[2]	50	8	4	48	4	56
No lifetime history of rhinosinusitis[2]	48	19	0	38	13	56
Frequent genital HSV-2 recurrences[1]	52	25	0	44	15	64
Severe chronic adult periodontitis[1]	37	22	0	54	8	76
Subjects from general population[3]	150	18 / 17	1 / 1	39 / 41	10 / 10	57 / 56

[1]Results obtained by genotyping. [2]Results obtained by allotyping. [3]Results obtained by allotyping / genotyping.

8.1 Frequently recurring intraoral herpes caused by HSV-1

In Study I, the three successive patients suffering from exceptionally active intraoral HSV-1 infection were all found to be homozygous for their *HLA-A**, *-B**, *-C**, and *DR1** alleles. They also had a total *C4A* (1 patient) or *C4B* (2 patients) deficiency and reduced serum C4 levels. The patient with total *C4A* deficiency had reduced serum CH50, and it was below the detection limit in the two patients with total *C4B* deficiency. In Finns, the prevalence of *HLA* homozygosity for the 9 most common haplotypes is known to be in total 0.03. All three patients had low Ig (sub)class values. Patients 1 and 3 had low levels of the IgG1 subclass. Patient 2, a *HLA A1*,B8*,DR3** homozygote, had a slightly decreased level of IgA.

8.2 Frequently recurring genital herpes simplex type 2 infection

The 52 consecutive adult patients included in the Study III all had highly frequently recurring genital herpes caused by herpes simplex virus type 2 cultured from typical lesions (Table 4.4).

Table 4.4 Characteristics of 52 patients suffering from frequently recurring genital herpes

Characteristic	Value
Male sex	19 (37)
Mean age at diagnosis (range) -yr	38.2 (23-62)
Mean duration of clinical disease at study entry (range) - yr	9.1 (2-32)
Mean number of recurrences (range) / yr	20 (10-48)
Severe first symptomatic genital infection	22 (42)
After first clinical episode, patient-reported typical recurrences	
Frequent recurrences within months	14 (27)
Frequency accelerating slowly over the years	28 (54)
Frequent recurrences delayed for over 2 years	10 (19)
Factors predisposing to recurrences	
Stress	40 (77)
Disturbed diurnal rhythm	22 (42)
Sun exposure	16 (31)
Respiratory infections	15 (29)
Mechanical irritation	6 (12)
Premenstrual period (of women)	18 (55)
Mid-period (of women)	9 (27)
Atypical (aborted) lesions	11 (21)
Radicular neuralgias:	31 (60)
Only during prodrome	8 (15)
Duration > 24 hours after vesicle formation, not chronic	17 (33)
Chronic	6 (12)
Immunoglobulin G antibodies against herpes simplex virus type 1	23 (44)

NOTE. Values are n (%), if not otherwise specified. From Study III, ref.[327], with permission from University of Chicago Press.

Recurrent extragenital vesicles were frequently seen (71%), most commonly on the lower back (37%). Postherpetic polyarthralgia, a rarely reported complication of genital herpes, was reported by 15% of the patients. Recurrent herpes in first-degree relatives was reported by 42% of patients. Age, duration of clinical disease, severity of the first clinical episode, or any other reported clinical manifestation – with the exception of neuralgias – did not associate with any measured immunologic variable.

Immunologic tests

The results of immunologic tests were then compared between the patients with genital herpes and the unselected control subjects. Total levels of plasma IgG, C3, C4, CH50, IgG1, and IgG3 were lower in the patients than in the control subjects (Table 4.5). Levels of IgM, IgA, IgG2, and IgG4 did not differ between the groups. A decreasing linear trend in the levels of C3 ($P = 0.016$), CH50 ($P = 0.023$), IgG ($P = 0.030$), IgG1 ($P < 0.001$), and IgG3 ($P = 0.003$, Jonkcheere-Terpstra for all) was noted in the order: seronegative control subjects > seropositive control subjects > patients.

Table 4.5 Total plasma levels of complement-fixing immunoglobulin subclasses G1 and G3 and complement studies in patients with frequent genital herpes recurrences and in control subjects.

Factor	Patients (n = 52)	Controls (n = 150)	P[1]
IgG1, g/L (ref 4.9.-11.4 g/L)	5.96 (4.04-11.90) [4.05-11.07]	6.60 (2.88-13.30) [4.00-10.41]	0.004
IgG3, g/L (ref 0.2-1.1 g/L)	0.24 (0.05-0.61) [0.06-0.59]	0.32 (0.11-0.84) [0.13-0.67]	0.001
C3, g/L (ref 0.5-1.5 g/L)	0.91 (0.65-1.45) [0.66-1.39]	1.01 (0.49-1.58) [0.66-1.46]	0.002
C4, g/L (ref 0.15-0.5g/L)	0.17 (0.05-0.37) [0.06-0.36]	0.19 (0.04-0.34) [0.10-0.33]	0.040
CH50 IU/ml (ref 50-130 IU/ml)	93 (20-200) [25-187]	110 (30-200) [45-200]	0.005

NOTE. Data are median (range) [2.5th-97.5th percentile], unless otherwise specified. CH50 was measured in serum, other parameters in plasma. CH50 = serum classical pathway hemolytic activity, values ≥ 200 counted as 200, ref = reference value. [1]Mann-Whitney 2-tailed test. From Study III, ref.[327], with permission from University of Chicago Press.

Of control subjects, 80 (53%) were HSV seropositive, and 19% were HSV-2 seropositive. The frequencies of low levels of IgG1, IgG3 in patients and control subjects, as well as in the HSV seropositive and HSV seronegative control subjects are depicted in Table 4.6.

Table 4.6 Total plasma immunoglobulin levels associated with frequently active genital herpes simplex virus (HSV) type 2 infection in logistic regression analysis

Plasma Ig level < 2 SD	Patients (n = 52)	All control subjects (n = 150)			HSV-seropositive control subjects (n = 80)			HSV-seronegative control subjects (n = 70)		
	No.(%)	No.(%)	OR (95%CI)	P	No.(%)	OR (95%CI)	P	No.(%)	OR (95%CI)	P
IgG1	13 (25)	10 (7)	4.7 (1.9-11.5)	0.001	7 (9)	3.5 (1.3-9.4)	0.014	3 (4)	7.4 (2.0-27.8)	0.003
IgG3	18 (35)	21 (14)	3.4 (1.6-7.2)	0.001	12 (15)	3.0 (1.3-6.9)	0.010	8 (11)	4.1 (1.6-10.4)	0.003
IgG1 or IgG3	28 (54)	28 (19)	5.1 (2.6-10.1)	<0.001	19 (24)	3.7 (1.8-7.9)	0.001	9 (13)	7.9 (3.3-19.2)	<0.001

Comparison of patients with control subjects and control subject subgroups with or without antibodies against herpes simplex HSV viruses type 1 or 2. CI = confidence interval, OR = Odds ratio. From Study III, ref.[327], with permission from University of Chicago Press.

