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MICROBIAL EVASION OF INNATE IMMUNITY BY UTILIZATION OF SOLUBLE COMPLEMENT REGULATORS

TARU MERI

Department of Bacteriology and Immunology

Haartman Institute

UNIVERSITY OF HELSINKI

HELSINKI, FINLAND

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Supervisors

Seppo Meri

Professor of Immunology, MD, PhD
Department of Bacteriology and Immunology
Haartman Institute
University of Helsinki
Finland

Sakari Jokiranta

Clinical Teacher, MD, PhD
Department of Bacteriology and Immunology
Haartman Institute and HUSLAB
University of Helsinki and Helsinki University Central Hospital
Finland

Reviewers

Esa-Matti Lilius

Senior Teacher, PhD
Department of Biochemistry and Food Chemistry
University of Turku
Finland

Olli Vainio

Professor, MD, PhD
Department of Medical Microbiology
University of Oulu
Finland

Opponent

Sven Bergström

Professor of Molecular Biology, MD, PhD
Department of Molecular Biology
Umeå University
Sweden

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What we know is just a drop, what we don't know
is an ocean.

– Isaac Newton –

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ORIGINAL PUBLICATIONS

This thesis is based on the following original publications:

I Hellwage, J., **Meri, T.**, Heikkilä, T., Alitalo, A., Panelius, J., Lahdenne, P., Seppälä, I. and Meri, S. 2001: The complement regulator factor H binds to the surface protein OspE of *Borrelia burgdorferi. Journal of Biological Chemistry* 276 (11):8427-8435.

II **Meri, T.**, Jokiranta, T.S., Hellwage, J., Bialonski, A., Zipfel, P. and Meri, S. 2002: *Onchocerca volvulus* microfilariae avoid complement attack by direct binding of factor H. *Journal of Infectious Diseases* 185(12):1786-93.

III **Meri, T.**, Hartman A., Lenk, D., Würzner, R., Hellwage, J., Meri, S., Zipfel, P. 2002: The yeast *Candida albicans* binds complement regulators factor H and FHL-1. *Infection and Immunity* 70(9):5185-5192.

IV **Meri, T**, Blom A. M., Hartman, A., Lenk, D., Meri, S. and Zipfel, P. 2004: The hyphal and yeast forms of *Candida albicans* bind the complement regulator C4BP. *Infection and Immunity* 72(11):6633-6641.

V **Meri, T.**, Cutler, S.J., Blom, A.M., Meri, S. and Jokiranta, T.S. 2005: Relapsing fever spirochetes *Borrelia recurrentis* and *B. duttonii* utilize C4b binding protein for complement evasion. Submitted to *Molecular Microbiology.*

ABBREVIATIONS

ACA	acrodermatitis chronica atrophicans
AP	alternative pathway
BSA	bovine serum albumin
C4BP	C4b-binding protein
C	complement
C1INH	C1-inhibitor
CP	classical pathway
CR1	complement receptor 1
CRP	C-reactive protein
DAF	decay-accelerating factor
EDTA	ethylenediaminetetraacetic acid
FB	factor B
FD	factor D
FH	factor H
FHL-1	factor H-like protein
FHR	factor H-related protein
FI	factor I
FITC	fluorescein isothiocyanate
GPI	glycophosphatidylinositol
HI-NHS	heat-inactivated NHS
HUVEC	human umbilical vein endothelial cells
HUS	hemolytic uremic syndrome
HRP	horse-radish peroxidase
IC	immune complex
IFL	immunofluorescence
LBRF	louse-borne relapsing fever
LP	lectin pathway
LPS	lipopolysaccharide
MAC	membrane attack complex
MBL	mannan-binding lectin
MCP	membrane cofactor protein
MPGN	membranoproliferative glomerulonephritis
mf	microfilariae
mw	molecular weight
NHS	normal human serum

NHS-EDTA	normal human serum containing EDTA
SCR	short consensus repeat domain
TBRF	tick-borne relapsing fever
TCC	terminal complement components
TP	terminal pathway
RCA	regulators of the complement activation gene cluster
RF	relapsing fever

