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Studies on hereditary C2 deficiency: Frequent occurrence of severe infections, atherosclerosis and rheumatological manifestations

Jönsson, Göran

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Frequent occurrence of severe infections, atherosclerosis and rheumatological manifestations

Göran Jönsson

LUND
UNIVERSITY
Faculty of Medicine

Department of Medical Microbiology
Section of Clinical Immunology
Lund University
Sweden

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To my family

PUBLICATIONS

This thesis is based on the following articles:

Paper I.

Göran Jönsson, Lennart Truedsson, Gunnar Sturfelt, Vivi-Anne Oxelius, Jean-Henrik Braconier, and Anders G. Sjöholm. *Hereditary C2 deficiency in Sweden: frequent occurrence of invasive infection, atherosclerosis, and rheumatic disease.* Medicine (Baltimore), 2005, 84(1), 23-34.[1]

Paper II.

Göran Jönsson, Vivi-Anne Oxelius, Lennart Truedsson, Jean-Henrik Braconier, Gunnar Sturfelt, and Anders G. Sjöholm. *Homozygosity for the IgG2 subclass allotype G2M(n) protects against severe infection in hereditary C2 deficiency*. The Journal of Immunology, 2006, 177(1), 722-8.[2]

Paper III.

Göran Jönsson, Anders G. Sjöholm, Lennart Truedsson, Anders A. Bengtsson, Jean-Henrik Braconier, and Gunnar Sturfelt. *Rheumatological manifestations, organ damage and autoimmunity in hereditary C2 deficiency*. Rheumatology (Oxford), 2007, 46(7), 1133-9.[3]

Paper IV.

Göran Jönsson, Eva Holmström, Barbro Selander, Vivi-Anne Oxelius, Jean Henrik Braconier, Gunnar Sturfelt, and Lennart Truedsson. *Vaccination against infections with encapsulated bacteria in hereditary C2 deficiency: great variation in antibody response*. Manuscript.

ABBREVIATIONS

Commonly used abbreviations:

AMI, Acute myocardial infarction
AP, Alternative pathway
C1-INH, C1-inhibitor
CP, Classical pathway
CPS, Capsular polysaccharides
CR, Complement receptor
CRD, Carbohydrate recognizing domain
CRP, C-reactive protein
C2D, Deficiency of the second complement component
DAF, Decay-accelerating factor
ELISA, Enzyme-Linked ImmunoSorbent Assay
Fab, Fragment antigen binding
Fc, Fragment crystallisable
FcγR, Fc gamma receptor
FcR, Fc receptor
GM allotypes, Genetic variants of the immunoglobulin heavy G chains
Hib, *Haemophilus* (*H.*) *influenzae* type b
HLA, Human leukocyte antigen
HRF, Homologous restriction factor
Ig, Immunoglobulin
IGHG, Immunoglobulin heavy G chains
LP, Lectin pathway
LPS, Lipopolysaccharide
MAC, Membrane attack complex
sMAP or **MAp19**, Small MBL-associated protein
MASP, MBL-associated serine protease
MBL, Mannan-binding lectin
MCP, Membrane cofactor protein
N. meningitidis, *Neisseria meningitidis*
PAMPs, Pathogen associated molecular patterns
PCR, Polymerase chain reaction
PRMs, Pathogen recognizing molecules
PRPs, Pathogen recognizing receptors
SLE, Systemic Lupus Erythematosis
S. pneumoniae, *Streptococcus pneumoniae,* pneumococci
TD, Thymus-dependent
TI, Thymus-independent
TLR, Toll-like receptor
TNF, Tumor necrosis factor
UCTD, Undifferentiated connective tissue disease

ABSTRACT

The complement system is a part of the innate immunity and is essential in the defence against microorganisms. Hereditary C2 deficiency (C2D) is one of the most common complement deficiency states with an estimated prevalence of 1:20,000 in persons of Western descent. In the present investigation, the identification of more than 40 C2D persons at a single centre combined with long observation periods provided a unique basis for assessment of C2D-associated manifestations and diseases. The predominant clinical manifestation was severe bacterial infections. The infections were mainly caused by *Streptococcus pneumoniae*. Repeated infections occurred primarily during infancy and childhood. On the other hand, about 25-30 % of the C2D persons remained healthy during the observation period. Immunological factors as IgG subclass levels, GM allotypes, complement proteins, and Fc receptors were assessed to explain this difference. Homozygosity for the *G2M*n* allele was strongly associated with protection against severe infections ($p<0.001$). This indicated that an efficient antibody response to polysaccharide antigens is of great importance in C2D. Mannan-binding lectin deficiency also contributed to the susceptibility to infection. The association between C2D and systemic lupus erythematosus (SLE) was confirmed, but notably the severity of SLE in patients with C2D was similar to that of other SLE patients. Another novel finding was a high occurrence of anti-cardiolipin antibodies (aCL) and antibodies to the collagen-like region of C1q. Both autoantibodies have a pro-atherosclerotic effect that might explain the high occurrence of cardiovascular disease found in the cohort. Interestingly, anti-phospholipid syndrome was not observed despite the high occurrence of aCL. Vaccination in 25 C2D persons resulted in antibody responses which show that C2D persons benefit from vaccination against infections caused by encapsulated bacteria such as pneumococci.

INTRODUCTION AND BACKGROUND

It has been traditional to arrange host responses to infection into separate parts of the immune system, such as complement, phagocytes, cytokines, cell-mediated immunity, and humoral immunity. A more modern approach is to consider two larger categories: innate and acquired immunity. The former incorporates the more rapid and phylogenetically more primitive non-specific responses to infection, such as surface defences, complement activation, cytokine amplification, and phagocytic responses (Figure 1). As a first-line defence against pathogens, innate immunity is critically important in impeding microbial invasion and in alerting other components of the body's defence system. Innate immunity relies on a limited number of germline-encoded proteins (1, 2). The main targets for pathogen associated molecular patterns or PAMPs (mannan-binding lectin; MBL, ficolins, C-reactive protein, C1q and natural immunoglobulin M; IgM) are binding to conserved molecules unique to the infectious microbes, for example certain carbohydrates or glycolipids from bacteria or double-stranded RNA encoded from viruses. The recognition molecules may be cell-associated receptors (pathogen recognizing receptors, PRPs) or soluble pathogen recognizing molecules (PRMs). Toll-like receptors are included in PRMs and play a major role in pathogen recognition, initiation of inflammatory and immune responses through a signal cascade called the NFκB pathway (3-8). Included in the PMRs are the collectins, among which MBL attracts special interest due to its ability to bind to several microorganisms (9, 10). The complement system, a key component of the innate immune response, protects mucosal surfaces and is present in human serum at high concentrations. The complement system has the functional capacity to bridge innate and acquired immunity. Acquired immunity involves more slowly developing, long-lived, and highly antigen-specific responses, such as antibody production and cell-mediated immunity (11). Innate and acquired immunity engage in a range of interactions that are extremely diverse and complex (Table 1).

Innate and acquired immunity

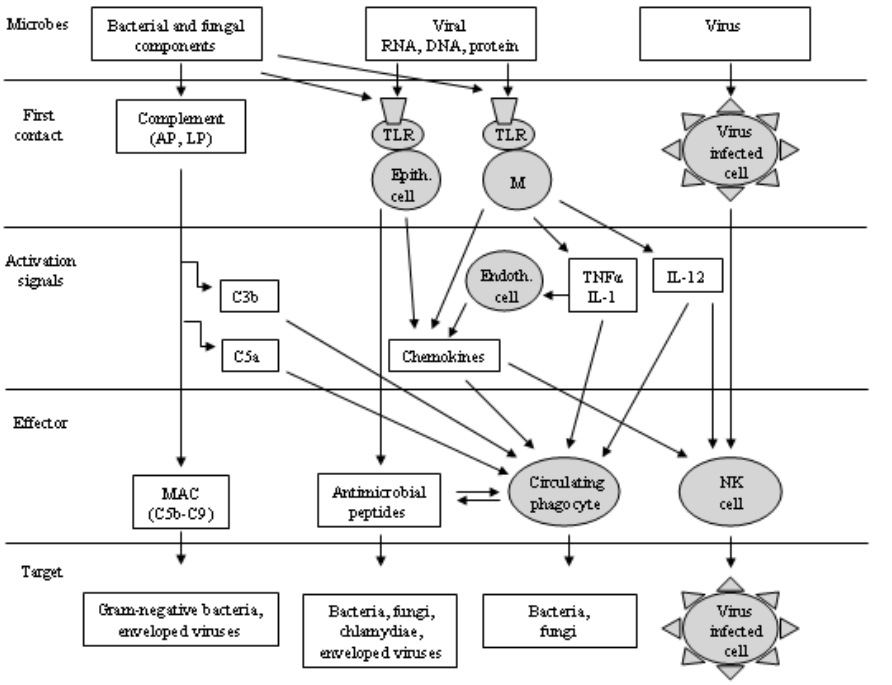

Figure 1. Schematic outline of different effector pathways in innate immunity.
Abbreviations: AP, alternative pathway; IL, interleukin; LP, lectin pathway; M, macrophage; MAC, membrane attack complex; TLR, toll-like receptor; TNF, tumor necrosis factor.

Table 1. Properties of the innate and acquired immunity.

	Innate immunity	**Acquired immunity**
Encoding of receptors	Germline	Somatic
Distribution of receptors	Not clonal	Clonal
Repertoire of receptors	Limited	Very large
Target	Invariable	Variable
Self- No self discrimination	Perfect	Not perfect
Activation speed	Fast	Slow
Long-lasting memory	No	Yes

Primary immunodeficiencies

The World Health Organization recognizes about 100 primary immunodeficiency disorders. Some of these deficiency states affect only a single cell or protein of the immune system, while others may affect more than one component. Since the functions of the immune system are critical for survival many of them can be performed by more than one component of the system. This redundancy acts as a back-up mechanism.

The primary immunodeficiencies result mainly from defects in T cells, B cells, phagocytic cells or the complement system. Immune deficiency disorders characterized by defective antibody production are the most common, accounting for about 70% of all primary immunodeficiencies (12). Deficiency states of the complement system are rare forms of primary immunodeficiencies, accounting for only 1% to 3% of these diseases (13, 14). However, these figures might not be representative since data on the prevalence of different complement deficiency states in the population at large is generally lacking.

It has also become increasingly evident that innate immune functions contribute greatly to host defence (15). An increased susceptibility to severe infections has been described in most forms of inherited complement deficiency states (13). There is also an association between complement deficiencies and immunological diseases such as Systemic lupus erythematosus (SLE) and glomerulonephritis but this association still provides a challenge for continued investigation (14).

THE COMPLEMENT SYSTEM

Complement was discovered at the end of the 19th century as a heat-labile component of blood plasma with bactericidal properties and the capacity to lyse erythrocytes from other species (16). In the 1960s many of the individual components of the system were characterized. Today, more than 30 proteins are known in the system, including soluble complement proteins, membrane-bound regulators, and cellular complement receptors (Table 2, Table 3, Figure 2) (17). Most of these proteins are produced in the liver or, to a lesser extent, by mononuclear phagocytes, lymphocytes, and fibroblasts (18). The complement system is an integral part of the innate immune defence and bridges innate and acquired

immunity by interactions within the immune response, including chemotaxis, opsonization, lysis of microbes and cells, augmentation of antibody production, and B and T cell responses (13). The most important function of complement, in the defence against bacterial infection, is probably to serve as a mediator of antibody-dependent immunity (19). It is well established that specific antibodies can activate both the classical and the alternative pathways of complement (20). Another important physiologic activity of complement is disposing of immune complexes and actions through the products formed during complement activation that create inflammatory injury (14).

The complement system has three pathways of activation that act at a target surface: the classical pathway, the alternative pathway, and the lectin pathway. These pathways all lead to the formation of the membrane attack complex (C5b-C9), which is a unique protein complex that perforates the cell membrane.

Table 2. Complement proteins involved in complement activation and their characteristics.

Protein	Genomic location	Number of polypeptide chains	µg/mL in plasma	Cellular origin	Function
Classical pathway					
C1q	1p34.1-p36.3	6 x 3 (A, B, C)	80	HC, MNPH, FB, GIEC	Activates the classical pathway. Binds to IgM, IgG, and CRP.
C1r	12p13	1	50	HC, MNPH, FB, GIEC	Cleaves C1s.
C1s	12p13	1	50	HC, MNPH, FB, GIEC	Cleaves C4 and C2.
C4 (C4A, C4B)	6p21.3	3 (α, β, γ)	250	HC, MNPH, FB, GUEC, PAC type II	C4b is acceptor for C2, binds to activating surfaces and protection from maturation of self-reactive B cells. C4a is an anaphylatoxin.
C2	6p21.3	1	20	HC, MNPH, FB, GUEC, PAC type II	Provides a catalytic subunit for formation of the classical pathway C3 and C5 convertase.
Alternative pathway					
Factor B	6p21.3	1	210	HC, MNPH, FB, AdiC, EC, EndoC	Catalytic subunit for formation of the alternative pathway C3 and C5 convertase.
Factor D	19p13.3	1	1-2	MNPH, AdiC	Cleaves factor B bound to C3b or C3(H_2O).
Properdin	Xp11.4-p11.2	1-4	25	MNPH	Stabilizes the alternative pathway C3 convertase. Positive regulator and initiator of the alternative pathway.
C3	19p13.3-p13.2	2 (α, β)	1300	HC, MNPH, FB, EC, EndoC	C3 is involved in all three complement activation pathways. C3b binds to activated surfaces, mediate opsonisation, phagocytosis and cytolysis. C3b binds factor B. C3b is a part of the C3 and C5 convertase. C3a functions as an anaphylatoxin. C3d stimulate B cells.

Lectin pathway					
MBL	10q11.2-q21	2-8 x 3	1-5	HC, K, SI, T, AstC	Binds to sugar structures of microbes. Activates the lectin pathway.
MASP-1	3q27-28	2	6	HC, K, SI, H, L, AstC, Plac	Cleaves C3 with low efficacy.
MASP-2	1p36.21	2	6	HC, SI, T	Activates complement by cleaving C4 and C2. Mediates ficolin complement activation.
MASP-3	3q27-28			Widely expressed	Inhibits MASP-1 and MASP-2 activity.
sMAP/ Map19	1p36			HC, SI, T	Part of complexes with MBL or ficolins.
L-ficolin	9q34.3	2-4 x 3	10	HC	Forms complexes with MASPs and activates the lectin pathway. Opsonin.
H-ficolin	1p36.11	4-6 x 3	15	HC, PAC type II, BEAS	Forms complexes with MASPs and activates the lectin pathway. Opsonin.
M-ficolin	9q34	4 x 3		MB, NPH, PAC type II.	Opsonin.

Abbreviations: AdiC, adipocytes; AstC, astrocytes; BEAS, bronchial epithelial cells; EndoC, endothelial cells; EC, epithelial cells; FB, fibroblasts; GUEC, genitourinary epithelial cells; GIEC, gastrointestinal epithelial cells; H, heart; HC, hepatocytes; KC, keratinocytes; K, kidney; L, lung; MNPH, mononuclear phagocytes; NPH, neutrophil phagocytes; MB, myoblasts; PAC, pulmonary alveolar cells; SI, Small intestine; T, testis.

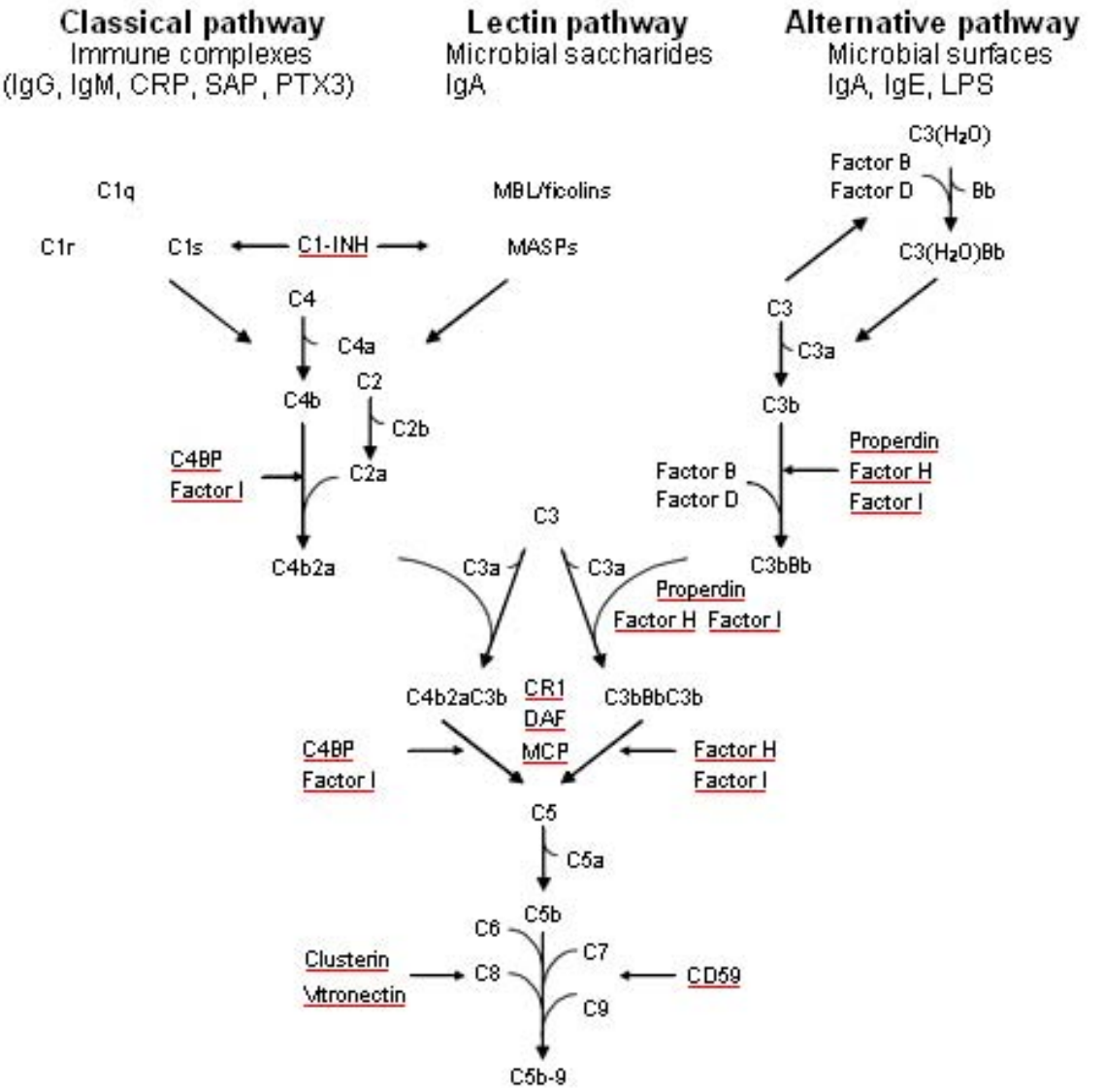

Figure 2. The complement system and regulatory proteins. The classical pathway is activated by binding of C1q to antibody-antigen complexes or other structures (e.g. CRP, SAP, PTX3) which results in formation of the classical C3 convertase C4b2a. Activation of the lectin pathway by MBL or ficolins results in an identical classical C3 convertase. The alternative pathway is activated on a surface that may promote spontaneous hydrolysis of the internal thioester bond of native C3, resulting in binding of factor B, which is cleaved by factor D, generating the alternative pathway C3 convertase C3bBb. The convertase is stabilised by properdin. C3b participates in the self-amplification loop of complement activation via the alternative pathway. Recruitment of further C3b molecules leads to the formation of C5 convertase and initiation of the lytic pathway. Sequential assembly of C5b to $C9_n$, ends with

formation of the membrane attack complex. Complement regulators are indicated by underlining.

Abbreviations: C1-INH, C1-inhibitor; CR1, complement receptor 1; DAF, decay-accelerating factor; MASP, MBL-associated serine proteases; MBL, mannan-binding lectin; MCP, membrane cofactor protein; PTX3, pentraxin 3; SAP, serum amyloid P component.

Classical pathway

The classical pathway comprises the Ca^{2+}-dependent $C1qr_2s_2$ complex (Figure 3), C4, C2, and C3. The pathway is initiated by the binding of the recognition molecule C1 to immune complexes via the Fc regions of the antigen-bound immunoglobulins to target cells or in fluid phases (Figure 4) (17).

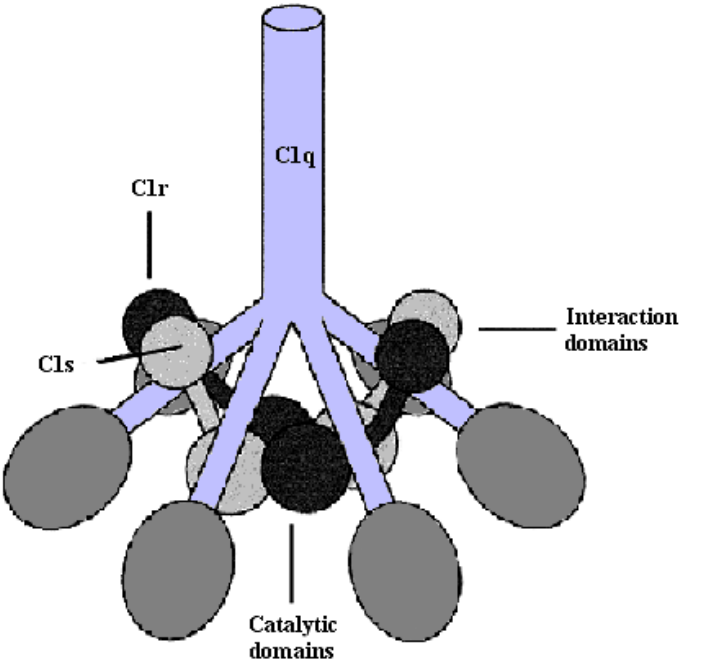

Figure 3. Modular structures of C1q, C1r and C1s and macroscopic model of the C1 complex. In the presence of Ca^{2+}, C1r and C1s bind to each other to form $C1r_2$-$C1s_2$. This complex then binds between the globular heads of C1q. The inactive C1 complex also consists of two C1 inhibitor molecules. Modified from (21).

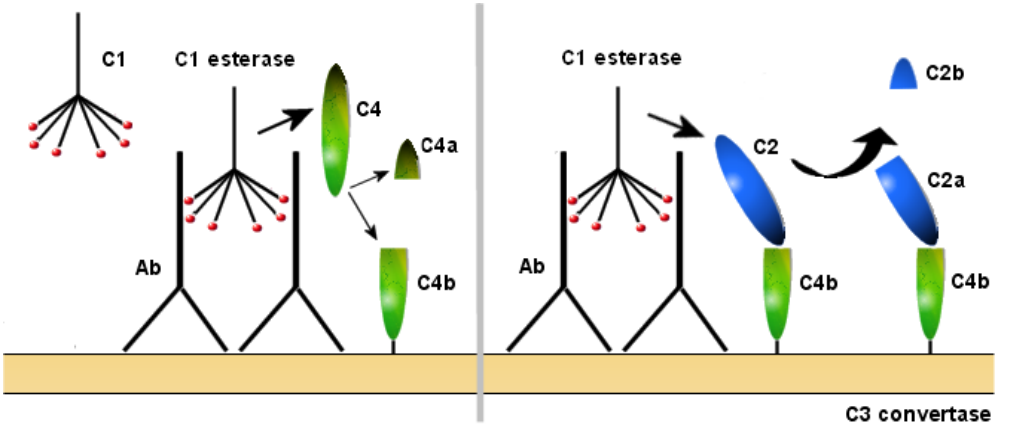

Figure 4. Activation of the C1 complex ($C1qr_2s_2$) is initiated by multipoint binding of the C1q molecule to the Fc portion of IgG or IgM. The binding is believed to cause a conformational change of the globular domains of C1q, and release of the C1 inhibitor molecules. This allows the autocatalytic activation of C1r which cleaves and activates C1s. C1s cleaves C4 from fluid-phase into C4a (smaller fragment) and C4b (larger fragment). C4b binds via a thioester group in close proximity to the initiating esterase. The next enzyme in the cascade is formed (C4b2a) after C1s also has cleaved C2 bound to C4b.
Abbreviations: Ab, antibody.

IgM and IgG3 are the strongest activators followed by IgG1 and IgG2. IgG4 does not activate C1q. Other molecules that also activate C1 in the same manner are, for instance, lipopolysaccharide (LPS), apoptotic cells, nucleic acids, and CRP (Table 3) (22). CRP exhibits multiple functional similarities to antibodies such as activating complement via the classical pathway, binding to receptors on phagocytic cells, induction of cytokine synthesis, and enhancement of phagocytosis. These functions are mainly explained by the shared ability of CRP and IgG to interact with complement component C1q and with Fcγ receptors (FcγR) I and II (23).

The binding of C1q to a target surface causes a conformational change in the collagenous region of C1 and activates the C1-associated serine protease dimer of C1r, which in turn activates the co-associated serine protease dimer of C1s. Activated C1s consecutively cleaves C4 and C4b binds C2 to generate the C3 convertase, C4b2a. The formation of C4b2a requires

the presence of Mg^{2+}. The C4b2a convertase can convert native C3 to C3b. After C4 and C3 are cleaved and C4b and C3b are formed they expose a highly reactive thioester with the ability to bind to hydroxyl or amide groups on the target surface. C4b and C3b that remain in fluid phase are immediately inactivated by hydrolysis.

Table 3. Antibody-independent activation of the classical pathway.

Type	**Activator**
Gram-positive bacteria	Polysaccharide structures of pneumoccoci and streptococci.
Gram-negative bacteria	The lipid A component of lipopolysaccharide (LPS) of the cell wall.
Viruses	Epstein-Barr virus, murine retroviruses
Plasma	Plasmin, tyrosin, CRP, SAP, PTX3.
Mitochondrial membranes	Human heart mitochondrial membranes after acute myocardial infarction. Probably mediated via cardiolipin.
Cytoplasmatic intermediate filaments	Vimentin-type
Nucleic acid and chromation	

Abbreviations: CRP, C-reactive protein; SAP, serum amyloid P component; PTX3, pentraxin 3.

Lectin pathway

At the end of the 1980's another activation pathway, the lectin pathway was discovered (24-26). The lectin pathway resembles the classical pathway in many respects. The pathway consists of MBL/MASP-complexes, C4, C2, and C3. MBL is a member of the collectin family of proteins and is composed of collagenous structures and C-type carbohydrate recognizing domains (CRD). CRD binds with high specificity, in presence of $C2^{2+}$, to sugar structures of many microorganisms (mannose, *N*-acetyl-glucosamine, *N*-acetyl-mannosamine, fucose, and glucose) (9). MBL shares structural similarity with C1q, the first component of the classical pathway (27). Various oligomeric structures of MBL have been visualised by electron microscopy (28). However, fully functional activity including both binding to microbial surfaces and activation of complement requires higher order of structures such as tetramers (29). Other members in this family are surfactant proteins A and D (SP-A and SP-D) (30, 31), and the liver protein CL-1 (collectin liver 1) (32). MBL exists in complex in plasma with MBL-associated serine proteases (MASPs), MASP-1 (25), MASP-2 (33), and MASP-3 (34) and with a smaller spliced variant of MASP-2 called small MBL-associated protein (sMAP or MAp19) (35). These circulating complexes work as opsonins and on binding to pathogens via MBL, the MASPs convert from inactive proenzyme to activated proteolytic enzyme. MASP-1 has proteolytic activities against C3 and C2 (36) and MASP-2 cleaves C4 and C2 with formation of the classical pathway convertase C4b2a (33, 36, 37). MASP-3 has inhibitory activity against MASP-2 (34). Under physiological conditions, MASP-1 and MASP-2 have also been shown to have synergetic activation effects and MASP-3 an inhibitory effect on the lectin pathway, independent of alternative pathway activity (38).

Closely related to the collectin family are the ficolins. They have a CRD that is a fibrinogen-like domain instead of a C-type lectin domain as found in collectins. In human serum, two ficolins (L-ficolin/P35 and H-ficolin, Hakata Ag) form complexes with MASPs or MAp19 and activate the lectin pathway (39, 40).

Alternative pathway

Components that are unique to the alternative pathway are factor B, factor D, and properdin. The alternative pathway lacks a recognition molecule analogous to C1. Instead, the alternative pathway is activated by various cell surfaces that favour the binding between C3b and factor

B and prevent the binding of the negative regulator factor H. The pathway provides a strong amplification loop of complement activation (Figure 5). It has been shown that polyanionic carbohydrates, such as sialic acid, on surfaces inhibit alternative pathway activation and promote the binding of factor H rather than factor B to C3b (41-43). An important function of host sialic acid is to regulate the innate immunity, and microbes have evolved various strategies for subverting this process by decorating their surfaces with sialylated oligosaccharides that mimic those of the host.

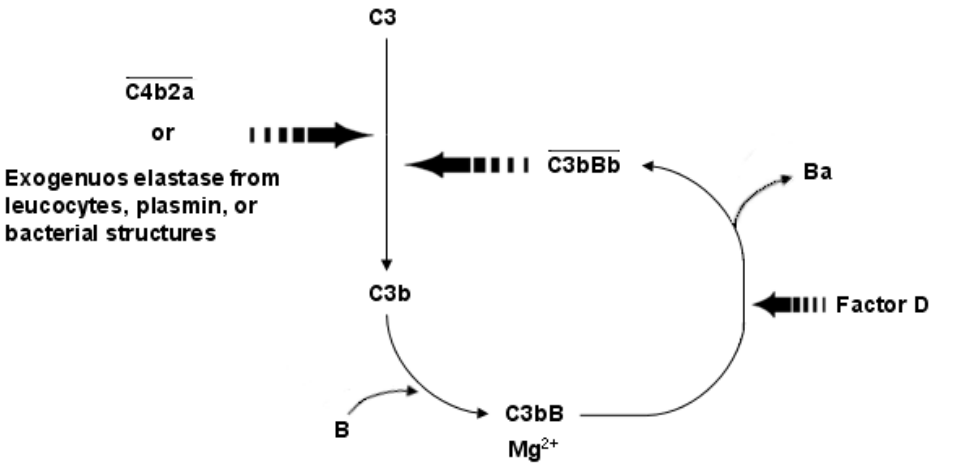

Figure 5. The alternative complement pathway and the amplification loop of C3 cleavage.

Under certain conditions immune-complexes are able to activate the alternative pathway (44). IgA and (20, 45) IgG2 may also activate the alternative pathway but requires a high epitope density (20). However, IgM, IgG1 and IgG3 are not significant activators of the pathway (20).

The “tick-over” mechanism, which also may initiate the pathway, involves spontaneous hydrolysis of the internal thioester and/or activation by proteolysis of small amounts C3, forming C3b or unstable intermediate C3i (Figure 6). These products may bind factor B and are then cleaved by factor D to form the alternative pathway C3 convertase, C3iBb. The enzyme cleaves more C3 to C3b and forms a surface-bound focus for the formation of more C3bBb. To avoid complete consumption of C3 from plasma, the mechanism is controlled at the target surface by factor H and factor B (46).

It has resently also been demonstrated that properdin can directly bind to microbial surfaces and initiate *in situ* assembly of a functional alternative pathway C3 convertases (47). Thus, properdin have two alternative functions; stabilizer and activator of the alternative pathway.

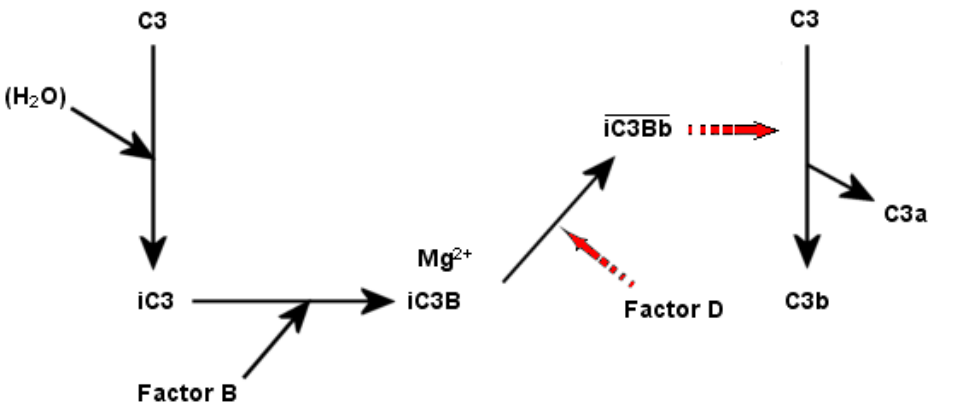

Figure 6. The "tick-over" mechanism of the alternative pathway. The mechanism may be initiated by proteolysis and/ or by hydrolysis of the C3 molecules internal thioester. C3b or unstable intermediate iC3b bind factor B and serve as a substrate for the enzyme factor D which cleaves factor B to form the alternative pathway C3 convertase.

Recently, the existence of an MBL-dependent C2 bypass mechanism for alternative pathway-mediated C3 activation has been demonstrated (48). The findings emphasize the role of the lectin pathway in antibody-independent complement activation by LPS. The possible impact of MBL in complement deficiency states is a field for further investigations.

The lytic pathway

The lytic pathway is initiated by deposition of multiple C3b molecules in close proximity to the C3 convertases generated by the classical, lectin or alternative pathway. The recruitment of further C3b causes a switch in substrate specificity in the C3 convertase and the C5 convertase may be created $C4b2a(C3b)_n$ or $C3bBb(C3b)_n$ (Figure 7) (49). These convertases

can convert native C5 to C5a and C5b. Structurally the C5 protein resembles C4 and C3, but lacks an internal thiolester bond (50).

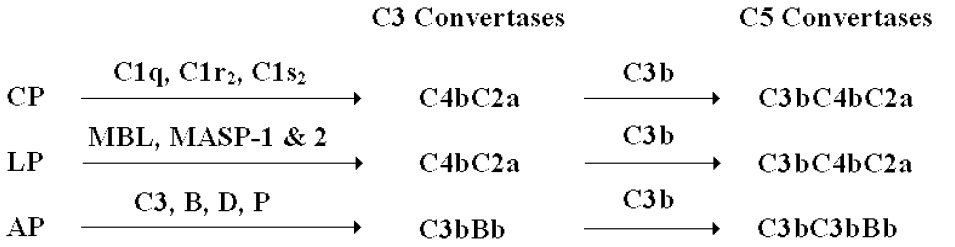

Figure 7. Assembly of the C5 convertase after activation of the complement pathways (49). Abbreviations: AP, alternative pathway; CP, classical pathway; LP, lectin pathway.

C5b has the ability to bind in a noncovalent manner to exposed hydrophobic sites on cell membranes and to serve as an anchor for assembly of the membrane attack complex (MAC, C5b, C6, C7, C8 and C9). The binding between these proteins is stable and lacks enzymatic activity. Addition of C8 to the membrane-bound C5b7 forms C5b8, which becomes more deeply integrated in the membrane and causes the cell to become slightly leaky. A polymerization sets off when C8 binds to additional C9 molecules, forming a tube of as many as 18 C9 molecules that disrupts the cell membrane causing an influx of water and ions into the cell (Figure 8) (51). This process induces cell death with features similar to apoptosis (52).

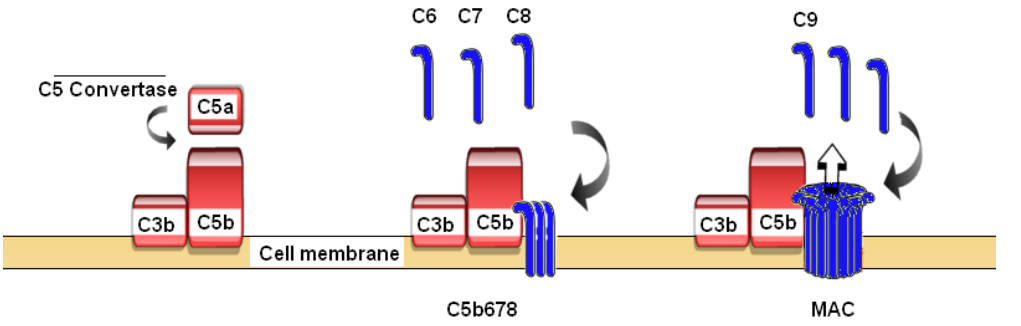

Figure 8. Assembly of the C5b-9 membrane attack complex (MAC). Recruitment of a further C3b into the C3 convertase generates a C5 convertase. Once C5b is membrane bound, C6, C7

and C8 attach themselves to form the stable complex, C5b678. This unit has some effect in disrupting the membrane, but primarily causes the polymerization of C9 to form tubules traversing the membrane. Disruption of the membrane by MAC leads to cell lysis.

Anaphylatoxins

The small cleavage fragments of C3, C4 and C5 are known as anaphylatoxins (C3a, C4a, C5a). They play a powerful role in the regulation of immunity and control the local pro-inflammatory response. Their actions stimulate histamine release from mast cells and basophils (C3a), increase vascular permeability (C3a), and promote vasodilatation (C3a and C4a). C5a is the strongest agonist; it is approximately 100-fold more effective than C3a and 1000-fold more effective than C4a and C5a-des-arg (C5a which has lost its carboxy-terminal arginine residue).

C3a is reported to be capable of suppressing both specific and polyclonal lymphocyte responses to mitogens and antigens, probably via the C3a-receptor on T cells (53, 54). Thus, the stimulating effect of C5a may to some extent be moderated by C3a. However, the anaphylatoxins are more efficiently regulated by carboxy-peptidase N (55).

C5a exerts numerous proinflammatory effects, such as chemotactic responses of neutrophils (56), release of granular enzymes from phagocytic cells (57), neutrophil production of superoxide anion (58), vasodilatation, increased vascular permeability (59), and induction of thymocyte apoptosis during sepsis (60, 61).

In conditions where massive complement activation occurs, such as sepsis, severe trauma, and most likely in allergic asthma, the effects of the anaphylatoxins may be lethal. For instance, in patients with sepsis, C5a was elevated and associated with significantly reduced survival rates and with multiorgan failure, as compared to less severely septic patients and survivors (62-64).

Complement regulation

The complement proteins are activated in a sequential manner resulting in the generation of products that have important biological activities. However, unwanted activation of complement can injure the host and may be life threatening. These toxic effects are mediated primarily by the anaphylatoxins C3a and C5a and by the formation of membrane attack complex on the host cell membrane. Many inflammatory diseases, including systemic lupus erythematosus, rheumatoid arthritis and glomerulonephritis are thought to involve excessive activation of the complement system. Uncontrolled complement activation is also implicated in post-ischemic inflammation with tissue damage and in sepsis. It is therefore well recognised, that the host, in order to prevent autologous complement-mediated attack, expresses a variety of both fluid-phase and membrane-bound complement regulatory proteins which limit cell damage. The complement regulators are presented in table 4.

Regulation of the complement system occurs both in plasma and at the cell membrane. The regulation acts at different levels in the system: initiation of activation, amplification, and at the effector function. The activation of the classical and the lectin pathways is inhibited by C1 esterase inhibitor (C1-INH) through binding in fluid phase to C1 and MASP-2, respectively. C1-INH can also irreversibly inhibit the enzymatic function the C1-complex by binding to activated C1r and C1s.

Factor H is a plasma protein that provides co-factor activity for factor I, but has a decay accelerating function as well, which makes it the major soluble protein that regulates the half-life of the C3 convertase in the alternative pathway (49). In addition, factor H discriminates between self and non-self by recognizing surface polyanions. Thus, lack of factor H causes uncontrolled alternative pathway activation resulting in secondary C3 deficiency. Properdin is known as the single physiological positive regulator of the alternative pathway and operates by stabilizing the C3 convertase against the intrinsic decay (65).

The most important membrane complement regulators are decay-accelerating factor (DAF), membrane cofactor protein (MCP), complement receptor-1 (CR1) and CD59. In humans DAF accelerates the decay of both the classical and alternative pathway C3 and C5 convertases (66). In addition, DAF acts as a hijacked receptor for echoviruses (67). MCP is a widely expressed complement regulator in humans that acts as a cofactor for factor I-mediated

cleavage of C3b and C4b deposited on self-tissue (68). CR1 shares many functional properties with DAF and MCP, and also promotes phagocytosis and binding of immune complexes to erythrocytes (69). Relatively recent investigations have shown that CR1 enhances B cell immunity (70) and mediates the binding of HIV to blood cells (71).