Low total plasma IgG1 (4.7 [1.9-11.5] 0.001) and IgG3 (3.4 [1.6-7.2] 0.001) values were more common in the patients than in the control subjects. Differences remained in subgroup analyses. The biggest difference was seen in the frequencies of low IgG or IgG3 between patients and HSV-seronegative control subjects (7.9 [3.3-19.2] < 0.001; Table 4.6). The levels of anti-HSV-2 subclass antibodies against gC-2 were not associated with frequent recurrences.

Genetic studies

In genetic studies, the $G3m^{g}$,$G1m^{a/a(x)}$ haplotype was more common in patients than in seronegative control subjects (P = 0.047, χ^2 test). In linear-by-linear association analysis of diploid genomes, a trend appeared toward more common $G3m^{g}$,$G1m^{a/a(x)}$ homozygosity in patients and $G3m^{b}$,$G1m^{f}$ homozygosity in the HSV-seronegative control subjects (P = 0.056). Of the 11 $G3m^{g}$,$G1m^{a/a(x)}$ homozygous patients, 10 also had low IgG3 levels.

Compared with the Finn90 historical control group *A*32* (in 9 patient vs. 4 control genomes, P = 0.018), the haplotype *B*15, DRB1*11* (4 vs. 0, P = 0.017), and their combination *A*32, B*15, DRB1*11* (3 vs. 0, P = 0.048) were associated with frequent genital herpes. The haplotype *A*2, B*15, DRB1*04* (0 vs. 10, P = 0.015), and its part *B*15, DRB1*04* (1 vs. 12, P = 0.036, Fisher's exact 2-sided test without statistical correction for multiple comparisons for all) were more common in the control subjects. The frequencies of *C4* nulls did not differ between the groups. The numbers of C4 genes obtained by C4 allotyping and *C4* genotyping, when the 2-bp insertion in exon 29 (codon 1213) of the *C4A* gene was counted as missing, were largely concordant (Table 4.7). In the testing of C4A gene numbers, discordant results were obtained in 2% of patients and 9% of control subjects, for C4B the corresponding frequencies were 4% and 5%.

Table 4.7 C4 gene numbers IV in immunophenotyping based on protein levels in serum compared with *C4* gene numbers by real-time polymerase chain reaction. Data from Studies III and IV

Group	C4 A or B number in phenotyping	*C4A* number in genotyping[1]					*C4B* number in genotyping			
		0	**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**
Severe genital herpes patients (n = 52)	**0**	0	0	0	0	0	8	0	0	0
	1	0	13	0	0	0	0	15	1	0
	2	0	0	23	0	1	0	1	28	0
	3	0	0	0	14	0	0	0	0	0
	4	0	0	0	0	1	0	0	0	0
Unselected control subjects (n = 150)	**0**	2	0	0	0	0	15	0	0	0
	1	0	21	4	0	0	0	42	2	0
	2	0	2	75	4	1	0	4	86	0
	3	0	0	1	36	1	0	0	0	1
	4	0	0	0	1	2	0	0	0	0
Severe chronic adult periodontitis patients (n = 37)	**0**	0	0	0	0	0	3	0	0	0
	1	0	8	0	1	0	0	16	0	0
	2	0	0	13	0	0	0	0	17	0
	3	0	0	0	13	0	0	1	0	0
	4	0	0	0	0	2	0	0	0	0

Concordant results are highlighted. [1]2-bp insertion in exon 29 (codon1213) of the C4A gene counted as missing.

Compared with the control subjects, *C4* nulls were more frequent in patients without neuralgias (3.3 [1.1-10.4] 0.037) and without prolonged or chronic neuralgias (3.4 [1.3-9.3] 0.016) after adjustment for low IgG1 and IgG3. Within the patient group, *C4* nulls were negatively associated with neuralgias (0.2 [0.06-0.81] 0.022) and prolonged or chronic neuralgias (0.2 [0.05-0.57] 0.004). In forward stepwise logistic regression analysis, the frequencies of *C4* deficiencies and $G3m^{g}$,$G1m^{a/a(x)}$ homozygosity were not significantly different between the patients and the control subjects.

Low IgG1 (4.9 [2.0-12.5] 0.001) and low IgG3 (3.6 [1.7-7.8] 0.001) were more frequent in the patients than in the control subjects (Table 4.8). Compared with seropositive control subjects, low IgG1 (4.2 [1.5-11.7] 0.007) and IgG3 (3.5 [1.5-8.3] 0.005) were more commonly found in the patients. In addition, low IgG1 (7.3 [1.9-28.2] 0.004) and low IgG3 (4.1 [1.5-10.6] 0.005) were more frequent in patients than in seronegative control subjects. Only low IgG1 was more common when all infected persons (patients and seropositive control subjects) were compared with seronegative subjects (4.0 [1.1-13.9] 0.030; Table 4.8).

Table 4.8 Total plasma immunoglobulin levels as risk factors for frequently active genital herpes, based on forward logistic regression analyses between patients with herpes simplex virus type 2 infection (n = 52), controls (n = 150), and control subgroups with (n = 80) or without (n = 70) antibodies against herpes simplex viruses type 1 or 2

Plasma levels < 2SD	Patients vs. all control subjects			Patients vs. HSV-seropositive control subjects			Patients vs. HSV-seronegative control subjects			Patients and HSV-seropositive control subjects vs. HSV-seronegative control subjects		
	OR	(95% CI)	P	OR	(95% CI)	P	OR	(95% CI)	P	OR	(95% CI)	P
IgG1	4.9	(2.0-12.5)	0.001	4.2	(1.5-11.7)	0.007	7.3	(1.9-28.2)	0.004	4.0	(1.1-13.9)	0.030
IgG3	3.6	(1.7-7.8)	0.001	3.5	(1.5-8.3)	0.005	4.1	(1.5-10.6)	0.005			0.054

CI = confidence interval, OR = odds ratio. From Study III, ref.[327], with permission from University of Chicago Press.