ABSTRACT

The complement system is an important part of human innate immunity. It consists of approximately 35 proteins in plasma and on cell membranes. Complement is activated via three pathways, alternative, classical or lectin pathway, and activation can lead to a formation of lytic membrane attack complexes. During the activation complement proteins opsonize the target for phagocytosis, enhance inflammatory response and attract phagocytes to the site. To protect own structures complement is tightly regulated by membrane-bound and fluid phase regulators. The main regulator of the alternative pathway in plasma is factor H, while the key regulator of the classical pathway is C4BP. The major function of complement is protection of the host against microbial evasion. Pathogenic microbes, however, have developed multiple mechanisms to escape the complement attack. One widely used and obviously effective mechanism is utilization of host fluid phase complement regulators factor H, FHL-1, which is a truncated form of FH, and C4BP.

In this thesis complement evasion of different kinds of microbes have been studied. The Lyme disease causing *Borrelia burgdorferi* sensu stricto is shown to bind factor H and FHL-1 and the main ligand for factor H was identified as the outer surface protein E. Acquisition of factor H enhances survival of *B. burgdorferi* sensu stricto in non-immune human serum. Two major borreliae species that cause relapsing fever, *B. recurrentis* and *B. duttonii*, are shown to utilize both factor H and C4BP. Both the regulators maintain their functional activity in regulating the complement system on the surface of the relapsing fever borreliae.

Also microbes other than bacteria can utilize complement regulators, which is shown with the clinically most important pathogenic yeast *Candida albicans* and microfilariae of a parasitic nematode *Onchocerca volvulus.* Despite being frequently a harmless commensal *C. albicans* causes occasionally systemic life threatening infections. *O. volvulus* is the causative agent of river blindness, and 18 million patients are infected with this parasite. Both the microbes are shown to specifically bind fluid phase complement regulators. *O. volvulus* microfilariae bind factor H, while *C.*

albicans binds also C4BP and FHL-1. The bound regulators maintain their complement regulatory activity on the microbial surface, which results in premature termination of the complement activation cascade. The microbial interaction sites on the complement regulators were mapped using recombinantly expressed deletion mutants.

Taken together, in this thesis, new microbes, representing bacteria, yeast and parasites, are shown to utilize soluble complement regulators for protection from the antimicrobial effects of the complement system.

1 INTRODUCTION

Humans are constantly surrounded by microbes. Microbes can enter the human body in numerous ways, for example in food and drink, in inhaled air, via wounds and during contacts with other humans. Many microbes are able to use vectors for invasion, for example malaria is transmitted by mosquitos and *Borrelia* spirochetes by ticks and lice. Some microbes are very active in the invading human body, even through intact skin, like cercariae of a blood trematode *Schistosoma mansoni* do. Microbes that infect humans belong to viruses, procaryotic bacteria, eucaryotic fungi and parasites. The enormous variety of hostile microbes, starting from a 25 nm virus to a 25 meter long tapeworm, is encountering multiple protection mechanisms in humans. Intact skin, mucus secreted from the epithelial cells, low pH of the gastric juice and bactericidal agents secreted in body fluids (e.g. lysozyme in tears) are some physiological barriers, which prevent microbes from entering the human body. Humans also maintain so called normal microbial flora on skin and several mucosal membranes. These commensal microbes can create an environment where the pathogens do not survive (e.g. low pH) and compete with the pathogenic ones. Some commensals are symbiotic, for example some participate in production of vitamin K in the intestine.

More specific protection against micro-organisms is provided by the human immune system. The immune system is divided into two different parts; the innate immunity and the acquired immunity. The innate immunity includes phagocytes (polymorphonuclear neutrophils, macrophages and natural killer cells) and the complement (C) system. T-cells, B-cells and antibodies are parts of the acquired immunity. The immune response from the innate immunity is quick, non-specific and does not require a previous contact with the microbe. The acquired immunity, on the other hand, is slower in reacting, much more specific and has the ability to remember the invader when encountering it again. With these mechanisms humans are relatively well equipped for protection against micro-organisms.