The thioester group found hidden in both C4 and C3 is crucial for the covalent and irreversible binding of the cleavage products C3b and C4b to membranes or other surfaces (72, 73). Surface bound C3b and C4b may propagate continued activation of the complement cascade. Thus, regulation of these components and their reactive internal thioester group is essential to avoid complement induced tissue damage. Decay of C3b is mediated by factor I in the presence of an appropriate cofactor (factor H, CR1 or MCP). Factor I cleaves C3b at two sites in the α-chain yielding C3f and iC3b (inactive C3b). A further cleavage by factor I is catalysed only by CR1 resulting in the fragments C3c and C3dg. C3dg is further broken down by serum proteases to C3g and membrane bound C3d (Figure 9). Decay of C4b follows similar patterns. Factor I cleaves C4b at either side of the thioester in presence of an appropriate cofactor (C4BP, MCP or CR1) releasing C4c and leaving C4d on the membrane.

In some situations the complement activation overcomes the first line of defence against complement-mediated cell damage. The regulation then becomes dependent on inhibition of the actions of MAC. Nucleated cells can, to some extent, resist lysis by MAC. However, sublytic activity of MAC can promote cell proliferation, generation of pro-inflammatory mediators, and production of extracellular matrix (74-83). Predominantly, MAC is inhibited by the membrane-bound CD59. Two fluid phase proteins, vitronectin and clusterin inhibit MAC formation *in vitro* (84, 85).

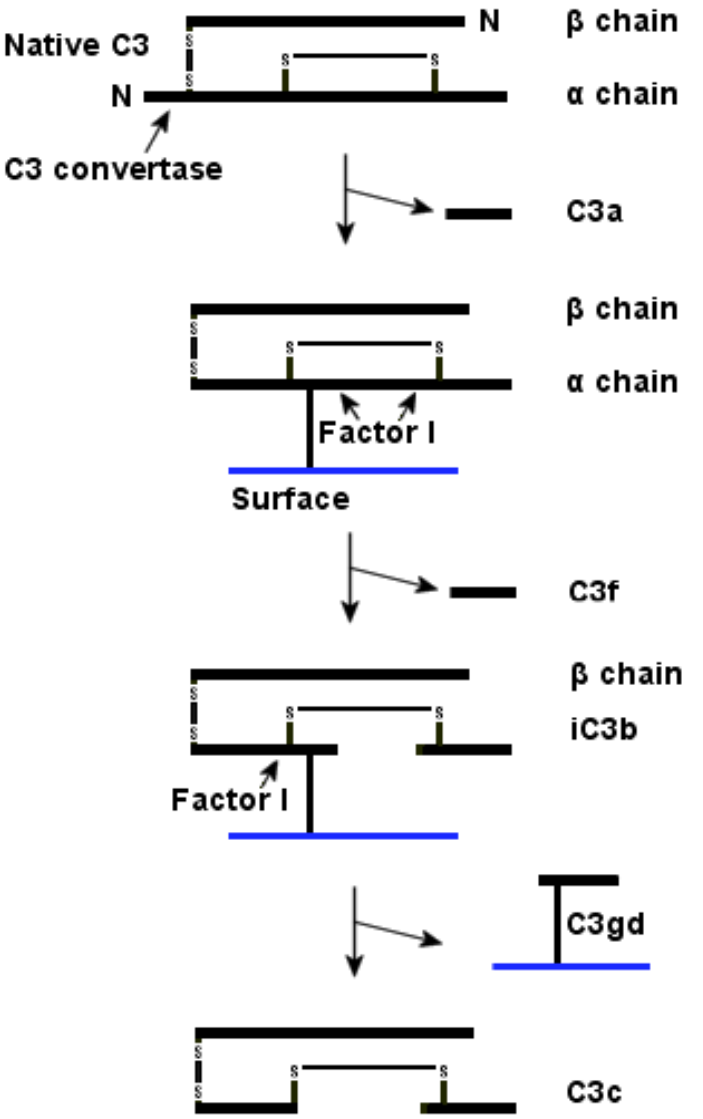

Figure 9. Activation and decay of C3 and C4. In principle, the decay of C4b follows the same break down as C3b with the exception that there is no fragment equivalent to iC3b. Factor I cleaves C4b and C4d remain on the membrane while C4c is released into fluid phase. Modified after (86).

Table 4. Fluid phase and cell membrane regulators of complement activation.

Location and protein	Ligand	Function(s)
Fluid phase in plasma		
C1-inhibitor	C1r, C1s MASPs	Dissociates C1r and C1s from C1 (87). Inhibits MASP-2 (88).
Formation of C3/C5 convertase		
C4BP	C4b	Cofactor for factor I in cleavage of C4b, decreases C3b deposition on a target surface (13).
Factor H	C3b	Cofactor for factor I in cleavage of C3b (13).
Factor I	Factor H, C4BP, MCP, CR1	Proteolytic inactivation of C3b and C4b (13).
Formation of MAC		
Clusterin (APO J, SP-40, 40)	C7, C8, C9	Clusterin binds to C7 and a β-subunit of C8 and C9. Prevents assambly of MAC (89).
Protein S (Vitronectin)	C5b-7	Prevents attachment of C5b-7 and C5b-9 to membranes (90).
Cell membrane		
Formation of C3/C5 convertase		
DAF (CD55)	C3b, C4b	Inhibits the activation of C3 and C5 by preventing the formation of new convertase. Also accelerates the decay of performing C3 and C5 convertases (66).
MCP (CD46)	C3b, C4b	Cofactor for factor I in cleavage of C4b and C3b (68).
CR1 (CD35)	C1q, C3b, C4b, iC3b, MBL	Mediates phagocytosis. Inhibits assembly and accelerates decay of C3 convertase, cofactor for factor I in cleavage of C4b and C3b (69).
CR2 (CD21)	iC3b, C3d, C3dg	Enhances B cell immunity (91).
CR3 (CD11b/CD18)	iC3b	Mediates phagocytosis (92, 93).
CR4 (CD11c/CD18)	iC3b	Mediates phagocytosis (92).
CRIg	iC3b	Mediates phagocytosis by Kupffer cells in the liver (94).
Formation of MAC		
CD59 (MIRL, Protectin)	C5b-8, C9	Prevents binding of MAC to cell membrane (95).

HRF	C5b-8, C9	Prevents binding of MAC to cell membrane (96).

Abbreviations: APO J, apolipoprotein J; bp, binding protein; C1- INH, C1-inhibitor; CD, Cluster of differentiation (i.e. cell surface antigen); CR, complement receptor; DAF, decay-accelerating factor; HRF, homologous restriction factor; MAC, membrane attack complex; MCP, membrane cofactor protein; MIRL, membrane inhibitor of reactive lysis; SP-40, 40, serum protein 40 kDa, 40 kDa.

Complement and genetics

Most complement proteins are inherited in an autosomal codominant pattern. Typical components with this form of inheritance include C1-INH, C2, C3, C5, C6, C7, and C9. The genetics of C1, C4 and C8 are more complex with involvement of multiple genes for each component and the properdin gene is located on the X chromosome. Several of the complement proteins also display a fairly large genetic variation, C4 being the most polymorphic. The genes encoding the proteins of the complement system are located on at least six different chromosomes (Table 2). The genes for C2, factor B, and the genes for C4 (C4A and C4B) are found on the short (p) arm of chromosome 6 within the major histocompatibility complex (MHC, Figure 10) (97). C4 is encoded by two closely located linked genes which produce two isotypic variants, C4A and C4B (98, 99). The two variants differ by only 6 amino acids but give rise to significantly different functions. C4A binds preferentially to amino groups after cleavage while C4B binds to hydroxyl groups. C4B is also more efficient in propagating continued activation of complement.

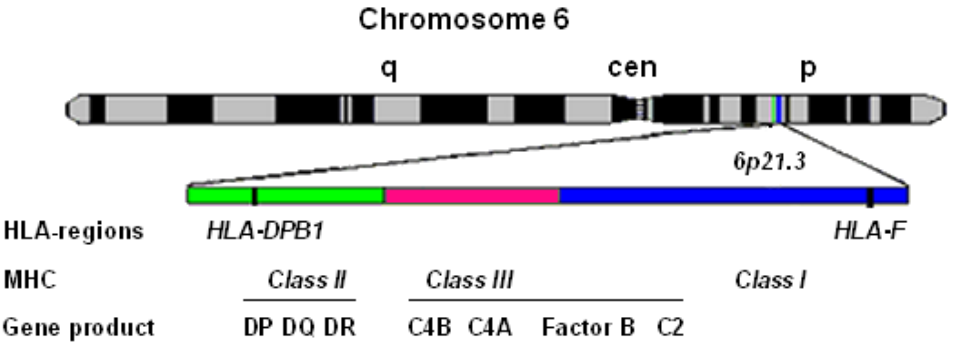

Figure 10. Simplified outline of chromosome 6 and the HLA complement gene cluster. Three different host-defence systems are controlled from this chromosome region: self-regognition (HLA-A, B, and C), immune response (HLA-DR, DP, and DQ), and complement (C4B, C4A, factor B, and C2).

Similar to the HLA class I and class II genes, the complement genes located in the HLA complex express a considerable polymorphism both at DNA level and with regard to the

produced proteins. These genes are inherited as haplotypes (single linkage group) and their polymorphism are characterized as complotypes (100).

Complement component C2 and factor B provide the functional catalytic subunit of the classical and alternative pathway C3/C5 convertases, respectively. These two proteins share a relatively high degree of sequence similarities and each protein is about 100kD in size. The *C2* and *Bf* genes are separated by only 421bp (Figure 10).

Laboratory analysis of complement

A recently published review by Mollnes et al. (2007) summarizes the latest considerations regarding complement analysis in a most comprehensive manner (101). Hemolytic assays have traditionally been used to assess the functional activity of the complement system. These types of analyses were first described by Mayer et al., 1961 (102) and Rapp et al., 1970 (103). The basic concept is the use of serial dilutions of the sample to be analyzed and incubation with antibody-sensitized sheep erythrocytes (classical pathway) at a defined temperature. The assay may be performed either in tubes or in agarose plates in the presence of Ca^{2+} and Mg^{2+} ions. The results can be expressed as reciprocal dilutions of the sample required to produce 50 or 100 % lysis (CH50 or CH100, respectively). Tests for the functional activity of the alternative pathway (AP50) use erythrocytes from guinea pig, rabbit or chicken as target cells and require a buffer with Mg^{2+} ions only and Ca^{2+} chelating ethylene glycol bis-amino tetraacetate (EGTA) buffer to block the classical and lectin pathway activation. Hemolytic assays have also been developed for analysis of the lectin pathway (104). Numerous modifications and improvements have been made over time for larger-scale clinical screening of complement deficiency states (105, 106). These analyses use a single serum dilution and a large number of erythrocytes. Furthermore, a complement function Enzyme-Linked ImmunoSorbent Assay (ELISA) was developed (107) and later also modified to include the lectin pathway (108). Based on this principle, a combined ELISA (Wieslab AB, Lund, Sweden) has been developed for functional analysis of all three complement pathways at the same time (109).

Individual complement components, irrespective of functional activity, can be measured by immunoprecipitation tests (nephelometer techniques or radial immunodiffusion), ELISA, and Western blotting. Thus, immunoprecipitation is the technique of precipitating an antigen out

of solution using an antibody specific to that antigen. Importantly, if an immunochemical assay does not reveal any complement deficiency state, the component may still be functionally inactive and only a functional assay can verify the diagnosis. This problem may occur in for example C1-INH-deficient patients with a non functional protein (110).

For an even more detailed analysis of individual complement activation products, highly specific monoclonal antibodies may be used for detection of neoepitopes only exposed upon activation-induced conformational changes without interference of the inactive components (111). These highly specific antibodies can be utilized in ELISA (112) or high-capacity immunosorbent techniques (113, 114).

COMPLEMENT-MEDIATED DEFENCE

Complement is activated by its capacity to distinguish between self and non-self, primarily as a defence against microorganisms. Several of the complexes and fragments generated during complement activation influence the cellular immune response. C3 and the fragments C3b and iC3b enhance the mitogen-induced proliferative response of B and T cells via complement receptors (CR1, CR2, and CR3) on phagocytic cells and lymphocytes (115). C3 fragment also markedly enhances the response of B cells to antigen (116). The C3b/C3d fragments are strong adjuvants that profoundly influence the B cell response to thymus-independent antigen (117).

However, the activation may also induce an inflammatory reaction which is harmful to the host. Complement-mediated inflammation is involved not only in specific immunological defence reactions, but also in the induction of tissue injury by ischemia, hypothermia or other general tissue-damaging factors. For instance, myocardial ischemia/reperfusion injury is accompanied by an inflammatory response primarily orchestrated by the complement cascade contributing to reversible and irreversible changes in tissue viability and organ function. Major trauma leads to systemic complement activation and complications to trauma may enhance the activation and increase the risk of a generalised inflammatory reaction with a fatal outcome.

Complement and infection

The complement system is fairly efficient in destroying microorganisms. However, many microorganisms have evolved a whole array of highly specific complement-modulating strategies (Table 5). In this way they can either stop or delay the effects of an innate immune attack, thereby creating a window of opportunity that allows their survival. In many cases the ability to avoid complement attack acts as a virulence factor, markedly aggravating host conditions.

There are at least three critical functions of complement that must be intact to stop host invasion of microbes: (a) opsonization and phagocytosis, (b) direct lysis, and (c) stimulation of inflammation via protein fragments generated by the complement cascade.

The mechanism of opsonization, where the antigen is bound to an opsonin (acute phase reactant, antibody or complement protein) to enhance phagocytosis, needs to identify the pathogen and recognize the receptors on the phagocytic cell. The pathogen is by this means bound to the surface of the phagocyte and then engulfed. Opsonization and phagocytosis is probably the most important process by which complement limits severe infections.

Direct lysis of complement by formation of transmembrane channels (MAC) is another way of destroying infectious agents. The MAC can kill Gram-negative bacteria that have a thinner outer lipid membrane. Some Gram-negative bacteria have developed resistance to complement-mediated lysis that frequently correlates to the outer membrane structure of the organism. For instance, a high concentration of sialic acids in the outer membrane protects from C3 deposition and activation of the alternative complement pathway (42, 118). In contrast, Gram-positive bacteria resist this attack by a thick cell wall and a capsular structure.

The anaphylatoxins, C3a, C5a and to a lesser extent C4a, induce release of histamine from mast cells and basophils, and attract neutrophils and monocytes to the site of tissue damage. Complement activation may also affect mucous secretion, smooth muscle contraction, and dilatation of blood vessels (119). Moreover, anaphylatoxins are intimately involved in directing activation of the inflammatory cells, the expression of adhesion molecules for cell to cell interactions and migration of cells from the bloodstream to tissues (120, 121). Microorganisms may express proteases on the bacterial surface and secrete these into the fluid phase, causing a dose-dependent reduction in C5a chemoattractant activity. This mechanism has been shown for *Serratia marcescens* (122) and similar mechanism for *Streptococcus pyogenes* (123).

Table 5. Evasion of complement-mediated defence against infection.

	Gram-negative bacteria	
Microbial component	***Mechanism of survival***	***Examples***
Elastase	Anaphylatoxins C3a and C5a are inactivated.	*Pseudomonas aeruginosa* (124)
Outer membrane structure	Inhibition of MAC insertion.	*Neisseria meningitidis* (125)
Sialylated lipooligosaccharide (LOS) and porin	Inhibition of MAC insertion, binding of C4BP to Por1 A or B, and binding factor H to LOS.	*Neisseria gonorrhoeae* (125, 126)
Long polysaccharide chains in the cell wall	Disassociation of MAC from bacterial membrane.	*Escherichia coli* (127), *Salmonella montevideo* (128)
Secreted protein, StcE	Inhibits C1 complex by binding to the activating surface and C1-INH.	*Escherichia coli* O157:H7 (129)
Outer membrane protein A, OmpA	Binds C4BP and is thereby protected against C3b deposition.	*Escherichia coli* K1 (130)
Surface protein, CD59-like protein	A natural membrane inhibitor of MAC	*Borrelia burgdorferi* (131)
	Gram-positive bacteria	
Membrane protein; Mrp and Enn	Disrupts complement activation by blocking IgG and IgA.	Group A Steptococci (GAS) (132)
Degrading enzymes; IdeS and IdeB	Remove the Fc region from IgG attached to the bacterium.	GAS (133)
Streptococcal inhibitor of complement, SIC	Functions as the human MAC regulators, clusterin and protein S.	GAS (134)
M protein family members Arp and Sir	Bind C4BP and are thereby protected against C3b	GAS (135, 136)

	deposition.	
Protein G	Bind IgG and thereby blocks FcR mediated phagocytosis.	Group G Steptococci (137)
Staphylococcal protein A, SPA	Similar function as Protein G.	*Staphylococcus aureus* (138, 139)
Staphylokinase	Anti-opsonin (C3b, iC3b) properties. IgG cleavage from bacterial cell wall.	*S. aureus* (140)
Extracellular fibrinogen-binding molecule, Efb	Bind to C3b and inhibits complement activation.	*S. aureus* (141)
Staphylococcal complement inhibitor, SCIN	Stabilizes both C3bBb and C4b2a and prevents additional generation of C3 convertases.	*S. aureus* (142)
Bacterial capsule, pneumococcal surface proteins	Provides a barrier between C3b deposition and complement receptors on phagocytic cells.	*Streptococcus pneumoniae* (136)
	Other microbes	
Proteins that mimic complement regulatory proteins	Proteins presented by various bacteria, viruses, fungi, and protozoans inhibit complement.	*Trypanosoma cruzi*, *Candida albicans, Aspergillus fumigates*, Epstein-Barr virus and other herpes viruses (143-146)

The importance of phagocytosis and opsonophagocytosis

The engulfing of microorganisms, other cells, and foreign particles by phagocytic cells is called phagocytosis and is perhaps the key goal to which the activity of white blood cells such as neutrophils and macrophages is directed. The dendritic cell, another type of phagocytic cell, is located in tissues particularly in those in contact with the external environment. They interact with T cells and B cells to initiate and shape the adaptive immune response. Among the phagocytes, the neutrophil is probably the most efficient cell-type at phagocytosis (147). Neutrophils triggered to leave the blood stream, migrate through the extracellular matrix and are guided to infected sites in order to kill the microbes after phagocytosis. A number of particles with various biological structures may be directly phagocytosed by neutrophils. However, an important defence mechanism is opsonization where particles are coated with acute phase reactants (e.g. CRP), antibodies and complement proteins. Opsonization enhances identification of the particles and potentiates the rate of phagocytosis (148-150). For instance, *Haemophilus* (*H.*) *influenzae* type B, *Neisseria* (*N.*) *meningitidis* and pneumococci are protected against direct phagocytosis by their polysaccharide capsule. After they have been coated with an opsonin they are more readily recognized and destroyed by the phagocyte.

In addition to immunoglobulins, microbes are opsonized by the complement proteins C1q, MBL, C4b and C3b/iC3b (151). C3b is probably the major opsonin of the complement system. Phagocytic cells, as well as other cell types, express complement receptors (CR1, CR3, and CR4) that bind MBL, C3b, C4b, or iC3b (table 4). Phagocytic cells also express Fc receptors on their surface that may bind to the Fc region of antibodies. CR1 is found on erythrocytes, B cells, monocytes, neutrophils, eosinophils, and dendritic cells; CR3 on monocytes, macrophages, neutrophils, granulocytes, dendritic cells, and NK cells; CR4 has not been as well characterized as the other complement receptors (152). CR1 has not yet been described as a phagocytic receptor. CR1 binds to a broad spectrum of opsonized microbes but similar to the integrins CR3 and CR4 is unable to mediate internalization of a particle without additional signals or preactivation of the phagocyte. It has been observed that complement receptors and Fc recptors produce cooperative effects for internalization (153). More recently reported data have shown that different phagocytic receptors produce synergistic effects. For example, Fc receptors induce internalization of particles coated with suboptimal concentrations of IgG together with MBL that binds to CR1 (154). Inflammatory cytokines

(TNFα), microbial products (LPS), adhesion factors (fibronectin) promote phagocytosis through CR3.

ANTIBODY RESPONSES TO POLYSACCHARIDE ANTIGENS

B cells

The principal function of B cells is to produce antibodies against soluble antigens. B cells are therefore an essential component of the adaptive immune system. The abbreviation "B" comes from bursa of Fabricius which is an organ in birds where avian B cells mature. B cells are produced in the bone marrow by hematopoietic bone marrow stem cells. The human body makes millions of different types of B cells each day that circulate in the blood or migrate to lymphoid organs and tissues (spleen, lymph nodes, tonsils, Peyer's patches, and mucosal surfaces). They do not produce antibodies until they become fully activated. Each B cell has a unique receptor protein, the B cell receptor (BCR), on its surface that will bind to a particular antigen. The BCR is a membrane-bound immunoglobulin, and it is this molecule that allows the distinction of B cells from other types of lymphocytes, as well as being the main protein involved in B cell activation. The B cells may either become plasma cells or memory B cells directly or they may undergo an intermediate differentiation step, the germinal center reaction, where the B cell will hypermutate the variable region of its immunoglobulin gene and undergo class switching (Figure 11).

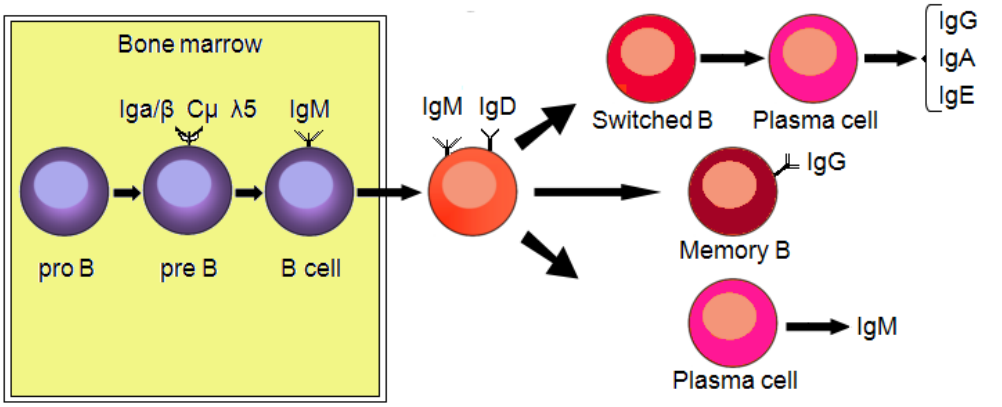

Figure 11. Maturation of B cells. The pre B cells express Iga/β, which is a B cell receptor-associated heterodimer responsible for intracellular signalling. They also express the heavy chain (Cμ) and pseudo-light chain (λ5) of IgM. In germinal centers of lymphoid organs, the B cells go through an isotype-switch and become immunoglobulin producing plasma cells (155).

Memory B cells are able to live for a long time, and can respond quickly following a second exposure to the same antigen. Some B cells express IgM or IgD in greater quantities than IgG and their receptors show polyspecificity with preference for other immunoglobulins, self antigens and common bacterial polysaccharides. These B cells are present in low numbers in the lymph nodes and spleen and are instead found predominantly in the peritoneal and pleural cavities. Other B cells are found circulating in the blood (156).

Antibodies

Antibodies are present on the B cell membrane and are secreted by plasma cells. Secreted antibodies circulate in the blood, serving as effectors of the humoral immunity by neutralization of antigens or by marking them for elimination. The immunoglobulins make up about 10-20 % of the plasma protein concentration (157). Antibodies are more frequently involved in supporting other components of the immune system than acting in isolation (Figure 12).

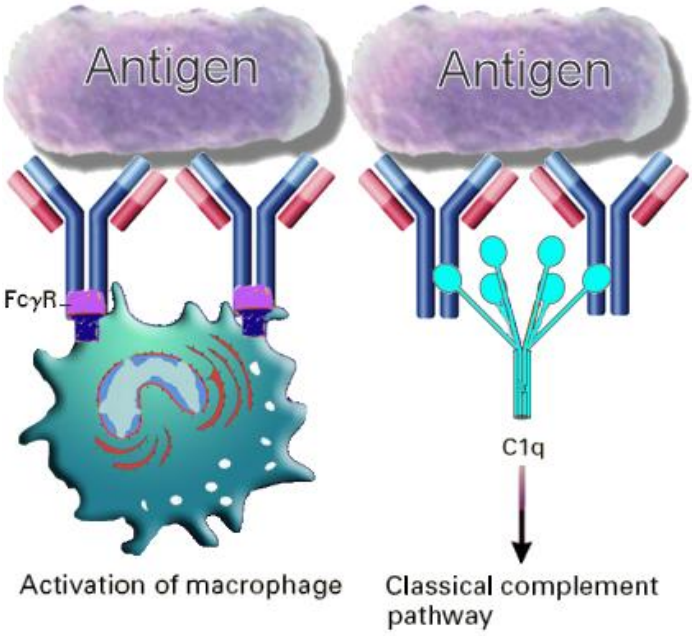

Figure 12. Two important functions of antibodies are opsonization and activation of the complement system. Modified after (158).

A typical antibody is composed of two immunoglobulin (Ig) heavy chains and two Ig light chains. Several different types of heavy chains exist that define the class or isotype of an antibody (159). All heavy chains contain a series of immunoglobulin domains, usually with one variable domain important for binding antigen and several constant domains. In humans, there are five known types of immunoglobulin heavy chains: γ (IgG), δ (IgD), α (IgA), μ (IgM) and ε (IgE) (157). The heavy chains α and γ have approximately 450 amino acids, while μ and ε have approximately 550 amino acids (Figure 13) (157).

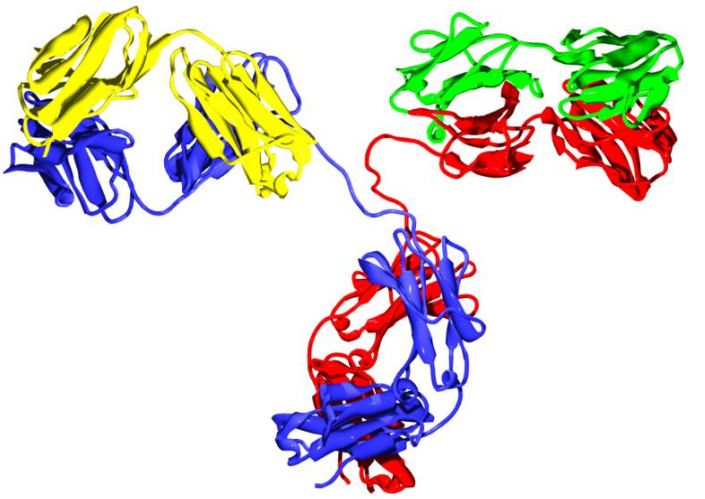

Figure 13. Structure of an antibody. The two heavy chains are red and blue, and the two light chains green and yellow. Modified after (160).

Each heavy chain has two regions: a constant region which is the same for all immunoglobulins of the same class and a variable region, which differs between different B cells, but is the same for all immunoglobulins produced by the same B cell or B cell clone (Figure 14). Heavy chains γ, α and δ have a constant region composed of three immunoglobulin domains in a row and a hinge region for added flexibility (161). Heavy chains μ and ε have a constant region composed of four immunoglobulin domains (157). The variable domain of any heavy chain is composed of a single immunoglobulin domain. These domains are about 110 amino acids long (157).

There are two types of light chains in humans, κ and λ. In each antibody, the two light chains are structurally identical. Each light chain has two successive domains: one constant and one variable domain. The approximate length of a light chain is from 211 to 217 amino acids (157).

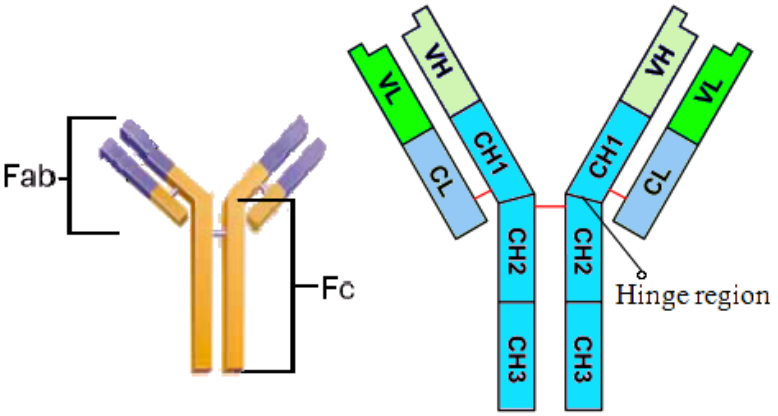

Figure 14. An antibody digested by papain yields three fragments, two Fab fragments and one Fc fragment, all of about 50 kDa. Heavy chains γ, α and δ have a constant region composed of three immunoglobulin domains in a row (CH1-CH3) and a hinge region for added flexibility. Abbreviations: Fab, Fragment antigen binding; Fc, Fragment crystallizable; V, variable; C, constant; H, heavy; L, light.

The Fc region (Fragment, crystallizable), is derived from the stem of the Y-shaped Ig molecule and is composed of two heavy chains that each contribute with two to three constant domains depending on the class of the antibody. Fc binds to various cell receptors and complement proteins. In this way, antibodies can mediate different physiological effects.

Each end of the forked portion of the antibody is called the Fab region (Fragment, antigen binding). It is composed of two constant and two variable domains of each of the heavy and the light chains (162). The antigen binding site is shaped by two variable domains which can bind to a specific antigen (Figure 14).

Somatic recombination, also known as V(D)J recombination, of immunoglobulins involves the random selection and combination of genes encoding each segment of the immunoglobulin variable region in a manner that generates a huge repertoire of antibodies with different paratopes. These segments are called variable (V), diversity (D) and joining (J) segments (163). The Ig heavy chains are determined by the V, D and J segments and the Ig light chains by the V and J segments. In the human genome, multiple copies of the V, D and J

segments exist in a tandem arrangement. Their selection for recombination within the individual B cell is called gene rearrangement (164). When a B cell successfully produces a functional immunoglobulin gene during its V(D)J recombination, this gene will suppress the expression of any other variable region gene by a process known as allelic exclusion (165, 166). Thus, the variable regions of all the immunoglobulin molecules within one given B cell will be identical, although the constant domains of the heavy chains may differ (157). The diversity generated by this mechanism in the variable region of the heavy chain - to be specific, in the complementarily determining region 3 (CDR3) - provides the human immune system with its ability to bind many distinct antigens. Isotype switching (or class switching) occurs after the process of V(D)J recombination and the following activation of the mature B cell generates the different antibody classes, all with the same variable domains as the original immunoglobulin generated in the immature B cell during recombination, but possessing distinct constant domains in their heavy chains (164).

A further mechanism for generating antibody diversity exists for the mature B cell after antigen stimulation. Activated B cells are more prone to somatic hypermutation in their immunoglobulin variable chain genes (167). This generates slight changes in the amino acid sequence of the variable domains of both the light and heavy chains between clones of the same activated B cell, and ultimately, differences in the affinity or strength of interaction between the B cell and its specific antigen (168). Thus, B cells expressing immunoglobulins with higher affinity for the antigen will outcompete B cells with lower affinity immunoglobulins for function and survival in a process known as affinity maturation (169).

Human serum contains “natural” antibodies which are present prior to the infection. IgM antibodies constitute the major component of the natural antibodies. These antibodies are an essential part of the first line defence against hematogenously spreading infections.

Polysaccharide antibodies

Polysaccharide encapsulated bacteria such as *Streptococcus (S.) pneumoniae*, *H. influenzae* type b, and *N. meningitidis* are among the most prevalent bacterial pathogens of humans. Carbohydrate antigens exhibit a large degree of antigenic variation. For example, today over 10 different serogroups of *N. meningitidis* and over 90 different serotypes of *S. pneumoniae* have been identified based on the capsular polysaccharides (CPS, Table 6). Immune responses to CPSs can occur in the absence of a functional thymus and the antigens are therefore designated as thymus-independent (TI). In infants and young children up to the age of 2 years the antibody response to CPS is inadequate resulting in an increased frequency of infections such as purulent otitis and pneumonia (170). A higher incidence of severe invasive infections such as septicemia and meningitis is also observed in this age group (171). A majority of carbohydrates are categorized as TI antigens by nature. In contrast, the thymus-dependent (TD) antigens give rise to long lasting immunological memory due to formation of memory B and T cells. Memory responses are characterized by the production of high-avidity antibody, i.e., antibodies that bind strongly to the antigen and of multiple isotypes (IgA, IgM, IgG_1, IgG_{2a}, IgG_{2b}, and IgG3).

Table 6. Clinically important bacteria and their serogroups/serotypes.

Bacteria	Capsular polysaccharide	O-antigen/ immunotype
Gram-positive		
Streptococcus pneumoniae	>90	
Staphylococci	>10	
Group B streptococci	>6	
Gram-negative		
Neisseria meningitidis	>10	>10
Haemophilus influenza	6 (a-f)	
Salmonellae	1 (Vi antigen)	>40
Escherichia coli	>70	>170
Vibrio cholera	1 (O139)	>200
Klebsiellae	>80	>10
Citrobacter		>40
Hafnia		>60
Proteus		>60

Thymus-independent antigens

Investigations in mice have revealed that TI antigens can be further divided into TI type 1 and TI type 2 based on their interaction with B cells (172, 173). TI type 1 antigens are defined as antigens capable of inducing proliferation and differentiation of both naive and mature B cells (157). These antigens activate B cells and may induce immune responses in neonates and adults (157, 172, 174). Examples common of the TI type 1 antigens are the bacterial LPS (157, 174).

The TI type 2 antigens share several common features of their immune response such as late development of antibody synthesis in ontogeny, no memory formation and a restricted isotype (IgM, IgG2) and idiotype usage. The regulatory T cells may influence the magnitude of the antibody response to capsular polysaccharide antigens. Conjugation of bacterial

polysaccharide to a protein carrier converts the immune response to a TD form and significantly improves the immunogenicity (175, 176).

In contrast to the mouse, the human peripheral B cell compartment displays a large population of $CD27^+$ memory B cells that represent up to 40% of all circulating B cells. They share characteristics similar to the splenic marginal zone B cells (SMZ) (177). Human SMZ B cells exhibit a rapid activation and immunoglobulin secretion response to TI antigens (178-180). The classical isotype-switched B cells and IgM^+ B cells are included among the $CD27^+$ memory B cells (155, 181-183). The $CD27^+$ memory B cells account for antibody responses to polysaccharides and show evidence of antibody diversification at early age before immune responses to antigens might be expected to have occurred (177).

The IgG allotype

In the middle of the 1950's, two research articles were published almost at the same time indicating the presence of genetic polymorphisms in immunoglobulins of rabbits and humans (184, 185). The discovery was initially met with scepticism, although there was no specific criticism of either the methodologies or the interpretation of data. During the following years studies of the immunoglobulin genetic markers contributed to our understanding in many areas such as transfusion sciences (186), fetal production of IgG (187), tolerance *in utero* (188), and molecular evolution (189, 190). The system was shown to be unique in its ability to characterize human populations by specific sets of haplotypes (191-194). The immunoglobulin genetic markers are associated with variation in susceptibility to several autoimmune and infectious diseases (195-202). They also influence immune responsiveness to infectious epitopes as well as to certain autoantigens (203-207).

Immunoglobulin allotypes can be observed by their antigenic determinants specified by allelic forms of the immunoglobulin genes. Between individuals, there are slight differences in the amino acid sequences of heavy or light chains. Even a single amino acid difference can give rise to an allotypic determinant, although in most cases several amino acid substitutions have occurred. Allotypic differences may be detected by using antibodies directed against allotypic determinants. There are also polymerase chain reaction (PCR) analysis for assessment of the different genetic markers of the immunoglobulins (208-210).

Thus, GM allotypes are genetic variants of the immunoglobulin heavy G chains (IGHG) of IgG molecules, coded from genes localized in the human IGH locus on chromosome 14 at 14q32.33. These genetic markers are inherited according to the Mendelian law (211). In a B cell producing immunoglobulins, only one of the alleles is permitted to express itself within a clone due to allelic exclusion. GM allotypes have been described in IgG1, IgG2 and IgG3, but not in IgG4. At present, 18 GM allotypes are known: G1M (1, 2, 3, 17) or G1M (a, x, f, z), G2M (23) or G2M (n), G3M (5, 6, 10, 11, 13, 14, 15, 16, 21, 24, 26, 27, 28) or G3M (b1, c3, b5, b0, b3, b4, s, t, g1, c5, u, v, g5) (193). The allotypes are inherited in fixed combinations called GM haplotypes (212).

The presence of the IgG2 allotype G2M(n) is associated with efficient IgG2 antibody responses to polysaccharide antigens in both adults and young children (213, 214). G2M(n) differs from

G2M(n-) by the presence of methionin instead of a valine residue at the CH2 position 52 in the Fc part of the IgG2 molecule (215). Of great interest is that the two IgG2 allotypes differ with regard to physicochemical properties, maturation during childhood, and catabolic rate (216-218). For instance, the GM haplotypes influence the IgG subclass concentration (217). The effect of the *G2M* n* allele on antipolysaccharide antibody responses is gene dose-dependent (213, 214).

The IgG2 allotypes are found in different combinations with other GM markers in the Northwestern Europen population principally depending on precence of the GM haplotypes *GM b;f;n*, *GM b;f;n-*, *GM g;a;n* and *GM g;a;n-* (Table 7). Caucasian children lacking the G2M(n) allotype are predisposed to infections caused by *H. influenzae* type b (219). This finding was later challenged by Takala et al. (1991) in a study of 178 Finnish children with invasive *H. influenzae* type b infections (220). The contrasting outcome between these studies could perhaps be explained by differences in the investigated population and the coexistence of several immune defects (221).

Table 7. Distribution of G2M phenotypes and their GM haplotypes in 430 Swedish children.

G2M phenotype	Homozygosity for G2M(n)	Heterozygosity for G2M(n) and G2M(n-)		Homozygosity for G2M(n-)		
GM haplotypes	*b;f;n/b;f;n*	*b;f;n/g;a;n-*	*b;f;n/b;f;n-*	*b;f;n-/ g;a;n-*	*g;a;n-/ g;a;n-*	*b;f;n-/ b;f;n-*
(n=430)	19.3%	27.2%	17.4%	17.7%	9.8%	6.7%

Modified after (218).

The role of Fc receptors

Fc receptors (FcR) are found on the surface of many immunological cells such as mast cells, natural killer cells, neutrophils, macrophages, monocytes and dendritic cells. The name is derived from the binding specificity to the Fc region. FcRs binds to antibodies that are attached to infected cells, invading pathogens or other foreign particles. Their activity stimulates phagocytic or cytotoxic cells to destroy microbes or infected cells by antibody-mediated phagocytosis or antibody-dependent cell-mediated cytotoxicity (ADCC). FcR

activity also stimulates release of various cytokines.

All FcγRs belong to the immunoglobulin superfamily of proteins and are the most important Fc receptors for inducing phagocytosis of opsonized microbes. These receptors are defined as molecules that contain domains with sequence similarity to the variable or constant domains of antibodies (222). The following receptors are included in the family: FcγRI (CD64), FcγRIIA (CD32), FcγRIIB (CD32), FcγRIIIA (CD16a), FcγRIIIB (CD16b). They differ in their antibody affinities and molecular structure (Table 8) (223). For instance, FcγRI binds to IgG more strongly than FcγRII and FcγRIII, and has an extracellular part composed of three immunoglobulin-like domains, which is one more domain than FcγRII and FcγRIII contains. These properties allow activation of FcγRI by a single IgG molecule. FcγRII and FcγRIII are low-affinity receptors binding mainly to IgG immune complexes (224).

The clinical relevance of FcγR polymorphisms has been intensively investigated in many case-control studies. FcγR polymorphisms are associated with either increased disease susceptibility or altered disease course. For instance, the FcγRIIIa-V158 allele is associated with rheumatoid arthritis (225), the FcγRIIIa-F158 loci with systemic lupus erythematosus (226), and the FcγRIIa-R/R131, FcγRIIIa-F158 genotype with increased disease relapses in Wegener's granulomatosis (227). FcγR polymorphisms predispose for infections caused by encapsulated bacteria (228). The antibody-mediated defence against these bacteria relies mainly on IgG2 subclass antibodies. The only human FcγR capable of efficiently interacting with IgG2 is FcγRIIa-H131. Thus, homozygosity for FcγRIIa-R131, the variant with poor IgG2 reactivity, is associated with impaired phagocytosis of encapsulated bacteria (229, 230). Furthermore, combined effects of FcγRIIa and FcγRIIIb polymorphisms influence susceptibility to meningococcal disease in patients with terminal complement component deficiencies (231).