8.3 Adult chronic or recurrent rhinosinusitis

According to the severity classification methods we used, the recruited 48 consecutive voluntary patients all suffered from severe treatment-refractory chronic or recurrent rhinosinusitis (CRRS). The most common pathogens cultured from their sinus cavities were *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis*. Repeatedly cultured *S. aureus* was more commonly found in patients with nasal polyposis than in those without it (P = 0.016, Fisher's exact test).

Subjects in the unselected group were on average 8.3 years younger than subjects in the other groups. CRRS patients had comorbidities more frequently than ARS patients (Table 4.9). Plasma and serum values in study groups are shown in Table 4.10.

Table 4.9 Comorbidities and clinical findings in patients with chronic or recurrent rhinosinusitis (CRRS) compared with acute rhinosinusitis patients (ARS)

	CRRS (n = 48) no. (%)	ARS (n = 50) no. (%)	Fisher's exact test P-value
Any hypersensitivity	34 (71)	14 (28)	< 0.001
Allergy	15 (31)	7 (14)	0.053
Nonallergic rhinitis	19 (40)	7 (14)	0.006
Nasal polyposis	29 (60)	3 (6)	< 0.001
Bronchial asthma	27 (56)	4 (8)	< 0.001
Nonsteroidal anti-inflammatory drug hypersensitivity	14 (29)	0 (0)	< 0.001
Current or previous smoking	13 (27)	29 (58)	0.002
Septal deviation	10 (21)	2 (4)	0.014
Irritant rhinitis	6 (13)	5 (10)	0.471
Upper molar infection	1 (2)	15 (30)	< 0.001
Nasal fractures	0 (0)	3 (6)	0.243

P-values < 0.05 are in bold type. Reproduced from Study II[328], with permission from Blackwell Publishing.

We searched for relevant differences, especially between the ARS and CRS patients. In multivariate logistic regression analysis of C4 deficiencies, C4AQ0s were more common in the CRS than in the ARS patients (odds ratio [OR] 6.9; 95% confidence interval [95% CI] [2.0-30.1]; P = 0.001) (Table 4.3). In the CRRS patients, C4AQ0s (2.7 [1.3-5.6] 0.006) and any C4Q0s (3.7 [1.6-8.5] 0.003) were also more frequent than in the unselected group. Further, C4Q0s (3.9 [1.4-11.3] 0.005) were more common in the CRRS than in the healthy group, but in C4AQ0s (2.6 [0.9-7.5] 0.044) they did not reach significance. We found no associations between C4AQ0s and any clinical comorbidities. When the American Rhinosinusitis Classification, published after our study commenced (Table 3.3), was applied, C4AQ0s were not more common in either CRS or recurrent rhinosinusitis subgroup. C4AQ0 was less frequent in ARS patients than in the combined group, but the difference did not reach statistical significance (P = 0.088, Fisher's exact test).

M = male, F = female, P = plasma, S = serum, C = complement factor, Ig = immunoglobulin, [1]Kruskal-Wallis, [2]Jonkcheere-Terpstra, [3]2-sample t test with Bonferroni correction for multiple comparisons, [4]Fisher's exact t test, [5]Fisher's exact test with Bonferroni correction, [6]n= 46, [7]CH50 = classical pathway hemolytic activity, values ≥ 200 counted as 200,[h]n= 47, CH50 was measured in serum, other parameters in plasma. P-values < 0.05 are in bold type. Reproduced from Study II[328], with permission from Blackwell Publishing.

Table 4.10 Plasma and serum values in patients with chronic or recurrent rhinosinusitis (CRRS), acute rhinosinusitis (ARS), unselected population and healthy subjects with no self-reported history of rhinosinusitis

	CRRS (C) n = 48	ARS (A) n = 50	Unselected (U) n = 150	Healthy (H) n = 48	Between groups	Linear trend between groups	Concentrations between groups	Values below reference	Values below reference, between groups		
Value	Median (Range)				P-value, global[1]	P-value[2]	P-value[3]		P-value, global[4]	P-value[5]	
Unit	10-90th percentile										
Reference range:(M/F)	Values below reference: n (%)						C vs. A	C vs. U+H		C vs. A	C vs. U+H
C3	1.05[6] (0.58-1.82)	1.28 (0.93-1.75)	1.01 (0.49-1.58)	0.92 (0.66-1.30)	**< 0.001**	A>C>U>H, **< 0.001**	**< 0.001**	**0.006**	1.000	1.000	1.000
(g/L)	0.86-1.43	1.03-1.59	0.81-1.32	0.76-1.13							
0.5-1.5	0 (0)	0 (0)	1 (1)	0 (0)							
C4	0.19[6] (0.06-0.47)	0.26 (0.09-0.40)	0.19 0.12-0.28	0.19 (0.09-0.37)	**< 0.001**	A>C>U>H, **< 0.001**	**< 0.001**	0.738	**0.022**	0.090	1.000
(g/L)	0.13-0.31	0.19-0.33	(0.04-0.34)	0.12-0.28							
0.15-0.5	8 (17)	2 (4)	32 (21)	10 (21)							
CH50[7]	130[h] (0-200)	198 (75-200)	110 (30-200)	105 (50-175)	**< 0.001**	A>C>U>H, **< 0.001**	**< 0.001**	0.068	0.117	0.102	0.994
(IU/ml)	57-200	126-200	56-175	70-145							
50-130	4 (9)	0 (0)	10 (7)	1 (2)							
IgA	1.71 (0-4.15)	1.84 (0.52-5.15)	1.89 (0-4.35)	1.80 (0.92-4.11)	0.475	-	0.272	0.154	**0.002**	**0.030**	**0.002**
(g/L)	0-2.67	0.98-2.75	0.95-2.79	1.13-2.77							
0.88-4.84 / 0.52-4.02	8 (17)	1 (2)	5 (3)	0 (0)							
IgM	1.27 (0-2.65)	1.01 (0.44-3.54)	1.11 (0.25-3.82)	1.04 (0.46-5.23)	0.604	-	0.998	1.000	0.118	0.228	0.180
(g/L)	0.49-2.06	0.59-1.81	0.55-1.98	0.70-1.95							
0.36-2.59 / 0.47-2.84	3 (6)	0 (0)	3 (2)	0 (0)							
IgG	9.78 (0-22.65)	9.25 (4.81-14.50)	11.20 (7.14-17.60)	10.70 (7.94-19.50)	**< 0.001**	H>U>A>C, **< 0.001**	1.000	**0.012**	**< 0.001**	0.390	**< 0.001**
(g/L)	0.85-14.56	7.25-12.26	8.63-13.59	8.81-12.74							
6.8-15	7 (15)	3 (6)	0 (0)	0 (0)							
IgG1	5.47 (0-17.00)	6.05 (3.44-11.40)	6.60 (2.88-13.30)	5.85 (3.23-10.50)	**< 0.001**	-	0.710	0.128	**< 0.001**	**0.044**	**< 0.001**
(g/L)	0.33-10.26	4.38-7.95	5.12-8.58	4.84-7.24							
4.9-11.4	18 (38)	8 (16)	10 (7)	6 (13)							
IgG2	2.41 (0-7.95)	2.96 (0.64-6.33)	3.03 (1.39-7.49)	2.98 (1.34-8.52)	**0.004**	H>U>A>C, **0.029**	**0.034**	**< 0.001**	**< 0.001**	0.564	**< 0.001**
(g/L)	0.65-4.02	1.24-4.78	1.98-4.40	1.66-5.04							
1.5-6.4	10 (21)	6 (12)	3 (2)	3 (6)							
IgG3	0.30 (0-2.05)	0.24 (0.10-0.68)	0.32 (0.11-0.84)	0.28 (0.13-0.52)	**0.010**	-	0.344	1.000	**< 0.001**	1.000	**0.002**
(g/L)	0.09-0.68	0.12-0.48	0.18-0.52	0.16-0.42							
0.2-1.1	18 (38)	17 (34)	20 (13)	9 (19)							
IgG4	0.32 (0-2.44)	0.42 (0-3.13)	0.45 (0-2.72)	0.35 (0-1.09)	0.139	-	0.610	0.464	0.080	0.234	**0.022**
(g/L)	0.05-1.14	0.12-1.18	0.12-1.29	0.10-0.83							
0.08-1.4	8 (17)	3 (6)	8 (5)	2 (4)							