The C system consists of fluid phase and membrane bound proteins. C can be activated nonspecifically or via antibodies already specifically bound to the target. After the initial activation the proteins of the C system co-

operate in a cascade-like manner and if the activation continues, cytolytic membrane-attack complexes (MAC) are formed. During C activation the target is also opsonized for phagocytosis. To protect own cells and structures the C system is tightly controlled by regulators on membranes and in the fluid phase.

Microbes have evolved to circumvent the immune responses of the host in many ways. Nonimmunogenic surface structures, antigenic variation, secreted enzymes for inactivation of proteins of the immune system and several other mechanisms enable some microbes to be pathogenic and invasive. One specific mechanism for immune evasion is utilization of host molecules. Microbes have been shown to bind, for example, soluble C regulators of the host and prevent C-mediated phagocytosis and lysis. In this thesis C evasion mechanisms of different microbes have been studied. Bacteria, (*Borrelia* spirochetes causing either Lyme disease or relapsing fever), a yeast *Candida albicans* and a nematode parasite *Onchocerca volvulus* are studied to show that structurally and functionally very different pathogenic micro-organisms have evolved to use a similar mechanism for their own protection.

2 BACKGROUND OF THE COMPLEMENT SYSTEM

2.1 History and overview

The C system is evolutionarily the oldest part of the immune system. Components of C have been found in many animal phyla, e.g. reptiles, amphibians and bony fishes. A C3/C4 –like protein with an intrinsic activity for covalent binding (an internal thioester bond) has been described in nonvertebrates, such as ascidians, sea urchins and even arthropods. Adaptive immunity, on the other hand, is found only in jawed vertebrates. Many C proteins resemble each other. For example C3, C4 and C5 belong to the same family. Gene duplications during the evolution have most probably been important for the development of C to the functional and structural complexity it nowadays presents in humans (222).

The C system in humans was discovered in the 1890's, when Nuttall and others discovered a heat-sensitive component in immune human serum. This component was found to be essential for the killing of bacteria. The name complement for this system was given by Paul Ehrlich in 1899, when C was thought to complement antibodies in the target lysis. As early as then Ehrlich thought that C consists of a group of factors, which affect each other. At that time C was seen only as an effector mechanism for the antibodies. Alternative pathway (AP) and the idea of C activation in the absence of antibodies was proposed in the 1950's by Louis Pillemer, who originally named AP as the properdin pathway (200).

The C system consists of approximately 35 proteins in plasma and on cell surfaces. It is activated via three different pathways, classical pathway (CP), lectin pathway (LP) and AP. In the absence of regulation activation of any of the three pathways leads to the formation of C3-convertases, which enzymatically cleave intact C3 molecules. C3 is cleaved to C3b, which binds covalently to targets. If the activation continues, proteins of the terminal pathway (TP) form massive MAC. MAC can penetrate the target cell membrane and form pores that subsequently lyse the target cell. The surface-attached C3b molecules are recognized by C receptors on phagocytes, and small peptides (C3a and C5a) cleaved from C3 and C5 attract macrophages and neutrophiles to the site. Taken together, C can

directly lyse the targets, mark them for phagocytosis and participate in generation of an inflammatory response.

2.2 Complement activation pathways

2.2.1 Alternative pathway

C3 is the central protein of the C system, firstly because of its role in the activation, but also by its large amount in the blood. The serum concentration of C3 is 0.7-1.5 mg/ml (Table 1). C3 has a molecular weight (mw) of 185 kD and it consists of 2 chains, α and β, which are held together by one disulphide bond and noncovalent forces (190). Disruption of an internal thioester bond in an activated C3 molecule allows it to bind covalently to target surfaces.