Table 8. Human Fc receptors and their functions.

Type of Fc receptor	Principal antibody ligand	Cell	Function following binding to antibody
FcγRI, (CD64)	IgG1 and IgG3	Macrophages	Phagocytosis
		Monocytes	Cell activation
		Neutrophils	Activation of respiratory burst
		Dendritic cells	Microbial killing
FcγRIIa, (CD32)	IgG1, IgG3>IgG2, IgG4	Neutrophils	Phagocytosis
		Eosinophils	Platelet aggregation
		Macrophages	
		Monocytes	
		Platelets	
		B cell	
FcγRIIb1, (CD32)	IgG1, IgG3>IgG2, IgG4	B cell	
		Mast cells	Inhibition of cell activity
			Gatekeeper for B cell self tolerance
FcγRIIb2, (CD32)	IgG1, IgG3>IgG2, IgG4	Macrophages	Phagocytosis
		Neutrophils	Inhibition of cell activity
		Eosinophils	
FcγRIIc2, (CD32)	IgG1, IgG3>IgG2, IgG4	Macrophages	ADCC, platelet aggregation
		Monocytes	
		Neutrophils	
		NK cells	
		Platelets	
FcγRIIIa, (CD16a)	IgG1, IgG3>IgG2, IgG4	NK cells	ADCC
		T cell	
FcγRIIIb, (CD16b)	IgG1, IgG3>IgG2, IgG4	Macrophages	Microbial killing
		Neutrophils	
		Mast cells	
		Eosinophils	
		Follicular dendritic cells	
FcεRI	IgE	Mast cells	Degranulation
		Eosinophils	
		Basophils	
FcεRII (CD23)	IgE	B cells	Possible adhesion molecule
		Eosinophils	

		Langerhans cells	
FcαRI	IgA	Monocytes	ADCC
(CD89)		Macrophages	Phagocytosis
		Neutrophils	Endocytosis
		Eosinophils	Microbial killing
		Dendritic cells	Cytokine production
Fcα/μR	IgA and IgM	B cells	Endocytosis
		Mesangial cells	Microbial killing
		Macrophages	
FcRn	IgG	Monocytes	Transfers IgG from mother to fetus
		Macrophages	through the placenta
		Dendritic cells	Transfers IgG from mother to infant
		Epithelial cells	in milk
		Endothelial cells	Protects IgG from degradation
		Hepatocytes	

Data from references (223, 224, 232-240).
Abbreviation: ADCC, antibody-dependent cell-mediated cytotoxicity.

Host defence to encapsulated bacteria

Sequencing of the human genome has demonstrated that many genes are polymorphic, including a number of genes that have been implicated in the development of sepsis. In other words, a genetic predisposition to development of sepsis may exist. Investigations in patients with selective immune defects have provided evidence of the specific host factors that mediate defence against infections caused by encapsulated bacteria. Thus, the frequency of these infections may be increased among patients with deficiencies of complement components (13), decreased phagocyte function or number (including neutropenia and aspleni) (241, 242), certain polymorphic variants of FcγRIIa allotypes (229), impaired antibody production (e.g. hypogammaglobulinemi, IgG2 subclass deficiency, and selective unresponsiveness to polysaccharides) (219, 243, 244), and in diseases affecting the immune system like sickle cell disease (350), nephrotic syndrome (245), neoplasms, and underlying medical conditions such as diabetes (246, 247) and alcohol induced liver disease (248). More recently, inherited disorders of human toll-like receptor signaling have also been associated with infections caused by encapsulated bacteria (8, 249).

Antibody and complement-dependent opsonophagocytosis is regarded as the major host defence to encapsulated bacteria such as *S. pneumoniae* (127, 250). Complement activation leads to deposition of C3b and iC3b that can be recognized by complement receptors CR1 and CR3 on phagocytic cells. IgG bound to the surface of the capsule may provide additional binding sites for C3b, strengthening the opsonic effect (251). The pneumococcal serotypes differ in the amount and site of covalently bound C3b and also in influence on degradative processing to iC3b and C3d. It has not been determined which of the complement pathways that are most important in the defence against *S. pneumonia* in humans. Experiments in genetically engineered mice suggest involvement of natural antibodies and a functional classical pathway (252) while earlier animal studies emphasized the alternative pathway (253).

It has recently been shown in a mouse model that protection from *S. pneumoniae* infection by C-reactive protein and natural antibody requires complement activation but not involvement of Fc gamma receptors (254). Impaired function of the classical pathway can limit antibody production which is explained by the adjuvant effect of C3d fragments on the immune response (117, 255). A substantial part of the human anticapsular antibodies to *S. pneumoniae* consists of polymeric IgA (256) and these antibodies support phagocytosis involving IgA receptors and the alternative pathway (257). There are also considerations to be made regarding the involvement of IgG2 in high epitope density, which may activate the alternative pathway (258).

H. influenzae is a non-motile Gram-negative coccobacillus first described in 1892 by Richard Pfeiffer. There are six generally recognized serotypes of *H. influenzae*: a, b, c, d, e, and f (259). The main pathogenic strain in human is *H. influenzae* type b (Hib). Its capsule contains poly(ribosyl) ribitolphosphates that protect the bacteria from phagocytosis. Antibodies directed against the capsular polysaccharide poly(ribosyl) ribitolphosphates or the outer membrane proteins of Hib promote bactericidal activity, C3 binding, and ingestion by phagocytic cells (260). *In vitro* studies have shown that Hib can activate both the classical and alternative pathways of complement and generate complement-dependent opsonic and bactericidal activities (261). The alternative pathway, however, requires presence of anticapsular antibodies for activation and killing of Hib (262, 263).

N. meningitidis, also known as meningococcus, is a Gram-negative bacterium. The bacterium is adapted to the human host and is part of the normal flora of the nasopharynx, which is its sole reservoir. Meningococci may causes severe to life-threatening infections such as septicaemia and meningitis. Other more rare manifestations of meningococcal diseases are pneumonia, purulent pericarditis and septic arthritis. It is the only form of bacterial meningitis known to cause epidemics. Strains isolated from patients are almost always encapsulated and mainly caused by serogroups A, B, C, W135 and Y (90%) (264).

The complement system is vital for protection against *N. meningitidis*. Bacterial structures such as polysaccharide capsule and those which mimic (lacto-*N*-neotetraose moiety, identical to a human blood group antigen) or bind (factor H, C4BP) host molecules function to prevent complement-mediated lysis and phagocytosis. *N. meningitidis* is more resistant to complement-mediated killing than many other Gram-negative bacteria such as *Escherichia coli* and *Shigella flexneri* (265). This is likely to be a key contribution to the high levels of bacteraemia seen in patients with meningococcal sepsis and the fulminant nature of the infection (266).

Bactericidal antibodies are also considered to be important for protection against disease caused by *N. meningitidis* (267-269). IgM antibodies are regarded as more efficient than IgG antibodies belonging to the IgG1 and IgG2 subclass (270). There is evidence that anticapsular antibodies support killing of meningococci through the alternative pathway (271-273) . Paradoxically, meningococcal antibodies of IgA class may block binding sites of IgM or IgG leading to reduced bactericidal action and increased susceptibility to meningococcal disease (274, 275).

COMPLEMENT DEFICIENCY STATES

Most inherited complement deficiencies are associated with susceptibility to severe bacterial infections (13). The invasive infections are commonly caused by encapsulated bacteria such as *S. pneumoniae*, *H. influenzae*, and Neisseria species. Isolated *N. meningitidis* strains from patients with complement deficiency are often of uncommon serotypes such as Y and W135. It is estimated that the prevalence of an inherited complete complement deficiency is about 0.03% in the general population, excluding deficiency of MBL which is common. Furthermore, a deficiency might occur 150-300 times more frequently among patients with invasive neisserial infection as compared to the general population (276). Complement deficiency states have been described for most of the known complement components. C1-INH deficiency occurs principally only in a heterozygous state. No patient with inherited total factor B deficiency has yet been confirmed.

Approximately 14% of the Swedish population have a genetically defined MBL deficiency (277). A large proportion of these persons are asymptomatic. On the other hand, patients with MBL deficiency have been described with an increased susceptibility to severe infections (10). Therefore MBL deficiency alone may not cause susceptibility to infection but may act as a cofactor in some persons. Thus, other coexisting factors may be needed to render the MBL deficiency a clinically significance (278).

Late complement component deficiencies (LCCD) are associated with infections caused by *N. gonorrhoeae* and *N. meningitidis* (50-60%), implicating the importance of the bactericidal effect of C5b-$C9_n$. LCCD increases the risk of a meningococcal disease by a 1000-fold. Similar to the findings in LCCD, meningococcal disease occurs in more than half of properdin deficient persons. The first patient with properdin deficiency was identified in a Swedish family by Sjöholm et al. in 1982 (279). Properdin deficiency was later confirmed to be an X-linked trait (280). The first episode of meningococcal disease in properdin deficiency occurs in males usually during the teenage years. The clinical course is, however, more fulminant with a higher mortality rate than observed in LCCD. Complement deficiency states may also involve lack of complement receptors as in the case of leukocyte adhesion syndrome where a functional CR3 may be missing (93). Patients with this disorder suffer from life-

threatening bacterial infections, and in its severe form, death usually occurs in early childhood unless bone marrow transplantation is performed.

Because inherited complement deficiency states are relatively rare with exception for MBL, C1-INH, and C2, information about them have been derived through accumulated case reports (13, 281). These meta-analyses may have suffered from an ascertainment bias since most reported persons came from surveys of rheumatological disease. Importantly, the ethnic background is a major determinant for both the prevalence of complement deficiency states as well as their associated diseases. On the other hand, these analyses have provided important information about the immune defence and contributed to development of new concepts about pathogenetic mechanisms in SLE and other diseases (13, 14, 255, 282).

Deficiencies of the classical pathway have mainly been associated with autoimmune disease and the manifestation with an increased susceptibility to bacterial infection has gained less attention (13, 283). The association of SLE with complement deficiency states has been described as most evident in persons lacking one of the early components of the classical pathway. Although homozygous C1 and C4 deficiencies are quite rare, reported persons with these deficiencies have uncommonly been accompanied by a history of severe infections (14). In a review of 109 hereditary C2 deficiency (C2D) persons described in the literature it was found that 22 % had at least one episode of meningitis or septicaemia caused by encapsulated bacteria (13).

Deficiency of the second component of complement is inherited as an autosomal codominant trait and is one of the most common genetic complement deficiency states with a frequency of 1:20,000 among persons of European descent. Heterozygous deficiency of C2 is present in about 1% of the Caucasians (284). A number of human traits are the result of two alleles that are equally expressed. Such traits are said to be codominant. When a person is heterozygous for such traits, the resulting phenotype or expression of these two traits is a blending, because both traits are expressed equally. Hence, the pedigree pattern of human codominant traits resembles that of autosomal dominant inheritance except that both alleles can be distinguished.

The predominant type (type I) of C2D is associated with HLA haplotype *A25 B18 DR2* and complotype *S042 (BfS, C2Q0, C4A4, C4B2*). The majority (~90%) of type I C2D is the result

of a 28-bp deletion at the 5´splice junction at exon 6 of the *C2* gene. This leads to skipping of exon 6 during RNA splicing and therefore a deletion of 134 bp in the *C2* transciption, resulting in a frame shift mutation generating a premature termination at the N-terminal domain of the C2 protein (285). A second cause for type I deficiency is 2-bp deletion in exon 2. This leads to a frame shift and a stop codon, resulting in undetectable C2 protein synthesis (Table 10) (286).

In contrast to type I, persons with type II C2-deficiency are rare and represent about 10% of all cases. It is characterized by a selective block of C2 protein secretion with retention of the protein in the intracellular compartment. Type II deficiencies are associated with a different HLA haplotype than found in type I and are caused by missense mutations of amino acid residues apparently important for the folding of the C2 polypeptide. A total of three missense mutations have been described so far (287, 288).

Table 10. Genetic and molecular basis of human C2 deficiency.

Type I deficiency		**No C2 protein produced**	**Reference**
1	Defect	28-bp deletion in exon 6	(285)
	MHC haplotype	HLA *A25 B18 DR2 BfS, C2Q0, C4A4, C4B2*	
2	Defect	2-bp deletion in exon 2	(286)
	MHC haplotype	HLA *A3 B35 C2Q0 BFF C4A32 BQ0 DR4 DR53*	
Type II deficiency		**The C2 protein is produced but not secreted**	
1	Mutation	Exon 5, C566T	(287)
	Substitution	Ser 189 Phe	
	MHC haplotype	HLA *A11 B35 DRw1 BFS C4A0B1*	
2	Mutation	Exon 11, G1930A	(287)
	Substitution	Gly 444 Arg	
	MHC haplotype	HLA *A2 B5 DRw4 BFS C4A3B1*	
3	Mutation	Exon 3, G392A	(288)
	Substitution	Cys 111 Tyr	
	MHC haplotype	HLA *A28 B58 DR12*	

Modified after (97).

C2D is associated with susceptibility to infection caused by encapsulated bacteria and development of autoimmune conditions such as SLE. Furthermore, SLE associated with C2D is described as a generally mild clinical subset of the disease (289). Skin and joint disease are abundant in these patients, while severe manifestations such as serositis, neuropsychiatric SLE, and glomerulonephritis are infrequent.

Primary deficiency of complement component C3 has been described in 27 persons from 19 different families. Deficiency of C3, the major opsonin, results in severe recurrent pyogenic infections with onset shortly after birth. Sometimes the deficiency is followed by development of immune complex-mediated disease. Secondary C3 deficiency may occur with factor H or factor I deficiencies or in the presence of C3 nephritic factors, predisposing the patient to the same risk of disease development (290).

Complement deficiency states may be acquired or inherited. Both disorders are associated with increased susceptibility to infection. Acquired deficiencies are more common than inherited and are found in association with other diseases such as severe SLE. Acquired deficiencies are classified in accord with the underlying mechanism responsible for the defect: consumptive, synthetic, or catabolic (291). Acquired deficiencies with consumption are seen in patients with SLE, vasculitis, burn injury, and in patients with autoantibodies to C3 convertases (nephritic factors). Decreased synthesis of complement components is frequently found in patients with severe liver disease (292-294) while catabolism has been described in patients with nephrotic syndrome, sickle-cell anaemia and inflammatory bowel disease (291, 295).

COMPLEMENT AND AUTOIMMUNITY

Immunoregulatory effect of complement

Danger signals are generated to defend the host and triggered by exogenous invasive microorganisms, endogenous tissue injury, and the intercellular inflammatory mediators. Since these mediators are released and/or secreted in response to danger, in reality they act as 'warning' signals that alert innate and adaptive immune host defence mechanisms. These warning signals interact with receptors including those that activate antigen-presenting cells (296). The complement system and toll-like receptors are essential to discriminate between conserved patterns on pathogens and on altered/damaged cells and to provide critical danger signals instructing the immune response.

In addition to this important role in danger recognition, the complement system has the ability to transform the danger signals into adequate cellular innate or adaptive immune responses. Complement also mediates the ability to distinguish between physiological and pathological danger, i.e., physiological cell death and death in response to injury. In the former situation, cells are merely marked for enhanced phagocytosis by C3 fragments without accompanying inflammation through CR3, whereas in the latter scenario inflammatory signals are accessorily triggered by the release of anaphylatoxins, which recruit and activate neutrophils and eosinophils.

Autoimmune manifestations and complement deficiency

SLE is a chronic inflammatory disease that can affect the skin, joints, kidneys, lungs, nervous system, and/or other organs of the body. The most common symptoms include skin rashes and arthritis, often accompanied by fatigue and fever. The clinical course of SLE varies from mild to severe, and typically involves alternating periods of remission and relapse.

During the 1950s it became widely recognised that measurements of complement proteins C3 and C4 were a useful diagnostic tool for SLE as well as for monitoring disease activity (297, 298). The pathogenesis of active SLE involved reduced serum levels of complement with abundant deposition of complement components in damaged tissue (299). The identification of antinuclear antibodies and other autoantibodies characteristic of SLE led to the

development of the immune complex theory. The model postulated that immune complexes of autoantibodies and their related autoantigens activate complement resulting in complement consumption and tissue damage.

The immune complex theory was later challenged by the discovery of deficiency states of the classical pathway of complement. These patients were found to be highly susceptible to development of autoimmune disease, especially SLE (14, 300). In fact, homozygous deficiencies of the classical complement proteins are now known to be the strongest genetic susceptibility factor for SLE in humans (Table 11). A hierarchy that predicts both strength and severity of disease has been established in accord with the position of the missing component so that the more early component have a stronger disease association. The classical pathway, including C1q, C4 and C2, is now regarded to be important for disposing apoptotic cellular autoantigens and/or the induction of B cell tolerance in the bone marrow. The immune complex theory has also been contradicted by the findings of both immune complexes and complement components in unaffected and normal tissues in SLE patients (14).

Possible roles for complement proteins, particularly C1q in regulation of cytokine release have also been suggested in SLE (301, 302). Regulation of type I interferons has been shown to be important in this disease (303).

Table 11. Association between complement deficiency and susceptibility to SLE or SLE-like disease.

Complement component	Percentage of patients with homozygous deficiency associated with SLE or SLE-like disease
C1q	>90%
C1r/s	57%
C4	75%
C2	10%
C3	13%
C5-9	Sporadic cases
MBL	Have been reported as a risk factor.
Alternative pathway	No known association.

Modified after data from (14).

In summary, defective immune regulatory mechanisms, such as the clearance of immune complexes and apoptotic cells, are important contributors to the development of SLE. The increased antigenic load, loss of immune tolerance, surplus T cell help, defective B cell suppression, and the shifting of T helper 1 (Th1) to Th2 immune responses lead to B cell hyperactivity and the creation of pathogenic autoantibodies. In addition, environmental factors, such as chemicals and drugs, dietary factors, ultraviolet light, viruses, and oestrogen may precipitate the onset of the disease (304).

An important consideration in the management of SLE patients is the bimodal pattern of mortality with early deaths related to active disease and/or sepsis and late deaths due to acute myocardial infarction (305). In follow-up studies, 30% of deaths were found to be attributable to coronary artery disease (306), and the relative risk of acute myocardial infarction in women with SLE aged 35–44 years was 50-fold higher than in the general population, with two-thirds of events occurring in those less than 55 years old (307).

Systemic lupus erythematosus and C2 deficiency

Hereditary C2 deficiency is associated with development of autoimmune manifestations such as SLE, cutaneous vasculitis, and undifferentiated connective tissue disease (UCTD). In contrast to homozygous C1q deficiency, the majority of affected persons are probably healthy. SLE may occur in about 10% of the C2D persons and the severity of the illness is reported to be milder than that seen in genuine SLE patients (14). Organ damage involving renal and cerebral disease appears less common while skin and joint manifestations are more typical (300, 308). There are also serological differences compared to genuine SLE with rarity of double stranded DNA and anti-nuclear autoantibodies (ANAs) while the frequency of anti-Ro (SSA) antibodies is higher (300, 309). It appears unlikely that partial C2 deficiency is a disease-susceptibility factor for the development of SLE (284).

MANAGEMENT OF COMPLEMENT DEFICIENCY

C1-inhibitor deficiency is a rare syndrome clinically characterized by recurrent episodes of angioedema or swelling of subcutaneous tissue. The syndrome may involve the skin, upper respiratory airways and abdomen. Treatment of these patients includes antifibrinolytic agents, anabolic steroids, and infusion of C1-INH concentrate. Fresh frozen plasma is an option to be considered for short term prophylaxis or treatment of the acute attack (310). New therapy with recombinant C1-INH and also drugs based on inhibition of the kallikrein induced bardykinin effect are developed and started to being used (311).

Hypocomplementemic urticarial vasculitis syndrome (HUVS) may cause several symptoms that are initially difficult to interpret including fever, angio-edema, dermal ulceration, iritis, uveitis and cardiac valve disease (312). The most common manifestations are arthralgia and arthritis (50%). HUVS also causes manifestations involving interstitial inflammation of lungs and kidneys (20-30%). HUVS may be triggered by underlying diseases such as infections, hematological diseases, connective tissue diseases, and malignancy. The challenge in these cases is to treat the underlying disease. Additional treatment may include indomethacin, dapsone, hydroxychloroquine, and corticosteroids.

Many of the autoimmune conditions related to complement deficiency states require treatment with various immunosuppressive drugs. Immunosuppressive treatment may cause severe infections that need prompt diagnosis and administration of antibiotics. It is important that clinicians are aware of the associations between SLE and immunodeficiency to ensure optimal investigation and management.

Substitution treatment

There is no specific treatment against C2D that reconstitutes the production of the protein. Nor is there any medication that could stimulate the secretion of the protein in type II C2D. Steinsson et al. described in 1989 a successful long-term plasma infusion treatment to a patient with severe SLE and C2 deficiency without any adverse effects (313). On the other hand, severe hypotension has been reported in patients given plasma infusion (314). Plasma infusion is contraindicated in patients with severe anemia, congestive heart failure, or increased blood volume. There may also be a risk of development of autoantibodies and of a serum sickness-like reaction. Recombinant human complement component C2 has been developed for laboratory research but not yet for treatment of humans. In selected cases, the use of a purified C2 protein could be of great value.

Antibiotics and chemotherapy against recurrent infections

Persons with a complement deficiency state have an increased risk of contracting severe infections. The infections are usually caused by pneumococci, *H. influenzae*, and meningoccoci. The empiric choice of antibiotics should therefore be directed against these pathogens. Long-term antibiotic prophylaxis has been widely used and of proven benefit (315). However, poor compliance and the risk of developing resistant strains are well known side effects of this strategy.

Vaccination

It is well accepted that innate immunity serves as a natural adjuvant in enhancing and directing the adaptive immune response. Immunization of persons with complement deficiency states may be a way of preparing the immune system against severe infections. Therefore, immunization has been recommended in complement deficiency states although

the efficacy has not been exhaustively investigated (13). Vaccination of properdin deficient persons has been observed to give normal antibody response to the meningococcal tetravalent vaccine (273). The acquired antibodies will efficiently opsonize and lyse meningococci. In a work by Fijen et al., (1998) immunization with a meningococcal polysaccharide vaccine in a cohort of 53 complement deficient persons (7, C3; 19 properdin; 27 terminal component) was successful in generating an antibody response and a bactericidal effect against strains of serotype A, C, Y, and W135 (316). Similar findings have been presented by Platonov et al. using a tetravalent meningococcal vaccine in 18 persons with late complement deficiency states (317).

Patients deficient of the classical complement components have an abnormal antibody response to T cell-dependent antigens. C2-deficient guinea pigs produce a lower concentration of antibodies after immunization than normal controls. After a secondary immunization they fail to develop amplification and to switch from IgM to IgG. This defect is antigen dose dependent and may be overcome by increasing the antigen dose or by injecting normal guinea pig serum at the time of the primary immunization (318). In humans, the impact of complement dysfunction on antibody responses to vaccination remains unclear. There are reports that vaccination responses in C2D persons range from severely impaired to a normal response (319, 320).

PRESENT INVESTIGATION

Aims of the study

1. To investigate clinical manifestations and diseases associated with C2D (Paper I).

2. To explore the immunological features of the increased susceptibility in C2D (Paper I and II).

3. To investigate the risk for development of atherosclerosis in C2D (Paper I and III).

4. To characterize rheumatological disease manifestations in C2D (Paper III).

5. To examine the autoantibody profile in persons with C2D (Paper III).

6. To investigate the vaccination responses to pneumococcal and Hib antigens in persons with C2D (Paper IV).

MATERIALS AND METHODS

Screening for complement deficiency states

Since 1977 analysis to identify complement deficiencies has been a routine part of complement analysis at the Clinical Immunology Unit, University Hospital of Lund, Lund, Sweden. About 46,000 consecutive patient samples have been analysed until 2006 with coverage of a broad spectrum of clinical conditions (Figure 15).

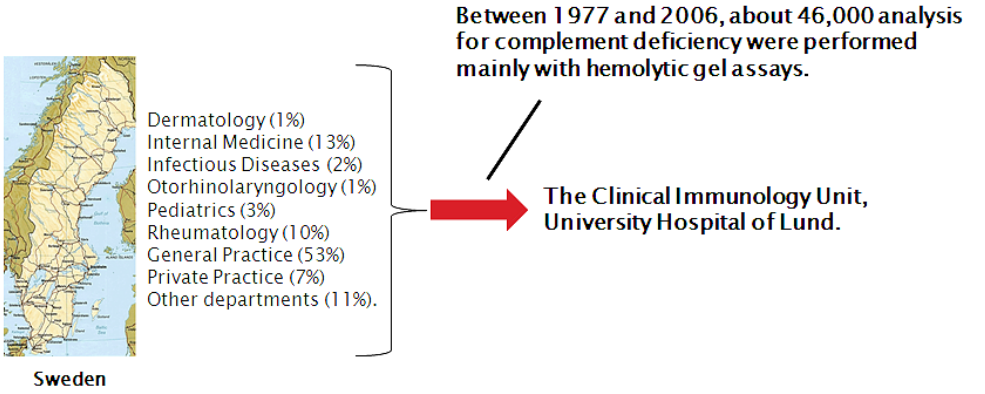

Figure 15. Schematic carton of the ascertainment of C2D persons.

The screening for complement deficiency states was mainly done with hemolysis in gel (19, 106). The hemolytic gel assays used sensitized sheep erythrocytes for the classical pathway and guinea pig erythrocytes for the alternative pathway. During 2006 and 2007 some of the referred blood samples were screened for deficiency with an ELISA (Wieslab, Lund, Sweden).

Study subjects

After initial screening detected a person with a complement deficiency state, further investigations were initiated as follows (Papers I-IV):

A). A new blood sample was retrieved in collaboration with the referring physician. This was done to ensure that the blood sample corresponded to the person identified during the screening process.

B). The new blood sample was again analysed with hemolysis in gel. If the result indicated a classical pathway deficiency, further measurements were performed to confirm the deficiency.

C). DNA was extracted from whole blood (321) for analysis of HLA-type and the common 28-bp deletion found in type I C2D (284). DR typing was performed using a PCR-technique (Olerup SSP AB, Saltsjöbaden, Sweden).

D). Written informed consent was obtained from every participant in the studies.

E). Medical records were retained in collaboration with the responsible physician.

F). If applicable, a family investigation was performed in collaboration with the responsible physician.

A majority of the blood samples confirmed to be C2D were referred from general practitioners (53%, Paper III). Twenty of 49 C2D persons were from southern Sweden. The number of included C2D persons in the different studies is given in table 12. The studies were approved by the Lund University Research Ethics Committee (Studies I, II, and III, LU 513-01; Study IV, LU 350-93).

Table 12. The Swedish C2D cohort in relation to the performed studies after initiation in 1977.

Investigation	Year of study	Number of persons detected with C2D	Included in the study
Study I	2002	40	40
Study II	2005	44	44
Study III	2006	45	45
Study IV	2007	49	25

Laboratory studies

Blood samples were obtained from patients and first-degree relatives. In 5 patients the amount of samples was limited and a priority of further analysis had to be made. All available serum and EDTA plasma samples were stored in aliquots at -80°C. A brief summary of laboratory methods are given in the following sections. A more detailed description is found in the papers I-IV, respectively.

Complement analysis

Most of the other complement proteins were performed by electroimmunoassay (322) and turbidimetry (Cobas Mira, Roche Diagnostica, Basel, Switzerland). C2D was considered when C2 was not detected in serum (<0.5 mg/L). The C2D persons were given consecutive patient numbers.

Autoantibodies

A fairly extensive amount of analysis was done regarding autoantibodies. This was partly facilitated by the use of a commercial kit to determining antibodies against ribonucleoprotein (RNP), histone, Scl-70, Sm, Sm B subunit, SS-A 52/ 60, SS-A 52, and SS-A 60 (INNO-LIA ANA, Innogenetics, Gent, Belgium). The autoantibodies were mainly investigated in relation to the rheumatological manifestations and atherosclerosis observed in C2D (Paper III). The used analysis with references are given in table 13.

Table 13. Autoantibodies presented in paper I and III.

Autoantibody	Reference interval	Methodological reference
ANA	<14 U/ml (WHO reference serum 66/233)	Immunofluorescence (IFL) with Hep-2-cells, serum dilution 1:400, Euroimmun, Lübeck, Germany.
Rheumatoid factor (RF)	<14 IU/ml	ELISA, (323).
Anti-cardiolipin antibodies (aCL)	<20 GPLU/ml	ELISA, (324).
Anti-DNA (dsDNA)	Negative	*Crithidia luciliae* IFL assay, serum dilution 1:10, Euroimmun, Lübeck, Germany (325).
Anti-C1qCLR	<16 AU/l	ELISA, (326).
Antineutrophil cytoplasmic autoantibodies (ANCA)	Negative.	BIOCHIP Mosaic IFL, Euroimmun, Lübeck, Germany.
Anti-proteinase 3 (PR3-ANCA)	<14 U/ml	ELISA, Wieslab AB, Lund, Sweden.
Anti-myeloperoxidase (MPO-ANCA)	<100 U/ml	ELISA, Wieslab AB, Lund, Sweden.

Immunoglobulins

The immunoglobulins IgM, IgG, and IgA were determined by turbidimetry using age related reference areas (327, 328). IgE was established by flouroenzyme-immunometric assay (UniCap, Pharamacia, Sweden). IgG subclasses were determined by single radial immunodiffusion using age-related reference intervals expressed as 2.5-97.5 percentiles (218). IgG4 concentrations were measured with ELISA (Bindazyme, The Binding Site, UK).

DNA analysis

MBL genotypes were analysed as previously described (329, 330). The MBL genotypes were classified according to table 14. The polymorphisms of FcγRIIa and FcγRIIIb were in principal done in accord with Edberg et al. (2002) (226). *G2M*n* and *G2M*n-* alleles were identified by PCR technique combined with pyrosequencing (Paper II), (215, 331).

Statistical methods

Non-parametrical tests such as Mann-Whitney *U* test and Kruskal-Wallis test were used for analysis of statistical relations between patient groups and immunological markers. Jonckheere-Terpstra test was employed in paper II to determine whether a dose-dependent trend was present for *G2M*n* and the stratified C2D persons. Fisher´s exact test was utilized for analysis of differences between C2D groups I-IV and in relation to controls. Distributions were compared with CHI^2 test. In paper I and III, standard mortality/morbidity ratio (SMR) was used for comparison between the C2D cohort and data obtained from the Swedish National Board of Health and Welfare Registries regarding age-related acute myocardial infarction (AMI) incidences. To calculate correlations, the Spearman rank test was performed in paper II. Wilcoxon signed rank test was applied in the investigation of vaccination responses (Paper IV). Results were considered significant when $p<0.05$.

Table 14. The C2D persons were classified as MBL deficient or sufficient depending on the findings of promoter haplotype in relation to the structural MBL gene variant.

MBL	MBL gene haplotype
Sufficient	AHY AHY
	AHY ALX
	AHY ALY
	ALY ALY
	ALY ALX
	ALX ALX
	AHY BLY
	AHY CLY
	AHY DHY
	ALY BLY
	ALY CLY
	ALY DHY
Deficient	ALX BLY
	ALX DHY
	ALX CLY
	BLY BLY
	BLY CLY
	BLY DHY
	CLY DHY
	DHY DHY

RESULTS AND DISCUSSION

Prevalence of C2D and genetics background

Hereditary C2 deficiency is a fairly common form of complement deficiency among persons of European descent with an estimated frequency of 1:20.000 (13, 14, 281, 332). The frequency of C2D equals that of common variable immunodeficiency (CVID) in Western countries (333). However, the number of identified persons with C2D is much lower than is the case of CVID patients. Our estimations point to a total of 450 C2D persons in Sweden, which suggest that only 11% have been identified (Paper IV). The corresponding number of identified CVID patients in Sweden were at least 200 patients (~44%) in 2002 (334). This difference might partly be due to the fact that the disease manifestations found in C2D have not been fully appreciated, partly to the problem with a lack of a specific treatment related to C2D compared with CVID. On the other hand, a majority of the C2D persons would certainly benefit from a correct diagnosis.

Deficiency in complement C2 is due to either obliteration of C2 protein synthesis by mini-deletions that cause frameshift mutations (type I), or blocked secretion of the C2 protein by single amino acid substitutions (type II). The genetic background to C2D is very uniform compared to many other immunodeficiencies (97). This is perhaps not so surprising since the predominant C2D type I (~90%) is caused by the homozygous presence of a 28-bp deletion of the C2 gene which is situated in the major histocompatibility complex (MHC, Figure 10, Paper I) (14). The consequence of this genetic characteristic is that most C2D persons share the same MHC haplotype or closely related haplotypes (Paper I and III). C2D type I has a homozygosity for *DRB1*15* with the C4 phenotype *C4A*B*2*. The frequency of C2D type I in paper III was 84%. In at least three C2D persons (Patient 19, 37, 38) a new MHC haplotype was established in conjunction with C2 null genes (Paper I) (14). Patient 45 may also have a different MHC haplotype than usually found in type I C2D, since she was found to be heterozygous for the 28-bp deletion (Paper III).

MHC is the most gene-dense region of the human genome and plays an important role in the immune system, autoimmunity, and in the reproductive process (335, 336). The unusually uniform genetics observed in C2D may therefore have consequences for disease expression,

antibody production, and antibody responses. In our survey of diseases associated with C2D we found a low frequency of allergy (*n*=1, Paper I). The mechanism behind this observation might be related to the documented IgG4 subclass deficiency (median 0.02 g/L; reference interval , 0.06-1.2 g/L, Paper II) that in turn could be connected to an impaired switch in IgE antibody production (Paper I and II). Thus, only one patient of 44 had a clearly high concentration of IgE (Paper II).

Type 1 diabetes mellitus and gluten-sensitive enteropathy are MHC class II-associated diseases (337). Interestingly, none of these diseases was recorded in the cohort suggesting that the C2D persons might carry MHC protective MHC alleles against these diseases. In contrast, the well-known association with SLE and SLE-like disease was confirmed (Paper I and III).

Antibody production may be influenced by the MHC haplotype. As mentioned above, we found low levels of IgG4 and IgE. Furthermore, 15 of the 44 patient investigated in paper II had low IgG2 concentrations. Perhaps surprisingly, these IgG subclass deficiencies did not correlate to an increased susceptibility to severe infection. Alper et al. (2003) have previously described similar findings in 13 C2D persons with recorded infections (338). They also found an increased frequency of IgD deficiency without correlation to documented infections. In paper IV, we investigated vaccination responses to pneumococcal antigen 6B, 7F, 23F, and *Haemophilus influenza* type b (Hib). The responses in C2D persons varied from equal to controls for (pneumococcal antigen 6B and 23F) to clearly impaired to pneumococcal antigen 7F and Hib. The explanation for this diverse response might be associated to other genetic markers found in C2D.

Further evidence for a restricted immune response governed by MHC genes was described in paper III. In this study we could confirm a low frequency of ANA and anti-dsDNA in C2D patients with SLE (14, 289, 339). As expected they also showed a higher frequency (45% in SLE patients) of autoantibodies to RNP (339). A novel finding was a high occurrence of anti-cardiolipin antibodies (aCL) and antibodies to the collagen-like region of C1q (anti-C1qCLR).

Invasive infections in C2D

Perhaps the most striking observation in our four studies regarding C2D was the high frequency of invasive infection in the cohort (Paper I-IV). About 57 % of the C2D patients

had experienced at least one episode of invasive infection (Paper I). The severe infections were caused by encapsulated bacteria such as *S. pneumoniae*, Hib, and *N. meningitidis*. The predominant etiologic agent was *S. pneumoniae*. The bacteria was found in 64% of the cases with meningitis and in 52% of the patients with septicemia. The invasive infections showed a bimodal pattern of morbidity with a majority (~66%) of documented infections occurring before the age of 13, and reoccurrence among C2D patients over 40 years of age. During the observed period, 6 patients died due to severe infections (Paper III). An increased susceptibility to infections was not observed among patients with rheumatological manifestation compared to other C2D persons (Paper I and III). Investigations concerning secondary immunodeficiency gave support for C2D as a susceptibility factor for infection.

The term invasive infection was defined to include septicemia, meningitis, osteitis, pyelonephritis, and peritonitis. The C2D persons were divided into 4 groups in accord with severity of recorded infections (Paper I). If a patient, for example, was documented for septicemia and meningitis at the same time, the invasive infections were counted as one episode (Paper I). The statements found in the medical records by the managing physicians concerning a patient was never challenged or changed. For instance, in a 7 year old boy (Patient 37), the patient's physician described a severe infection diagnosed as septicemia of unknown origin and cause. The invasive infection was counted as one episode. In patient 17, a severe umbilical infection with septicemia was recorded, the causative agent was suspected to be *Streptococcus agalactiae*. All other documented episodes of invasive infections in the C2D cohort, were verified by blood or cerebrospinal fluid culture (Paper I and II). In paper II, the stratification was kept to facilitate the analysis of why some of the C2D persons (25%) remained healthy (no recorded invasive infection) during long-term follow-up. Also in paper IV, the stratification was kept to determine the influence of previously encountered infections on vaccination responses.

In paper II, we evaluated a number of potential genetic modifiers of the C2 phenotype including IgG subclass levels, GM allotypes, complement components (factor B versus properdin, factor B versus factor H), and polymorphisms of MBL and the FcγRs (FcγRIIa and FcγRIIIb). C2D has primarily been associated with SLE (14, 300). C2D has also been described as an immunodeficiency associated with recurrent infections (13, 281). However, it was not known why some C2-deficient persons suffered from severe infections. Investigation of a rational assortment of potential gene modifiers, indicated that G2M*n homozygosity was

associated with protection from infection. MBL deficiency was shown to serve as a potential cofactor for susceptibility to invasive infections. In other words, evidence was provided for that antibody-dependent immunity can overcome susceptibility to infection in C2D.

One important consideration in paper II was that the numbers of C2D persons were fairly small when the clinical subsets were distributed. This, coupled with that no correction for multiple comparisons in the statistical analysis was made, could make the data difficult to interpret in terms of biological significance. Nevertheless, the finding remains statistically significant even after changing the significance level from 0.05 to 0.0071, which would be the standard Bonferroni adjustment for the fact that 7 different immunological factors were investigated. Furthermore, an independent statistical analysis regarding the presence of the *G2M*n/G2m**n genotype and severity of infection showed significance (p=0.02). Jonckheere-Terpstra Test, which tests for differences among several independent samples, is more powerful than the Kruskal-Wallis or other median tests (e.g. Mann-Whitney's test).

Due to the findings in paper II, we concluded that an antibody mediated defence is of great importance in C2D. Thus, C2D persons could be expected to benefit from vaccination against *S. pneumoniae, N. meningitidis,* and Hib (Paper I and II). In paper IV, 25 C2D persons were immunized with 23-valent pneumococcal vaccine (Pneumo23®) and twenty-one with a Haemophilus b conjugated vaccine, ActHIB®. The C2D persons responded with a significant increase in antibodies to the presented antigens (pneumococcal serotype 6B, 7F, and 23F, and Hib).

Compared to a control group, the C2D persons had a lower response to pneumococcal serotype 7F and Hib. The reason for this difference is not known. Structurally, the pneumococcal serotype 7F is characterized by a branched molecular form while 6B and 23F display at least one side with a strait repeated polysaccharide structure (340). This structural difference might affect the functions of pattern recognition in innate immunity and thereby change its adjuvant effect on adaptive immunity responses.

Perhaps the T and/or B cell functions influence the vaccine response in C2D. The 23-valent pneumococcal vaccine induces a T cell-independent response whereas the vaccination response to Hib is considered to be T cell-dependent since it contains a protein conjugate (Tetanus Toxoid Conjugate).

Another factor that might be of importance in explaining the difference in vaccine response is the ability of recognition molecules to activate the alternative pathway or the lectin pathway of complement. Activation of the complement cascades generates C3b that works as an enhancer for immunoglobulin production from B cell. Both MBL and properdin are of interest for this hypothesis. It has recently been demonstrated that properdin can bind to specific target surfaces and act as an initiator of the alternative pathway (47). Furthermore, the existence of a MBL-dependent C2 bypass mechanism for alternative pathway-mediated C3 activation has been demonstrated (48). Whether the solution to this mystery depends on the biochemical property of the antigen, recognition molecules, T and B cell functions, or on other genetic variation influencing the response, remains to be seen.