The concentrations of C3, C4, IgA, IgM, IgG, IgG1, IgG2, IgG3, IgG4, and CH50 levels, frequencies of low values, and the results of statistical analyses between the groups are depicted in Table 4.10. There were significant differences in global P-values between the groups in plasma concentrations of C3, C4, IgG, IgG1, IgG2, IgG3, and CH50 levels. Levels of C3, C4, and CH50 had linear trends with the highest values in the ARS patients followed by CRRS, unselected, and healthy subjects. IgG and IgG2 levels had linear trends with the highest values in healthy subjects followed by unselected, ARS, and CRRS subjects. Plasma levels of C3, C4, CH50, and IgG2 were lower in the CRRS than in the ARS patients (Table 4.10).

Low IgG1, IgG2, IgG3, and IgG4 levels were common in all study groups. Global P-values between the groups reached significance in low C4, IgA, IgG, IgG1, IgG2, and IgG3 values. Low IgA, IgG, IgG1, IgG2, IgG3, and IgG4 were all more frequent in the CRRS patients than in the combined group subjects. However, only low IgA and low IgG1 were more frequent in CRRS than in ARS (Table 4.10).

We then analyzed whether any combinations of low Ig class or subclass levels in plasma would more significantly associate with CRRS than with ARS. Having a combination of low IgG4 with low IgG1 or IgG2 was significantly more frequent in CRRS than in ARS patients (P = 0.030, Fisher's exact t-test with Bonferroni correction). The same significance was reached with low IgA (Table 4.10).

The relative contribution of low immunoglobulin levels and C4A nulls, taken into multiple logistic regression analysis as independent variables, were then studied. When CRRS patients were compared with the combined group, differences in C4AQ0 (2.89 [1.3-6.3] 0.007, low IgG1 (6.23 [2.7-14.5] 0.001), low IgG2 (6.19 [1.8-21.0] 0.003), and low IgG3 (2.38 [1.04-5.4] 0.039) remained significant. When CRRS and ARS patients were compared, only C4AQ0 (7.65 [2.3-25.7] P = 0.001) and low IgG1 (3.6 [1.3-9.9] 0.013) remained significantly more frequent in the CRRS patients.

We then took all the questioned comorbidities (Table 4.11) and all the performed laboratory measurements into forward stepwise logistic regression analysis. In it, only four factors distinguished CRRS from ARS patients: nasal polyposis, asthma, C4AQ0, and low IgG4 together with IgG1 or low IgG2 (Table 4.11). Having one of these factors differentiated a CRRS patient from an ARS patient with a sensitivity of 87.5% and specificity of 80%. The specificity rose to 96% if two factors were present.

Table 4.11 Clinical and immunologic risk factors for chronic or recurrent rhinosinusitis group (CRRS), based on forward logistic regression analysis with acute rhinosinusitis group (ARS) as control subjects

Risk factor	OR	(95% CI)	P
Nasal polyposis	10.64	(2.5-45.7)	0.001
Bronchial asthma	8.87	(2.3-34.9)	0.002
Complement factor C4A null	5.84	(1.4-24.9)	0.017
Low immunoglobulin G4 with decreased G1 or G2	15.25	(1.4-166.8)	0.026

OR = odds ratio, CI = confidence interval. Reproduced from Study II[328], with permission from Blackwell Publishing.

8.4 Severe chronic adult periodontitis

According to the severity classification methods we used, the recruited 37 consecutive non-immunocompromised patients all suffered from severe treatment refractory chronic adult periodontitis despite the regular (interval between visits: 5 +/- 1.5 SD [range 3-12] months) long-term treatment during their follow-up (15 +/- 5.1 [5-27] years). The patients were older ($P < 0.001$) and more often males ($P = 0.004$, Fisher's exact 2-sided test) than the unselected control subjects. Of the 37 patients, 19 (51%) smoked.

After age adjustment, the patients' plasma concentrations of C3 were higher, and the CH50 levels lower than in the control subjects (Table 4.12). In control subjects, the levels of plasma C3, C4, and serum CH50 correlated with each other ($P < 0.001$ for all comparisons, Spearman's rho). In patients, only the levels of C3 and C4 correlated with each other ($P = 0.001$, Spearman's rho).

Table 4.12 Differences between patients with severe adult chronic periodontitis and control subjects in complement levels

Parameter g/L	Reference range g/L	Patients (n = 37)	Control subjects (n = 150)	P
C3	0.5-1.5	1.22 (0.78-1.98) [1.12-1.30]	1.03 (0.49-1.58) [0.99-1.07]	0.001
C4	0.15-0.5 g/L	0.21 (0.11-0.48) [0.19-0.23]	0.20 (0.04-0.34) [0.19-0.21]	0.260
CH50	50-130 IU/ml	80 (25-200) [63-97]	116 (30-200) [109-123]	<0.001

NOTE. Data are age-adjusted means (range) [95% confidence interval], unless otherwise specified. CH50 = classical pathway hemolytic activity, values ≥ 200 counted as 200. CH50 was measured in serum, other parameters in plasma. [1]ANCOVA.