AP activation starts when the internal thioester bond in C3 is spontaneously hydrolyzed in the fluid phase resulting in the formation of C3(H_2O) (233). Functionally the C3(H_2O) form as well as C3b can bind factor B (FB) and make it susceptible to a cleavage by factor D (FD) to Ba and Bb. FB is a single chain serum glycoprotein. The fluid phase C3-convertase C3(H_2O)Bb can cleave C3-molecules into C3b and C3a. The small (9 kD) single chain C3a peptide (144), cleaved from the alpha-chain of C3, acts as an anaphylatoxin (318). The activated C3b can bind covalently to the surfaces nearby via the thioester bond (191, 288). Surface-attached C3b binds FB, which is cleaved by FD, and C3bBb, the AP C3-convertase essential for the C3b amplification loop, is formed. Binding of FB to C3b needs the presence of Mg^{2+} ions. AP convertases cleave new C3 molecules, and within minutes the system can attach billions of C3b-molecules to the target surface. Surface-bound C3b can bind C5, which is susceptible to a cleavage by C3bBb to C5b and C5a. C5a is another anaphylatoxin released to the surrounding environment. C5b attached to the surface can bind C6, which initiates the TP activation.

2.2.2 Classical pathway

The first component of the CP is the C1-complex, which consists of one C1q and two C1r and C1s molecules each. C1q is formed of six identical

subunits with a globular domain at one end and an elongated collagen-like part at the other end. According to its appearance the structure has been visualized as a bunch of tulips. C1q interacts with C1r and C1s via the elongated parts and binds to a target via the globular domains. The main targets for C1 are Fc-regions of IgG or IgM antibodies (147). C1q can bind to the antibodies only when at least two IgGs are attached close to each other on the target. Therefore, relatively high surface density of Igs is needed for the CP activation. In the absence of antibodies C1q can bind to bacterial lipopolysaccharides (LPS), nucleic acids, immune complexes (IC), some viruses and C-reactive protein (CRP) resulting in CP activation (154, 193). Ca^{2+}-ions are needed to assemble the C1-complex together (328). When C1q is bound to the target via multiple globular domains, its conformation changes allowing C1r to activate C1s, that subsequently cleaves C4 and C2 (123).

Table 1. Properties of the components of the AP, CP and LP.

Component	Size (kD)	Serum conc.	Structural features
ALL PATHWAYS:			
C3	185	500-1200	2 chains
C3b	175	(μg/ml)	alpha-chain 115 kD
			beta-chain 75 kD
C3a	9		single chain
ALTERNATIVE PATHWAY:			
Factor B	93	140-400	serine protease
Ba	33		
Bb	60		
Factor D	24	1-5	serine protease
Properdin	2-5x53	25	polymer of 53 kD chains
CLASSICAL PATHWAY:			
C1	780		
C1q	460	100-180	hexamer of 73 kD subunits
			serine protease
C1r	80	30-40	serine protease
C1s	80	50	
C4	102	200-600	3 chains
C4b			alpha-chain 97 kD
C4a			beta -chain 75 kD
			gamma-chain 33 kD
C2	102	20-25	single chain
LECTIN PATHWAY:			
MBL	3-6x32	0-5	polymer of 32 kD chains,
			collectin
MASP-1	100	1-10	serine protease
MASP-2	76		serine protease

C4 consists of three chains kept together by disulphide bonds. Like C3b, it has an internal thioester bond which, upon disruption, enables C4b to attach covalently to a surface (279). C4 is cleaved at a single point to C4b and C4a by C1s. C4a acts as a weak anaphylatoxin (120). C4b is hydrolyzed in fluid phase by body fluids or gets covalently attached to a surface.

C2 is a single-chain plasma protein. It binds to the target-attached C4b in the presence of Mg^{2+} ions and is thereafter cleaved by C1s to C2b and C2a resulting in formation of the classical pathway C3-convertase C4b2a (168). C2a contains the catalytic site of this enzyme. Activation now continues with a cleavage of C3 in the same manner as in the AP activation by the AP-convertase C3bBb. The CP C5-convertase (C3b4b2a) is formed when C3b binds to the CP C3-convertase (305). This complex cleaves C5 resulting in initiation of TP.