Rheumatological manifestations and atherosclerosis in C2D

In paper I, the association of C2D and autoimmune manifestations was confirmed (341). However, there was no extensive description of the rheumatological manifestations, organ damage, and autoimmune responses. There were also questions to be addressed regarding the increased frequency of atherosclerosis found in paper I. Statistical calculations had confirmed that there was a significantly increased in frequency of AMI in the C2D cohort compared with the Swedish population in general.

In paper III, the C2D cohort was enlarged with 5 new C2D persons (*n*=45). Medical records were supplemented with a questionnaire concerning Framingham-related risk factors. The rheumatological diseases found in the study were SLE (*n*=12), undifferentiated connective tissue disease (UCTD, *n*=5), and vasculitis with skin manifestation (*n*=3).

New issues that had never before been analysed in persons with complement deficiency were SLICC/ACR DI (Systemic Lupus International Collaborating Clinics/American College of Rheumatology- Damage Index) and working capacity. SLICC/ACR-DI is a validated tool for measurement of present organ damage in SLE patients. The index enables also comparison of organ damage between different SLE patients. SLICC/ACR-DI includes items to evaluate organ damage and biological systems affected by the disease itself as well as by therapy or intercurrent diseases (342, 343).

Organ damage in the SLE patients was mainly due to cardiovascular disease (CVD) manifestations. The investigations suggested that SLE in C2D patients run virtually a similar risk of developing severe disease as patients with genuine SLE. The mean SLICC/ACR-DI in the C2D patients with SLE was 3.8 at 10 years, which equals the annual organ damage score found during course of disease in an epidemiological recruited cohort of SLE patients (344). Analysis of working capacity showed that the rheumatological diseases had a marked impact on the general health status and not on the frequently recorded infections in the cohort. These findings challenged to some extent the established hierarchy of the classical complement pathway deficiencies and the severity of disease expression (Table 11). Furthermore, the true prevalence of SLE in C2D or complete C3 deficiency is not known. Thus, there is a need for long-term prospective cohort studies of these complement deficiency states to ascertain more correct prevalences.

Medical records and the mailed questionnaire regarding CVD risk factors failed to explain the high frequency of CVD damage. Thus, the cardiovascular damage seemed more likely to be directly linked to the complement deficiency. A novel finding was a high prevalence of aCL and anti-C1qCLR. The anti-C1qCLR antibodies were found in several children (*n*=6, median, 11 years, range 1.3-17 years) indicating the importance of the genetic predisposition for development of this autoantibody. Interestingly, C1q-containing immune complexes may have a pro-atherogenic activity by inhibiting the function of cholesterol 27-hydoxylase in human arterial endothelium and macrophages (345). Patients with hereditary deficiency of cholesterol 27-hydroxylase develop premature atherosclerosis despite normal serum lipids (346, 347). Thus, C1q-containing complexes may provide an explanation for the development of cardiovascular damage observed in C2D (Paper I and III).

Antiphospholipid syndrome (APS) is a disorder characterized by recurrent venous or arterial thrombosis and/or spontaneous abortions. APS is associated with elevated levels of antibodies directed against membrane anionic phospholipids (ie, anticardiolipin antibodies, antiphosphatidylserine, and beta-2 glycoprotein I) or their associated plasma proteins for example circulating anticoagulant. APS may occur in patients without any associated disease or in association with an autoimmune disorder such as SLE.

Studies that use a murine model of antiphospholipid syndrome have demonstrated a critical role for complement activation that leads to fetal and placental injury in the presence of antiphospholipid antibodies. Treatment with heparin reduces the risk of spontaneous abortions in pregnant BALB/c mice by inhibiting complement activation both *in vivo* and *in vitro* (348). Patients with APS have complement activation with cleavage of C2 (349). Our study of C2D patients with aCL did not show any manifestations of APS. This might suggest that C2D persons are protected against some of the manifestations seen in APS. The lack of documented APS in C2D also gives some support to the applicability of the murine model of APS to humans.

CONCLUSIONS

A large Swedish cohort of persons with deficiency of the second component of complement (C2D) with long-term follow-up provided a unique basis for evaluation of disease manifestations and mechanisms associated with impaired classical pathway and lectin pathway functions.

Retrospective analysis of the C2D cohort revealed a high rate of severe infections. The causative agent was mainly *Streptococcus pneumoniae*. C2D should be considered more often in the context of immunodeficiency states.

Homozygosity for the *G2M** allele is known to promote antibody responses to polysaccharide antigens. G2M(n) was strongly associated with protection against severe infections in C2D. Mannan-binding lectin (MBL) deficiency was found to be a cofactor for increased susceptibility to severe infection in C2D.

The well-known association between SLE and C2D was confirmed as well as a low prevalence of anti-nuclear antibodies (ANA) and antibodies to native DNA (dsDNA). However, C2D patients with SLE run the same risk of developing severe illness as observed in genuine SLE. The main reason for this was a high rate of cardiovascular disease.

A novel finding was the association of C2D and atherosclerosis. The mechanism behind this might be the exceptional autoantibody profile found in the cohort with a high occurrence of anti-C1qCLR.

A high prevalence of anti-cardiolipin antibodies was also found in the cohort. However, no cases of antiphospholipid syndrome (ASP) were documented suggesting a protective role of C2D.

Vaccination responses in C2D showed restrictions that might be attributed to the biochemical property of the antigen, to recognition molecules like MBL or propedin, to T and B cell functions, or to other genetic variants in C2D. The biochemical basis for target recognition of the complement system is incompletely understood. Vaccination against infections caused by encapsulated bacteria is recommended in C2D.

SAMMANFATTNING PÅ SVENSKA

Populärvetenskaplig

Människans försvar mot främmande ämnen (t.ex. bakterier och virus) är uppbyggt kring den medfödda och förvärvade immuniteten. Till den medfödda immuniteten räknas olika barriärer såsom huden och slemhinnorna. Andra viktiga delar är komplementsystemet och ätarceller (fagocyter). Komplementsystemet är uppbyggt av ett 30-tal proteiner som både kan vara bundna till cellytor eller cirkulerar fritt i blodet. Systemet har många uppgifter och hjälper bl.a. till med att avlägsna bakterier och skadade kroppsegna celler. En betydelsefull uppgift är att leda fagocyter till platsen där en skada har uppstått. Komplementsystemet påverkar också den förvärvade immuniteten genom stimulering till produktion av skyddande antikroppar. Den medfödda immuniteten utgör ofta ”den första linjens försvar” medan den förvärvade immuniteten kräver mera tid för mobilisering mot en inkräktare.

Komplementsystemet initieras via tre s.k. aktiveringsvägar som benämns den klassiska vägen, alternativa vägen och lektinvägen. De främmande ämnenas ytstruktur på t.ex. en bakterie känns igen av de proteiner som är först i respektive komplementväg. Antikroppar som fastnat på en yta kan också starta en komplementaktivering. De tre aktiveringsvägarna sammanstrålar till en knutpunkt som utgörs av det tredje komplementproteinet, C3. Den vidare aktiveringen fortsätter därefter i en gemensam terminal väg som avslutas med uppbyggnaden av kanal som går tvärs igenom cellmembranen på en bakterie eller en skadad cell. Själva kanalen består av proteiner som fogats samman till ett komplex, det s.k. membran attack komplexet (MAC). Under aktiveringens gång har ett flertal proteinfragment bildats som har stor betydelse i att dirigera och förstärka det fortsatta immunologiska försvaret mot inkräktaren.

Ärftlig komplementbrist, dvs. att någon av komplementfaktorerna saknas, förekommer i sällsynta fall och leder till ökad infektionskänslighet och någon gång till utveckling av vissa typer av reumatism och andra immunologiska sjukdomar. Å andra sidan är många personer med komplementbrist helt friska, något som sannolikt beror på att immunförsvaret på olika sätt kan kompensera brister inom komplementsystemet. Infektionskänsligheten vid komplementbrist kan vara mest framträdande under barnaåren för att sedan minska i vuxen ålder. Infektionerna orsakas ofta av bakterier som har en skyddande yttre kapsel. Klassiskt är

luftvägsbakterier som *Streptococcus pneumoniae* (pneumokocker) och *Haemophilus influenzae* typ b (Hib). Dessa bakterier kan orsaka olika övre luftvägsinfektioner såsom halsfluss, öroninflammation och lunginflammation. De kan även ge upphov till svåra livshotande infektioner som blodförgiftning (sepsis) och hjärnhinneinflammation (meningit). En annan kapslad bakterie som orsakar svåra infektioner hos personer med ärftlig komplementbrist är *Neisseria meningitidis* (meningokocker**)**.

I denna avhandling beskrivs olika kliniska och immunologiska aspekter på en ärftlig komplementbrist som sätter funktionen hos den klassiska aktiveringsvägen ur spel. Ärftlig brist på komplementprotein C2 (C2-brist) förekommer hos en person på 20,000 med västerländsk härstamning. Två varianter av C2-brist har beskrivits och benämns vanligen typ I respektive typ II. Vid typ I finns det en genetisk skada som leder till att proteinet inte tillverkas. Denna variant är vanligast (~90%) och är kopplad till en speciell vävnadstyp. Orsaken till denna koppling är att C2-brist genen finns i samma del av kromosom 6 som gener för vävnadstypen. Vid typ II finns också en förändring i komplementprotein 2 genen men denna avvikelse leder till att proteinet inte kommer ut från den producerande cellen till blodbanan. Vävnadsantigenet vid typ II är mera heterogent än vad som ses vid typ I. Vid C2-bristår det således ett komplementprotein i den klassiska aktiveringsvägen som saknas och inte går att mäta i blodet. Komplementsystemet fungerar i denna situation i huvudsak via den alternativa vägen och under vissa betingelser via lektinvägen.

I det första arbetet beskrivs en grupp bestående av 40 svenskar med C2-brist och deras sjukdomar. Personerna hade identifierats efter att behandlande läkare skickat ett blodprov för komplementanalys till klinisk immunologi i Lund. Totalt hade cirka 46,000 komplementanalyser gjorts mellan åren 1977 till 2006. En majoritet (53 %) av personerna i gruppen hade fått sitt blodprov tagit vid sin vårdcentral för vidarebefordran till laboratoriet. I samarbete med behandlande läkare kunde en noggrann sammanställning göras av varje enskild person sjukdomshistoria under en mycket lång observationstid (1560 personår).

Det mest slående fyndet i första arbetet var en mycket hög förekomst av infektioner. Inte mindre än 57 % av patienterna hade drabbats av minst en svår infektion såsom blodförgiftning eller hjärnhinneinflammation. Upprepade svåra infektioner hos en och samma patient förekom också i hög utsträckning. De svåra infektionerna drabbade i första hand barn men förekom även hos vuxna. För att få en bättre överblick av alla dokumenterade infektioner

delades personerna in i fyra undergrupper beroende på svårighetsgraden av genomgångna infektioner (Tabell 1). Vid de flesta episoder med svåra infektioner fanns det taget minst en bakterieodling från blodet eller från ryggmärgsvätska som ytterligare stärkte allvaret av de behandlande läkarnas beskrivningar av infektionerna. Speciellt en patient verkar till att ha varit mycket svårt drabbad av infektioner och kunde dokumenterats för 57 lunginflammationer varav 15 tillfälle krävt sjukhusvård. Utöver denna omfattande sjuklighet hade patienten även två episoder med blodförgiftning.

Tabell 1. Indelning av personer med C2-brist efter svårighetsgrad av dokumenterade infektioner samt förekomst av reumatologisk sjukdom.

Svårighetsgrad av genomgånga infektioner	Antal personer i gruppen	SLE	UCTD	Vaskulit
Grupp I, mindre allvarliga infektioner	10 (25 %)	2	1	1
Grupp II, en eller flera lunginflammationer samt mindre allvarliga infektioner	7 (17,5 %)	4	1	0
Grupp III, haft en svår invasiv infektion	11 (27,5 %)	2	2	0
Grupp IV, upprepade svåra invasiva infektioner	12 (30 %)	2	0	2
Totalt	40	10	4	3

Förkortningar: SLE, Systemisk lupus erythematosus; UCTD, Undifferentiated Connective-Tissue Disease, Vaskulit, vaskulit med framförallt hudmanifestationer.

De svåra infektionerna orsakades i huvudsak av pneumokocker (57 %). Någon koppling till någon annan sjukdom eller behandling som skulle kunna förklara de frekventa infektionerna gick inte att få fram vid genomgång av de insamlade uppgifterna. Infektionerna orsakat totalt fem dödsfall under observationstiden.

I litteraturen har det sedan tidigare beskrivits ett samband mellan komplementbristen och utveckling av bindvävssjukdom som systemisk lupus erythematosus (SLE), Undifferentiated Connective-Tissue Disease (UCTD) och kärlinflammation (vaskulit) med hudengagemang. Detta samband gick att konfirmera hos tio patienter med SLE, fyra hade UCTD och tre hade hudvaskulit. En ny upptäckt var en ökad förekomst av hjärt-kärlsjukdom. Risken för hjärt-kärlsjukdom vid C2-brist visade sig var 4-5 gånger högre jämfört med normalbefolkningen vilket är likvärdigt med riskökningen vid tobaksrökning.

Efter första arbetets sammanställning kunde följande slutsatser göras:

1. C2-brist är sannolikt underskattat när det gäller förekomsten av infektioner. Vid utredning av en person som haft flera infektioner bör undersökning av komplementsystemet vara med i utredningen.
2. Bindvävssjukdom förekommer vid komplementbristen.
3. Personer med bristen har en ökad risk för hjärt-kärlsjukdom och bör om möjligt minska på andra kända riskfaktorer såsom rökning, dåligt reglerad sockersjuka och högt blodtryck.
4. Vidare studier behövdes för att få fram orsaken till varför vissa inte drabbades av svåra infektioner, bakomliggande mekanism till åderförkalkningen och möjligheten att skydda komplementbristarna mot infektioner med hjälp av vaccination.

Det andra arbetet i avhandlingen handlar om skillnaderna i infektionskänslighet mellan personerna med komplementbristen. Studien kom till att omfatta ytterligare fyra personer som identifierats vid screeningverksamheten vid immunologen i Lund. Indelningen av personerna i förhållande till svårighetsgrad av dokumenterad infektion utgjorde basen för de fortsatta undersökningarna av andra immunologiska faktorer som kunde tänkas påverka infektionskänsligheten.

Ett av fynden från den första undersökningen hade visat att de svåra infektionerna i huvudsak orsakades av pneumokocker. Det förekom även en rad andra kapslade bakterier i odlingsisolaten. Försvaret mot pneumokocker är bl.a. beroende av skyddande antikroppar och god funktion hos fagocyterna. Några dokumenterade fall med nedsatt fagocytfunktion fanns inte hos personerna i gruppen.

Patienter med antikroppsbrister har en känd ökad risk för infektioner orsakade av pneumokocker och mätningar av de olika typerna av antikroppshalter får därför anses vara rationellt. De antikroppstyper som kommer ifråga benämns immunoglobulin G (IgG), immunoglobulin A (IgA) och immunoglobulin M (IgM). IgG förekommer i fyra varianter som betecknas IgG1, IgG2, IgG3 och IgG4. Hos vuxna är halten av IgG2 i blodet av intresse i försvaret mot pneumokocker. Denna halt är styrd av genetiska faktorer som beskrivits i GM systemet. Anlaget som styr mängden IgG2 kallas för *G2M*n* och kan förenklat sägas förekomma i tre varianter. Den genotyp som har uppsättningen *G2M*n/ G2M*n* ger högst koncentration av IgG2 i blodet. *G2M*n/ G2M*n-* utgör en mellanvariant jämför med den

svagaste varianten som heter *G2M*n-/ G2M*n-*. Som synes är det ”*n*” kontra ”*n-*” som anger vilken variant en person har.

Andra faktorer som kunde tänkas spela roll var koncentrationen av mannan-bindande lektin (MBL) i blodet. MBL fungerar i likhet med en antikropp som en igenkänningsmolekyl av ett flertal främmande ämnen däribland en rad mikroorganismer. Även här går det med genetik att avgöra en persons förmåga att producera MBL.

C2-brist leder till avsaknad av funktion av den klassiska vägen. Kvar finns den alternativa vägen och möjligen lektinvägen som initieras av MBL. Den alternativa vägens funktion påverkas av regulatoriska proteiner. Faktor H verkar hämmande medan faktor B kan öka alternativa vägens funktion. Properdin är också involverat och räknas som en förstärkande regulator. Brist på någon av regulatorerna skulle kunna medföra en störning av alternativa vägens funktion.

Fagocyterna har på ytan receptorer för skaftet på antikroppar (Fc del). Inbindningen stimulerar till uppslukande av antikroppen som i sin tur kan var bunden i andra ändan till en mikrob. En ökad känslighet för meningokocker har tidigare beskrivits i relation till kvalitén på Fc receptorns bindningsförmåga. Även här går detta att få fram receptorns funktion genom att titta på olika genetiska varianter för receptorn.

Den enskilt viktigaste faktorn visade sig vara G2M(n). Genotypen *G2M*n/ G2M*n* visade sig var skyddande mot svåra infektioner hos personer med komplementbristen. Mekanismen bakom detta fynd är inte helt lätt att förstå. Den enkla förklaringen ligger i att genotypen medför ett kraftfullt antikroppssvar som skyddar mot infektioner. Man kan också tänka sig att genotypen leder till bättre antikroppsproducerande celler och att dessa finns redan tidigt utvecklade i barndomen. Det kan inte heller uteslutas att genotypen står i samklang med en annan gen eller gener som verkar gynnsamt för immunförsvaret mot kapslade bakterier.

MBL-gener som leder till uttryck av lägre halter av MBL i blodet visade sig vara en faktor för ökad risk för infektioner. Ingen av antikroppstyperna eller IgG subklasser korrelerade till ökad mottaglighet för infektioner. Övriga undersökta faktorer gav ingen ytterligare information.

I det tredje arbetet studerades organskada till följd av SLE och bakgrunden till den ökade risken för hjärt-kärlsjukdom. Av intresse var också att göra en sammanställning av förekomsten av olika s.k. autoantikroppar. Autoantikroppar bildas vid olika sjukdomstillstånd och anses numera vara den direkta orsaken till t.ex. organskada. Gruppen bestod nu av 45 personer från 33 olika familjer. Återigen gjordes en uppdatering av alla medicinska data med tillägg av ett frågeformulär rörande riskfaktorer för utveckling av hjärt-kärlsjukdom. Antalet observerade personår hade nu gått upp till 1772 år.

SLE är en autoimmun sjukdom som kan påverka olika organ i kroppen eller organsystem, särskilt huden, leder, blodet och njurar. Sjukdomen går ofta skovvis med perioder av försämring följt av förbättring och vise versa. Autoimmun betyder att det är en felreglering i immunsystemet, som i stället för att skydda kroppen från bakterier och virus, attackerar patientens vävnader och organ. Vanligen går det att hitta en rad olika skadliga autoantikroppar både i blodet och i olika vävnader. Exempel på sådana är antinukleära antikroppar (ANA) och antikroppar mot DNA. Orsaken till sjukdomen är okänd.

SLE vid komplementprotein 2 brist har tidigare beskrivits som ett väsentligen milt tillstånd med i huvudsak problem från hud och leder. Djupa manifestationer med organskada på t.ex. centrala nervsystemet (CNS), hjärta eller njurar har befunnits vara mera sällsynta. Vidare har förekomsten av autoantikroppar som ANA och anti-DNA varit låg.

För att mäta organskadan hos SLE patienterna i gruppen tillämpades ett validerat index som kallas för SLICC/ ACR DI (Systemic Lupus International Collaborating Clinics/American College of Rheumatology- Damage Index). Förenklat går SLICC/ ACR DI ut på att summera poäng av olika dokumenterade organskador hos en SLE patient för att kunna göra jämförelser mellan olika SLE patienter eller grupper av SLE patienter. Indexet har i flera undersökningar visat sig vara mycket pålitligt.

Närmare analys av de tolv SLE patienterna med SLICC/ ACR DI visade att organskadan var lika uttalad och svår som hos vanliga SLE patienter. Den främsta orsaken till detta var att många SLE patienter med komplementbristen hade hjärt-kärlsjukdom. Men även organskada på CNS och njurar förekom . Patienter med reumatisk sjudom var också i större utsträckning förtidspensionerade jämfört med övriga personer med komplementbristen.

Vid genomgång av journaler och frågeformulär sågs ingen förklaring till översjukligheten i hjärt-kärlsjukdom. Benägenheten för åderförkalkning verkade till att vara en följd av komplementbristen. Ytterligare stöd för detta återfanns i den ovanliga autoantikroppsprofil som komplementbristarna uppvisade. Förekomsten av ANA och anti-DNA var låg medan andra autoantikroppar som mot ribonukleärt protein förekom hos 5 av 11 (45 %) SLE patienter. Sannolikt bottnar komplementprotein 2 bristarnas speciella autoantikroppsprofil i deras likartade genetiska bakgrund.

Vi fann en hög förekomst av antikardiolipinantikroppar (69 %) och antikroppar mot komplementfaktor C1q (anti-C1q, 43 %) som inte bara förekom hos patienter med reumatisk sjukdom. Båda autoantikropparna kan vara involverade i risken för utveckling av åderförkalkning. Intressantast är dock anti-C1q antikropparna som i antikroppskomplex har visat sig kunna hämma kolesterol 27-hydroxlas i fagocyter och kärlväggar. Enzymet anses utgöra det först försvaret mot inlagring av kolesterol i kärlväggen och förhindrar därmed åderförkalkning. Hos personer med avsaknad av enzymet har man funnit en tidig utveckling av åderförkalkning trots normala blodfetter.

Antifosfolipidsyndrom (APS) är en störning av blodkoaguleringen vilket orsakar blodproppar i både artärer och vener men även graviditetsrelaterade problem såsom missfall, för tidig födelse eller allvarlig havandeskapsförgiftning. Antifosfolipidantikroppar som antikardiolipin och lupusantikoagulans är antikroppar som ger en ökad benägenhet för blodpropp. Dessa antikroppar är särskilt vanliga hos patienter med bindvävssjukdomar och framförallt vid SLE. Speciellt antikardolipinantikroppen korrelerar väl till sjukdom och anses vara drivande för syndromet. Ju högre koncentration av autoantikroppen som uppmätts i blodet desto större är risken för att drabbas av APS manifestationer. Märkligt nog hade personerna med komplementbristen en hög förekomst av antikardiolipinantikroppar (69 %) men ingen person dokumenterades med APS.

I djurförsök på gravida möss med antifosfolipidantikroppar har behandling med blodförtunnande medicin (Heparin) minskat risken för spontanabort. Mekanismen bakom heparineffekten har visat sig vara en hämning av komplementaktiveringen. Hos människor med APS har man uppmätt en ökad komplementaktivering med klyvning av komplementprotein C2. Man skulle därför kunna dra slutsatsen att C2-brist medför ett skydd mot APS eftersom den klassiska vägen inte går att aktivera. Möjligen är det därför som APS

inte återfinns vid komplementbristen trots hög förekomst av en sjukdomsframkallande autoantikroppar.

I det avslutande manuskriptet har vaccinationssvar hos 25 personer med komplementprotein 2 brist studerats. Vaccination med ett 23-valent polysackaridvaccin mot pneumokocker och ett protein konjugerat *Haemophilus influenzae* typ b vaccin gavs även till 51 friska frivilliga kontrollpersoner. Specifika antikroppar mot pneumokocker serotyp 6B, 7F och 23F samt mot Hib mättes för IgG, IgA och IgM före och efter vaccinationen. Även koncentrationer av IgG2 analyserades. C2-bristarna indelades i fyra undergrupper efter svårighetsgrad av genomgångna infektioner för att se om infektionerna påverkade vaccinsvaret.

Vaccination till personer med proteinbrist i den alternativa vägen (properdinbrist) har tidigare visats sig vara lyckat med normala svar mot meningokocker. Properdinbrist medför en ökad risk för livshotande infektioner orsakat av meningokocker och vaccination är självklart att rekommendera. Vid komplementbrister i den terminala vägen ses en ökad risk för meningokocksjukdom. Vaccination har visat sig vara att rekommendera även i dessa fall. En del undersökningar på ett mindre antal personer med komplementprotein 2 brist har genomförts med ett mera blandat resultat. Kända experter inom komplementområdet har ändå förordat vaccination mot pneumokocker, Hib och meningokocker med tanke på den ökade infektionskänsligheten.

Personerna med komplementbristen svarade generellt bra på vaccinationen. För både serotyp 6B och 23F var vaccinsvaren fullt likvärdiga mot kontrollgruppen. Många uppvisade en koncentration (>1 mg/L) av specifika antikroppar som anses vara skyddande mot svåra infektioner orsakade av pneumokocker och Hib.

Komplementbristen medförde därmed ett sämre antikroppssvar mot 7F och Hib. Orsaken till detta är oklar men skulle kunna tillskrivas de olika biokemiska egenskaper som dessa antigen uppvisar. Antigenet 7F har en grenad molekylär struktur medan 6B och 23 F är mera raka. Hib utgörs av ett proteinantigen som även involverar vita blodkroppar för skapande av minnesceller och antikroppar. Möjligen kan komplementsystemets igenkänningsmekanism för aktivering påverkas av skillnader hos antigen. Det är nämligen sedan tidigare känt att en komplementaktivering leder till ett kraftfullare antikroppssvar mot det aktiverande antigenet.

Vaccinsvaret mot pneumokockantigenen följdes även hos fyra personer under 4-6 års tid. Märklig nog sågs stora individuella skillnader. Som ett exempel kan ett syskonpar nämnas där vaccinsvaren var helt olika. Det ena syskonet hade ett antikroppssvar som varade över flera år med relativt stor mängd skyddande antikroppar. Det andra syskonet hade ett svar som var precis det motsatta, dvs. mycket kort varaktighet och med låg koncentrationer av specifika antikroppar.

Dessa data tyder sammantaget på att det troligen inte enbart är komplementbristen i sig som ger upphov till de olika vaccinationssvaren. Sannolikt inverkar andra faktorer inom immunförsvaret till dessa skillnader. Flera undersökningar krävs för att komma vidare i denna fråga.

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I

Hereditary C2 Deficiency in Sweden

Frequent Occurrence of Invasive Infection, Atherosclerosis, and Rheumatic Disease

Göran Jönsson, MD, Lennart Truedsson, MD, PhD, Gunnar Sturfelt, MD, PhD, Vivi-Anne Oxelius, MD, PhD, Jean Henrik Braconier, MD, PhD, and Anders G. Sjöholm, MD, PhD

Abstract: Although frequently asymptomatic, homozygous C2 deficiency (C2D) is known to be associated with severe infections and rheumatic disease. We describe the clinical findings in 40 persons with C2D from 33 families identified in Sweden over 25 years. Medical records covering 96% of the accumulated person-years were reviewed, giving a mean observation time of 39 years (range, 1–77 yr). Severe infection was the predominant clinical manifestation in the cohort: 23 patients had a past history of invasive infections, mainly septicemia or meningitis caused by *Streptococcus pneumoniae*, and 12 patients had repeated infections of this kind. Nineteen patients had at least 1 episode of pneumonia, and recurrent pneumonia was documented in 10 patients. Repeated infections occurred mainly during infancy and childhood. Systemic lupus erythematosus was found in 10 patients. Another 7 patients had undifferentiated connective tissue disease (n = 4) or vasculitis (n = 3). We found no correlation between susceptibility to invasive infection and rheumatologic disease. Cardiovascular disease occurred at a high rate, with a total of 10 acute myocardial infarctions and 5 cerebrovascular episodes in 6 patients. Causes of death among the C2D patients were infection (n = 5), acute myocardial infarction (n = 3), and cancer (n = 1). We suggest that severe infection may be the principal clinical manifestation of C2D. We also provide novel evidence for a possible role of C2D in the development of atherosclerosis consistent with findings in mannan-binding deficiency and experimental C3 deficiency. In addition, we confirm the well-known association between C2D and systemic lupus erythematosus.

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Abbreviations: ACR = American College of Rheumatology, AMI = acute myocardial infarction, ANA = antinuclear antibodies, C2D = homozygous C2 deficiency, MASP = MBL-associated serine protease, MBL = mannan-binding lectin, MHC = major histocompatibility complex, PCR = polymerase chain reaction, SLE = systemic lupus erythematosus.

INTRODUCTION

Homozygous C2 deficiency (C2D) is an important and fairly common form of complement deficiency in individuals of European descent[23,52,74]. The C2 gene is located in the middle of the major histocompatibility complex (MHC) class III region together with the genes for C4 and factor B[75]. Two principal variants of C2D have been distinguished. C2D type I is characterized by the absence of detectable C2 synthesis, while C2D type II is caused by a selective block of C2 secretion. The main cause of C2D type I is a 28-bp deletion in the C2 gene of the human leukocyte antigen-B*18,S042,DRB1*15 MHC haplotype[10,34]. This mutation is thought to account for more than 90% of all C2D cases. Rare C2D alleles have been identified in conjunction with other MHC haplotypes[48].

The function of C2 in the complement cascade is to provide the catalytic subunit of the C3 convertase C4b2a[72]. C4b2a can be generated through the classical pathway ($C1qr_2s_2$, C4, C2), which is the principal mechanism for antibody-dependent activation of complement, and through the lectin pathway, which supports innate immunity. The lectin pathway uses mannan-binding lectin (MBL) and ficolins for recognition of defined carbohydrate structures[41,71]. MBL-associated serine proteases (MASPs) form complexes with MBL and ficolins and are activated when the recognition molecules bind to appropriate targets. The C1s moiety of the $C1qr_2s_2$ complex and MASP-2 in MBL/MASP

From Department of Infectious Diseases (GJ, JHB), Department of Pediatrics (VO), and Department of Rheumatology (GS), University Hospital of Lund; and the Institute of Laboratory Medicine, Section of Microbiology, Immunology and Glycobiology (GJ, LT, AGS), Lund University, Lund, Sweden.

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Address reprint requests to: Dr. Göran Jönsson, Department of Infectious Diseases, University Hospital of Lund, S-221 85, Lund, Sweden. e-mail: goran.jonsson@insatnet.nu.

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or ficolin/MASP complexes specifically cleave C4 and C2[53]. Thus, activation of complement through the classical pathway and lectin pathway is impaired in C2D. By contrast, the alternative activation pathway (factor B, factor D, and properdin) is intact[72], and C1-dependent and MBL-dependent C2 bypass mechanisms may contribute to complement activation in C2D[36,40].

C2-deficient persons may be healthy[1], but C2D is primarily known to be associated with systemic lupus erythematosus (SLE) and SLE-like disease, and with susceptibility to invasive infections caused by encapsulated bacteria[23,48,52,62,74]. Information concerning disease associations in C2D is based mainly on descriptions in the literature of individual patients and families, which implies that conclusions may be biased at many levels[3]. Among 107 reported cases of C2D, 32% of the patients had SLE or SLE-like disease and 22% had at least 1 episode of invasive infection caused by encapsulated bacteria[23,52]. Other diseases have also been reported in conjunction with C2D.

This investigation concerns the clinical findings in 40 persons with C2D identified in Sweden between 1977 and 2002. Medical records were reviewed, giving long observation times for most patients. Although effects of patient selection were certainly operative, the identified cohort should provide an improved basis for discussion of the clinical consequences of C2D. The findings suggest that the importance of severe infection is underrated in C2D. Furthermore, the patients demonstrated an increased rate of cardiovascular disease that appeared to be independent of rheumatic disease manifestations.

PATIENTS AND METHODS

Patients

Screening for detection of complement deficiency by hemolysis in gel[64,70] as a routine part of complement analysis was initiated at the Clinical Immunology Unit, University Hospital of Lund, Lund, Sweden, by the end of the 1970s. Since that time, screening has been performed with samples from about 40,000 consecutive patients covering a broad spectrum of clinical conditions. Between 1977 and 2002, 40 Swedish patients with C2D were identified (Tables 1 and 2). To our knowledge, no other Swedish patients with C2D were diagnosed during this period. The patients were retrieved from hospital departments of internal medicine, infectious diseases, rheumatology, dermatology, pediatrics, and otorhinolaryngology, and from general and private practice. Seventeen C2D patients were found in Scania, a province in southern Sweden for which the laboratory provides primary service with regard to complement analysis. Patients 1, 19, 20, 21, 24, 25, 27, and 30 were treated at the University Hospital of Lund; Patients 2, 3, 17, and 18 at the Hospital of Ängelholm; Patients 4 and 28 at the University Hospital of Malmö; Patients 8 and 9 at the Hospital of Kristianstad; and Patient 35 at the Hospital of Helsingborg.

From the rest of Sweden, samples either were sent directly to our laboratory for complement analysis or were referred from Clinical Immunology laboratories (Karolinska Hospital, Huddinge University Hospital, Sahlgrenska University Hospital, and the University Hospital of Örebro) following initial screening for detection of complement deficiency. In the Stockholm area, Patient 7 was treated at the Sachs Children's Hospital, Patient 11 at the South Hospital, Patient 23 at the Karolinska Hospital, and Patient 40 at the Astrid Lindgren Children's Hospital. Patient 36 was treated in private practice. Patients 14, 26, 31, 32, and 33 were treated at Sahlgrenska University Hospital, Gothenburg; Patients 37, 38, and 39 at Skövde Hospital; Patient 29 at Uddevalla Hospital; Patient 12 at Trollhättan Hospital; Patient 16 at Jönköping Hospital; Patient 34 at Växjö Hospital; Patient 6 at Norrköping Hospital; Patient 22 at Örebro Hospital; Patient 5 at Boden Hospital; Patient 10 at Umeå University Hospital; Patient 13 at Skellefteå Hospital; and Patient 15 at Härnösand Hospital.

Medical records were reviewed and discussed with patients' physicians. The multicenter study was approved by the Lund University Research Ethics Committee (LU 513-01), and ethics committees of the 6 other centers involved. The study was based on written informed consent. One patient did not permit review of his medical records beyond the age of 11 years.

Information concerning the number of inhabitants in the province of Scania and in Sweden was obtained from Sweden's Statistical Databases, Stockholm, Sweden. Data from the Swedish National Board of Health and Welfare registries of disease and causes of death were also used.

Laboratory Studies

Blood samples were obtained from the patients and from first-degree relatives. Serum and EDTA plasma were stored in aliquots at −80°C. In 5 C2D patients, the small sample volumes available restricted extended analysis. Screening for detection of complement deficiency was performed with hemolytic gel assays using sensitized sheep erythrocytes for the classical pathway and guinea pig erythrocytes for the alternative pathway[70]. C2 and most other complement proteins were quantified by electroimmunoassay[35,37]. C3 and C4 were determined by turbidimetry (Cobas Mira, Roche Diagnostica, Basel, Switzerland). C2 concentrations were given in mg/L assuming that the pooled normal serum used for reference contained C2 at 26 mg/L[17]. The immunoglobulins IgM, IgG, and IgA were determined by turbidimetry using age-related reference areas[6,67]. Screening for antinuclear

TABLE 1. Clinical Manifestations in Patients With Homozygous C2 Deficiency (C2D), 1977–1990*

Patient No.	Family No.	Sex	Identification of C2D (year/age of patient in yr)	Reason for Investigation	Clinical Findings
1	1	F	1977/26	SLE	Recurrent tonsillitis, sinusitis, AOM, and UTI. STD with *N. gonorrhoeae*. SLE (21 yr†). Severe atherosclerosis, CVA, dissecting aorta aneurysm, and AMI × 2. Died of AMI (34 yr‡).
2	2	F	1977/29	SLE	Recurrent sinusitis, URTI, gastroenteritis, and UTI. Pneumonia. SLE (29 yr).
3	2	M	1977/26	Family investigation	Recurrent URTI and AOM. Pneumonia × 2. UTI. SLE (34 yr). Sjögren syndrome. AMI.
4	3	F	1974/32	SLE	SLE (28 yr). Died of *Staph. aureus* septicemia (59 yr).
5	4	F	1980/55	Osteitis	Recurrent tonsillitis and wound infections with pyoderma gangrenosum. Osteitis. Erysipelas. Pneumonia × 3. FUO. UCTD. Angina pectoris and AMI × 3. CHF. Pulmonary embolism and venous thrombosis × 3. Aortic valve insufficiency. Died of AMI (75 yr).
6	5	F	1985/37	Meningitis	*S. pneumoniae* meningitis × 2, sinusitis, orbital phlegmon, subperiosteal abscess, and recurrent URTI. Surgery for mandibular osteitis. AOM and peritonsillitis.
7	6	M	1983/11	Meningitis	*H. influenzae* meningitis. *S. pneumoniae* septicemia. Pneumonia, recurrent AOM, and sinusitis.
8	7	F	1985/27	Arthralgia	Recurrent AOM and skin abscesses. Salpingitis. FUO. SLE (44 yr).
9	7	M	1985/25	Family investigation	Recurrent URTI.
10	8	M	1984/76	Exanthema	Hypertension. Died of AMI (77 yr).
11	9	M	1985/62	SLE	*Staph. aureus* septicemia with epidural abscess. Osteitis in the jaw bone. Recurrent pneumonia × 57 and conjunctivitis. Recurrent UTI and diarrhea. FUO. *S. maltophilia* septicemia and pyelonephritis. SLE (57 yr). Pacemaker for Adam-Stoke syncope. Mitral valve insufficiency and asymptomatic atrial fibrillation. Died of cancer (76 yr).
12	10	M	1985/44	SLE	Pneumonia. SLE (44 yr). Hypertension, mitral valve insufficiency, and CHF. Died of *S. pneumoniae* septicemia and meningitis (51 yr).
13	11	F	1985/6	Septicemia	Streptococcal and *N. meningitidis* septicemia. Pneumococcal pneumonia, pyelonephritis × 3, and severe varicella. Recurrent AOM and URTI.
14	12	F	1985/65	Not known	Hypertension. Died of pneumonia (67 yr).
15	13	M	1987/16 mo	Meningitis	*S. pneumoniae* meningitis × 2. *S. pneumoniae* septicemia.
16	14	M	1986/17 mo	Meningitis	*S. pneumoniae* meningitis × 2. Recurrent tonsillitis, AOM, and URTI.
17	15	F	1989/14	Meningitis	Umbilical infection with septicemia. *S. agalactiae* and *N. meningitidis* meningitis.
18	15	F	1989/17	Family investigation	Recurrent sinusitis and URTI.
19	16	F	1990/45	SLE	Pneumonia × 2 and recurrent URTI. External otitis. SLE (47 yr).

Abbreviations: AMI, acute myocardial infarction; AOM, acute otitis media; CHF, congestive heart failure; CVA, cerebrovascular accident; FUO, fever of unknown origin; SLE, systemic lupus erythematosus; STD, sexually transmitted disease; UCTD, undifferentiated connective tissue disease; URTI, upper respiratory tract infection; UTI, urinary tract infection.

*See Table 2 for 1993–2002; no cases were diagnosed 1991–1992. Clinical data are restricted to rheumatologic, cardiovascular, and infectious diseases.

†Age at onset of SLE.