In linear regression analysis, *C4A* gene numbers ($P < 0.001$), *C4B* gene numbers ($P < 0.001$), and age ($P < 0.001$), but not gender ($P = 0.153$) were associated with the level of plasma C4 in the study subjects. Each *C4A* gene raised the level of plasma C4 on average by 0.036 g/L, *C4B* gene by 0.044 g/L, and each year of age by 0.001 g/L. No associations were found between *C4* gene numbers and CH50 levels.

C4 gene numbers estimated by allotyping and genotyping were again largely concordant (Table 4.7). Frequencies of total *C4A* or *C4B* deficiencies did not differ between the groups. Having ≤1 *C4A* or *C4B* was not associated with the age of the subject, and was more frequent in patients than in control subjects (2.4 [1.1-5.5] 0.032). However, plasma C4 levels did not differ between the groups.

9. Discussion

9.1 Frequently recurring intraoral herpes caused by HSV-1

Due to the infrequent nature of HLA homozygosity and to the extreme rarity of frequently recurring intraoral herpes, the probability that consecutive patients suffering this infection would, by coincidence, have the two combined seems minimal. HLA homozygosity is known to predispose to chronic viral infections and may limit the range of epitopes presented by infected cells and professional APCs[67]. This may lead to a less efficient immune response, already impaired by the various immune evasion mechanisms of HSV-1. HSV-1 is known to have immune evasion molecules that protect it from the HLA-mediated presentation of its antigens on the surface of the infected cell[250]. It also has effective immune evasion molecules against complement[206]. *C4* isotype deficiency may further contribute to the host's susceptibility, because HSV-1, even more effectively than HSV-2, evades from the AP of complement[105, 152, 206, 340, 364].

Clinically, the patients suffered from a highly debilitating disease. Their treatment with antiviral medication titrated to a high enough dose was highly effective. However, the treatment was complicated by its high cost. That the patients appear to suffer from an impairment of cell-mediated (HLA) or humoral (complement) immunity or both seems to support a decision to treat them actively. Due to the rarity of this disease form, it is unlikely that controlled treatment trials would become available soon.

9.2 Frequently recurring genital herpes simplex type 2 infection

Compared with previous literature, male sex was found only infrequently (37%) in our highly symptomatic patients[387]. Radicular neuralgias in sacral or lumbar dermatomes or both were disturbingly common and had developed into chronic neuralgia in 12% of the patients (Table 4.4).

For the first time in literature, we found genetic polymorphisms of Ig genes associated with frequently recurring genital herpes in a well-characterized patient population[125]. Of the 11 $G3m^{g},G1m^{a/a(x)}$ homozygous patients, 10 also had low IgG3 levels. This suggests that $G3m^{g},G1m^{a/a(x)}$ is a susceptibility haplotype, but only when it is present in conjunction with low

IgG3 (with which *G3m*^{g} is associated)[326]. Both *in vitro* data on the function and allotype-specificity of vFcR and genetic data from animal studies support this hypothesis[21, 24, 246]. Susceptibility to complicated or chronically active virus infection that is immunoglobulin allotype-specific has previously been suggested only in association with respiratory syncytial and hepatitis C virus infections, respectively[29, 264].

Normal IgG1 levels were associated with HSV seronegativity. Since total plasma IgG1 and IgG3 levels were negatively associated with the disease or its activity or both, we may not yet know the epitope against which potential vaccines may need to be directed. Protective vaccines may also need to evoke, not only cellular immunity, but also IgG1- and IgG3-specific humoral immunity and ADCC[90, 184].

C4 nulls were negatively associated with neuralgias. It may thus be that the complement-mediated inflammation evoked by recurrences is sometimes harmful to the host[121]. In frequently recurring genital herpes, neuralgic pain was surprisingly common and often the most distressing complication of the disease[89, 91]. It also interfered with the patient's ability to work and seemed to have a tendency to become chronic. In animal models, antibody-mediated immunity seems important in neuroprotection[138, 229]. In addition, the viral FcR formed by gE/I is required for axonal localization and cell-to-cell spread[279, 380]. Antibody-mediated immunity may thus have a role in neuroprotection. However, no association between neuralgias and low antibody levels was found. The patient group studied was small, and low IgG1 and IgG3 levels were common in our patients. To study the role of antibodies in neuroprotection with sufficient statistical power would require a larger study. The effect of vaccines and antiviral medication on specific prevention of chronic neuralgia should be tested in the future. Whether eczema herpeticum could serve as an easy clinical marker of reduced antiherpetic immunity should also be tested in the future.

Due to the slow recruitment process, patients suffering from frequently recurring oral-facial HSV-1 could not be studied. Whether the roles of different arms of humoral immunity (antibodies, complement) in defense against HSV-1 and HSV-2 differ could, therefore, not be addressed in this study. Patients with frequently active HSV-2, who suffer from a highly debilitating disease form that impairs self-esteem, every day activity, and sexual well-being, may often have a genetic predisposition to the disease. Their treatment with antiviral medication, with the treatment titrated to sufficient doses, was highly effective but complicated by the high cost of medication.

9.3 Adult chronic or recurrent rhinosinusitis

The high frequency of low Ig subclass levels in the general population and in ARS patients potentially influences their use in the clinical practice[27, 192]. The reference values of commercial tests used to measure IgG subclass levels are based on mostly unpublished data. Since the frequencies of especially low IgG1 and low IgG3 clearly exceeded the expected 2.5%, the usefulness of manufacturer's reference values in the Finnish population may be limited. Though low IgG subclass levels were frequent in all our groups, their frequency seemed to be positively associated with the clinical severity of rhinosinusitis. There were no clear cut-off points which would have differentiated reliably between our study groups. Due to the complexity and redundancy of immunity, any "correct" reference values may be difficult to determine and would require standardized methods and studies in clinically well-characterized and large populations[262].