‡Age at death.

antibodies (ANA) was performed by indirect immunofluorescence with HEp-2 cells (Euroimmun, Lübeck, Germany) at patient serum dilutions 1/100 and 1/400. This corresponds to detection of ANA at 3.5 and 14 IU/mL (World Health Organization reference serum 66/233). The diagnostic ANA titer was 400, as established by determination of the 96.5 percentile for negativity in healthy blood donors (98 females, 98 males). DNA was extracted from whole blood according to standard procedures[43]. Detection of the 28-bp deletion associated with C2 deficiency type I was done by polymerase chain reaction (PCR) amplification[69]. DR typing was performed using a PCR-technique (Olerup SSP AB,

TABLE 2. Clinical Manifestations in Patients with C2D, 1993–2002*

Patient No.	Family No.	Sex	Identification of C2D (year/age of patient in yr)	Reason for Investigation	Clinical Findings
20	17	F	1993/5	Hematuria	Recurrent tonsillitis, AOM (treated > 17 times), and URTI. Conjunctivitis, varicella, and gastroenteritis.
21	17	F	1993/44	Family investigation	*S. pneumoniae* septicemia and pneumonia. Recurrent tonsillitis. Hypertension.
22	18	M	1993/6	Septicemia	*S. agalactiae* meningitis. Septicemia and epiglottitis × 2 (*K. kingae* and *H. influenzae*). Varicella.
23	19	F	1995/57	SLE	Recurrent tonsillitis, skin abscesses, and pneumonia (> 3 times). FUO. Sjögren syndrome. SLE (25 yr).
24	20	F	1995/40	Severe pneumonia	Pyelonephritis × 3 and UTI. Recurrent sinusitis and bronchitis. *S. pneumoniae* septicemia and pneumonia. FUO. Erythema nodosum. Cutaneus vasculitis.
25	20	F	1995/35	Family investigation	*N. meningitidis* meningitis. Pneumonia × 2. Recurrent sinusitis and AOM. UCTD.
26	21	M	1996/70	Erysipelas	Skin abscesses and erysipelas. Hypertension. AMI × 3 times and CHF. CVA at 4 times. Died of acute peritonitis (71 yr). A first-degree relative died of meningitis.
27	22	M	1996/41	Myalgia and arthralgia.	Pneumonia × 4. Recurrent AOM and URTI. *Salmonella* gastroenteritis. UCTD.
28	23	M	1996/2	Septicemia	*S. pneumoniae* septicemia. Recurrent tonsillitis, acute peritonsillitis, AOM, and URTI. Pyelonephritis. Ethmoiditis.
29	24	F	1996/53	SLE	Enterococcal species septicemia. SLE (10 yr)
30	25	F	1997/2	Minor infections	Pneumonia. Recurrent URTI, AOM, and UTI. FUO. Gastroenteritis.
31	26	F	1997/41	Skin disease	Vasculitis.
32	26	F	1997/38	Family investigation	Recurrent URTI.
33	27	F	1998/50	Alveolitis and lung fibrosis	UCTD.
34	28	M	1999/47	Vasculitis	*S. pneumoniae* septicemia and pneumonia. *Staph. aureus* septicemia, skin abscesses, and phlegmon. Venous thrombosis.
35	29	M	1999/5	Septicemia	*S. pneumoniae* septicemia. Pneumonia. Recurrent sinusitis and AOM. URTI.
36	30	F	1999/76	Septicemia, recurrent pneumonia	*S. pneumoniae* septicemia. Recurrent pneumonia, bronchitis, and UTI. Pleural tuberculosis.
37	31	M	2001/8	Septicemia	*S. pneumoniae* septicemia and pneumonia. Recurrent AOM and URTI.
38	31	M	2002/5	Family investigation	URTI.
39	32	F	2002/10	Septicemia, meningitis	Died of septicemia and meningitis caused by *S. pneumoniae* (10 yr).
40	33	M	2002/16 mo	Septicemia, meningitis	*S. pneumoniae* septicemia and meningitis. Ethmoiditis.

*See Table 1 for abbreviations and notes.

Saltsjöbaden, Sweden). C4 was phenotyped according to established procedures[69].

Statistical Analysis

To assess the risk of acute myocardial infarction (AMI) in C2D, we had to take into account the known influence of age and gender. Therefore, persons in the C2D cohort between 30 and 79 years of age during the follow-up period 1940–2000 were used as the observed population (Figure 1). Twenty-five persons could be observed and attributed with person-time at risk during this period. The number of person-years at risk during the follow-up period was summarized for men, women, all persons, and those diagnosed with SLE. A person was considered as being at risk only until his or her first AMI was recorded. The 5 patients with AMI had their first AMI between 1975 and 1999. Age- and gender-specific data on mean AMI incidence available during 1987–2001 were used to obtain the number of cases with their first AMI that could be expected to

occur in the C2D cohort during the follow-up period if the cohort had the same incidence of AMI as an imaginary cohort from the general Swedish population with the same size and the same age and gender distribution. Calculations of the standardized mortality/morbidity ratios were made with exact confidence intervals based on the Poisson distribution. Statistical significance is considered when the lower limit of the 95% CI is 1.0 or above[54,55].

RESULTS

C2D Cohort

A cohort of 40 Swedish citizens with C2D belonging to 33 apparently unrelated white families was identified in the course of 25 years (see Tables 1 and 2): 23 females and 17 males. The mean age at the time C2D was diagnosed was 31 years (range, 17–76 yr; median, 34 yr). At the end of the study, the mean age was 52 years (range, 1–79 yr; median, 44 yr). Nine patients were younger than 18 years when C2D was diagnosed (range, 1–16 yr). Medical records were reviewed covering a total of 1560 person-years (see Figure 1) including laboratory results, radiologic findings, clinical physiology investigations, and autopsy reports. Medical records covering 96% of the accumulated person-years were reviewed, giving a mean observation time of 39 years (range, 1–77 yr).

Laboratory Findings

C2 was not detectable in sera (<0.5 mg/L) from the 40 persons with C2D, while other complement proteins were present at essentially normal concentrations. Heterozygous first-degree relatives (n = 25) showed C2 in the range between 7.5 and 19.5 mg/L (reference area, 20.0–41.3 mg/L).

Thirty-three persons with C2D were found to be homozygous for the 28-bp deletion[34] and were also homozygous for DRB1*15 and C4A*4 B*2. For 1 patient (Patient 39), who died of fulminant infection, DNA was not available. However, the 28-bp deletion was present in both parents, which implied that the patient was homozygous for this defect. Three persons with undetectable C2 (Patients 19, 37, 38) were heterozygous for the 28-bp deletion indicating compound heterozygosity for C2D involving a second mutation, distinct from the 28-bp deletion. Patient 19 showed DRB1 15, 11 and the C4 phenotype C4A 3,4 B 1,2, suggesting that the second mutation was present on a haplotype containing DRB1*11 and C4A*3 B*1. Patients 37 and 38 were brothers, and showed DRB1 1,15 and the C4 phenotype C4A4 B2. Thus, the second mutation was probably present on a haplotype containing DRB1*1 and C4A*4 B*2. The mutations in Patient 19 and in the brothers, Patients 37 and 38, did not appear to be part of MHC haplotypes that have been described previously in conjunction with C2 null genes[48]. In 3 deceased patients (Patients 1, 10, 14) investigations for the 28-bp deletion and MHC typing could not be performed.

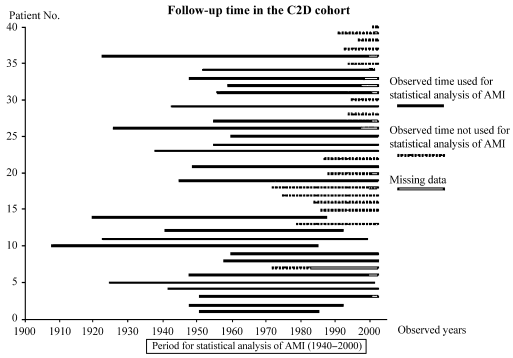

FIGURE 1. Observation time and availability of clinical documentation for the C2D patients. See Methods section for description of statistical analysis of AMI.

The concentrations of IgM, IgG, and IgA were normal in 35 patients from whom appropriate samples were available. According to medical records the 5 remaining patients did not have hypogammaglobulinemia. ANA at a diagnostic level (titer $\geq$ 400) were found in 3 patients with SLE. ANA at a low level (titer 100) was found in 4 SLE patients.

Infections

The occurrence of infection was the most prominent clinical manifestation in the C2D cohort. In an attempt to describe the wide range of severity of infection in individual patients, we divided the 40 patients into 4 groups. One group (n = 10) consisted of patients (Patients 1, 8, 9, 10, 18, 20, 31, 32, 33, 38), who had a history of minor infections including recurrent otitis media, sinusitis, throat infections, and infections of the respiratory tract. A second group of patients (n = 7) (Patients 2, 3, 14, 19, 23, 27, 30) had documented minor infections and at least 1 episode of pneumonia. A third group (n = 11) (Patients 4, 5, 12, 21, 25, 26, 29, 35, 36, 39, 40) had 1 invasive infection combined with a history of pneumonia and other infections. The fourth group (n = 12) (Patients 6, 7, 11, 13, 15, 16, 17, 22, 24, 28, 34, 37) had at least 2 invasive infections.

Eleven female patients and 8 males had a history of pneumonia. In 6 patients the bacteriologic cause was established by blood culture demonstrating *Streptococcus pneumoniae*. Recurrent pneumonia (2 episodes or more) was documented in 7 female patients and 3 males. One patient (Patient 11) was treated for 57 episodes of pneumonia, requiring hospitalization 15 times.

A history of meningitis was obtained in 4 female patients and 6 males. Four of these patients had 2 episodes of meningitis (Table 3). The predominant cause of meningitis was *S. pneumoniae* (64%), followed by *Neisseria meningitidis* (14%), *Streptococcus agalactiae* (group B streptococci)

TABLE 3. Age at Infection and Etiology (Cerebrospinal Fluid Culture Result) of C2D Patients With Meningitis

Patient No.	Age	Etiology
6	12 yr	*S. pneumoniae*
	40 yr	*S. pneumoniae*
7	7 yr	*H. influenzae* type b
12	51 yr	*S. pneumoniae*
15	6 mo	*S. pneumoniae*
	16 mo	*S. pneumoniae*
16	17 mo	*S. pneumoniae*
	20 mo	*S. pneumoniae*
17	1 mo	*S. agalactiae*
	14 yr	*N. meningitidis*
22	53 wk	*S. agalactiae*
25	12 yr	*N meningitidis*
39	9 yr	*S. pneumoniae*
40	1 mo	*S. pneumoniae*

(14%), and *Haemophilus influenzae* (7%). The mean age of the patients at the time of meningitis episode was 10 years (range, 3 wk–51 yr; median, 4 yr).

Septicemia was documented in 8 female patients and 10 males (Table 4). Two episodes or more were found in 5 patients. Positive blood cultures were obtained in 21 of the 23 episodes. The predominant etiologic agent was *S. pneumoniae* (52%), followed by *Staphylococcus aureus* (13%). The other bacteria found were *S. agalactiae*, *N. meningitidis*, *H. influenzae* type b, *Kingella kingae*, *Stenotrophomonas maltophilia*, and an Enterococcal species, each as a single isolate. The origin or infectious focus could be established in 17 of the 23 septic episodes. The mean age of the patients at the time of septicemia episode was 26 years (range, 1 mo–75 yr; median, 24 yr). In 3 patients, the precise age at the time of septicemia was not recorded.

The invasive infections among the 23 patients were not restricted to septicemia or meningitis (n = 21). Osteitis (Patients 5, 6, 11), pyelonephritis (Patients 13, 24), and peritonitis (Patient 26), were also classified as invasive infections. Some of these patients were also documented as having 1 or several episodes of septicemia or meningitis (Patients 6, 11, 13, 24).

Documented minor infections usually occurred during infancy (1–23 mo) and childhood (2–12 yr), and ceased during adolescence (13–18 yr). Nine of the 18 patients with pneumonia had their first pneumonia during infancy or childhood. Among the 18 patients with septicemia, the first episode occurred during infancy and childhood in 10 patients. Nine of the 10 patients with meningitis experienced the first episode during infancy and childhood. Eighty percent of the patients with recurrent episodes of septicemia or meningitis were under the age of 18 years. During adolescence, only 1 invasive infection (meningitis) was recorded. Among adults, invasive infections or pneumonia occurred in 18 patients.

Rheumatologic Disease

Ten patients (7 female and 3 male) fulfilled 4 or more of the 1982 American College of Rheumatology (ACR) classification criteria for SLE. The mean age at onset of SLE was 34 years (median, 32 yr). ANA were demonstrated at diagnostic levels in 3 and at a low level in 4 of the SLE patients. In Patient 10, the presence of ANA was documented in the medical records. The low prevalence of ANA in SLE patients with C2D agrees with results of previous investigations[48].

Four patients (Patients 5, 25, 27, 33) had undifferentiated connective tissue disease or incomplete SLE (<4 ACR criteria). Another 3 patients (Patients 24, 31, 34) had vasculitis with skin manifestations, verified by biopsy in 2 cases.

TABLE 4. Age at Infection, Etiology, and Related Infectious Foci in Conjunction With Septicemia Episodes in Patients With C2D

Patient No.	Age at Septicemia (yr)	Etiology	Related Infectious Focus
4	60	*Staph. aureus*	Probably skin lesions
7	10	*S. pneumoniae*	Sinusitis
11	57	*Staph. aureus*	Epidural abscess
	74	*S. maltophilia*	Pyelonephritis
12	51	*S. pneumoniae*	Meningitis
13	Child	Streptococcal species	
	Child	*N. meningitidis*	
15	2	*S. pneumoniae*	
17	Infant	*S. agalactiae**	Umbilical infection
21	48	*S. pneumoniae*	Pneumonia
22	21 mo	*H. influenzae*	Epiglottitis
	30 mo	*K. kingae*	Epiglottitis
24	40	*S. pneumoniae*	Pneumonia
28	2	*S. pneumoniae*	
29	53	Enterococcal species	Cholecystitis and pancreatitis
34	37	*Staph. aureus*	Skin abscesses and phlegmon
	44	*S. pneumoniae*	Pneumonia
35	5	*S. pneumoniae*	
36	75	*S. pneumoniae*	Pneumonia
37	5	*S. pneumoniae*	Pneumonia
	7		
39	9	*S. pneumoniae*	Meningitis
40	1 mo	*S. pneumoniae*	Meningitis

*Suspected but not verified by blood culture (compare Table 3).

TABLE 5. Relationship Between Severity of Infection and Presence of Rheumatologic Disease in C2D*

Severity of Infection	All C2D Patients No. (%)	No Rheumatologic Disease No. (%)	SLE No. (%)	UCTD No. (%)	Vasculitis† No. (%)
Minor infections	10 (25.0)	6 (26.1)	2 (20.0)	1 (25.0)	1 (33.3)
Pneumonia (1 or more) + minor infections	7 (17.5)	2 (8.7)	4 (40.0)	1 (25.0)	0 (0)
Invasive infection (1) + pneumonia or other infections	11 (27.5)	7 (30.4)	2 (20.0)	2 (50.0)	0 (0)
Invasive infections (2 or more)	12 (30.0)	8 (34.8)	2 (20.0)	0 (0)	2 (67.7)
Total	40 (100)	23 (100)	10 (100)	4 (100)	3 (100)

*No significant correlation was found between documented invasive infections and the presence of rheumatologic disease (p = 0.34, RR = 0.72, 95% confidence intervals: 0.4–1.3, Fisher exact test).
†Vasculitis: vasculitis with skin manifestations verified by biopsy in 2 patients.

Severe infections occurred in some of the patients, and 1 patient with SLE (Patient 12) died of an invasive pneumococcal infection. In 4 SLE patients, serious infections predated the onset of SLE by several decades. There was no relationship between susceptibility to infections and the presence of rheumatologic disease (Table 5).

Cardiovascular Disease

Cardiovascular disease occurred at a high rate. Three male patients (Patients 3, 10, 26) and 2 females (Patients 1, 5) had a total of 10 AMI episodes. Calculations concerning the risk for AMI in the C2D cohort showed a statistically significant increase that was about 4.0 times higher than that found in the general Swedish population (Table 6). Two AMI episodes were documented in a woman with SLE (Patient 1). AMI also occurred in a male patient with SLE (Patient 3). The increased AMI rate was statistically significant in the group of patients with SLE, undifferentiated connective tissue disease, or vasculitis, but not in the group with SLE or in patients without rheumatologic disease (see Table 6). None of the 5 patients with AMI was a tobacco smoker, or had diabetes or hyperlipemia[11,16,32,42]. Two patients (Patients 10, 26) had well-regulated hypertension[11]. The mean age at the first AMI was 58 years (range, 33–77 yr; median, 57 yr). A cerebrovascular accident occurred once in a female with SLE (Patient 1) and 4 times in a male without SLE (Patient 26). These 2 patients had additional cardiovascular manifestations including acute aorta dissection, AMI, congestive heart

TABLE 6. Acute Myocardial Infarctions (AMI) in C2D in Patients Aged 30–79 Years: Patients Have an Increased Frequency of AMI Compared With the General Swedish Population Diagnosed With AMI in the Same Age Group and During the Same Follow-Up Period Between 1940 and 2000

No. of Observed Patients	C2D Patients	No. of Patients With AMI	Person-Years in the C2D Cohort	AMI Incidence in the C2D Patients per 100,000	Expected No. of AMI Cases in the C2D Cohort*	SMR in the C2D Cohort	95% CI†
8	Males	3	204	1500	0.88	3.4	0.77–9.9
17	Females	2	391	510	0.38	5.2	0.63–19
25	Total	5	595	840	1.26	4.0‡	1.3–9.2
10	SLE	2	235	850	0.46	4.3	0.52–16
15	Not SLE	3	360	830	0.80	3.8	0.77–11
17	SLE, UCTD, and Vasculitis	3	372	810	0.59	5.1‡	1.0–15
8	Not SLE, UCTD, or Vasculitis	2	223	900	0.67	3.0	0.36–11

Abbreviations: See previous tables. SMR, standard mortality/morbidity ratio.
*The expected number of C2D persons with AMI based on the supposition that the incidence for AMI was the same in the C2D cohort as in the Swedish reference population.
†Exact confidence intervals for SMR were calculated based on the Poisson distribution.
‡Considered significant since the lower limit of the CI is 1.0 or above (references 54, 55).

TABLE 7. Spectrum of Disease Categories*, Apart From Infectious, Cardiovascular, and Rheumatologic Diseases, in Patients With C2D†

Disease Category	No. of Patients	Disease Category	No. of Patients
Asthma/allergy	6	Oncology	4
Dental/maxillofacial diseases	0	Ophthalmology	3
Dermatology	3	Psychiatry	3
Endocrinology	2	Pulmonary/respiratory diseases	4
Gastroenterology	6	Abdominal surgery	7
Hematology	2	Cardiothoracic surgery	1
Nephrology/urology	5	Ear, nose, and throat surgery	2
Neurology	5	Neurosurgery	0
Obstetrics/gynecology	4		

*World Health Organization. The International Statistical Classification of Diseases and Related Health Problems, 10th revision. Geneva: WHO; 1992.

†A patient can belong to several categories but is recorded only once in each category.

failure, and hypertension. Cardiac valve insufficiency was seen in 3 patients (Patients 5, 11, 12). Angina pectoris (Patient 5), atrial fibrillation (Patient 11), and venous thrombosis (Patient 34), were each seen once. Hypertension was found in 5 patients (Patients 10, 12, 14, 21, 26).

Other Disease Manifestations

Disease categories of clinical manifestations other than those related to infectious, cardiovascular, or rheumatologic diseases are summarized in Table 7. In the cohort, 1 male (Patient 35) and 2 female patients (Patients 8, 30) had asthma. The prevalence of allergy and eczema was low. One patient was diagnosed with pyoderma gangrenosum. Abdominal surgery had been performed in 7 patients (appendicitis, cholecystitis, and inguinal hernia). Six patients had gastroenterology-related manifestations: gastritis (2/40), pancreatitis (2/40), proctitis (1/40), and chronic diarrhea (1/40). Cancer was documented in 4 patients (Patients 11, 14, 26, 34).

Cause of Death

Nine C2D patients died during the observation period. Infections (pneumonia, meningitis, and septicemia) accounted for death in 5 patients. AMI was established as a cause of death in 3 patients, and 1 patient died of breast cancer. Of the 9 patients who died, 4 patients had SLE: 2 died of infection, 1 died of AMI, and 1 died of lung cancer.

Manifestations in First-Degree Relatives

Family studies were performed in 18 families, resulting in identification of 7 nonindex persons with C2D among the first-degree relatives (Table 8). A female SLE patient (Patient 2) had a C2-deficient brother (Patient 3), who had a history of minor infection and pneumonia at the time of the initial investigation, but developed SLE 10 years later. Another index patient (Patient 24) with severe pneumococcal pneumonia had a sister with a past history of meningococcal meningitis (Patient 25). A 5-year-old girl (Patient 20) was investigated for complement function due to transient hematuria, the cause of which was never established. Her history included recurrent otitis media. Her mother, who was also found to be C2 deficient (Patient 21), had a history of septicemia. Four additional nonindex cases of C2D had a history of minor infections, but no significant manifestations of disease. One of these was a 5-year-old child with a short observation time.

Family histories revealed the occurrence of severe infections in family members, most of whom were not investigated for complement function. Patients 6 and 26 had first-degree relatives who had died of meningitis. The father of Patient 20 was a heterozygous carrier and had a history of meningitis as an adult. The patient's grandfather died of meningitis.

TABLE 8. Clinical Findings in First-Degree Relatives With Documented C2D*

Index Patient			First-Degree Relative		
Patient No.	Clinical Finding	Observation Time (yr)	Patient No.	Clinical Finding	Observation Time (yr)
2	SLE	44	3	SLE	49
8	SLE	44	9	Minor infections	42
17	Septicemia and meningitis × 2	27	18	Minor infections	27
20	Minor infections	12	21	Septicemia	53
24	Pyelonephritis × 3 and vasculitis	47	25	Meningitis	42
31	Vasculitis	44	32	Minor infections	38
37	Septicemia × 2	9	38	Minor infections	5

See previous tables for abbreviations and notes.

*Two additional first-degree relatives died of meningitis, but were not investigated for complement function.

With the exception of the father of Patient 20, none of the 25 heterozygous carriers identified had a history of invasive infection or rheumatologic disease conditions.

Secondary Immunodeficiency

Even in the absence of immunosuppressive treatment, 79% of patients with SLE develop serious infections during the course of their disease[51]. Treatment with corticosteroids further increases the incidence of infections[28]. Two SLE patients (Patients 11, 12) were treated with corticosteroids at 2.5–20.0 mg per day at the time of the invasive infections. Patient 12 developed proliferative glomerulonephritis (World Health Organization grade IV) during his last year of life. Plasma exchange and intravenous pulse cyclophosphamide treatment were tried without success. He died of septicemia and meningitis shortly after. Patient 34 had received treatment with corticosteroids and cyclophosphamide for glomerulonephritis, but not at the time of the 2 septicemia episodes that he experienced. Patients 4 and 5 developed invasive infections during corticosteroid therapy.

DISCUSSION

The principal finding in the current study is that severe infection was the predominant clinical manifestation among C2D patients: 57% of the patients had a past history of invasive infections, and 30% had repeated infections of this kind. In addition, pneumonia was a frequent finding. To some extent, the predominance of infections in the current study compared with previously published data[23,52] may be explained by patient selection at the clinical level and by long observation times. More likely, the importance of C2D as a basis for susceptibility to infection has not been appreciated fully in the literature. Effects of patient selection were probably stronger for SLE, which was present in 25% of the cohort, in contrast to the prevalence of 10% for SLE in C2D proposed in the year 2000[48]. It is also noteworthy that 8 of our SLE patients were identified in 1977–1990 (see Table 1) and only 2 SLE patients were identified in 1993–2002 (see Table 2). Another 18% of our patients had undifferentiated connective tissue disease or vasculitis. The association we found between C2D and cardiovascular disease could hardly have been influenced by patient selection. Some clinical manifestations that have been reported in C2D and other complement deficiency states, such as anaphylactoid purpura[23,52,65], acute glomerulonephritis[27], and membranoproliferative glomerulonephritis[23,52], were not observed among our patients.

Many persons with C2D are known to be essentially healthy or to have limited clinical problems with a questionable relationship to C2D. Consistent with this, we found that 4 of 7 first-degree relatives with C2D did not have major clinical problems, with reservation for a short period of observation in at least 1 of the nonindex cases. Severe infections were found in a few additional first-degree relatives. The data suggest that C2D might be associated with significant disease in perhaps 50% of the cases, and that the most important category that tends to be overlooked is the group of patients with severe infections. The absence of conditions such as classical rheumatoid arthritis in C2D[62] might be due to protective effects of linked MHC genes[2] or of the complement deficiency.

Estimates of the prevalence of C2D have been made by determining the allele frequency of C2 null genes, either by measurement of C2 or by detection of the 28-bp deletion of the C2 gene in western countries. The results have suggested prevalences of C2D in the approximate range between 1:13,500 and 1:40,000[48]. Of the 17 patients with C2D retrieved from Scania, 6 had SLE. Considering current diagnostic practices in Scania, it is possible that all or nearly all Scanian SLE patients with C2D were found. Pickering et al[48] have proposed that development of SLE in C2D occurs in the order of 10%. With the finding of 6 SLE patients with C2D in Scania, this suggests that about 60 persons might have C2D in the province, which has 1.2 million inhabitants, corresponding to a C2D prevalence of about 1:20,000. Extrapolated to the Swedish population of about 9 million, prevalences ranging between 1:40,000 and 1:20,000 would correspond to 225–450 Swedish C2D cases. We found 40 patients, which further emphasizes that C2D is often overlooked.

The reasons for the development of SLE in classical pathway deficiencies have been subject to extensive study[48,68]. Current data favor the hypothesis that autoimmunity in the disease is triggered by impaired complement-dependent elimination of apoptotic cells. Impaired handling of immune complexes might also play an important pathogenetic role.

The role of complement in cardiovascular disease is ambiguous. Animal experiments suggest that deficiency of late complement components protects against the development of atherosclerosis[8,26]. Conversely, genetically engineered C3-deficient mice have been shown to develop atherosclerosis at an increased rate[14]. MBL deficiency has been reported to be implicated in the disease process, either through increased susceptibility to infection caused by *Chlamydia pneumoniae* or by involvement of the lectin pathway in inflammation[39,57]. In recent prospective studies, MBL deficiency has been shown[7] to be associated with coronary artery disease in American Indians and with arterial thrombosis in SLE[47]. C2 participates in C3 activation through the lectin pathway[71], which might be the cause for the development of atherosclerosis in C2D. The 4-fold increase of the risk for a first AMI found in the C2D cohort is comparable to the increased risk of a coronary event in tobacco smokers[42], which is one of the strongest independent predictors of premature coronary heart disease[15]. Patients with established

atherosclerotic disease have a 5- to 7-fold increased risk of recurrent AMI compared with the general population[15]. The frequent occurrence of cardiovascular disease in C2D was unexpected, and due to the design of the present study, analysis according to the Framingham coronary risk profile was not performed[5,18].

Experimental studies in genetically engineered C1q-deficient mice have emphasized the importance of genetic background on disease expression of complement deficiencies[44]. Background genes are likely to contribute to the heterogeneity of the human C2D phenotype, but this has not yet been demonstrated. As C2D is nearly always caused by the homozygous presence of a C2 gene containing a specific mutation[3,75], immunologic properties are probably more uniform in C2D than in other complement deficiencies due to the presence of strongly linked MCH genes[69]. C2D and other deficiencies of the classical pathway are associated with IgG subclass aberrations[9] that do not function as markers for susceptibility to infection or other manifestations of C2D[4]. Common variable immunodeficiency[61], IgG allotypes[63,76], and impaired alternative pathway function[45,58] have been discussed in relation to susceptibility to infection in C2D.

In accordance with previous studies[22,23], *S. pneumoniae* was found to be the predominant cause of infection in C2D. *N. meningitidis*, *H. influenzae* type b, and other bacteria were identified in comparatively few patients. Two patients, 1 of whom has been reported before[59], had neonatal infections with *S. agalactiae*. Another child with *S. agalactiae* infection and C2D has also been described[20]. It is noteworthy that repeated episodes of pyelonephritis occurred in 4 patients belonging to the group with a history of septicemia and meningitis.

The reasons for impaired immunity in C2D are not altogether clear. Conversely, immunity is evidently sustained by C2-independent mechanisms in many patients. Experiments with genetically engineered mouse strains suggest that the classical pathway supports innate immunity to *S. pneumoniae* and *S. agalactiae*[13,73]. Earlier studies of C4-deficient guinea-pigs suggested that innate immunity to *S. pneumoniae* is a function of the alternative pathway[12]. The lectin pathway, which involves activation of C3 by C4b2a might also contribute to innate immunity mechanisms[71]; deficiency of MBL has been reported to be associated with susceptibility to pneumococcal[56] and meningococcal disease[31]. Furthermore, a recently described patient with MASP-2 deficiency had repeated severe pneumococcal infections[66]. On the other hand, the findings of Brown et al[13] do not suggest that the lectin pathway contributes to defense against *S. pneumoniae*.

The frequent restriction of severe infections to infancy and childhood in C2D indicates that acquired immunity is operative, and in accord with this it has been suggested that vaccination might be helpful[22,23]. Acquired immunity in C2D could be accomplished by antibodies capable of recruiting the alternative pathway[33,38,50,59]. Target-bound IgG can serve as a protected site for assembly of alternative pathway C3 convertase[25], and antibodies to capsular sialic acid of *S. agalactiae* have been shown to promote opsonization by blocking sialic acid-mediated down-regulation of the alternative pathway[21]. Antibodies could also contribute to immunity by C1-dependent C2-bypass activation of the alternative pathway[36]. On the other hand, the importance of the alternative pathway in acquired immunity to *S. pneumoniae* might be questioned, based on animal experimental data[12].

Antibodies might also support immunity through complement-independent mechanisms. Among these, phagocyte Fc-receptors interacting with antibodies of the IgG2 isotype have been shown to be important in deficiencies of the late complement components[24,49].

Impaired function of the classical pathway can limit antibody production[46], which is explained by the adjuvant effect of C3d fragments on the immune response[19]. The significance of this for immune defense in C2D is not known. However, available evidence does not suggest that antibody responses to encapsulated bacteria are grossly impaired in C2D[29,59,60].

In conclusion, retrospective analysis of Swedish patients with C2D revealed that invasive infections occurred at a high rate. We believe the importance of C2D as an immunodeficiency predisposing to severe infection is not fully recognized. The findings also confirm the well-known association between C2D and SLE, and provide novel evidence for a possible role of C2D in the development of atherosclerosis. C2D has approximately the same prevalence as common variable immunodeficiency[30], and perhaps should be considered more often in the context of immunodeficiencies, as well as in atherosclerotic and inflammatory diseases.

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II

Homozygosity for the IgG2 Subclass Allotype G2M(n) Protects against Severe Infection in Hereditary C2 Deficiency[1]

Göran Jönsson,[2]*‡¶ Vivi-Anne Oxelius,† Lennart Truedsson,‡¶ Jean Henrik Braconier,* Gunnar Sturfelt,§ and Anders G. Sjöholm‡¶

Homozygous C2 deficiency (C2D) is the most common deficiency of the classical complement pathway in Western countries. It is mostly found in patients with autoimmune disease or susceptibility to bacterial infections and in healthy persons. We wished to assess to what extent other immunological factors might explain differences of susceptibility to infections in C2D. For this reason, 44 Swedish patients with C2D were stratified with regard to the severity of documented infections. Investigations of IgG subclass levels, IgG subclass-specific GM allotypes, concentrations of factor B, properdin, and factor H, and polymorphisms of mannan-binding lectin and the Fc receptors FcγRIIa and FcγRIIIb were performed. Homozygosity for the *G2M*n* allele, which is known to promote Ab responses to polysaccharide Ags, was strongly associated with the absence of severe infections ($p < 0.001$) in the patients, suggesting a major protective role. The combination of mannan (or mannose)-binding lectin and C2 deficiency was found to be a minor susceptibility factor for invasive infection ($p = 0.03$). Low concentrations of IgG2 and factor B might sometimes contribute to susceptibility to infection. Other factors investigated did not appear to be important. In conclusion, the findings indicated that efficient Ab responses to polysaccharides are protective against severe infection in C2D. Implications with regard to vaccination should be considered. *The Journal of Immunology,* **2006, 177: 722–728.**

Studies of inherited immunodeficiency states have strongly contributed to the current knowledge of immunological in vivo functions (1). An aspect that has only been partly explored is the influence of the modifying effects that coincident genetic factors may have on disease expression. Homozygous C2 deficiency (C2D)[3] is a well-defined deficiency of the complement system, with an estimated prevalence of ~1:20,000 in Western countries (2). C2D is associated with the susceptibility to infection caused by encapsulated bacteria and with the development of autoimmune conditions such as systemic lupus erythematosus, and it may also be a risk factor for atherosclerosis (2–4). Moreover, many persons with C2D are healthy (2–4). The phenotypic heterogeneity encountered in C2D probably indicates that other genes influence disease expression in the patients.

C2 supplies the catalytic moiety of the C3 convertase C4b2a, which can be generated through activation of the classical pathway, the principal mechanism for Ab-dependent recruitment of complement (5), or through the lectin pathway, which is an important constituent of innate immunity (6). The lectin pathway involves the recognition molecules mannan-binding lectin (MBL), L-ficolin, and H-ficolin, which form complexes with MBL-associated serine proteases and bind to microbial carbohydrates and other targets. Impaired functions of the classical pathway and the lectin pathway could both account for the clinical consequences of C2D. Complement-mediated defense in C2D mainly relies on the alternative pathway, C3 convertase C3bBb (5), the recruitment of which is usually intact in C2D.

In mice with experimental C1q deficiency, another classical pathway deficiency state, the expression of autoimmune disease, is strongly influenced by the genetic background (7). Most likely, the genetic background also influences susceptibility to infection. In patients with C2D and infections, individual case reports have described coincident findings of common variable immunodeficiency (8), low IgG2 concentrations combined with lack of the G2M(n) allotype (9, 10), and impaired alternative pathway function due to low factor B concentrations (11, 12) or properdin deficiency (13). Among the background genes in C2D, it is noteworthy that >90% of the cases are caused by the homozygous presence of a 28-bp deletion of the C2 gene in the MHC haplotype *HLA-B*18,S042,DRB1*15* and closely related haplotypes (2, 4). This implies that immune functions determined by the MHC might be expected to be unusually uniform in C2D as compared with many other immunodeficiencies.

We recently described a cohort of 40 Swedish patients with C2D in which invasive infection was the predominant manifestation (4). To date, this is the largest comprehensive study of C2-deficient patients reported by a single center. In the present investigation, 44 patients with C2D were stratified with regard to severity of infections. Selected immunological factors with potential influence on susceptibility to infection in C2D were analyzed, including IgG subclasses and their GM allotypes, concentrations of the alternative pathway proteins factor B, properdin, and factor H, and polymorphisms of MBL and the Fc receptors FcγRIIa (CD32) and FcγRIIIb (CD16).

*Department of Infectious Diseases, †Department of Pediatrics, ‡Department of Clinical Microbiology and Immunology, and §Department of Rheumatology, University Hospital of Lund, Lund, Sweden; and ¶Institute of Laboratory Medicine, Section of Microbiology, Immunology, and Glycobiology, Lund University, Lund, Sweden

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[2] Address correspondence and reprint requests to Dr. Göran Jönsson, Department of Infectious Diseases, University Hospital of Lund, SE-221 85 Lund, Sweden. E-mail address: goran.b.jonsson@skane.se

[3] Abbreviations used in this paper: C2D, homozygous C2 deficiency; IGHG, immunoglobulin constant heavy G chain; MBL, mannan (or mannose)-binding lectin.

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GM allotypes are markers of the Ig constant heavy G chain (IGHG) (14, 15). The IgG subclass-specific GM allotypes of IGHG1, IGHG2, and IGHG3 are well characterized and have important immunological functions (14–17). The homozygous presence of the IgG2 allotype G2M(n) is known to be associated with efficient IgG2 Ab responses to polysaccharide Ags both in adults and young children (18, 19). G2M(n) differs from G2M(n−), the alternative immunochemically distinguished IgG2 allotype, by the presence of methionine instead of a valine residue at CH2 position 52 in the Fc part of the IgG2 molecule (20). Furthermore, the two IgG2 allotypes differ with regard to physicochemical properties, maturation during childhood, and catabolic rate (21–23).

Among IgG subclass-specific GM allotypes, G3M(b) and G3M(g) are alternative markers for IgG3, whereas G1M(f)/G1M(a) and G2M(n)/G2M(n−) are alternative markers for IgG1 and IgG2, respectively. The alleles are inherited as haplotypes in fixed combinations, of which there are four principal variants in northwestern Europe: *GM*b;f;n*, *GM*b;f;n−*, *GM*g;a;n*, and *GM*g;a;n−*. Due to allelic exclusion, each B cell line only expresses genes from one haplotype (14, 15).

MBL polymorphism was examined on the assumption that combined C2 and MBL deficiency might be associated with increased susceptibility to infection (24, 25).

IgG receptors represent another group of factors involved in the defense against encapsulated bacteria. Homozygosity for the FcγRIIa-R131 allotype has been suggested to be a risk factor for pneumococcal infections in children and adults (26). Moreover, combined effects of FcγRIIa-R131/R131 and FcγRIIIb-NA2/NA2 have been shown to influence susceptibility to *Neisseria meningitidis* in patients with terminal complement component deficiencies (27).

Among the immunological factors investigated, we found that homozygosity for G2M(n) is protective against severe infection in C2D, indicating that efficient Ab responses to polysaccharides is of crucial importance in the patients. The impact of IgG2 levels, MBL deficiency, and components of the alternative pathway was less pronounced. There was no evidence for correlation between FcγRIIa or FcγRIIIb polymorphisms and susceptibility to infection in C2D.

Materials and Methods

Patients

Between 1977 and 2002, 40 Swedish patients with C2D were identified. Demographics and clinical manifestations have been previously described (4). A history of invasive infection, mainly septicemia and meningitis, was obtained in 57% of the patients. The predominant pathogen was *Streptococcus pneumoniae*. A diagnosis of systemic lupus erythematosus was made in 25% of the patients, and another 18% had undifferentiated connective tissue disease or vasculitis. An increased rate of atherosclerotic disease was also found. Another four patients, an essentially healthy 49-year-old male, a 36-year-old woman with undifferentiated connective tissue disease and invasive infection, a 63-year-old man with systemic lupus erythematosus, and a 12-year-old boy with ethmoiditis and an intracranial epidural abscess, were added to the study. A summary of data with stratification of the patients into four groups with regard to severity of infections is given (Tables I and II). The investigation was approved by the Lund University Research Ethics Committee (protocol LU 513-01). Written informed consent was obtained for each patient.

Igs and complement proteins

Serum and EDTA plasma were stored in aliquots at −80°C. Analysis of GM allotypes and IgG subclasses was performed as described in detail elsewhere (22, 28). In short, the IgG subclass allotypes G1M(f), G1M(a), G2M(n), and G3M(b) were quantified by a sensitive competitive indirect ELISA, whereas homozygosity and heterozygosity for G2M(n) and G2M(n−) were established by double immunodiffusion (29). Concentrations of the IgG subclasses IgG1, IgG2, and IgG3 were determined by single radial immunodiffusion using age-related reference intervals (22) expressed as 2.5–97.5 percentiles. IgG4 levels were measured with a commercial ELISA (Bindazyme; The Binding Site). IgE was determined by fluoroenzyme-immunometric assay (UniCAP; Phadia). IgM, IgG, and IgA were previously determined by turbidimetry (Cobas Mira; Roche Diagnostic Systems) in most of the patients (4), and the same method was used for the new patients included in the study. Factor B, properdin, and factor H were determined by electroimmunoassay (30). The pooled serum used for reference was assumed to contain factor B at 200 mg/L, factor H at 500 mg/L, and properdin at 25 mg/L (31). In four patients, concentrations of MBL were determined by sandwich ELISA (mAb 131-1; Immunolex) (32).

Ten patients were deceased and, in four of these, very limited amounts of serum were available for analysis. This explains why the number of patients varies somewhat for the parameters investigated.

Gene nomenclature

General guidelines were followed (33). For IGHG and the FcγRs FcγRIIa and FcγRIIIb, the HUGO Gene Nomenclature database was consulted (34), (www.gene.ucl.ac.uk/cgi-bin/nomenclature/searchgenes.pl). For allotypes of FcγRIIa and FcγRIIIb, we adopted the designations used by van Sorge et al. (26). For the GM allotypes of IGHG1, IGHG2, and IGHG3 we used the International Immunogenetics Information System database (35) (http://imgt.cines.fr) using alphabetical designations. However, asterisks for indication of subspecificity groups of the IGHG3 allotypes G3M(b) and G3M(g) were omitted. Alleles, haplotypes, and genotypes were italicized with an asterisk between the gene symbol and the allele or haplotype designations (e.g., *G3M*b, G2M*n−*; *GM*b;f;n, GM*b;f;n−*; *G2M*n/G2M*n, G2M*n/G2M*n−*, etc.). For MBL deficiency and MBL sufficiency genotypes, we used the simplified designations suggested by Kronborg et al. (36). Thus, MBL sufficiency genotypes were homozygous for the wild-type structural gene A (*A/A*). Genotypes containing a high or medium expression promoter haplotype together with a wild-type structural gene (*YA*) and another haplotype with a structural mutation (0) were also classified as MBL sufficiency genotypes (*YA/0*). MBL deficiency genotypes were those that were homozygous for a structural mutation (0/0) or contained a low expression promoter haplotype with a wild-type structural gene (*XA*) and another promoter haplotype associated with a structural mutation (*XA*/0).

DNA analysis

DNA was obtained from whole blood of 40 persons with C2D (37) and was not available in four of the deceased patients. A reference population of healthy blood donors ($n = 200$) was used for the polymorphisms investigated. MBL genotypes were analyzed as previously described (32, 38). The polymorphisms of FcγRIIa and FcγRIIIb were investigated according to Edberg et al. (39) with minor modifications. Primers for the FcγRIIa and MBL variants were synthesized by MWG Biotec, and primers for FcγRIIIb were synthesized by biomers.net. *G2M*n* and *G2M*n−* alleles were identified by PCR analysis combined with pyrosequencing (20, 40), confirming the results obtained by allotyping of the proteins.

Statistical analysis

Most of the statistics were analyzed using the computer program SPSS, version 10.0. Fisher's exact test, Mann-Whitney *U* test, and the Jonckheere-Terpstra test were used for analysis of statistical relations between patient groups and immunological markers. Distributions were compared with the CHI^2 test. Binomial probability distribution was used to ascertain differences between medians of IgG subclass concentrations. Spearman rank correlation was used in conjunction with analysis of factor B, properdin, and factor H levels. All p values were two-tailed.

Results

Patients with C2D were stratified into four groups according to severity of infections (Table I). Patients with rheumatologic manifestations were fairly evenly distributed among the patient groups (Table II). The results of GM allotyping in relationship to severity of infections are given in Table III. The patients were classified with regard to homozygosity for G2M(n), heterozygosity for G2M(n) and G2M(n−), homozygosity for G2M(n−), and the associated GM haplotypes.

The *G2M*n/G2M*n* genotype was found in nine persons, seven of whom belonged to group 1, the patient group that only had minor infections (Table III). Two patients with this genotype had a history of repeated invasive infections. Statistical analysis with

Table I. *Stratification of C2D patients (n = 44) according to the severity of infections encountered during the observation periods available*[a]

	Group 1: Minor Infections	Group 2: Pneumonia, Minor Infections	Group 3: Invasive Infection (1 Episode), Pneumonia, Other Infections	Group 4: Invasive Infections (2 Episodes or More), Other Infections
No. of patients	12	7	12	13
Episodes of:				
Pneumonia		14	12	>20[b]
Septicemia[c]			8	15
Meningitis[d]			4	10
Other invasive Infections			3	17
Total no. of invasive infections			15[e]	42[f]

[a] Major infections are specified with regard to the number of documented episodes.
[b] One patient showed 57 episodes of pneumonia.
[c] Fifty-seven percent of the septicemia episodes occurred before the age of 13, and 35% occurred after the age of 40.
[d] Seventy-nine percent of the meningitis episodes occurred before the age of 13, and 14% occurred after the age of 40.
[e] Septicemia and meningitis were documented at the same time in three patients.
[f] One patient had two episodes of septicaemia. In conjunction with one of these episodes, epidural abscess and pyelonephritis were documented.

regard to the presence of *G2M*n/G2M*n* revealed a highly significant difference between group 1 and groups 2–4 (relative risk = 9.3; confidence interval (95%) = 2.2–38.8; $p < 0.001$; Fisher's exact test). Expression of *G2M*n* was consistently associated with the *GM*b;f;n* haplotype. The rare *GM*g;a;n* haplotype was not found in the cohort. A *G2M*n* dose-dependent trend from susceptibility to infection toward resistance to infection was demonstrated in the patient groups 1–4 ($p = 0.02$; Jonkheere-Terpstra test).

None of the patients in the cohort originally described had low levels of IgM, IgG, or IgA (4). The concentrations of these proteins were also normal in the new patients added to the study. A patient with urticaria was the only patient with clearly raised concentrations of IgE (410 IU/L; reference interval, <100 IU/L). IgG1 concentrations were slightly decreased in four patients. IgG3 levels were essentially normal. In accordance with previous studies (41, 42), the levels of IgG2 and IgG4 were found to be low. Thus, low IgG2 concentrations were present in 15 of the 44 patients investigated (Fig. 1). In adults, the range was 0.56–5.1 g/L (median, 2.3 g/L; reference interval, 1.7–6.1 g/L). The decrease of IgG4 concentrations was even more pronounced (range, <0.002–0.54 g/L in adults; median, 0.02 g/L; reference interval, 0.06–1.2 g/L). When distributed according to IgG2 allotypes, all medians for IgG2 concentrations were below the medians of the age-related reference interval (Fig. 1). In the largest group, adults with *G2M*n/G2M*n−*, the difference was statistically significant ($p < 0.001$). The results also indicated a *G2M*n* dose-dependent effect on IgG2 concentrations (Fig. 1), similar to that reported in complement-sufficient persons (18, 19).

Eight adults had low IgG2 levels, and five of these had a history of invasive infections (Fig. 1). Adults with invasive infections did not differ from the other patients with regard to median IgG2 levels ($p = 0.11$; Mann-Whitney *U* test). Among the children investigated ($n = 12$), seven had moderately or slightly low IgG2 levels as defined by age-related reference intervals (not shown). Seven of the children had invasive infections, and four of these showed normal IgG2 concentrations. In conclusion, no consistent correlation was found between severity of the infections and the concentrations of IgG2. Similar conclusions were drawn with regard to the other IgG subclass proteins.

Concentrations of factor B are known to be comparatively low in C2D (41), as was also found in the present study (Fig. 2, *A* and *B*). The concentrations of properdin and factor H showed normal distribution. Factor B levels were moderately decreased in four patients, three of whom had a history of repeated invasive infections. Moreover, patient groups 3–4 showed a median factor B level (158 mg/L) that was somewhat lower than the median level in groups 1 and 2 (190 mg/L; $p = 0.02$; Mann-Whitney *U* test). We also examined the relationship between factor B and factor H concentrations, considering that this might influence alternative

Table II. *Demographic data of the CD2 patients*[a]

No Invasive Infections	Invasive Infections
Group 1 $n = 12$ (27%) Age at diagnosis of C2D (median) = 23 Person-years = 485 Person-years (median) = 43 Patients that died during the observation period ($n = 2$) Rheumatologic disease ($n = 5$)	Group 3 $n = 12$ (27%) Age at diagnosis of C2D (median) = 40 Person-years = 520 Person-years (median) = 52 Patients that died during the observation period ($n = 1$) Rheumatologic disease ($n = 5$)
Group 2 $n = 7$ (16%) Age at diagnosis of C2D (median) = 41 Person-years = 333 Person-years (median) = 49 Patients that died during the observation period ($n = 5$) Rheumatologic disease ($n = 5$)	Group 4 $n = 13$ (30%) Age at diagnosis of C2D (median) = 11 Person-years = 383 Person-years (median) = 22 Patients that died during the observation period ($n = 2$) Rheumatologic disease ($n = 3$)

[a] The patient groups are the same as those described in Table I.

Table III. *Homozygosity for the G2M*n allele and the GM*b;f;n haplotype confers resistance to invasive infection in C2D*

G2M Allotype[b]	G2M(n,n)	G2M(n,n−)			G2M(n−,n−)		
GM Haplotype	*b;f;n/b;f;n	*b;f;n/g;a;n−	*b;f;n/*b;f;n−	*n/n−	*b;f;n−/g;a;n−	*g;a;n−/g;a;n−	*b;f;n −/*b;f;n−
Group 1 (n = 12)	7[c]	2[d]	0[d]	1[e]	0	1	1
Group 2 (n = 7)	0	6	0		1	0	0
Group 3 (n = 12)	0	4	5		3	0	0
Group 4 (n = 13)	2	4	2		4	0	1
Total (n = 44)	20.9%	37.2%	16.3%		18.6%	2.3%	4.7%
Controls (n = 430)[f]	19.3%	27.2%	17.4%		17.7%	9.8%	6.7%

[a] The G2M(n) and G2M(n−) allotypes and the associated GM haplotypes are given in the C2D patients, who were divided into groups according to the severity of the infections (see Table I).
[b] In 40 patients, confirmatory investigation of *G2Mn* and *G2Mn−* was performed by DNA analysis.
[c] $p < 0.001$ (Fisher's exact test) for the homozygous presence of G2M(n) in group 1 compared with groups 2–4.
[d] As compared with the *GMb;f;n−* haplotype, the increased prevalence of the *GMg;a;n−* haplotype among heterozygous patients with noninvasive infections was not statistically significant (p = 0.15, Mann-Whitney U test).
[e] One patient could only be analyzed for G2M(n) due to lack of serum.
[f] The distribution of GM haplotypes in the C2D cohort did not differ (p = 0.72, CHI^2 test) from that found in a previously described control population (22).

pathway function in C2-deficient serum (43). However, factor B and factor H concentrations were fairly closely correlated in the patients (r = 0.64; confidence interval (95%) = 0.40–0.80; $p <$ 0.0001) in accord with previous findings in complement-sufficient persons (44). No correlation was found between concentrations of factor B and properdin (p = 0.21; r = 0.20; Fig. 2*B*).

Based on MBL genotypes, 40 C2D patients were classified as being MBL-sufficient or MBL-deficient (36). The six patients with MBL deficiency genotypes all had a history of invasive infection (Table IV). However, the difference was not statistically significant (p = 0.06; Fisher's exact test). The investigation was supplemented by measurements of MBL concentration in the sera of four patients, assuming MBL sufficiency at MBL concentrations >0.5 mg/L (45). The patients were clearly MBL-sufficient (range, 2.4–10.5 mg/L). With the inclusion of the four additional patients in the statistical analysis, the association between MBL deficiency and invasive infection in patients with C2D was found to be statistically significant (relative risk = 1.3; confidence interval (95%) = 1.1–1.6; p = 0.03; Fisher's exact test). No patient with combined C2 and MBL deficiency had rheumatologic disease.

No correlation was found between FcγRIIa or FcγRIIIb allotypes and severity of infections (Table V). The distribution of FcγR allotypes in C2D resembled that found in healthy controls.

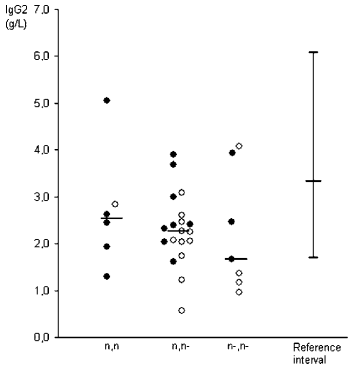

FIGURE 1. IgG2 levels in relationship to IgG2 allotypes (n,n, n,n−, n−,n−) and invasive infection in 32 C2-deficient adults. Open symbols indicate patients with invasive infections (patient groups 3 and 4, Table I). The children are divided into three age groups, each with its own symbol and reference interval. Medians are indicated with horizontal bars.

Combinations of FcγRIIa and FcγRIIIb allotypes were not informative. In a study of meningococcal disease, Platonov et al. (46) reported that FcγRIIa polymorphism influenced outcome, but not in patients below the age of 5 years. In our study, the exclusion of nine children with invasive infections that occurred below this age did not change the results.

Discussion

The *G2M*n/G2M*n* genotype was found to be protective against severe infection in C2D, suggesting the involvement of an Ig-dependent mechanism capable of compensating for the impaired immunity caused by the complement deficiency. Judging from the history of patients without severe infections, the protective function of *G2M*n/G2M*n* was already operative at early age, which implies that it did not require a mature immune system and was sustained during prolonged observation. Of note, two patients with the genotype had repeated invasive infections, which shows that the protective effect of *G2M*n/G2M*n* is sometimes insufficient. One of the patients was a child who was homozygous for the FcγRIIa-R131 and FcγRIIIb-NA2 allotypes, considered to be an unfavorable combination of FcγRs (27). However, the influence of FcγRIIa and FcγRIIIb polymorphisms was found to be low in C2D.

Basic defense mechanisms against *S. pneumoniae* are known to include specific Abs and complement. Experiments in genetically engineered mice suggest that innate immunity to *S. pneumoniae* involves natural Ab and a functional classical pathway of complement (47). Earlier animal studies have emphasized a role of the alternative pathway (48). Splenic marginal zone B cells are a likely source of natural Abs and can respond rapidly to thymus-independent Ags (49) such as capsular polysaccharides that can induce protective Ab responses (50). Furthermore, a subset of circulating CD27$^+$ memory B cells develops early in life and shares properties with splenic marginal zone B cells (51).

The strong impact of G2M(n) on immunity in C2D is difficult to fully understand. The most simple explanation is the established association between the homozygous presence of G2M(n) and the findings of quantitatively strong Ab responses to polysaccharide Ags (18, 19). Several mechanisms have been suggested through which *G2M*n* and the associated *GM*b;f;n* haplotype might promote Ab responses, including involvement of haplotype-linked genes and slow processing of Ag by macrophages (17, 18, 52). Circulating CD27$^+$ memory B cells account for Ab responses to polysaccharides and show evidence of Ab diversification at an early age before immune responses to Ag might be expected to

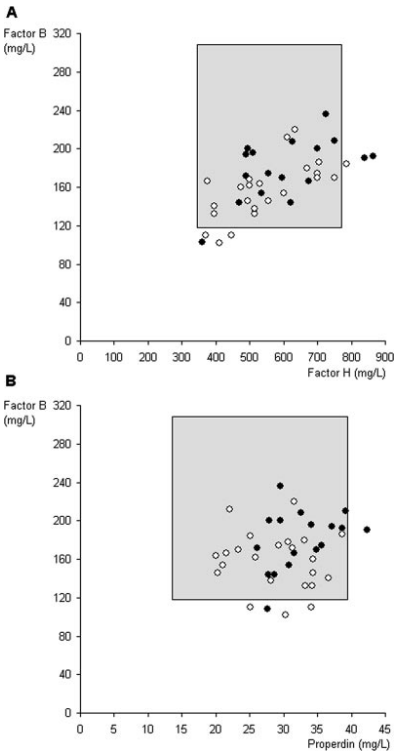

FIGURE 2. Concentrations of factor B vs concentrations of factor H (*A*) and concentrations of properdin (*B*) in 40 patients with C2D are depicted. The 95% reference areas are shaded. Open symbols indicate patients with invasive infections (patient groups 3 and 4, Table I). Factor B levels were correlated with the levels of factor H ($r = 0.64$; $p < 0.0001$), but not with those of properdin ($r = 0.20$). The median factor B concentration (158 mg/L) in patients with invasive infection was lower than the median concentration in the other patients (190 mg/L; $p = 0.02$, Mann-Whitney *U* test). Such statistically significant differences were not found for factor H and properdin.

Table V. *FcγRIIa and FcγRIIIb polymorphisms in 40 C2D patients*

	FcγRIIa Allotypes[a]			FcγRIIIb Allotypes[a]		
Severity of Infections	HH	HR	RR	NA1/1	NA1/2	NA2/2
Group 1 ($n = 10$)	3	5	2	2	1	7
Group 2 ($n = 6$)	2	3	1	0	2	4
Group 3 ($n = 11$)	2	7	2	0	3	8
Group 4 ($n = 13$)	3	7	3	1	9	3
Total ($n = 40$)	25.0%	55.0%	20.0%	7.5%	37.5%	55.0%
Controls ($n = 200$)[b]	22.5%	51.5%	26.0%	11.5%	47.0%	41.5%

[a] No correlation was found between severity of infections (see Table I) and FcγR polymorphisms.
[b] Healthy blood donors.

have occurred (51). There are four variants of B cells as determined by GM haplotypes (16). The possibility that the *GM*b;f;n* haplotype contributes to early Ab diversification in $CD27^+$ memory B cells might perhaps be considered.

Given the moderate size of the cohort investigated, statistically clear-cut results were expected for common variants of immunological factors with a strong impact on susceptibility to infection in C2D. The *G2M*n/G2M*n* genotype met these qualifications. We also assumed that the study would provide useful information concerning less frequent variants and factors with modest influence on disease expression. Based on previous reports (9, 10), we expected the *G2M*n−/G2M*n−* genotype to be associated with susceptibility to invasive infections (9, 10). A *G2M*n* dose-dependent trend from susceptibility to infection toward resistance to infection was found that supports this assumption to some extent.

Concentrations of IgG2 and IgG4 are low in deficiencies of the classical pathway, which probably reflects impaired maturation of Ig production (41, 42). Because the *GM*b;f;n* haplotype partly determines the concentrations of IgG2 (28), the question was asked of whether IgG2 levels might reflect susceptibility to infection in C2D. Indeed, low IgG2 levels were found in several patients with invasive infection, but correlations between IgG2 levels and patient groups were not statistically significant. In general accord with results of Alper et al. (41), IgG subclass concentrations did not predict the occurrence of infections in C2D.

With regard to other Igs, only one patient with C2D showed increased IgE concentrations. Considering the evidence for impaired isotype switching with very low IgG4 levels in C2D, it is conceivable that C2D might counteract development of atopic disease.

Table IV. *MBL polymorphisms in 40 C2D patients*[a]

	MBL Sufficiency Genotypes		MBL Deficiency Genotypes[b]		MBL Concentrations[c]	
Severity of infections	*A/A*	*YA/0*	*XA/0*	*0/0*	MBL-Sufficient	MBL-Deficient
Group 1 ($n = 12$)	6	4	0	0	2	0
Group 2 ($n = 7$)	5	1	0	0	1	0
Group 3 ($n = 12$)	4	3	4	0	1	0
Group 4 ($n = 13$)	8	3	0	2	0	0
Total ($n = 44$)[d]	57.5%	27.5%	10.0%	5.0%		
Controls ($n = 200$)[e]	58.0%	28.0%	7.0%	7.0%		

[a] Serum concentrations of MBL were determined by sandwich ELISA in four patients from whom DNA samples were not obtainable. The patients were divided into groups according to severity of infections (see Table I).
[b] All patients with MBL deficiency genotypes had invasive infections, but the difference was not statistically significant ($p = 0.06$; Fisher's exact test).
[c] The four patients showed MBL concentrations at 10.5, 10.0, 2.75, and 2.4 mg/L, respectively. Values >0.5 mg/L were considered to indicate MBL sufficiency (46).
[d] Combined analysis, using results of MBL genotyping and MBL measurements, indicated that the association between MBL deficiency and invasive infections in C2D was statistically significant (relative risk = 1.3, confidence interval (95%) = 1.1–1.6; $P = 0.03$, Fisher's exact test).
[e] Healthy blood donors.

Low factor B levels have been suggested to cause susceptibility to infection in C2D (11, 12). We found moderately low factor B concentrations in 13% of the patients with invasive infection and in 6% of the other patients, indicating that low factor B levels could be a minor susceptibility factor. Interestingly, a statistically significant association was found between combined C2 and MBL deficiency and the occurrence of invasive infections, suggesting that MBL has a C2-independent role in host defense (24, 25).

FcγRIIa-R131 and FcγRIIIb polymorphisms are associated with increased susceptibility to meningococcal disease in deficiencies of the terminal complement components (27). Perhaps surprisingly, no such effect was found in C2D. Phagocytosis with ligand binding to receptors for Fc and C3b/iC3b is considered to be a major defense mechanism against *S. pneumoniae* (50). Phagocytic killing of *N. meningitidis* involving Abs and the alternative pathway of complement has been described in experiments with C2-deficient sera (53). It is not known if FcγRs were required in the assay system. Antibody-dependent opsonophagocytosis of *S. pyogenes* was recently shown to require involvement of iC3b receptors (CD18/CD11b), but not FcγRs (54). Moreover, results of animal experiments indicate that FcγRs might not always be of critical importance in defense against *S. pneumoniae* (55, 56).

Abs might also mediate protective effects through other complement-dependent mechanisms in C2D. Anticapsular IgM and IgG Abs may trigger immune adherence of *S. pneumoniae* to CR1 by recruitment of C4 (57, 58). Repeated severe infections in children with C2D usually cease after adolescence (3, 4) indicating establishment of acquired immunity. Acquired immunity could involve IgA and IgG2 Abs capable of activating the alternative pathway (59). Alternative pathway-mediated serum bactericidal responses against *N. meningitidis* and *Haemophilus influenzae* type b have been documented in C2D following immunization with capsular polysaccharide vaccines (53). Janoff et al. (60) have emphasized the potential role of alternative pathway activation by anticapsular IgA Abs in defense against *S. pneumoniae*.

In a broad sense, our findings suggest that efficient Ab responses to polysaccharides are a principal cause for absence of severe infections in C2D. MBL deficiency and impaired alternative pathway function due to low factor B concentrations may increase susceptibility to infections, but they hardly have a major impact on disease expression. At the same time, the findings are consistent with the possibility that combinations of C2D with rare defects of immunity such as properdin deficiency (13) or common variable immunodeficiency (8) could be important in individual cases. Quite clearly, rheumatologic disease was not an important determinant of susceptibility to infection in the cohort investigated.

In conclusion, evidence was provided to suggest that the *G2M*n/G2M*n* genotype protects against severe infection in C2D. GM typing should be helpful for assessment of prognosis in C2D, including C2-deficient siblings of index patients presenting with infection. Vaccination with polysaccharide vaccines in C2D has been discussed (3), and it does promote bactericidal responses despite the absence of a functional classical pathway (53). The present findings indicate that Ab-dependent immunity can overcome susceptibility to infection in C2D and might thus contribute to the establishment of future rationales for vaccination in C2D and perhaps also in other complement deficiency states.

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Disclosures

The authors have no financial conflict of interest.

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Rheumatological manifestations, organ damage and autoimmunity in hereditary C2 deficiency

G. Jönsson[1,3], A. G. Sjöholm[3], L. Truedsson[3], A. A. Bengtsson[2], J. H. Braconier[1] and G. Sturfelt[2]

Objective. To analyse rheumatological manifestations, organ damage and autoimmune responses in a large cohort of patients ($n=45$) with homozygous C2 deficiency (C2D) and long-term follow-up.
Methods. Medical records were reviewed and were supplemented with a mailed questionnaire for assessment of cardiovascular disease (CVD) risk factors. Organ damage was evaluated using the Systemic Lupus International Collaborative Clinics/American College of Rheumatology Damage Index (SLICC/ACR DI). Causes for disability pensions were investigated. Autoantibodies were determined with established methods.
Results. Patients with rheumatological diseases had systemic lupus erythematosus (SLE, $n=12$), undifferentiated connective tissue disease ($n=5$) or vasculitis ($n=3$). Judging from annual SLICC/ACR DI, C2D patients with SLE run a similar risk of development of severe disease as other patients with SLE. An increased rate of CVD was observed not explained by Framingham-related risk factors. Disability pensions were mainly related to rheumatological disease. The prevalence of anti-nuclear antibodies in C2D with SLE and of anti-SS-A was 25% while anti-RNP was found in 45%. Only one patient showed antibodies to dsDNA. Formation of anti-cardiolipin antibodies (aCL) appeared to be increased in C2D despite the absence of an anti-phospholipid syndrome. The prevalence of antibodies to the collagen-like region of C1q (C1qCLR) was also remarkably high and was not related to rheumatological manifestations.
Conclusions. Severity of SLE in C2D is similar to that of SLE in other patients. Conventional risk factors do not explain the occurrence of CVD in C2D. The high prevalence of aCL and anti-C1qCLR indicates mechanisms through which impaired complement function promotes formation of autoantibodies.

Key words: Antiphospholipid syndrome, Autoantibodies, C2 deficiency, Cardiovascular disease, Complement, SLE.

Introduction

Systemic lupus erythematosus (SLE) is a B-cell-dependent autoimmune disease with strong familial aggregation [1]. A Mendelian mode of inheritance is not seen, but multiple candidate genes of susceptibility have been identified by association studies. These include major histocompatibility complex (MHC) alleles, complement deficiency genes, Fcγ receptor (FcγR) alleles and other genetic markers [2]. In experimental murine models, several genes and pathogenetic pathways have been shown to contribute to development of lupus-like disease [2, 3]. Furthermore, a broad variety of environmental factors have been suggested to be implied in the aetiology of the disease [4, 5].

The concept of complement involvement in the pathogenesis of SLE originates from the findings of hypocomplementaemia and deposition of complement proteins in target organs [6, 7] indicating that complement activation is important in the pathogenesis of SLE. During the 1970s, inherited complement deficiencies were surprisingly found to be associated with development of SLE [8]. This suggests that impaired complement function promotes autoimmune inflammation and does not protect against development of the disease.

C2 deficiency (C2D) has an estimated prevalence of $\sim$1/20 000 persons of European descent [6]. The structural gene for C2 is located in the MHC class III region together with genes for C4 and factor B [6]. Nearly all cases of C2D are caused by a 28-bp deletion in the C2 gene, a mutation associated with the *HLA-B*18,S042,DRB1*15* haplotype [9]. Deficiencies of C1q, C1r, C1s and C4 have a more heterogeneous genetic background [6].

C2 supplies the catalytic part of the C3 convertase C4b2a, which can be generated through the classical pathway or the lectin pathway of complement [10]. The classical pathway is initiated by interaction of C1q with IgM and IgG in immune complexes or with other C1q-binding structures [11]. In the lectin pathway, mannan-binding lectin (MBL) and ficolins that form complexes with MBL-associated serine proteases (MASPs), bind to target structures such as microbial carbohydrates [11]. C4b2a is generated through the actions of C1s and MASP-2. Hence, abnormal immune functions in C2D may be ascribed to impaired classical pathway or lectin pathway activity. The alternative activation pathway is usually intact in C2D and the recently reported MBL-dependent activation of C3 and the alternative pathway without involvement of C2 may play a role [12].

Among patients with C2D initially reported in the literature, about one-third showed SLE or SLE-like disease with predominance of cutaneous manifestations [6, 8]. Development of severe SLE with kidney involvement appears to be rare in C2D, but may occur [6, 8]. C2D is also known to be associated with susceptibility to invasive infections and a variety of immunological diseases, but many persons with C2D appear to be completely healthy [13, 14].

We recently described a large cohort ($n=40$) of C2-deficient patients emphasizing the high prevalence of invasive infections [14]. The C2D cohort has now been enlarged and in this investigation we have focused on rheumatological and cardiovascular manifestations in C2D. Most of the patients were subject to prolonged observation, which enabled analysis of organ damage and working capacity, issues that have not been previously addressed in patients with complement deficiency.

Patients and methods

Patients

Between 1977 and 2006, 45 Swedish persons from 33 families were identified through screening as a routine part of complement

[1]Department of Infectious Diseases and [2]Department of Rheumatology, University Hospital of Lund and [3]Institute of Laboratory Medicine, Section of Microbiology, Immunology and Glycobiology, Lund University, Lund, Sweden.

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Correspondence to: G. Jönsson, Department of Infectious Diseases, University Hospital of Lund, SE-221 85, Lund, Sweden. E-mail: goran.b.jonsson@skane.se

analysis at the Clinical Immunology Unit, University Hospital of Lund. During this period, about 46 000 analyses for complement deficiency were performed mainly with haemolytic gel assays [15]. The first collected serum samples were retrieved from hospital departments of Dermatology (1%), Internal Medicine (13%), Infectious Diseases (2%), Otorhinolaryngology (1%), Paediatrics (3%), Rheumatology (10%), General (53%) and Private Practice (7%) and from other departments (11%). More than one-third of the patients were found in southern Sweden, the rest were either sent directly to our laboratory or were referred after initial screening from other Clinical Immunology laboratories in Sweden. Seven non-index persons to a first-degree relative with C2D were identified through family studies in 18 families [14]. Among these non-index patients, one patient (Patient 3) developed SLE later in life and two patients (Patient 21 and 25) were documented for severe infection. To the previously described 40 C2D patients (Patients 1–40) [14], two female (Patients 42 and 45) and three male (Patients 41, 43 and 44) patients were added to the study. Of the 45 patients, 25 were females and 20 were males.

In the previous investigation, which included 40 of the patients, 33 were found to be homozygous for the 28-bp C2 gene deletion, *DRB1*15* and *C4A*4 B*2* [14]. Three patients (Patients 19, 37 and 38) were heterozygous for the 28-bp deletion and two of them (Patients 37 and 38) had MHC haplotypes not previously described in relation to the C2 null genes. Of the five additional C2D persons (Patients 41–45), four were homozygous for the 28-bp deletion and one heterozygous (Patient 45), but DRB1 and C4 variants were not determined.

The mean age at the time of C2D diagnosis was 32 yrs (median 35, range 1–76). The medical records contained at the time of this review a total of 1772 person-years. The average time of follow-up per person was 39 yrs (range 3–77). A control group consisting of patients with genuine SLE ($n=134$) of whom 28 had secondary anti-phospholipid syndrome (APS) was also available. Informed written consent was given by the participants and the study was approved by the Research Ethics Committee of the University of Lund and six other centres.

Supplementary data regarding clinical, genetic and autoantibody findings in the 45 C2D persons are available in Supplementary Table S1 published online.

Assessment of working capacity

Data from the Regional Social Insurance Office concerning working incapacity, i.e. temporary or permanent disability pension, were utilized for analysis of C2D-associated morbidity in 26 adult persons. Information concerning the number of inhabitants in the labour force was obtained from the Swedish Statistical Database, Stockholm, Sweden. The observation period was 1981–2003 and patients between 18 and 65 yrs with assessable data were investigated. During this observation period, the median year (1992) was chosen for registration. Data from 1992 regarding disability pension in the Swedish population and in the C2D cohort ($n=19$) were used for comparison and determination of the point prevalence on 31 December 1992.

Cardiovascular risk factors

A questionnaire modified from Bengtsson *et al.* [4] and medical records were used for assessment of traditional risk factors for development of cardiovascular disease (CVD). The following traditional risk factors for CVD were recorded: arterial hypertension (blood pressure $\geq$140/90 mmHg or treatment with anti-hypertensive drugs), diabetes mellitus (fasting glucose $\geq$7.0 mmol/l or treatment with insulin or oral hypoglycaemic agents), dyslipidaemia [high-density lipoprotein (HDL) cholesterol $\leq$ 1.6 mmol/l, low-density lipoprotein (LDL) cholesterol $\geq$ 3.4 mmol/l, or triglycerides $\geq$ 2.3 mmol/l or treatment for hyperlipidaemia], post-menopausal status, smoking, obesity [body mass index (BMI) $\geq 30\,kg/m^2$] and a family history of premature CVD in first-degree relatives. Premature CVD was defined as an acute myocardial infarction or sudden death before the age of 55 yrs in males and 65 yrs in females [16, 17]. The questionnaire was given to 25 patients ($\geq$18 years) and all 25 patients responded. Ten patients were <18 yrs of age and 10 patients were deceased. At the time when the questionnaire was distributed, only two of the six patients with a record of acute myocardial infarction (AMI) were alive. Blood samples for analysis of cholesterol and triglycerides were obtained in 13 females and 7 males. The CVD risk calculator programme published by Anderson *et al.* [18], was used to assess the risk of a cardiac event in the C2D patients. The upper limit for intervention is, according to this risk assessment model, $\geq$16%.

Laboratory studies

Available serum and EDTA plasma samples were stored in aliquots at −80°C. Assessment of anti-nuclear antibodies (ANA) was performed by indirect immunofluorescence with HEp-2 cells (Euroimmun, Lübeck, Germany) at a serum dilution of 1/400 corresponding to ANA at 14 IU/ml (WHO reference serum 66/233). Rheumatoid factors (RF) were measured by an enzyme-linked immunosorbent assay (ELISA) [19]. Anti-cardiolipin antibodies (aCL) were determined by ELISA [20], native DNA (dsDNA) antibodies with the *Crithidia luciliae* test [21] using a commercial kit (Euroimmun, Lübeck, Germany) and antibodies to the collagen-like region of C1q (anti-C1qCLR) as described by Mårtensson *et al.* [22]. Anti-C1qCLR values were given in arbitrary units (AU/l) with values <16 AU/l defined as negative. The aCL values were defined as negative (<20 IgG phospholipid units, GPLU/ml), low (20–40 GPLU/ml), medium (41–80 GPLU/ml) and high (>80 GPLU/ml). For reference, analysis of aCL ($n=100$) and anti-C1qCLR ($n=96$) was performed in healthy blood donors. Autoantibodies to ribonucleoprotein (RNP), histone, Scl-70, Sm, Sm B subunit, SS-A 52/60, SS-A 52 and SS-A 60 were determined by immunoblot analysis (INNO-LIA ANA, Innogenetics, Gent, Belgium). Indirect immunofluorescence for detection of antineutrophil cytoplasmic autoantibodies (ANCA) was performed with BIOCHIP Mosaic (Euroimmun, Lübeck, Germany). Antibodies against proteinase 3 (PR3) and myeloperoxidase (MPO) were determined by ELISA using commercial antigens provided by Wieslab AB, Lund, Sweden.

Assessment of SLE

SLE disease activity and cumulative organ damage were determined by using the SLE disease activity index (SLEDAI-2K) [23] and the SLICC/ACR DI [17], respectively. In addition, information on glucocorticoid treatment and immunosuppressive drugs was documented during the available observation period. Experienced rheumatology specialists carried out clinical evaluation of the patients. SLEDAI and the SLICC/ACR DI were established by information in the medical records.

Statistics

Differences between groups were analysed with Fisher's exact test, the χ^2 test and the Mann–Whitney test. The Kruskal–Wallis test was used to make comparisons of the aCL and anti-C1qCLR concentrations between the four patient groups given in Figs 1 and 2. All *P*-values were two-tailed. Standard mortality/morbidity ratio (SMR) was calculated in C2D persons considered at risk for AMI (30–79 yrs of age) during the follow-up period 1940–2005. Twenty-eight C2D persons in the cohort could be observed and attributed to person-time until their first AMI was recorded. The person-time found in the C2D persons was compared with data from the Swedish National Board of Health and Welfare Registries concerning age-related AMI incidences in

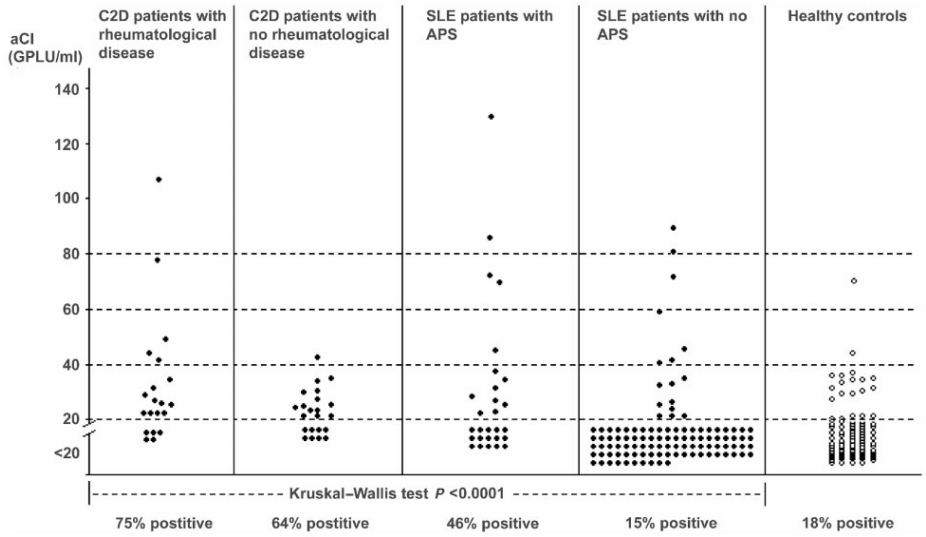

FIG. 1. Comparison between aCL concentrations in C2D patients ($n = 42$) and patients with genuine SLE ($n = 134$) using the earliest collected available serum samples. The aCL levels were defined as negative (<20 IgG phospholipid units, GPLU/ml), low (20–40 GPLU/ml), medium (41–80 GPLU/ml), and high (>80 GPLU/ml) indicated with broken lines. The C2D patients had higher concentrations of aCL than the SLE patients ($P < 0.0001$, Kruskal–Wallis test).

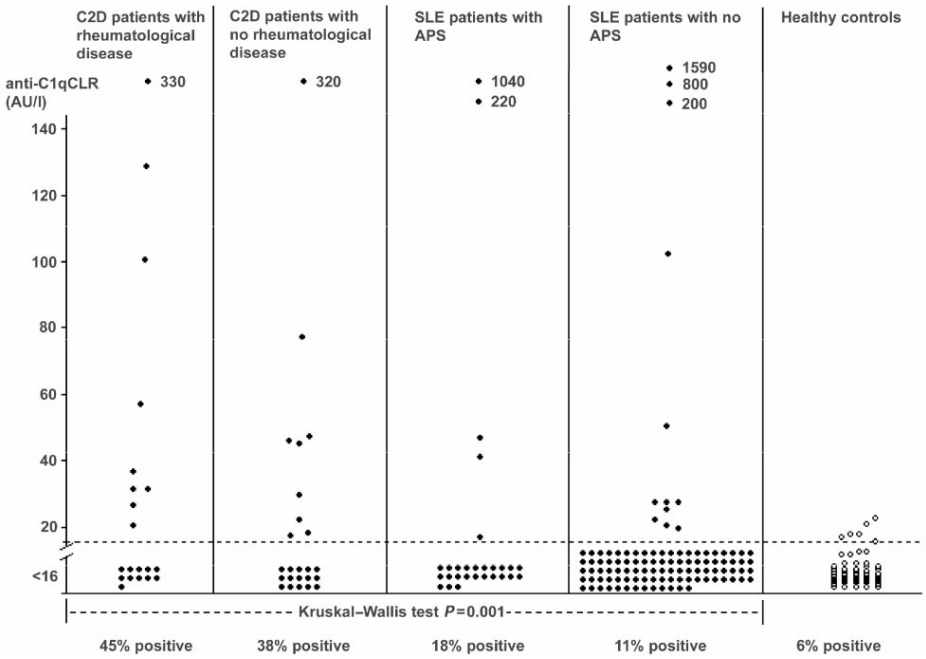

FIG. 2. Comparison between anti-C1qCLR concentrations in C2D patients ($n = 42$) and patients with genuine SLE ($n = 134$) using the earliest collected available serum samples. The upper limit of anti-C1qCLR levels defined as negative (<16 AU/l) is indicated with a broken line. The C2D patients had higher concentrations of anti-C1qCLR than the SLE patients ($P = 0.001$, Kruskal–Wallis test).

TABLE 1. Clinical findings in the 20 C2D patients with rheumatological disease

Patient no.	Rheumatological disease	ACR criteria for SLE	Other clinical manifestations
1	SLE	1, 2, 3, 5, 6, 8, 11	Alopecia, back pain, myalgia, Raynaud's phenomenon, SCLE, AMI x 2, died of AMI (34 yrs).
2	SLE	1, 5, 6, 9	Alopecia, fractures, gastrointestinal vasculitis, back pain, pancreatitis, Raynaud's phenomenon, ruptured Achilles' tendon.
3	SLE	5, 6, 10, 11	AMI, pernicious anaemia, Sjögren's syndrome.
4	SLE	1, 2, 3, 8, 10, 11	Osteoporosis, SCLE, died of septicaemia (59 yrs).
8	SLE	1, 3, 4, 5	Asthma.
11	SLE	1, 2, 3, 4, 5, 11	Alopecia, arthralgia, atrioventricular block II, diabetes, fractures, glaucoma, back pain, ruptured Achilles' tendon, died of pulmonary cancer (76 yrs).
12	SLE	1, 2, 5, 6, 7, 10, 11	Myalgia, pulmonary fibrosis, venous thrombosis and pulmonary emboli, vasculitis with skin manifestations, died of septicaemia and meningitis (51 yrs).
19	SLE	1, 2, 5, 10	SCLE, Raynaud's phenomenon.
23	SLE	1, 2, 3, 5, 10	Myalgia, Sjögren's syndrome.
29	SLE	1, 3, 4, 5, 8	Cholecystitis, back pain, myoma, pancreatitis, Raynaud's phenomenon.
43	SLE	1, 2, 3, 5, 6, 9	AMI x 2, prostate cancer, SCLE.
45	SLE	1, 2, 3, 9, 10, 11	Atrioventricular block II-III, parotid gland tumour, polyneuropathy, septicaemia.
5	UCTD	1, 2, 5	Alopecia, cholecystitis, osteoporosis, pyoderma gangrenosum, venous thrombosis and pulmonary emboli, died of AMI (75 yrs).
25	UCTD		Atopic dermatitis, meningitis, *Pustulosis palmaris et plantaris*, Raynaud's phenomenon.
27	UCTD		Arthralgia, myalgia.
33	UCTD	2, 6	Arthralgia, liver steatosis with fibrosis, alveolitis, pulmonary fibrosis.
42	UCTD	7	
24	Vasculitis		Arthralgia, erythema nodosum, pyelonephritis, septicaemia x 2, died of septicaemia (49 yrs).
31	Vasculitis		Myalgia.
34	Vasculitis	7	Malignant melanoma, venous thrombosis, septicaemia x 2.