Very little is previously known of the importance of complement-mediated immunity in defense against rhinosinusitis[194, 199, 252, 288]. In our study, the clinical (presumed) activity of sinus problems seemed to be associated with higher levels of the measured complement variables. Thus, deficiencies in either or both arms of humoral immunity (complement and antibodies) may contribute to the etiopathogenesis of the disease. *C4A* deficiency was more strongly associated with CRRS than any Ig value or combination of low Ig concentrations. It thus seems promising in patient assessment and needs to be tested further. Testing of subclass levels alone seems to be of limited value in the timely diagnosis and treatment of patients predisposed to chronic disease forms. We need immunologic parameters that would better predict end-organ damage in CRRS patients[6, 69, 168].

The higher frequency of *C4A* nulls in CRRS than in ARS patients may be explained by its ability to regulate antibody production, by its function in defense against encapsulated pathogens or in the clearance of immune complexes, necrotic and late apoptotic cells presumably generated during ARS[68, 84, 378]. In addition, the associated full MHC haplotypes and either the existence of another functional gene in linkage disequilibrium with *C4A* nulls or of multiple functional polymorphisms in these *MHC* haplotypes may play a role[168, 197]. Despite the assumed role of C4B in defense against encapsulated pathogens, its frequency was, somewhat surprisingly, similar in the two patient groups. Comorbidities associated with hyper-inflammation were common in patients with chronic forms of the disease, many of these (e.g. asthma, hypersensitivity) are known to have an immunologic basis. C4A, IgA, and IgG4 are thought to possess anti-inflammatory functions[2, 38, 84, 97]. Our findings suggest that both factors impairing and enhancing immune responses need to be present simultaneously. Not only effective forms of infection prevention, but also anti-inflammatory treatments may need to be clinically tested in rhinosinusitis patients.

9.4 Severe chronic adult periodontitis

Complement system appears important in the etiopathogenesis of chronic periodontitis. Plasma C3 concentrations were higher and CH50 levels lower in patients than in control subjects, but C4 concentrations did not differ between the groups. Low CH50 levels have previously been reported[15, 25, 167, 233]. Complement activation was not measured in our study. The levels of C4 and CH50 may reflect a higher consumption of CP components together with AP (and CP) upregulation. *C4* deficiencies may predispose to severe chronic periodontitis. The combination of more frequent *C4* deficiencies in the patients but similar levels of C4 may also imply that either the numbers of *C4A* and *C4B* genes need to be balanced in order to protect against periodontitis, or that the surplus of *C4* deficiencies in patients was found by chance.

The most common pathogen associated with periodontitis, *Porphyromonas gingivalis*, is known to be able to evade from and exploit complement[283]. C4 deficiencies were more frequent in periodontitis patients, and they are known to be more frequent in atherosclerosis patients[220, 354]. Hypothetically, these diseases may share common susceptibility genes. In addition, an impaired defense against oral pathogens may predispose to atherosclerosis[220, 354]. Quite likely, the predisposition to periodontitis depends on multiple polymorphisms of immunologically active genes present in an extended haplotype, and in linkage disequilibrium with each other[349, 356]. If genetic testing is attempted, to achieve good predictive value we would probably need to test multiple susceptibility genes[317]

9.5 General discussion: limitations and findings of special interest in our studies

The prevalence of *C4B* deficiency we found in the unselected group was the highest ever reported in any population. We conformed to the ethical regulations on genetic studies, and the control subjects were informed on the diseases we studied. Consequently, some of the control subjects in the unselected group may have suffered from these diseases, and may have been more willing to participate because of this. We had no information on any diseases suffered by the control subjects. This may have caused a selection bias: those affected may have been more willing to participate. Any such bias would, however, only diminish the difference in the prevalence of any deficiency with an effect on the course of a disease. However, the frequencies of C4 deficiencies or low Ig values did not differ between the unselected group and the healthy blood donor group in Study II.

In all our studies, the patients had more C4 null alleles (Table 4.3) and – when measured – also more IgG SCDs than the control subjects. This

did not reach significance in the genital herpes study. To study whether *C4* deficiencies are, in fact, overrepresented in herpes patients would require a larger study. The difference needed in the prevalences between groups of any studied clinical factor – to become potentially clinically relevant – is arbitrary, but should probably be larger than what was found in our study. Since immune evasion from complement by the closely related virus HSV-1 is more effective than by HSV-2, our findings may not be applicable to HSV-1.

In pilot case-control studies, reasonable numbers of recruited patients are small[133]. An association study is never able to prove any causal relationship between the factors studied[88]. In studies II and III, factors with statistically significant differences in prevalence were commonly independent in logistic regression analyses. Susceptibility to common infectious diseases is likely to be polygenic. Linkage equilibrium frequently occurs in the human genome and in the genes affecting immunity. It is likely that multiple genes, acting in concert as haplotypes, have effects on disease susceptibility[197]. This phenomenon further constrains the interpretation of our findings before further studies recruiting considerably larger numbers of study subjects are available. Multiple polymorphisms (e.g. in Toll-like receptor genes, cytokine genes) that are not in linkage disequilibrium may also, with the factors we studied, only together impart a clinically significant genetic effect on disease susceptibility[365].

The findings in studies I and IV are interesting, but their interpretations are limited by the small numbers of patients studied and require further confirmation. However, our findings from the two larger studies seem particularly interesting. Our rhinosinusitis study was the first study to compare the frequencies of low subclass levels in severely affected patients with patients having non-complicated disease forms or no disease. The methods and especially the reference values used in IgG subclass measurements vary greatly between manufacturers and clinical centers. Since subclass levels are commonly measured in clinical practice, these findings may spur further studies on their clinical usefulness. Interestingly, the deficiencies most strongly associated with chronic rhinosinusitis are also associated with not only impaired immune defense but also with hyper-inflammation. Since the majority of patients coming for sinus operations benefit from treatment, our findings are likely to be applicable only to those with treatment refractory disease. Attempts to treat early with, for example, surgery or anti-inflammatory medication or both seem particularly attractive in patients found to have multiple subtle predisposing immunologic disturbances. If supported by further studies, the measurement of levels of plasma Ig classes, IgG subclass levels, and C4 null alleles together might become a useful tool in clinical practice.

We were also able to associate disease activity and severity of genital herpes with a functionally relevant genetic polymorphism encoding an

allotypic form of IgG1[21, 24, 206]. Based on the results from basic immunology, herpes simplex virus type 1 is known to effectively evade from this allotypic IgG1[206]. Our findings may spur further research directed to find potentially important epitopes on HSV-2, against which vaccine-induced immunity would be effective[184]. It also encourages studies that would further test the ability of candidate vaccines to stimulate humoral immune responses against these epitopes[184]. Since these patients most likely have a genetic cause to a debilitating disease, this could have an effect on further clinical studies on disease management, on prevention of neurologic sequelae, and even on the right to receive reimbursable antiviral treatment.