AMI, acute myocardial infarction; SLE, systemic lupus erythematosus; SCLE, subacute cutaneous lupus erythematosus; UCTD, undifferentiated connective tissue disease; vasculitis, vasculitis with skin manifestations verified by skin biopsy in two patients; ACR, American College of Rheumatology. ACR criteria for SLE: 1. Malar rash, 2. Discoid rash, 3. Photosensitivity, 4. Oral ulcers, 5. Arthritis, 6. Serositis, 7. Renal disorder, 8. Neurological disorder, 9. Haematological disorder, 10. Immunological disorder, 11. Antinuclear antibody.

the Swedish population during the period of 1987–2003. Exact confidence intervals (CI) were calculated with the Poisson distribution. The SMR calculations were considered significant when the lower limit of CI was $\geq$1.0.

Results

Clinical manifestations

Among the 45 patients with C2D, 12 patients (8 females and 4 males) had a clinically diagnosed SLE and fulfilled four or more of the 1982 ACR classification criteria [24]. The distribution of ACR criteria for SLE and other clinical manifestations are given in Table 1. The most common ACR criteria were arthritis (83%), malar rash (92%), discoid lesions (67%), photosensitivity (67%) and serositis (42%). The mean age at diagnosis of SLE was 37 yrs (median 39, range 10–57). Seventy-five per cent of the SLE patients were above the age of 30 yrs at the time of their SLE diagnosis. Patient 12 developed a diffuse proliferative glomerulonephritis (WHO class IV) with progression to renal failure in spite of aggressive immunosuppressive treatment including plasma exchange. Two patients had involvement of the central nervous system with psychosis (Patient 4) and myelitis (Patient 29). Subacute cutaneous lupus erythematosus (SCLE) was found in four patients (Patients 1, 4, 19 and 45).

Undifferentiated connective tissue disease (UCTD) and/or incomplete SLE (<4 ACR criteria) were found in five patients (Patients 5, 25, 27, 33 and 42). The principal manifestations were skin disease (3/5) and arthritis (3/5). Patient 42 developed membranous glomerulonephritis (WHO, class V) with proteinuria and haematuria at the age of 35 yrs without progression to renal failure.

Another three patients (Patients 24, 31 and 34) had widespread vasculitis with skin manifestations that were mainly localized to the trunk or limbs. In two patients, the vasculitis diagnosis was histopathologically verified. One patient (Patient 24) had verified anti-Scl-70 antibodies and was also repeatedly positive for anti-PR3. Patient 34 was treated for a suspected Wegener's granulomatosis although repeated biopsies from the mucous membrane of the nose did not confirm the diagnosis. However, the clinical picture was characterized by almost constant rhinorrhea, sinus pain, cough, shortness of breath, muscle pain, arthritis and palpable purpura and blister-like vasculitis lesions of the skin. Evidence of kidney involvement with haematuria and proteinuria was also found, but a kidney biopsy was never performed. The patient did not develop renal failure and analysis of ANCA was negative.

Organ damage

SLICC/ACR DI was assessed in the 12 C2D patients with SLE. The mean SLICC/ACR DI score was 3.8 at 10 yrs after diagnosis. A main cause of damage was cardiovascular manifestations (Table 2). Documented cardiovascular manifestations included five AMI in three SLE patients (Patients 1, 3 and 43). Two patients had valvular disease (Patients 11 and 12), atrioventricular block II-III was found in two patients (Patients 11 and 45) and pericarditis with duration for >6 months was documented in two patients (Patients 3 and 12). Patient 43 had gone through surgery with a three-vessel coronary bypass grafting. The autopsy report concerning Patient 1, a 34-yr-old woman, revealed severe atherosclerosis, a cerebrovascular accident, a dissecting aorta aneurysm and two myocardial infarctions.

Patients with documented AMI (Patients 1, 3, 5, 10, 26 and 43) did not have high levels of anti-C1qCRL (median < 16 AU/l, range < 16–31 AU/l) or aCL (median < 20 GPLU/ml, range < 20–26 GPLU/ml). The patients had their first AMI at a mean age of 56 yrs (median 56, range 33–77 yrs). In general, the frequency of conventional Framingham risk factors was fairly low in the C2D cohort and the calculated percentage risk of suffering a cardiac event during the following 10 yrs was also found to be low in 20 accessible adult C2D patients (mean 6%, median 5%, range 0–16%). Thus, only one patient reached the limit for intervention against development of CVD (Patient 27, 16%).

During the available observation period, the SLE patients were treated with an average dose of glucocorticoids of 2.5 mg/day (range 0–20) with a maximum dose of 40 mg/day. Three SLE patients (Patients 1, 4 and 12) received periodical treatment with azathioprine and hydroxychloroquine. One SLE patient (Patient 45) was treated with pulses of cyclophosphamide for extensive skin manifestations. A cushingoid appearance developed in four patients (Patients 1, 2, 11 and 12).

Calculations concerning the risk of AMI in the C2D cohort ($n = 28$) showed a statistically significant increased SMR of 4.1

TABLE 2. Predominant organ damage found in C2D patients with SLE was mainly related to CVD and skin manifestations. For each patient, the SLICC/ACR DI scores are given for each category after 10 yrs of observation

Patient no	1	2	3	4	8	11	12	19	23	29	43	45	
SLICC/ACR DI categories													Sum per category
Ocular	1					1							2
Neuropsychiatric	2		1	1								1	5
Renal							3						3
Pulmonary							3						3
Cardiovascular	2		2			2	2				2	1	11
Peripheral vascular													0
Gastrointestinal													0
Musculoskeletal		3		1		3		1					8
Skin	2	1		2		2	1	1				2	11
Premature gonadal failure													0
Diabetes						1							1
Malignancy						1					1		2
SLICC/ACR DI scores	7	4	3	4	0	10	9	2	0	0	3	4	

TABLE 3. Presence of antibodies against nuclear antigens in adult C2D patients in relation to diagnosis given as number and percentage of patients

	SLE	UCTD	Vasculitis	No rheumatological disease	All C2D patients
	($n=12$)	($n=5$)	($n=3$)	($n=11$)	($n=31$)
ANA[a]	3 (25%)				3 (10%)
Anti-dsDNA[b]	1 (8%)				1 (3%)
Antibodies[c] to:	($n=11$)	($n=5$)	($n=3$)	($n=10$)	($n=29$)
Histone	1 (9%)		2 (66%)	2 (20%)	5 (17%)
RNP	5 (45%)			1 (10%)	6 (21%)
Scl-70			1 (33%)		1 (3%)
Sm	2 (18%)				2 (7%)
Sm B subunit	1 (9%)				1 (3%)
SS-A 52/60	1 (9%)	1 (20%)			2 (7%)
SS-A 52		1 (20%)			1 (3%)
SS-A 60	2 (18%)				2 (7%)

[a]Determined by indirect immunofluorescence on HEp2 cells.
[b]Determined by the *Crithidia luciliae* test.
[c]Determined by immunoblot analysis (INNO-LIA ANA).

(95% CI, 1.5–8.9). Continuous long-term glucocorticoid treatment (>5 yrs) for rheumatological disease was observed in eight patients (Patients 1, 2, 4, 11, 12, 34, 42 and 45). There was no statistically significant increased risk for AMI in this group (SMR 2.3, 95% CI 0.06–13). In the 20 patients with no long-term treatment, the risk of AMI resembled that found in the C2D cohort in general (SMR 4.8, 95% CI 1.6–11). In conclusion, the risk of AMI is increased with four times in C2D as compared with the general Swedish population. Whether glucocorticoid treatment of rheumatological disease reduces the risk of AMI in C2D is more uncertain since our calculations are based on observations in only eight patients.

Working capacity

Nine females and one male of 26 investigated patients received disability pension. The median age for receiving disability pension was 43 yrs (range 37–63). Among C2D patients, the underlying cause of the pension was SLE in 7/9 patients and other rheumatological diseases (UCTD and vasculitis) in 2/6 patients. One of the 11 C2D patients without rheumatological disease received a disability pension because of severe chronic obstructive pulmonary disease. Thus, rheumatological disease (SLE, UCTD and vasculitis) was the principal cause of disability in C2D ($P=0.01$, RR = 2.4, 95% CI 1.1–4.7, Fisher's exact test). On 31 December 1992 (point prevalence), about 8% of the labour force received disability pension in Sweden compared with 21% of the investigated patients in the C2D cohort.

Infections

Invasive infections were documented in nine of the 20 patients (45%) with rheumatological disease, and three of them died of septicaemia (Patients 4, 12 and 24). Pneumonia was documented in six SLE patients, in three patients with UCTD, and in two patients with vasculitis. About 80% of the C2D patients with recurrent invasive infections were under the age of 18 yrs. Invasive infections among the patients without rheumatological disease ($n=25$) were documented in 17 patients (68%).

Autoantibodies

Findings with regard to ANA are summarized in Table 3. Only three SLE patients showed a positive immunofluorescence test for ANA with HEp-2 cells. Antibodies to dsDNA were only found in one patient (Patient 45). The rarity of anti-dsDNA antibodies was further verified by analysis of samples obtained during repeated SLE flares (SLEDAI-2K > 4). Single flares were investigated in Patients 1, 2, 8, 19 and 23; two flares were investigated in Patient 12. Anti-dsDNA antibodies were not found in these SLE patients. Antibodies to RNP and histone were each present in about 20% of all patients with C2D. Anti-RNP was more prevalent in SLE ($P=0.02$, RR = 8.2, 95% CI 1.1–61.2, Fisher's exact test) than in the other patient groups. All patients with SCLE (Patients 1, 4, 19 and 45) had antibodies to RNP or SS-A. In the three SLE patients with Raynaud's phenomenon, anti-RNP antibodies were present in two (Patients 1 and 19), and anti-SS-A in one (Patient 4). We found no anti-SS-A antibodies in the two SLE patients with sicca symptoms (Patients 3 and 23), but both had anti-Sm antibodies.

Three patients with SLE and one patient with vasculitis had increased concentrations of RF (median 38, range 16–76 IU/ml). The prevalence of anti-C1qCLR and aCL was high among the patients with C2D (Figs 1 and 2). In six patients (Patients 11, 12, 23, 24, 26 and 33), a medium or high level of aCL were found (median 47, range 42–107 GPLU/ml). The patients with increased aCL levels were significantly older than the aCL negative patients ($P=0.04$, Mann–Whitney test). SLE patients with infection have been reported to have transiently increased aCL levels [25]. However, we found no correlation between aCL and a history of invasive infection in the C2D cohort.

The first available blood sample in the SLE control group was used for analysis of aCL and anti-C1qCLR. Twenty-eight patients in the SLE control group had a clinical verified APS. The results found in the SLE control group were compared with the first available blood sample in the C2D patients. The C2D patients had higher concentrations of aCL and anti-C1qCLR as compared with the patients with genuine SLE (Figs 1 and 2, $P<0.0001$, $P=0.001$, respectively, Kruskal–Wallis test).

Despite the high frequency of aCL in the C2D group, very few patients had venous thrombosis (Patients 5, 12 and 34). Only one of these (Patient 12) had aCL (44 GPLU/ml). The significance of the aCL in Patient 12 is questionable, since the patient died of septicaemia with disseminated intravascular coagulation and severe uraemia that may have caused the pulmonary embolism that was found at autopsy. Prior to that, the patient had neither documented aCL nor APS-associated manifestations. In the two other patients, aCL was negative (Patient 5) or once weakly positive (25 GPLU/ml, Patient 34). Three patients (Patient 5, 11 and 12) had valvular disease and two (Patient 11 and 12) had aCL at a medium level (42 and 44 GPLU/ml, respectively). A Libman–Sacks endocarditis was not documented in these patients.

Longitudinal analysis (mean 7 yrs, range 1–15 yrs) of 20 serum samples from five C2D patients with SLE (Patients 1, 2, 3, 4 and 19) was performed in order to examine if ANA, anti-dsDNA and anti-C1qCLR varied in accordance with disease activity.

Autoantibody levels were stable over time despite moderate changes of the SLEDAI-2K score (range 0–6).

Causes of mortality

Ten patients with C2D died during the observation time. In six patients, death was due to severe infection. Three patients died of AMI and one patient died of breast cancer. Of the four patients with SLE, who died during the observation period, two died of invasive infection, one of lung cancer and one of AMI (Table 1).

Discussion

Deficiency states within the classical pathway of complement are the strongest known susceptibility factors for development of SLE [2]. We present here clinical and laboratory data from a large C2D cohort gathered at a single centre.

During recent years, a hierarchy within the classical pathway has been established [1] in that the risk for SLE development and disease severity is high in C1 and C4 deficiency states and more modest in C2 deficiency. SLE is rare in patients with complete C3 deficiency. Furthermore, SLE associated with C2D in early studies was described as a generally mild clinical subset of the disease [26]. Skin and joint disease predominated in the patients, while severe manifestations such as serositis, neuropsychiatric SLE and glomerulonephritis were mostly absent. The results of the present study suggest that C2-deficient patients with SLE run virtually a similar risk of development of severe disease as other patients with SLE. Thus, the mean annual organ damage score during course of disease equalled that found in an epidemiologically recruited cohort of SLE patients in southern Sweden [27]. The female predominance among SLE patients with C2D is well established [26] and resembles the ordinary female/male distribution in the disease [28]. This is in contrast to C1q deficiency where female/male distribution is almost equal.

Regarding complement deficiency states within the classical pathway and development of SLE, several issues could be addressed from an epidemiological point of view. Patients with deficiency of C1q or C4 usually develop SLE early in life, which facilitates the recognition in cross-sectional studies. We would like to stress that the true prevalence of SLE in C2D is not known, but has been estimated to be in the order of 10% [6]. We believe that this estimation is probably too low since SLE in C2D persons may develop later in life and cross-sectional surveys might underestimate frequencies. In our C2-deficient patients, SLE was diagnosed at a median age of 39 yrs, which is comparable to findings in epidemiologically recruited SLE patients [28]. Four of our patients developed SLE at an age above 50 yrs. In the C2-deficient patients, the predominate finding during infancy and childhood was recurrent severe infections [14]. Finally, SLE in complete C3 deficiency is considered to be rare, but this could be subjected to an underestimation due to lack of data. Thus, there is a need for long-term prospective cohort studies to assess the prevalence of SLE in complement deficiency states other than C1q and C4 deficiency,

Among the C2D SLE patients, the high frequency of severe organ damage was mainly due to cardiovascular damage resembling that seen in genuine SLE [27]. In an attempt to clarify this finding, medical records and a questionnaire concerning Framingham risk factors were used. However, assessment of Framingham-related risk factors failed to explain the high cardiovascular damage rate. Thus, the cardiovascular damage is likely to be a more direct consequence of the complement deficiency. In recent studies, MBL deficiency has been associated with coronary artery disease [29, 30]. Furthermore, the vascular damage has been shown to be enhanced in genetically engineered C3-deficient mice [31]. These data indicate that development of cardiovascular damage in C2D might be related to impaired function of the classical and also the lectin pathway.

Analysis of working capacity demonstrated a marked impact of C2D on the general health status. Rheumatological disease was the main cause of chronic illness among the adult patients. Thus, 21% of the cohort received a disability pension, which equals previous investigations (19%) of unselected SLE patients during long-term follow-up [32].

Low or absent ANA titre have been consistent findings in C2-deficient patients with rheumatological manifestations [6, 33]. Furthermore, antibodies to native DNA appear to be rare [6, 26]. These serological features were confirmed in the present study. Furthermore, we found no evidence of fluctuating antibody levels in conjunction with changes in disease activity. As compared with some earlier reports [6], the prevalence of anti-SS-A antibodies was not particularly high.

A novel finding was the high prevalence of aCL and anti-C1qCLR in C2D. The cause of this deviated autoimmune response is not known but might be related to the importance of complement for elimination of autoreactive lymphocytes [34] and elimination of potential autoantigens [6]. Our observation that patients with aCL had a higher frequency of anti-C1qCLR than patients without aCL supported this idea. The majority of C2D persons is homozygous for the *HLA-B*18,S042,DRB1*15* haplotype [35]. This implies that their immune responses governed by MHC genes are expected to show a restriction that might contribute to the antibody profile.

Among anti-phospholipid antibodies, aCL predominate and are strongly associated with the APS and development of thrombotic events [36]. The aCL have also been reported to play a role in development of atherosclerosis [37] and might well have contributed to cardiovascular events in C2D. However, in this study the C2D patients with aCL did not show recurrent thrombosis or other manifestations of APS. Fetal loss induced with anti-phospholipid antibodies in mice is prevented by inhibition of complement activation with heparin [38]. Moreover, complement activation with cleavage of C2 has been reported to be a characteristic finding in patients with an APS [39]. Most likely, C2D protects against some manifestations of the syndrome.

A potentially important immunological mechanism in atherosclerosis involves formation of immune complexes showing strong pro-atherogenic activity in animal models [40]. Interestingly, C1q-containing immune complexes may promote development of atherosclerosis by inhibiting the function of cholesterol 27-hydroxylase in human arterial endothelium and macrophages [41]. Even if we found no correlation between the anti-C1qCLR and cardiovascular damage, these antibodies may be regarded as indicators of *in vivo* formation of C1q-containing complexes providing a potential link between impaired classical pathway function and development of cardiovascular damage in C2D.

In conclusion, a large cohort of C2-deficient patients with long-term follow-up provided a partly unique basis for evaluation of disease manifestations and mechanisms associated with impaired classical pathway and lectin pathway functions. The severity of SLE in C2D does not differ from disease severity in genuine SLE patients. Novel findings included a high prevalence of aCL and anti-C1qCLR in C2D. The absence of APS manifestations suggests that complement dysfunction might partly prevent biological effects of aCL.

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IV

Vaccination against infections with encapsulated bacteria in hereditary C2 deficiency: great variation in antibody response

Göran Jönsson, Eva Holmström, Barbro Selander, Vivi-Anne Oxelius, Jean Henrik Braconier, Gunnar Sturfelt, and Lennart Truedsson.

From the Department of Infectious Diseases (GJ, JHB), the Department of Pediatrics (VO), and the Department of Rheumatology (GS), University Hospital of Lund, and the Institute of Laboratory Medicine, Section of Microbiology, Immunology and Glycobiology (GJ, EH, BS, LT), Lund University, Lund, Sweden.

Correspondence: Göran Jönsson, Department of Infectious Diseases, University Hospital of Lund, SE-221 85, Lund, Sweden. E-mail: goran.b.jonsson@skane.se

Abbreviations used in this paper: C2D, hereditary C2 deficiency or C2-deficient; GMC, geometric mean concentration; SLE, Systemic lupus erythematosus.

Abstract

Hereditary C2 deficiency (C2D) is a common form of complement deficiency in Caucasians (~1:20,000). C2D is recognized as an important susceptibility factor for invasive infections caused by *Streptococcus pneumoniae* and *Haemophilus influenzae* type b (Hib). The infections are most pronounced during childhood indicating that antibody-mediated acquired immunity may be operative despite the absence of a functional classical pathway. Previous reports concerning C2D persons also suggest that specific antibodies may be obtained through vaccination. To further investigate this contention, C2D persons were vaccinated with Pneumo 23® (n=25) and ActHIB® (n=21). Analysis of specific immunoglobulin levels to pneumococci serotype 6B, 7F, and 23F, and Hib, before and after vaccination, was performed. For reference, a vaccinated control group of 51 healthy persons was available. Post-vaccination of specific IgG antibodies at a level >1 mg/L was found in similar frequency in the C2D persons (n=21 and n=22, 84-88%, respectively) and controls (n=41 and n= 42, 81-83%, respectively) against pneumococci serotype 6B and 23F (p=1.0 and p=0.7, respectively). Conversely, the response to serotype 7F at a level of specific IgG>1 mg/L was impaired in C2D persons compared to controls (n=19, n=49, respectivly, p=0.01). Further analysis of C2D persons with a more than two-fold antibody increase or post-vaccination concentrations indicated an impaired vaccination response to pneumococci serotype 7B and Hib (p<0.001). In conclusion, the vaccination responses varied from equal to controls as for pneumococci serotype 6B and 23F and to clearly impaired as for pneumococci serotype 7F and Hib. However, most C2D persons appeared to benefit from the immunization.

Introduction

The complement system is part of the innate immunity and contributes to many immune functions that protect against severe infections as well as autoimmune manifestations. The system can be activated though three mechanisms, classical pathway ($C1qr_2s_2$, C4, C2, C3), the alternative pathway (C3, factor B, factor D and properdin), and the more recently recognized lectin pathway. The three activation pathways convey to formation of C3 convertases, C4b2a for the classical pathway and lectin pathway and C3bBb for the alternative pathway. The C3 convertases produces the principal opsonins C3b and iC3b that stimulate phagocytosis. The activation subsequently continuous with the late complement components (C5-C9) that assemble to a cell lysing membrane attack complex that may kill gram-negative bacteria such as *Neisseria (N.) meningitidis* and *Haemophilus influenzae* type b (Hib). Conversely, gram-positive bacteria like *Streptococcus (S.) pneumoniae* resist the bactericidal action of C5-C9. Complement activity still plays a central role in stimulating inflammation through the release of the pro-inflammatory mediators C3a and C5a, and in the improvement of the adaptive immune response to *S. pneumoniae.*

Hereditary deficiency of the second component of complement (C2D) is one of the most common complement deficiency states in populations of Western descent and has an estimated prevalence of at least 1:20,000 (1, 2). Two principal variants of C2D has been described (3, 4). The predominant variant of C2D is type I (90%), which is caused by homozygosity for a 28-base pair deletion in the C2 gene located within the major histocompatibility complex (MHC) haplotype *HLA-B18, SB42, DR2,* resulting in a complete lack of C2 synthesis (3-5). C2D is mainly associated with autoimmune diseases such as systemic lupus erythematosus (SLE) and with an increased susceptibility to infections caused by encapsulated bacteria such as *S. pneumoniae* and Hib (1, 2, 6, 7). C2D may also be a risk

factor for development of atherosclerosis (6). On the other hand, many persons with C2D are healthy (2, 6, 7). The infections are thought to result from impaired opsonization of the bacteria due to absence of C4b2a-mediated cleavage of C3. Although C3-dependent opsonization remains possible though the alternative pathway, it is less efficient (8). Thus, the immune responses to certain antigens may be partly impaired in C2D (2).

S. pneumoniae is globally recognized as a common pathogen in all age groups and causes infections such as otitis media, sinusitis, community-acquired pneumonia, septicemia, and meningitis. The severity of the infections caused by *S. pneumonia* is associated with age (children and the elderly) and with the burden of underlying diseases such as diabetes mellitus, lung disease and cardiac failure. It accounts for almost 50% of bacterial isolates from the cerebrospinal fluid and 30% from blood isolates of young infants with serious infections in developing countries (9). The polysaccharide capsule mediates virulence of both *S. pneumoniae* and Hib, and determines the serotype. At present 91 serotypes of *S. pneumoniae* are known (10-12). The antigenic variation of the serotypes is due to the heterogeneous structural and chemical composition of the capsular polysaccharides (12). Current data suggest that the 11 most common serotypes cause at least 75% of invasive disease in all regions. The diversity of the pneumococcus capsule poses a serious obstacle to the design of a universal vaccine. Pneumo23® contains 23 purified capsular polysaccharides from *S. pneumoniae* accounting for approximately 90% of serotypes associated with infections in the Western countries with an average protective efficacy of about 60%–70% (13).

Six serotypes of *Haemophilus influenzae* have been described (14). Before the introduction of conjugate Hib vaccine, children experienced very high rates of invasive Hib disease: 400–700

cases per 100,000. The use of vaccine has resulted in a 90% decline in the rate of invasive Hib disease (15). However, patients with immunoglobulin deficiencies, complement deficiency, reduced splenic function or other immunological disorder have an increased susceptibility to invasive infections caused by encapsulated bacteria (1, 2, 6, 16). The resistance to invasive infections causes by encapsulated bacteria is, besides complement function, dependent on presence of natural IgM, acquired antibodies, and on phagocytosis.

We recently described a cohort of 40 C2-deficient (C2D) patients with a high frequency of severe infections (57%) mainly caused by encapsulated bacteria (6). In a follow-up study of 44 C2D persons, the importance of *G2M*n/G2M*n* genotype was emphasized as protective against severe infections suggesting the involvement of an Ig-dependent mechanism (17). The C2D cohort has now been enlarged and in this study we have focused on antibody responses following vaccination with the 23-valent pneumococcal vaccine Pneumo23® and to Haemophilus b conjugate vaccine ActHIB®.

Results

Correlation of susceptibility to infection and specific antibodies to pneumococci and Hib

The C2D persons were stratified into four groups in accord to severity of infections, in order to facilitate further analysis of different vaccination responses in relation to documented infections (Table 1). Group I consisted of persons with only minor infections; group II had minor infections and at least one documented pneumonia; group III had one invasive infection combined with pneumonia and other infections. The fourth group had at least two invasive infections. About 65% of all episodes with invasive infections (meningitis and septicemia) occurred before the age of 13.

No significant difference in fold increase was found for the investigated antibodies, IgG, IgA and IgM between group I and groups II-IV. However, when we compared the pre-and post-vaccination geometric mean concentration (GMC) there were implications that group I had higher pre-vaccination level of IgG to pneumococcal serotype 6B and 23F than groups II-IV (Table 2). The reverse was found for the pneumococcal IgM antibodies. Thus, groups II-IV had higher levels of IgM antibodies to serotype 6B and 23F than group I. Furthermore, a significant fold increase was only found for IgM and in groups II-IV to serotype 23F ($p<0.05$, Mann-Whitney *U* test). When comparing group I against the combined groups II-IV no difference between pre-and post-vaccination GMC for any immunoglobulin isotype to serotype 7F or Hib was shown.

Analysis of immunoglobulin concentrations

All 25 vaccinated C2D persons had normal levels of IgG, IgA, and IgM. One female (no. 33) had a slightly increased IgG1 concentration. Three adult C2D persons (Patients 21, 42, and 43) had a fairly low IgG2 concentration (mean 1.2 g/L, range 1.2-1.3 g/L, reference interval 1.7-6.1 g/L). In all adult C2D persons, the mean IgG2 concentration was 2.5 g/L (range 1.2-5.1 g/L). Patient 21 and 43 showed a response that was below average to the pneumococcal antigens as compared to the other C2D persons. Patient 42 responded well to both vaccines. Patient 28, a two-year-old boy, had a moderately low IgG2 concentration of 0.39 g/L (age-related reference 0.43-2.54 g/L). However, he responded very well to the pneumococcal vaccination (6B, 73-fold increase, 7F, 38-fold increase, and 23F, 4-fold increase). The four other children had a normal IgG2 concentration in relation to their age-related reference intervals. No consistent correlation was found between severity of infection and the concentrations of IgG1 or IgG2. Among the 51 healthy controls, we found no abnormal immunoglobulin concentrations.

Antibody responses to Hib vaccination

In general, the 21 C2D persons responded fairly well to immunization with the *H. influenzae* type b conjugate vaccine (Figures 1D, 2D, and 3D) The vaccination gave a mean 82-fold increase (range 0.6-686 mg/L, $p<0.0001$, Wilcoxon signed rank test) in the C2D persons and in controls a mean 278-fold increase (range 1.2-1400 mg/L, $p<0.0001$, Wilcoxon signed rank test) of IgG anti-Hib antibodies. Pre-vaccination concentrations of anti-Hib IgG and IgA showed no difference between C2D persons and controls ($p=0.3$, $p=0.08$, respectively, Mann-Whitney *U* test). Perhaps surprisingly, the pre-vaccination concentrations of anti-Hib IgM were found to be significantly higher in C2D than compared with controls ($p=0.006$, Mann-Whitney *U* test). This finding could, however, not be explained by difference in resistance to severe infection (group I) or an increased susceptibility to infection (groups II- IV) found in the C2D persons ($p=0.2$, Mann-Whitney *U* test).

Before immunization 12 C2D persons (52%) and 22 controls (42%) had a pre-vaccination concentration of specific IgG>1.0 mg/L ($p=0.3$, Fisher's exact test). After vaccination against Hib, 19 (90%) C2D persons and 51 (100%) of controls reached this level ($p=0.08$, Fisher's exact test). The two remaining C2D persons (no. 42 and 47) had a post-vaccination concentration of 0.9 mg/L. In the C2D persons, the pre-vaccination GMC was 0.9 mg/L (range 0.05-12.0 mg/L) and in controls 0.6 mg/L (range 0.05-31.0 mg/L) of IgG anti-Hib antibodies. Post-vaccination GMC rose to 9.4 mg/L (range 0.9-48.0 mg/L) and in controls 35.2 mg/L (range 1.5-77.0 mg/L). The C2D persons also showed an increase in IgA and IgM antibody levels (both $p<0.0001$, Wilcoxon signed rank test). In conclusion, a majority of the C2D persons obtained a level of specific IgG>1.0 mg/L considered as protective, but the response measured as fold increase or post-vaccination concentration of IgG, IgA and IgM antibodies was significantly lower in the C2D persons compared with the controls ($p<0.01$,

Mann-Whitney *U* test). The vaccination response of IgG, IgM and IgA anti-Hib antibodies was not obviously influenced by previously encountered infections. Both the C2D persons and controls showed an IgG2 subclass predominance of antibodies to Hib (both had 62.5% of IgG2 pre-and post-vaccination, Figure 4D and 5D).

Antibody responses to 23-valent pneumococcal polysaccharide vaccine

The 25 C2D persons responded well to immunization with the 23-valent pneumococcal polysaccharide vaccine depending on the antigen. Among vaccine-related serotypes, there was evidence of a trend toward protection against serotype 6B and 23F but not compellingly for serotype 7F (Figure 1A-1C). The C2D responded with a statistically significant higher fold increase as compared to controls for the serotype 6B (Figure 1A, $p<0.005$, Mann-Whitney *U* test). Between the C2D persons and controls, there was no statistical difference found for any of serotypes in IgG pre-vaccination antibody concentrations ($p>0.07$, Mann-Whitney *U* test). Twenty-four of the C2D persons (96%) responded with a 2-fold increase or had a post-vaccination concentration of IgG>1.0 mg/L to at least one serotype and 20 of persons with C2D (80%) responded to all three serotypes. The corresponding rates for controls were 98% (50/51) and 84% (43/51) to all three investigated serotypes. However, statistical analysis of the number of persons with a 2-fold increase to only one of the three serotypes revealed a significant difference between C2D persons (n=14) and controls (n=47, $p=0.002$, relative risk 0.6, 95% CI 0.4-0.9, Fisher's exact test). The main explanation for this difference was a relatively low response rate to serotype 7F in the C2D persons (fold increase $p<0.0001$, post-vaccination concentration $p=0.0006$, Mann-Whitney *U* test). The number of responders to serotype 7F at a level of >1 mg/L was also shown to be lower in the C2D persons (19/25, 76%) compared to controls (50/51, 98%, $p=0.004$, relative risk 0.8, 95% CI 0.6-1.0, Fisher's

exact test). Similar to controls, more than 91% of the IgG anti-pneumococcal antibodies in the C2D persons were of the IgG2 subclass (Figure 4A-C, 5A-C).

The C2D persons achieved a good IgA response to serotype 6B and 23F, but not to serotype 7F (Figure 2A-2C). Pre-vaccination concentration of antibodies to serotype 6B and 7F was found to be the same as compared with controls ($p>0.8$ and $p>0.7$, respectively, Mann-Whitney *U* test). The C2D persons had a higher pre-and post-vaccination concentration of 23F anti-IgA antibodies compared to controls ($p=0.02$, $p=0.05$, respectivly, Mann-Whitney *U* test).

The IgM pre-and post-vaccination antibody concentrations for all serotypes showed no difference between C2D persons and controls ($p>0.08$, Mann-Whitney *U* test). On the other hand, there was a significant difference in fold increase that was most profound for serotype 7F with lower levels in the C2D persons (Figure 3A-3C).

Investigations of antibody responses over time in adult C2D persons

Two of the C2D persons (C2D no. 3 and no. 25) showed a relatively pronounced and lasting IgG response to the given pneumococcal vaccination (Figure 6B and 6D). C2D no. 3 and C2D no. 2 were siblings and both had SLE but in C2D no. 2 a weaker more rapidly declining response was seen. The reason for this difference is not readily explainable but suggests that C2D *per se* does not severely limit responses to vaccination with polysaccharide antigens. Patient no. 19 had also SLE and was recorded for two episodes of pneumonia. She responded with a high and long lasting level to serotype 7F, but not at all to serotype 6B and 23F. Perhaps could this response be attributed to her unique MHC haplotype not previously described in relation to the C2 null gene (6). C2D no. 25 was documented for meningitis

caused by *Neisseria meningitidis* at the age of 12 years. Before the age of 36 years she also experienced two episodes of pneumonia. In the analysis of her vaccination response, it cannot be excluded that we also measured previously acquired anti-pneumococcal antibodies (Table 1, Figure 6D). Nevertheless, the specific antibodies remained at a high level for all three serotypes with duration of at least 5 years.

Influence of G2M(n) allotype and antibody concentrations

GM allotypes are markers of the immunoglobulin constant heavy G chain (IGHG) (18, 19) and may influence the obtained specific antibody levels (20-22). However, we found no correlation between the presence of G2M(n) and antibody responses to the given vaccines. The low number of C2D persons with homozygosity for G2M(n-) (n=4) hindered statistical analysis.

Discussion

The vaccination responses to encapsulated bacteria in persons with complement deficiency states have previously been investigated in properdin deficiency, C5-C9 deficiency, and in C2 deficiency (23-26). Normal vaccination responses to capsular polysaccharides have also been reported in C3 deficiency (26, 27), implying that obviously impaired responses to such antigens should not be expected in C2D. Properdin deficiency predisposes to fulminant meningococcal infection (20-50%) and vaccination is strongly indicated. The vaccine response to polysaccharide antigens in a person with properdin deficiency is regarded to be normal. In C5-C9 deficiency vaccination increases phagocytosis and clinical studies give some support for protection (26). In C1, C4 and C2 deficiency, there is evidence that anticapsular antibodies can activate the alternative pathway (28-30). An important consideration is that impaired function of the classical pathway can limit antibody production which is explained by the adjuvant effect of C3d fragments on the immune response (31, 32). Nevertheless, vaccination responses to *S. pneumonia* and *H. influenzae* type b have so far not been investigated in a larger cohort of C2D persons.

Judging from our results in the present study, C2D persons are able to obtain an antibody response to the 23-valent pneumococcal vaccine and to the Haemophilus b conjugate vaccine. The response to pneumococcal serotype 6B and 23F equaled in some aspects that of healthy controls. The vaccination was also beneficial to a majority of the C2D persons in the sense that a concentration of more than 1 mg/L of specific IgG was achieved, which is considered to be protective in normal individuals (33-36). We could also conclude that some C2D persons preserve their specific antibodies for more than 5 years.

Due to a great variation in antibody responses, the low responses could hardly be ascribed to the C2D itself. The discrepancy might suggest involvement of other antibody isotypes or recognition molecules such as mannan-binding lectin (MBL) that could contribute to alternative pathway activation. For instance, the existence of a MBL-dependent C2 bypass mechanism for alternative pathway-mediated C3 activation has been demonstrated (37). Furthermore, it has been shown that a substantial part of the human anticapsular antibodies to *S. pneumoniae* consists of polymeric IgA (28) and that these antibodies support phagocytosis involving IgA receptors and the alternative pathway (29). There are also considerations to be made regarding the involvement of IgG2 in high epitope density, which may activate the alternative pathway (30). The homozygous presence of the IgG2 allotype G2M(n) is associated with efficient IgG2 antibody responses to polysaccharide antigens that perhaps might provide the right condition for activation of the alternative pathway. In a recently published paper, homozygous presence of G2M(n) allotype was shown to be protective against invasive infections in C2D (17). The combination of MBL and C2 deficincy was also found to be a susceptibility factor for invasive infection.

Complement-deficient guinea pigs (C4, C2) have been shown to get lower concentrations of antibodies and display an inability to maintain the antibody levels compared with normal controls to a the T cell-dependent antigen (38). After a secondary immunization they fail to develop amplification and to switch from IgM to IgG. The deviant response was overcome by increasing the antigen dose. The C2D persons also showed an impaired response compared to controls to a T cell-dependent antigen (Haemophilus b conjugate vaccine). We found no indications that they were unable to switch from IgM to IgG in relation to previously documented infection. However, for the pneumococcal antigens there were support for a lack of switch from IgM to IgG in the C2D persons how had experienced severe infections. They

responded with an increase of IgM antibodies while the C2D group without previously documented infections instead obtained higher levels of IgG. This might be attributed to a failure to build up an adequate B cell memory to relatively low stimulating antigens.

In conclusion, our findings extend previous observations and therefore provide evidence in support of pneumococcal vaccination of C2 deficient persons. Vaccination against *H. influenzae* type b was valuable to a majority of the C2D persons. Further investigations are needed to elucidate the concept of recruitment of the alternative pathway by anticapsular antibodies. C2D patients, especially children, may benefit from the development of improved polysaccharide vaccines.

Materials and methods

C2D persons

Between 1977 and 2007, 49 persons with C2D were identified in clinical routine analysis at the Clinical Immunology unit, University Hospital of Lund, Sweden. Since the initiation of the present study in 1993, 25 C2D persons were enrolled and a written informed consent was obtained from each person. Demographics and clinical manifestations of the vaccinated persons are show in table 1. The distribution of gender was equal between the C2D persons (F:M, 16:9) and controls (F:M, 39:12, p=0.3, Fisher's exact test). However, the C2D persons (median 41 years, range 2-63 years) were significant older than the control group (median 27 years, 16-61 years, p=0.02, Mann-Whitney U test).

The participants received an injection in the deltoid muscle of 0.5 mL of 23-valent pneumococcal vaccine, which contains 25 μg of each of the following type-specific capsular polysaccharide: 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, and 33F (Pneumo23®, Sanofi Pasteur MSD, SNC, France). The C2D persons were also vaccinated with Haemophilus b conjugate vaccine, tetanus toxoid conjugate (ActHIB®), also produced by Sanofi Pasteur MSD. In 4 of the C2D persons vaccinated with Haemophilus b conjugate vaccine, the pre-or post-vaccination blood samples were not technically handled in accord with the study protocol and therefore excluded from further analysis. A control group consisting of 51 healthy persons was also vaccinated with Pneumo23® and ActHIB®. The investigation was approved by the Lund University Ethics Committee (protocol LU 350-93).

Serum samples

Venous blood samples were collected from all subjects before vaccination, and at 4 to 6

weeks after vaccine administration. Serum samples were stored in aliquots at −80°C until they were analyzed for specific antibodies. In 4 C2D persons serum samples were collected over 4-6 years for long-term follow-up after vaccination.

Antibodies to pneumococcal and Hib capsular polysaccharides

Specific IgG, IgA, IgM, IgG1 and IgG2 to capsular polysaccharides of *S. pneumoniae* (serotypes 6B, 7F, and 23F) and Hib were determined by ELISA (39-42). Hib were measured by antibodies to the capsular polysaccharide in human sera using an antigen composed of Haemophilus b oligosaccharides conjugated to human serum albumin (HbO-HA) kindly provided by Dr. Moon Nahn, University of Rochester, NY, USA. Purified pneumococcal capsular polysaccharides were provided by Pasteur Mérieux Connaught, Marcy-l'Etoilt, France. Pneumococcal C-polysaccharide for preabsorption of serum samples was purchased from Statens Serum Institut, Denmark. In the assay, bound IgG, IgA and IgM was detected with correspondingly goat anti-human IgG, IgA and IgM alkaline phosphatase conjugate (γ-, α-, and μ-chain specific $F(ab')_2$ fragment, product no. A-3312 (IgG), A-3062 (IgA) and A-1067 (IgM), Sigma Biosciences, St. Louis, MO, USA). The following mouse monoclonal antibodies were used for detection of specific IgG1 and IgG2 antibodies: antihuman IgG1 (NL-16, Skibio, Bedfordshire, UK), antihuman IgG2 (HP6014; Skibio, Bedfordshire, UK). The detection of the monoclonal antibodies involved the use of affinity purified and alkaline phosphatase conjugated goat anti-mouse IgG antibodies (Dako, Glostrup, Denmark). The color reaction was developed with p-nitrophenylphosphate (1 mg/mL) in diethanolamine, pH 9.8, for one hour at room temperature. Absorbance was measured at 405 nm in a Multiscan Plus photometer (Labsystems Ltd., Helsinki, Finland). Values obtained were mean absorbance values from coated wells with subtraction of background absorbance in the uncoated well. For expression of antibody concentrations in mg/L, calibration of a local reference serum was

made against an international calibrator provided by Dr C Frasch, Bethesda. MD, USA (anti-pneumococcal antibodies lot 89 SF and anti-Hib antibodies serum pool lot 1983). The anti-Hib pool contained : IgG 60.9 µg/mL, IgA 5.6 µg/mL, IgM 3.5 µg/mL, IgG1 30.9 µg/mL, and IgG2 16.1 µg/mL. The detection limits for the ELISA tests were calculated to be between 0.01 mg/L to 0.03 mg/L when the limit was defined as the lowest point on the dilution curve significantly higher than baseline (>+2 SD). A post-vaccination concentration of specific IgG antibodies >1 mg/L were considered as long-term protective against infections caused by *S. pneumonia* and Hib (33-36).

Immunoglobulins and complement proteins

IgG, IgA and IgM were determined by turbidimetry using age-related reference areas (Cobas Mira; Roche Diagnostic, Basel, Switzerland) (43, 44). Concentrations of the IgG subclasses IgG1, IgG2 and IgG3 were determined by single immunodiffusion and 2.5-97.5 percentiles age-related reference intervals were used (45). IgG4 levels were measured with a commercial ELISA (Bindazyme, The Binding Site Ltd, Birmingham, UK.). Screening for detection of complement deficiency was mainly performed with hemolytic gel assays (46). C3 and C4 were determined by turbidimetry (Cobas Mira; Roche Diagnostic, Basel, Switzerland). C2 concentrations were given in mg/L assuming that the pooled normal serum used for reference contained C2 at 26 mg/L (47). C2D was defined as serum C2 concentration <0.5 mg/L.

Statistical analysis

Most of the statistics were analyzed with the computer program SPSS version 10.0. Wilcoxon signed rank test was used in conjunction with analysis of antibody responses to investigated antigens. Fisher´s exact test and Mann-Whitney *U* test were used for analysis of statistical relations between C2D persons and controls. Quotients of pre-and post-vaccination

concentrations were calculated for comparison between the C2D persons and controls (Mann-Whitney *U* test). The Mann-Whitney *U* test was used to compare post-vaccination concentrations of different antigens between the C2D person and controls. If a pre-and post-vaccination concentrations were not measurable, the result was set to be the detection limit for the used ELISA. All *p* values were two-tailed and considered significant at $p<0.05$. The Bonferroni method was valid for use in the analysis of the influence of G2M(n) allotype on antibody concentrations (calculated *p* value<0.0025).

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Table 1. Clinical manifestations in the vaccinated C2-deficient (C2D) persons. The C2D persons were stratified into groups I-IV in accord with documented infection ranging from minor infections to recurrent invasive infection.

Patient no.	Gender	Age at vaccination	Received vaccines	Documented infections	Isolated bacteria (blood or liquor)	Other disease manifestations
Group I						
8	F	41	Pnc/Hib	Minor infections		SLE. Asthma.
9	M	39	Pnc/Hib	Minor infections		
18	F	27	Pnc/Hib	Minor infections		
31	F	48	Pnc/Hib			Vasculitis. Erythema nodosum.
32	F	40	Pnc/Hib	Minor infections		
33	F	51	Pnc/Hib			UCTD. Lung emphysema. Chronic bronchitis. Pneumothorax.
41	M	50	Pnc/Hib			
43	M	63	Pnc/Hib			SLE. AMI x 2. Prostate cancer.
Group II						
2	F	46	Pnc	Pneumonia		SLE. Pancreatitis. Depression.
3	M	43	Pnc/Hib	Pneumonia x 2		SLE, AMI, Sjögren syndrome. Pernicious anaemia.
19	F	50	Pnc/Hib	Pneumonia x 2		SLE
27	M	42	Pnc/Hib	Pneumonia x 4		UCTD
47	F	11	Pnc/Hib	Pneumonia Epiglottitis		
Group III						
21	F	51	Pnc/Hib	Septicaemia. Pneumonia x 2	Pnc	Hypertension.
25	F	37	Pnc/Hib	Meningitis. Pneumonia x 2	Mnc	UCTD. Pustulosis palmaris et plantaris.
29	F	54	Pnc	Septicaemia Cholecystitis	Enterococcal species	SLE. Pancreatitis.
45	F	48	Pnc/Hib	Septicaemia	*S. aureus*	SLE. AV-block II-III. Parotid gland tumour.
Group IV						
13	F	20	Pnc/Hib	Septicaemia x 2. Pneumonia. Pyelonephritis x 3	Streptococcal species, Mnc	Appendicitis.
15	M	10	Pnc	Septicaemia Meningitis x 2	Pnc all 3 times	
16	M	15	Pnc/Hib	Meningitis x 2	Pnc 2 times	Eczema
17	F	24	Pnc/Hib	Septicaemia. Meningitis.	*S. agalatiae*, Mnc	Epilepsy
22	M	12	Pnc/Hib	Epiglottitis with septicaemia x2 Meningitis	Hib and *K. kingae*, *S. agalatiae*	
24	F	42	Pnc/Hib	Septicaemia Pneumonia Pyelonephritis x 3	Pnc	Vasculitis. Chronic bronchitis. Erythema nodosum.
28	M	2	Pnc	Septicaemia. Ethmoidit Pyelonephritis	Pnc	
42	F	38	Pnc/Hib	Pyelonephritis x 2		UCTD. Anorexia nervosa. Preeclampsia. Membranous glomerulonephritis (WHO, class V).

Abbreviations: AMI, acute myocardial infarction; Hib, *Hemophilus influensae* type b; *K. kingae, Kingella kingae;* Mnc, *Neisseria meningitidis*; Pnc, *Streptococcus pneumonia*; *S. aureus, Staphylococcus aureus; S. agalatiae, Streptococcus agalatiae;* SLE, systemic lupus erythematosus; UCTD, undifferentiated connective tissue disease.

Table 2. Stratification of the C2D persons according to severity of infection in relation to pre- and post-vaccination concentrations. Group I had a higher pre-vaccination concentration of anti-IgG antibodies to the pneumococcal serotypes 6B and 23F than groups II-IV. The C2D patients in groups II-IV, showed a tendency of higher levels of pneumococcal anti-IgM antibodies to serotypes 6B and 23F compared with group I.

	Severity of infection[A]		
Specific immunoglobulin isotype and antigen	**Group I, Pre-and post-vaccination GMC (mg/L)**	**Groups II-IV, Pre-and post-vaccination GMC (mg/L)**	***p* value**[B]
IgG Pnc 6B	9.4, 12.9	1.6, 3.7	0.03, 0.2
IgG Pnc 7F	3.9, 5.7	1.2, 2.3	0.08, 0.1
IgG Pnc 23F	3.5, 8.4	0.8, 2.2	0.03, 0.02
IgG Hib	0.7, 10.6	1.1, 8.8	0.5, 1.0
IgG2 Pnc 6B	7.2, 11.2	1.5, 2.9	0.02, 0.07
IgG2 Pnc 7F	4.9, 8.1	1.4, 2.7	0.07,< 0.05
IgG2 Pnc 23F	3.6, 7.0	1.0, 1.9	0.03, 0.02
IgG2 Hib	0.9, 9.2	0.5, 4.6	0.7, 0.2
IgA Pnc 6B	0.2, 0.4	0.1, 0.8	0.6, 0.5
IgA Pnc 7F	0.1, 0.3	0.1, 0.3	0.6, 0.4
IgA Pnc 23F	0.2, 0.9	0.1, 0.3	0.4, 0.2
IgA Hib	0.1, 0.4	0.1, 0.7	0.8, 0.3
IgM Pnc 6B	0.9, 1.7	2.5, 4.7	<0.05, 0.1
IgM Pnc 7F	0.5, 2.0	1.2, 2.3	0.1, 1.0
IgM Pnc 23F	0.3, 0.3	0.7, 1.4	0.07, 0.007
IgM Hib	0.1, 0.3	0.2, 2.2	0.2, 0.1

[A]C2D persons were divided into groups according to the severity of infections (see Table 1).

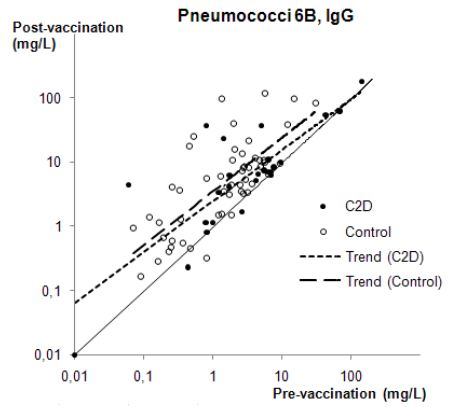
[B]Comparison between group I and the combined groups II-IV of pre- and post-vaccination concentrations. All *p* values were calculated with the Mann-Whitney *U* test. Abbreviations: GMC, geometric mean concentration; Pnc, *Streptococcus pneumoniae*; Hib, *Hemophilus influensae* type b.

Figure legends

Figure 1-5. Pre- and post-vaccination concentrations (mg/L) of C2-deficient (C2D) persons and controls (n=51) are depicted in a Log10 (x) to Log10 (y) scale; (A) pneumococci type 6B, (B) pneumococci type 7F, (C) pneumococci type 23F and (D) *H. influenzae* type b. Twenty-five C2D persons were vaccinated with the 23-valent pneumococcal vaccine (Pneumo 23®) and 21 were included after vaccination with *H. influenzae* type b conjugate vaccine (ActHIB®). Mean trend lines were added to facilitate interpretation of the data; C2D dotted lines, and controls broken lines. The non-broken lines represent a 1:1 response. For both C2D persons and controls, the post-vaccination geometric mean concentration (GMC) with range is presented. Closed symbols indicate C2D persons. Statistical results concerning antibody responses are shown for the C2D persons and controls (Wilcoxon). Comparisons between C2D persons and controls in fold increase (FI) and post-vaccination concentrations are also included (Mann-Whitney *U* test).

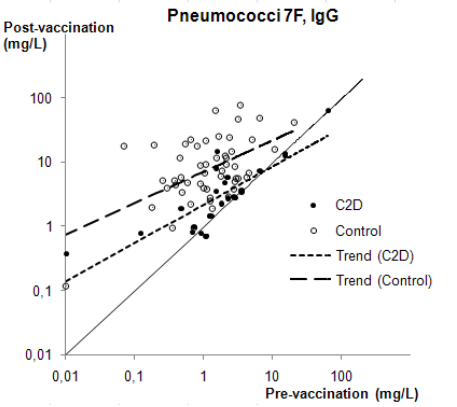
Figure 6A-D. Four C2D persons were followed over 4-6 years with measurements of IgG anti-pneumococcal antibodies. Arrows indicate time of vaccination with the 23-valent pneumococcal vaccine Pneumo23®. Note the different scale on y-axis.

A



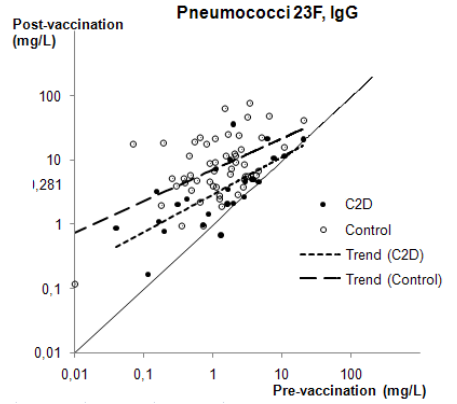
C2D GMC 5.4 mg/L, range 0.01-179.0 mg/L, p=0.0006 (Wilcoxon). Controls GMC 4.7 mg/L, range 0.07-30.0 mg/L, p<0.0001 (Wilcoxon). C2D compared to controls; FI p=0.005. Post-vaccination concentration p=0.5 (Mann-Whitney U test).

B



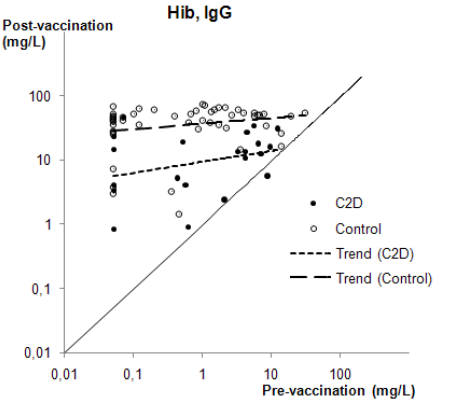
C2D GMC 3.0 mg/L, range 0.38-65.0 mg/L, p=0.004 (Wilcoxon). Controls GMC 7.7 mg/L, range 0.1-80.0 mg/L, p<0.0001 (Wilcoxon). C2D compared to controls; FI p<0.0001. Post-vaccination concentration p=0.0006 (Mann-Whitney U test).

C



C2D GMC 3.3 mg/L, range 0.2-36.0 mg/L, p<0.0001 (Wilcoxon). Controls GMC 3.5 mg/L, range 0.04-72.0 mg/L, p<0.0001 (Wilcoxon). C2D compared to controls; FI p=0.3. Post-vaccination concentration p=0.5 (Mann-Whitney U test).

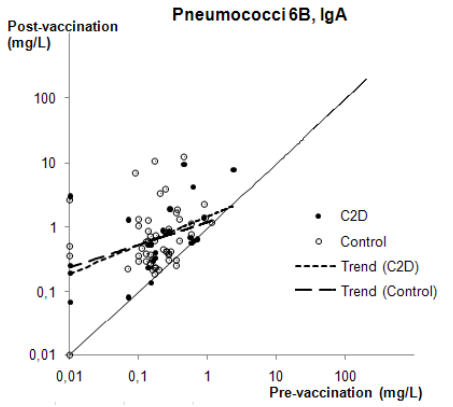
D



C2D GMC 9.4 mg/L, range 0.9-48.0 mg/L, p<0.0001 (Wilcoxon). Controls GMC 35.2 mg/L, range 1.5-77.0 mg/L, p<0.0001 (Wilcoxon). C2D compared to controls; FI p=0.0008. Post-vaccination concentration p<0.0001 (Mann-Whitney U test).

Figure 1.

A



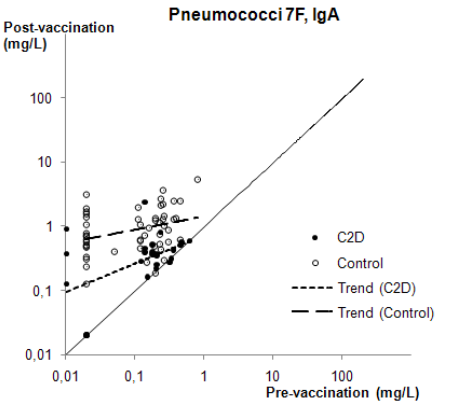
C2D GMC 0.7 mg/L, range 0.07-9.8 mg/L, p<0.0001 (Wilcoxon).

Controls GMC 0.6 mg/L, range 0.01-13.0 mg/L, p<0.0001

(Wilcoxon). C2D compared to controls; FI p=0.8.

Post-vaccination concentration p=0.9 (Mann-Whitney U test).

B



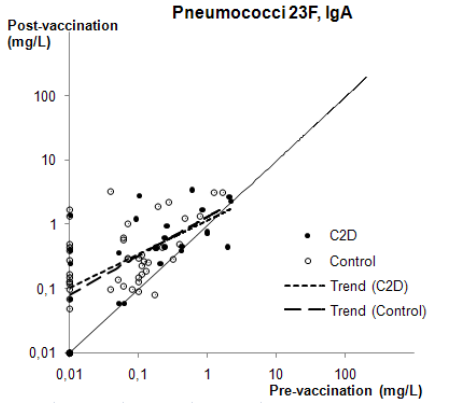
C2D GMC 0.3 mg/L, range 0.02-2.5 mg/L, p<0.0001 (Wilcoxon).

Controls GMC 0.9 mg/L, range 0.1-5.4 mg/L, p<0.0001

(Wilcoxon). C2D compared to controls; FI p<0.0001.

Post-vaccination concentration p<0.0001 (Mann-Whitney U test).

C



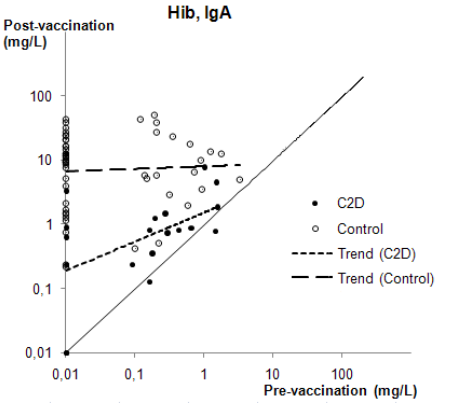
C2D GMC 0.4 mg/L, range 0.01-3.4 mg/L, p=0.002 (Wilcoxon).

Controls GMC 0.2 mg/L, range 0.01-3.4 mg/L, p<0.0001

(Wilcoxon). C2D compared to controls; FI p=0.1.

Post-vaccination concentration p=0.05 (Mann-Whitney U test).

D



C2D GMC 0.54 mg/L, range 0.01-13.0 mg/L, p=0.0004 (Wilcoxon).

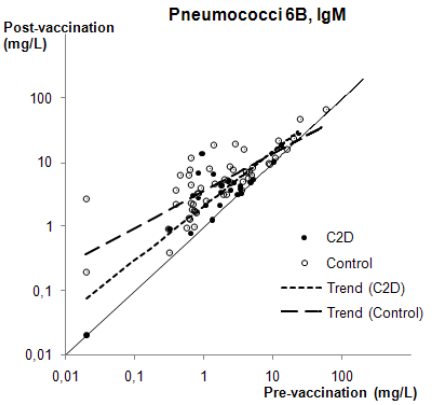
Controls GMC 7.1 mg/L, range mg/L, p<0.0001

(Wilcoxon). C2D compared to controls; FI p<0.0001.

Post-vaccination concentration p<0.0001 (Mann-Whitney U test).

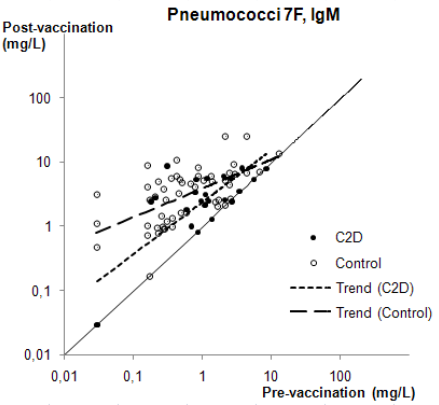
Figure 2.

A



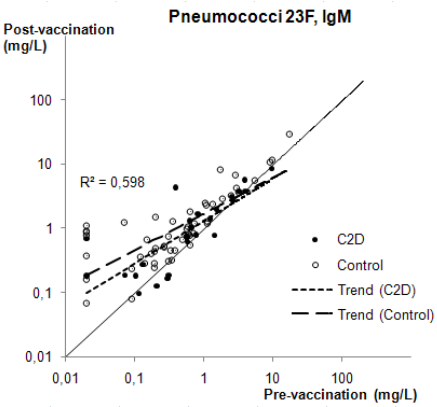
C2D GMC 3.4 mg/L, range 0.02-29.0 mg/L, p<0.0001 (Wilcoxon). Controls GMC 4.7 mg/L, range 0.2-67.0 mg/L, p<0.0001 (Wilcoxon). C2D compared to controls; FI p=0.01. Post-vaccination concentration p=0.3 (Mann-Whitney U test).

B



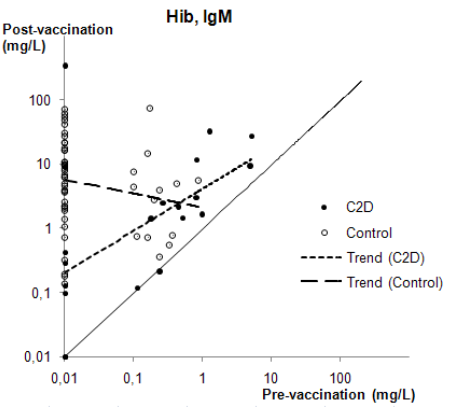
C2D GMC 2.4 mg/L, range 0.9-27.7 mg/L, p<0.0001 (Wilcoxon). Controls GMC 3.1 mg/L, range 0.2-26.0 mg/L, p<0.0001 (Wilcoxon). C2D compared to controls; FI p=0.0008. Post-vaccination concentration p=0.5 (Mann-Whitney U test).

C



C2D GMC 0.9 mg/L, range 0.1-8.9 mg/L, p=0.006 (Wilcoxon). Controls GMC 1.0 mg/L, range 0.07-30.0 mg/L, p<0.0001 (Wilcoxon). C2D compared to controls; FI p=0.01. Post-vaccination concentration p=0.8 (Mann-Whitney U test).

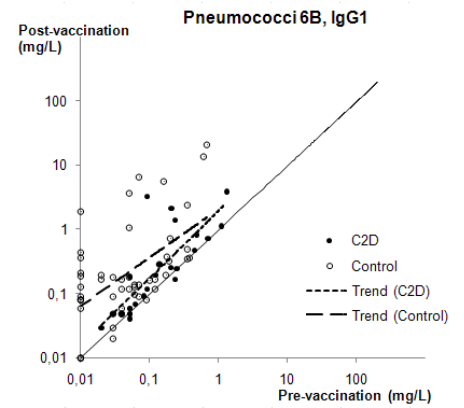
D



C2D GMC 1.0 mg/L, range 0.01-340.0 mg/L, p<0.0001 (Wilcoxon). Controls GMC 4.9 mg/L, range 0.1-77.0 mg/L, p<0.0001 (Wilcoxon). C2D compared to controls; FI p<0.0001. Post-vaccination concentration p=0.01 (Mann-Whitney U test).

Figure 3.

A



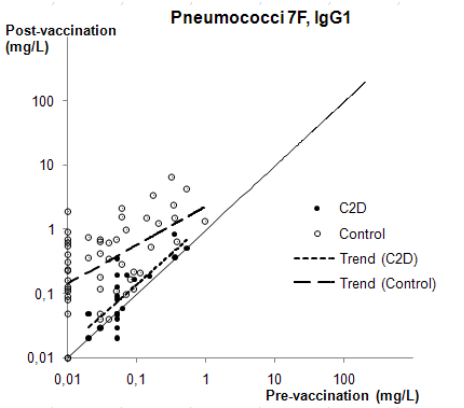
C2D GMC 0.2 mg/L, range 0.03-3.8 mg/L, p=0.0001 (Wilcoxon).

Controls GMC 0.2 mg/L, range 0.01-21.0 mg/L, p<0.0001

(Wilcoxon). C2D compared to controls; FI p=0.005.

Post-vaccination concentration p=0.1 (Mann-Whitney U test).

B



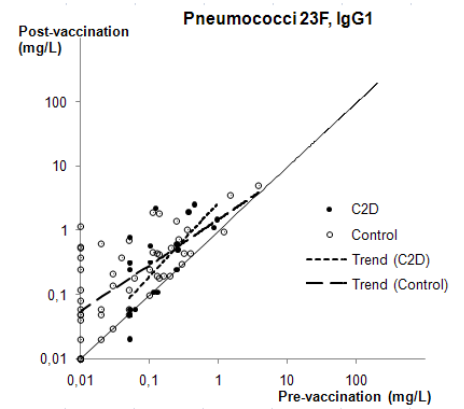
C2D GMC 0.1 mg/L, range 0.02-0.9 mg/L, p=0.004 (Wilcoxon).

Controls GMC 0.3 mg/L, range 0.01-6.7 mg/L, p<0.0001

(Wilcoxon). C2D compared to controls; FI p<0.0001.

Post-vaccination concentration p=0.001 (Mann-Whitney U test).

C

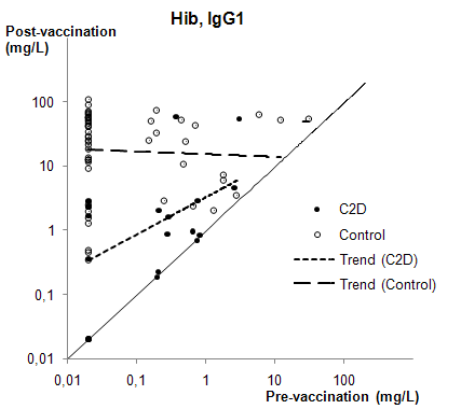


C2D GMC 0.2 mg/L, range 0.02-2.5 mg/L, p=0.0006 (Wilcoxon).

Controls GMC 0.2 mg/L, range 0.01-5.2 mg/L, p<0.0001

(Wilcoxon). C2D compared to controls; FI p=0.06.

Post-vaccination concentration p=0.7 (Mann-Whitney U test).



C2D GMC 1.0 mg/L, range 0.02-3.0 mg/L, p<0.0001 Wilcoxon).

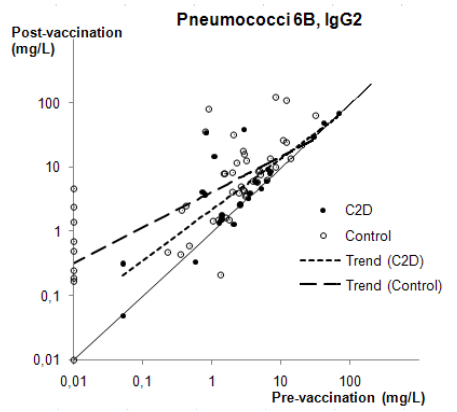
Controls GMC 17.2 mg/L, range 0.3-112.0 mg/L, p<0.0001

(Wilcoxon). C2D compared to controls; FI p<0.0001.

Post-vaccination concentration p<0.0001 (Mann-Whitney U test).

Figure 4.

A



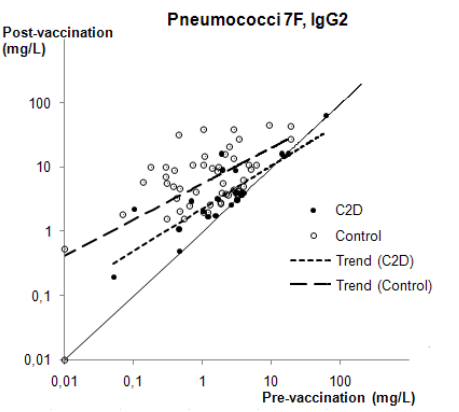
C2D GMC 4.5 mg/L, range 0.05-71.0 mg/L, p=0.0003 (Wilcoxon).

Controls GMC 4.0 mg/L, range 0.01-125.0 mg/L, p<0.0001

(Wilcoxon). C2D compared to controls; FI p=0.004.

Post-vaccination concentration p=0.9 (Mann-Whitney U test).

B



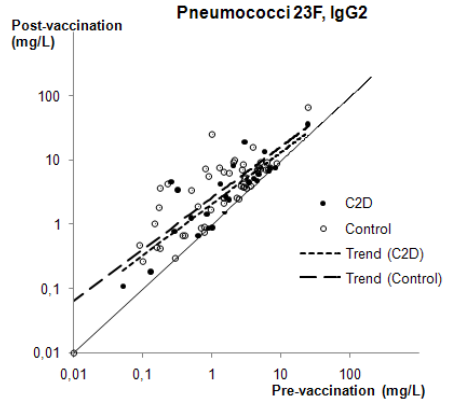
C2D GMC 3.8 mg/L, range 0.2-65.0 mg/L, p<0.0001 (Wilcoxon).

Controls GMC 5.8 mg/L, range 1.0-46.0 mg/L, p<0.0001

(Wilcoxon). C2D compared to controls; FI p<0.0001.

Post-vaccination concentration p=0.04 (Mann-Whitney U test).

C

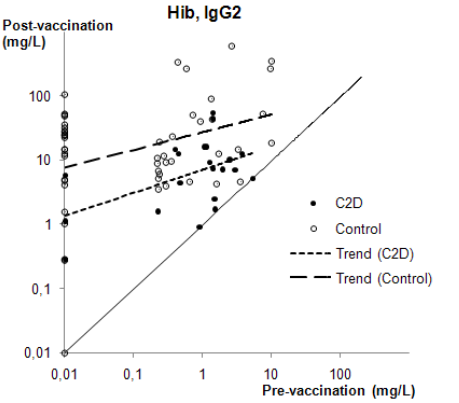


C2D GMC 2.9 mg/L, range 0.1-36.0 mg/L, p<0.0001 (Wilcoxon).

Controls GMC 3.1 mg/L, range 0.01-68.0 mg/L, p<0.0001

(Wilcoxon). C2D compared to controls; FI p=0.1.

Post-vaccination concentration p=0.7 (Mann-Whitney U test).



C2D GMC 5.9 mg/L, range 0.3-56.0 mg/L, p<0.0001 (Wilcoxon).

Controls GMC 14.7 mg/L, range 0.01-618.0 mg/L, p<0.0001

(Wilcoxon). C2D compared to controls; FI p<0.0001.

Post-vaccination concentration p=0.02 (Mann-Whitney U test).

Figure 5.

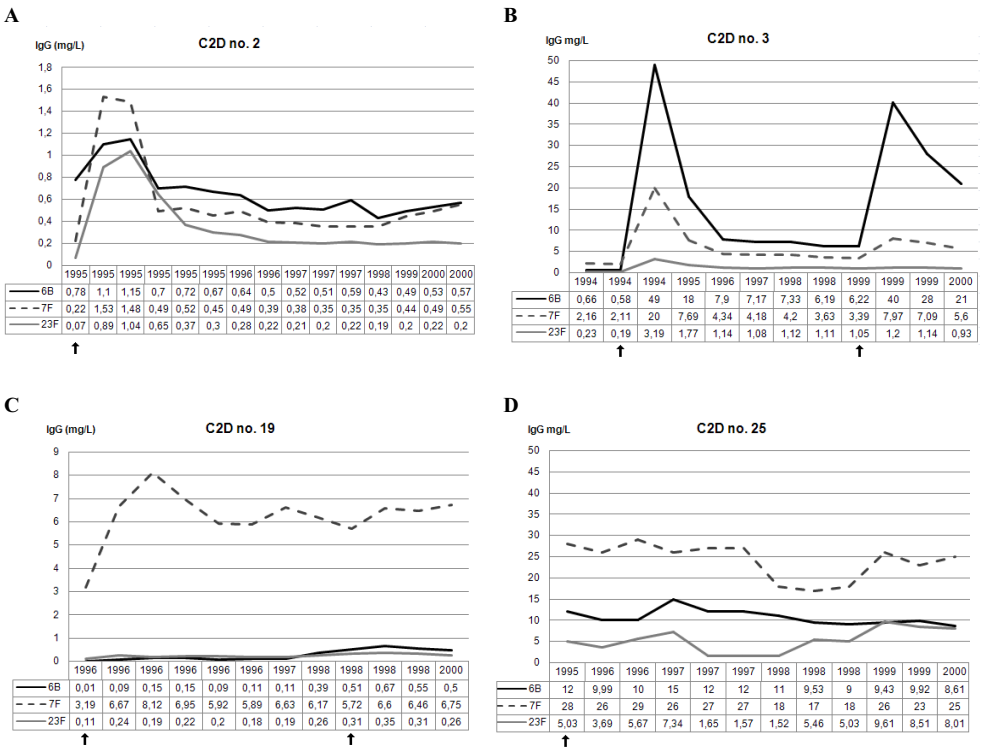

A
IgG (mg/L)
C2D no. 2
1,8
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
1995 1995 1995 1995 1995 1995 1996 1996 1997 1997 1997 1998 1999 2000 2000
6B 0,78 1,1 1,15 0,7 0,72 0,67 0,64 0,5 0,52 0,51 0,59 0,43 0,49 0,53 0,57
7F 0,22 1,53 1,48 0,49 0,52 0,45 0,49 0,39 0,38 0,35 0,35 0,35 0,44 0,49 0,55
23F 0,07 0,89 1,04 0,65 0,37 0,3 0,28 0,22 0,21 0,2 0,22 0,19 0,2 0,22 0,2
B
IgG mg/L
C2D no. 3
50
45
40
35
30
25
20
15
10
5
0
1994 1994 1994 1995 1996 1997 1998 1998 1999 1999 1999 2000
6B 0,66 0,58 49 18 7,9 7,17 7,33 6,19 6,22 40 28 21
7F 2,16 2,11 20 7,69 4,34 4,18 4,2 3,63 3,39 7,97 7,09 5,6
23F 0,23 0,19 3,19 1,77 1,14 1,08 1,12 1,11 1,05 1,2 1,14 0,93
C
IgG (mg/L)
C2D no. 19
9
8
7
6
5
4
3
2
1
0
1996 1996 1996 1996 1996 1996 1997 1998 1998 1998 1998 2000
6B 0,01 0,09 0,15 0,15 0,09 0,11 0,11 0,39 0,51 0,67 0,55 0,5
7F 3,19 6,67 8,12 6,95 5,92 5,89 6,63 6,17 5,72 6,6 6,46 6,75
23F 0,11 0,24 0,19 0,22 0,2 0,18 0,19 0,26 0,31 0,35 0,31 0,26
D
IgG mg/L
C2D no. 25
50
45
40
35
30
25
20
15
10
5
0
1995 1996 1996 1997 1997 1997 1998 1998 1998 1999 1999 2000
6B 12 9,99 10 15 12 12 11 9,53 9 9,43 9,92 8,61
7F 28 26 29 26 27 27 18 17 18 26 23 25
23F 5,03 3,69 5,67 7,34 1,65 1,57 1,52 5,46 5,03 9,61 8,51 8,01

Figure 6.

Appendix

Clinical and laboratory findings in 49 C2D persons.

Patient no.	Sex	Identification of C2D (year/age of patient)	Follow-up time (year)	Homozygous for HLA-*DRB1*15* and *C4A*4 B*2*, / C2D-type I[a]	Identified autoantibodies[f]	Rheumatological disease and severe infection
1	F	1977/26	34	Unknown[b]	RNP	SLE. Died of AMI (34yr).
2	F	1977/29	44	Yes/ yes	RNP, Sm	SLE. Pneumonia.
3	M	1977/26	54	Yes/ yes	ANA, RF, RNP, Sm, aCL, anti-C1qCLR	SLE. Sjögren syndrome. Pneumonia.
4	F	1974/32	60	Yes/ yes	RF, SSA, aCL	SLE. Died of *S. aureus* septicemia (59 yr).
5	F	1980/55	76	Yes/ yes		UCTD. Osteitis. Pneumonia. Died of AMI (75 yr).
6	F	1985/37	51	Yes/ yes	aCL, anti-C1qCLR	*S. pneumoniae* meningitis x 2. Orbital phlegmone, subperiosteal abscess and osteitis.
7	M	1983/11	11	Yes/ yes	anti-C1qCLR	*H. influenzae* meningitis. *S. pneumoniae* septicemia. Pneumonia.
8	F	1985/27	47	Yes/ yes	aCL	SLE. Salpingitis.
9	M	1985/25	42	Yes/ yes	aCL	
10	M	1984/76	77	Unknown[b]		Died of AMI (77 yr).
11	M	1985/62	76	Yes/ yes	aCL, anti-C1qCLR	SLE. *S. aureus* septicemia with epidural abscess. Osteitis. Recurrent pneumonia x 57. *S. maltophilia* septicemia and pyelonephritis. Died of cancer (76 yr).
12	M	1985/44	51	Yes/ yes	ANA, RF, Histon, aCL, anti-C1qCLR	SLE. Pneumonia. Died of *S. pneumoniae* septicemia and meningitis (51 yr).
13	F	1985/6	22	Yes/ yes	aCL, anti-C1qCLR	Streptococcal and *N. meningitidis* septicemia. Pneumococcal pneumonia. Pyelonephritis x 3. Severe varicella.
14	F	1985/65	67	Unknown[b]		Died of pneumonia (67 yr).
15	M	1987/1 year and 4 months	16	Yes/ yes	aCL, anti-C1qCLR	*S. pneumoniae* meningitis x 2. *S. pneumoniae* septicemia.
16	M	1986/1 year and 5 months	21	Yes/ yes		*S. pneumoniae* meningitis x 2.
17	F	1989/14	30	Yes/ yes	Histon	Umbilical infection with septicemia. *S. agalactiae* and *N. meningitidis* meningitis.
18	F	1989/17	32	Yes/ yes	Histon, anti-C1qCLR	
19	F	1990/45	59	Heterozygous/ heterozygous[c]	RNP, aCL, anti-C1qCLR	SLE. Pneumonia x 2.
20	F	1993/5	15	Yes/ yes	aCL	
21	F	1993/44	56	Yes/ yes	aCL, anti-C1qCLR	*S. pneumoniae* septicemia and pneumonia.

22	M	1993/6	15	Yes/ yes	aCL	*S. agalactiae* meningitis. Septicemia and epiglottitis x 2 (*K. kingae* and *H. influenzae*).
23	F	1995/57	64	Yes/ yes	Sm, aCL, anti-C1qCLR	SLE. Sjögren syndrome. Pneumonia > 3 times.
24	F	1995/40	47	Yes/ yes	RF, Histon, Scl-70, aCL	Vasculitis. Pyelonephritis x 3. *S. pneumoniae* septicemia and pneumonia x 2. Died of septicemia (49 yr).
25	F	1995/35	45	Yes/ yes	aCL	UCTD. *N. meningitidis* meningitis. Pneumonia x 2.
26	M	1996/70	71	Yes/ yes	aCL, anti-C1qCLR	Died of acute peritonitis (71 yr).
27	M	1996/41	45	Yes/ yes	anti-C1qCLR	UCTD. Pneumonia x 4.
28	M	1996/2	11	Yes/ yes	aCL	*S. pneumoniae* septicemia. Pyelonephritis. Ethmoiditis.
29	F	1996/53	59	Yes/ yes	SSA, aCL	SLE. Enterococcal species septicemia.
30	F	1997/2	10	Yes/ yes	aCL	Pneumonia.
31	F	1997/41	44	Yes/ yes	Histon, aCL, anti-C1qCLR	Vaculitis.
32	F	1997/38	41	Yes/ yes		
33	F	1998/50	53	Yes/ yes	SSA, aCL, anti-C1qCLR	UCTD.
34	M	1999/47	47	Yes/ yes	aCL, anti-C1qCLR	Vaculitis. *S. pneumoniae* septicemia and pneumonia. *S. aureus* septicemia.
35	M	1999/5	8	Yes/ yes	aCL	*S. pneumoniae* septicemia. Pneumonia.
36	F	1999/76	76	Yes/ yes	aCL	*S. pneumoniae* septicemia. Pleural tuberculosis.
37	M	2001/8	12	Heterozygous/ heterozygous[c]		*S. pneumoniae* septicemia and pneumonia.
38	M	2002/5	5	Heterozygous/ heterozygous[c]		
39	F	2002/10	10	Unknown[d]		Died of septicemia and meningitis caused by *S. pneumoniae* (10 yr).
40	M	2002/16 mo	1.5	Yes/ yes		*S. pneumoniae* septicemia and meningitis. Ethmoiditis.
41	M	2003/49	49	Unknown/ yes[e]	RNP, aCL, anti-C1qCLR	
42	F	2004/36	38	Unknown/ yes[e]	SSA	UCTD. Pyelonephritis x 2.
43	M	2005/63	63	Unknown/ yes[e]	RNP, aCL	SLE.
44	M	2005/13	13	Unknown/ yes[e]	aCL, anti-C1qCLR	Intracranial epidural abscess. Ethmoiditis.
45	F	2005/47	47	Unknown/ heterozygous[e]	ANA, anti-dsDNA, SSA, aCL	SLE. *S. aureus* septicemia.
46	F	2006/8	8	Unknown/ yes[e]	anti-C1qCLR	
47	F	2006/11	10	Unknown/ yes[e]		Pneumonia, epiglottis.
48	F	2006/18	15	Unknown/ yes[e]	ANA, aCL	
49	M	2007/15	13	Unknown/ yes[e]		

AMI, Acute myocardial infarction; SLE, Systemic Lupus Erythematosis; UCTD, Undifferentiated Connective Tissue Disease.

[a]The main cause of C2D type I is a 28 bp deletion in the C2 gene of the *HLA-B*18,S042,DRB1*15* MHC haplotype.

[b]In 3 deceased patients (no. 1, 10, 14) investigations for the 28-bp deletion and MHC typing could not be performed.

[c]Three persons had undetectable C2 (no. 19, 37, and 38). The MHC haplotypes found in these patients have not been previously described in conjunction with C2 null genes.

[d]The patient died of septicemia and meningitis caused by *S. pneumoniae*. Her parents were both heterozygous for the 28-bp deletion suggesting that the patient was homozygous for this defect.

[e]DRB1 and C4 variants were not determined.

[f]Findings of positive autoantibodies in relation to there individual reference intervals.