10. Conclusions

1. Genetic complement factor *C4A* and *C4B* deficiencies and low IgG subclass levels were common in the population. There were no significant differences in the frequencies of *C4A* and *C4B* deficiencies or low IgG subclass levels between the general population and blood donors without a history of rhinosinusitis, even though low IgG subclass levels were somewhat more common in the general population.

2. *C4A* deficiencies, low IgG1 levels, and the combination of low IgG4 together with low IgG1 or IgG2 were all more common in patients with chronic recurrent rhinosinusitis (CRRS) than in acute rhinosinusitis (ARS) patients.

3. Deficiencies of *C4* were also associated with chronic severe adult periodontitis. Low IgG subclass levels were associated with CRRS. Low IgG1 and IgG3 levels and the immunoglobulin heavy chain gene haplotype $G3m^{g},G1m^{a/a(x)}$ were associated with frequently recurring genital HSV-2 infection. *HLA-A**, *-B**, and *-DRB1** gene polymorphisms were associated with active genital herpes infection. *HLA-A**, *-B**, *-C** and *-DRB1** homozygosity, together with a total deficiency of *C4A* or *C4B* isotype, may be associated with frequently recurring herpetic gingivostomatitis.

4. Genetic deficiencies of *C4* were negatively associated with radicular neuralgias in genital HSV-2 infection.

5. The new PCR-based quantitative analysis of *C4A* and *C4B* genes complemented the traditional C4A and C4B protein allotyping with reproducible results in patient analyses.

11. Summary

The present study addressed whether plasma concentrations of complement factors C3, C4, or immunoglobulins, serum CH50, or polymorphisms in the class I and II *HLA* genes, isotypes and gene numbers of C4, or allotypes of IgG1 and IgG3 heavy chain genes were associated with severe frequently recurring or chronic mucosal infections. Frequencies of low levels in IgG1, IgG2, IgG3 and IgG4 were also tested, for the first time, from adult general population. According to strict clinical criteria, altogether 188 consecutive voluntary patients without a known immunodeficiency and 198 control subjects were recruited. In the patients, other chronic or recurrent infections were infrequent.

Frequently recurring intraoral herpes simplex type 1 infections, a rare form of the disease, was associated with a total deficiency of the C4A or C4B isotype, and with homozygosity in *HLA -A*, -B*, -C**, and *-DR** genes.

Low plasma levels of IgG subclasses were common in the voluntary subjects from the general adult population. We also tested, for the first time, the frequency of low subclass levels in acute rhinosinusitis. Low levels of IgA, IgG, IgG1, IgG2, IgG3, and IgG4 were all more frequent in patients with adult chronic or recurrent sinusitis than in presumably healthy control subjects. When patients having a chronic form of the disease were compared with those with an acute form, only low levels of IgG1 were more common in CRRS. The best immunologic parameter in differentiating between patients with complicated and uncomplicated forms of the disease was C4A deficiency, and the combination of low plasma IgG4 together with either low IgG1 or IgG2 performed almost equally. Clinically, nasal polyposis and bronchial asthma were associated with complicated disease forms. C4A, IgA, and IgG4 are known to possess anti-inflammatory activity. Their lack, together with a concurrent subtly impaired immunity caused by low subclass levels, may predispose to chronic disease forms.

Frequently recurrent genital HSV-2 infections were associated with low levels of IgG1 and IgG3, present in 54% of the recruited patients. This association was partly allotype-dependent. According to previous studies, HSV effectively evades the allotype $G1m^{a/ax}$ of IgG1, whereas $G3m^{g}$ is associated with low IgG3. The $G3m^{g},G1m^{a/ax}$ haplotype, in association with low levels of IgG3, was more common in patients than in control subjects who lacked antibodies against herpes simplex viruses. Certain *HLA* genes were more common in patients than in control subjects. Having more than one *C4A* or *C4B* gene was associated with neuralgias caused by the virus.

In severe chronic adult periodontitis, any *C4A* or *C4B* deficiency combined

was associated with the disease. Lower levels of plasma C3 or C4 or both, and serum CH50 were found in herpes and periodontitis patients, when compared with control subjects. In rhinosinusitis, there was a linear trend with the highest levels found in the order: ARS > CRRS > general population > blood donors with no self-reported history of rhinosinusitis. Complement is involved in the defense against the tested mucosal infections. The new quantitative analysis of C4 genes and the conventional C4 allotyping method complemented each other.

Seemingly immunocompetent patients with chronic or recurrent mucosal infections frequently have subtle weaknesses in different arms of immunity. Their susceptibility to chronic disease forms may be caused by these. Host's subtly impaired immunity often seems to coincide with effective immune evasion from the same arms of immunity by the disease-causing pathogens.

If subclass levels alone are tested without additional immunologic factors known to predispose to severe disease forms, their interpretation is difficult and of limited value in early diagnosis and treatment. To prevent organ damage caused by frequent mucosal infections, early initiation of therapy would probably be required. Further easily applicable immunologic parameters are needed.

12. Acknowledgements

This study was carried out at the Division of Infectious Diseases, Department of Medicine, Helsinki University Central Hospital and at the Department of Bacteriology and Immunology, Haartman Institute, University of Helsinki during the years 2001-2006. I am deeply grateful to Professor Ville Valtonen for providing excellent working facilities: the cozy room above Ward 4. I express my gratitude to all the skilled members of our staff, from my colleagues to our nurses to our hospital attendants, who together have made this such a pleasant place.

My warmest thanks are due to my supervisor Professor Ville Valtonen for his friendship, enthusiasm, and unfailing support, however rough the road turned during this work. He has guided me with care, given me much freedom in the choice of scientific topics, and provided me with the opportunity to test various hypotheses. His profound knowledge and understanding in the field of infectious diseases has been invaluable. It is a privilege to work with you! Your ability to fathom what I am trying to say, even before I know it myself, is admirable. I am most grateful to my co-supervisor Professor Seppo Meri for his encouraging and optimistic attitude, and for sharing with me his impressive knowledge and insight in the field of complement research. My finances would be much worse off, had he not helped me with the acquisition of grants.

I sincerely thank Docent Marja-Liisa Lokki for never stopping to believe in this work. She introduced me to the field of genetics; what began as an immunologic study was converted to immunogenetic research. To pull through, doggedness was required at all stages of this study. Yet her support and persistence was admirable. She also skillfully and meticulously performed all the C4 gene analyses.

I am indebted to the reviewers of this work, Docent Esa Rintala and Professor Olli Lassila, for fruitful criticism and for the most flexible and positive attitude toward the tight time limit. You made the governing of my time less demanding.

My sincere and warm-hearted thanks are due to Docent Irma-Leena Notkola for the invaluable and skilled statistical analyses. Her broad knowledge and long experience in medical statistics made my life so much easier. You not only knew the limits and the applicability of different statistical methods, but also critically assessed the validity of the results and reviewed the manuscripts. If there was a question there was an answer, whether or not it fitted our theories or pleased us. Your warm-heartedness, so familiar

even since my early childhood, is to be applauded. I am proud to be your cousin, Imma!

Jari Suvilehto, MD, did not spare his efforts when he recruited the acute rhinosinusitis patients. He was constantly on call; 24/7 is more than one should ask. Even more than any help I received from you during this study, I value your friendship that has lasted for well over 20 years. Yet Jari, my dear brother-in-law, I owe you the most for your loyalty, caring, and unselfish love toward my late sister.

I warmly thank all my other collaborators and coauthors. Docent Asko Järvinen patiently searched for new patients for my studies. Thanks are also due for his encouraging attitude, ideas, patience, and efforts not to stress me with any more clinical duties than absolutely necessary – not an easy task! Docent Kimmo Mattila kindly shared his expertise on dental infections and statistics. I am indebted to Docent Maija Lappalainen and Hanna Jarva, MD, PhD, for their on-line support. Though extremely busy, you always took the time for me and my questions and provided me with clear and concise answers. Whenever I needed encouragement I got it from you. Docent Ilkka Seppälä was always willing to help, to discuss any ideas, and answer any questions I posed. He meticulously performed all the Ig analyses and, together with Docent Heikki Sarvas, performed and believed in the worth of Gm allotyping in our patients. This happened at a time when no clinical course of a viral disease had been linked to host Ig allotypes – too bad we finished only third with HCV and RSV spurting ahead of us! Eija Hiltunen-Back, MD, PhD spared no effort when recruiting patients. Her optimistic attitude and cheerfulness is almost enviable. We share the common vision that medication to patients with severe manifestations of herpes should not be restrained by financial difficulties; I do hope that one day this will be true. Docent Henrik Malmberg and Olli Tahkokallio, MD helped in patient recruitment and shared their ideas and vast experience in chronic rhinosinusitis. Professor Sirkka Asikainen greatly encouraged me along the way. Her expertise on severe periodontitis is unsurpassable. I regard your timely and thorough reviews of our manuscript highly. Anja Nieminen, DDS and Marja Vesanen, DDS tirelessly recruited patients. Docent Tuomo Timonen was always friendly and willing to help. Hannamari Välimaa, MD, PhD, DDS set up and performed the anti-HSV-2 IgG subclass analyses, a time-consuming task, while finishing her own thesis. Despite this, she always managed to be helpful and in good spirits. Anil Palikhe, MD, also in the

midst of his thesis work, took the time to reconstruct the *HLA* haplotypes punctually and in an extremely pleasant manner. Professor Seppo Sarna helped with the statistical analysis of my first article.

I am deeply grateful to all the patients and control subjects that participated in my studies. I also admire the "never give up"- attitude of my patients. Their diseases are, by many, perceived to be almost trivial, but cause a great deal of personal suffering and a marked decrease in the quality of their lives. I acknowledge the financial support that I have received from the following organizations: Emil Aaltonen and Maud Kuistila Foundations, Special Finnish Governmental Subsidy for Health Sciences Research, the Finnish Society for Medicine (Duodecim), and the Finnish Red Cross Blood Transfusion Service.

The caring, optimistic, and ever helpful atmosphere in the Outpatient Clinic and Wards 4 and 131 helped me to carry on with this work. Helena Kautovaara, Leena Kero, Sirpa Kuusisto, Hanna Leena Leinonen, Päivi Pilli, Pia Puustinen, Mirja Pyhähuhta, Maija Sainio, Riitta Savolainen, Sirpa Tourunen, and so many others spared no efforts in helping me. I applaud Pirjo Mikkola for all the help I received, and I am also more than grateful to Leena Lajunen for patience and willingness to collect even the umpteenth article I needed. I am also indebted for the help received from Pirjo-Liisa Leivo and other friendly folks at Vita Laboratories; they recruited the unselected subjects. Thanks are rightfully due to Minna Haanpää, Pirkko Kokkonen, Tupu Kuismin, Marjaana Mustonen, and Marita Sirén for their skilled laboratory assistance and timely help.

I am deeply grateful to Eeva Ruotsalainen, MD, PhD, who has shared the same workplace with me for over 16 years, as well as the same study for seven years. I have grown to trust you utterly. Your warm, kind, patient, and gracious attitude towards me has been priceless, my friend! Maija Haanpää, Jouni Kerminen, Markku Paatelma, Ville Turpeinen, and Friskis&Svettis, you kept me going.

For general support, I owe greatly to all my friends and my dear godchildren Vivi, Sanni, Veera, Erkka, Lauri, and Robin, for being there. Auli deserves extra credit for her invaluable help. My "brur / Bruder / hermano" Jukka and Maria helped me in organizing the dissertation party, in choosing the wines, and with their admirable attitude in all other aspects of life.

I thank my parents Irja and Kari for fostering me, for their support and love, and for steering me towards an academic career. My late sister Kirsi Suvilehto, MD, PhD I thank for all the ways she supported and trusted

me till the very end. Without her I would never have started this project. All the more grieved I am for not having you beside me now; this thesis commemorates you. Jyry and Juulia, you are an integral part of my life and my heart. A big hug for your attitude(s) towards all the hardships in your lives, it is simply impressive. My dearest "twin-sister" Marja Eder-Seppänen – always encouraging and always loving – and Anselm, my beloved godson Manu, and equally dear ones Niko and Sonja: you are a constant source of joy to me. You always bring the sunshine with you. My parents-in-law Hannu and Irma, thank you for all your support and caring. Irma Muhonen, MA also revised the language of my thesis. All my dear in-laws: Maria, Timo, beloved Sampo and my (impressively organized and optimistic) goddaughter Malla; Katariina, Tuomo, dear godson Mika, Mia, and Milla – how can I thank you? Maria, Timo, Sampo, and Malla: I cherish in the memories of all our Christmases and travels together.

I thank God for giving me Taina. Through thick and thin, joys and sorrows we share our lives. As all should know, there are no words beautiful enough to thank You.

Helsinki, January 2007

Mikko Seppänen

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