2.2.3 Lectin pathway activation

LP is the most recently discovered pathway of C (314). Mannan-binding lectin (MBL) is the first component of LP. MBL has a C1q-like structure that consists of elongated collagenous α-helical parts and globular domains. In human serum it is present in different forms with different numbers of subunits. MBL binds to N-acetyl-glucosamine containing carbohydrates or mannose (267). In the circulation MBL makes complexes with MBL-associated serine proteases (MASPs) (199, 309), which can cleave C4 and C2. After cleavages of C4 and C2 the activation of the LP is similar to the activation of CP.

MBL and MASPs have very low serum concentrations. LP might have a role in preventing infections during early childhood, since children with homo- or heterozygous mutations of MBL appear to have a slightly increased risk for recurrent infections (314).

2.2.4 Terminal pathway

All three C activation pathways can initiate activation of TP. C5 is the first component of TP (Table 2). It consists of two chains linked together with one disulphide bond (304). Unlike its close homologues C3 and C4, C5 does not contain an internal thioester bond (128). CP and AP C5-convertases cleave C5 into C5a and C5b (62). C5a, a potent anaphylatoxin and chemotaxin (59), is released, and C5b binds to C6 (90). Binding of C6 to C5b has to occur within minutes, otherwise the conformation of C5b changes and C6 binding is no longer possible (85). If the association with C6 is successful, C7 can bind to the C5b6 complex. The hydrophobic complex formed (C5b67) attaches itself to a nearby membrane. The next component of the TP, C8, binds to C7 (174) and the complex inserts itself deeply into the target membrane. The binding of C9 to C5b678 allows the complex to disturb the membrane stability and when more C9 molecules are recruited a pore is formed (247). Upon binding to C8 a major conformational change takes place in C9 transforming it to an elongated molecule that spans across the membrane (312).

Table 2. Properties of the TP components.

Component	Size (kD)	Serum conc. (μg/ml)	Structural features
C5	192	75	2 chains
C5a	11		alpha-chain 118 kD
C5b	181		beta -chain 74 kD
C6	120	45-70	single chain
C7	105	55-60	single-chain
C8	152	80	3 chains alpha-chain 65 kD beta -chain 65 kD gamma-chain 22 kD
C9	70	80	single chain

Structurally the TP components C6, C7, C8 and C9 are relatively homologous to each other. C6, C7 and C9 are single-chain proteins (86) whereas C8 is formed from 3 chains (88, 89).

There are two hypotheses how TP causes cell lysis. One suggests formation of a hydrophilic channel through the cell membrane resulting in the exchange of small ions and water across the target cell membrane. The other hypothesis is based on formation of "leaky patches" on the target cell membrane (30, 95).

2.3 Regulation of the C system

As described above, C is potentially a very cytotoxic system. If not controlled, a destruction of self structures would follow. Regulators are needed to protect own cells and to avoid overconsumption of the C components. Most C regulators on the cell membranes and in the fluid phase act on C3b or prevent the formation of MAC.

Regulation of C at the C3-level occurs mainly by two different mechanisms. First is the enhanced inactivation of C3b and C4b. Serine protease factor I (FI) is the enzyme that cleaves C3b and C4b, but it needs a cofactor. Thus, many regulators possess a cofactor-activity for FI. The second regulatory mechanism at the C3-level is direct interference with an active C3-convertase either by binding to it and displacing the enzymatic component C2a or Bb, called the decay accelerating activity or by prevention of C3-convertase formation.

2.4 Membrane bound regulators

2.4.1 Complement receptor type 1 (CD35)

Complement receptor type 1 (CR1) is expressed by many circulating cells, like erythrocytes, polymorphonuclear leukocytes and lymphocytes (98). It is a single-chain glycoprotein with a transmembrane domain (Table 3). Most of CR1 in the circulation is found on the surfaces of erythrocytes and only trace amounts are found in a soluble form in plasma. CR1 regulates both AP and CP by acting as a cofactor in FI-mediated cleavage of C3b and C4b and by accelerating the decay of the AP and CP C3-convertases (100, 145). CR1 is the only regulator which can also act as a cofactor in further

cleavage of iC3b into C3c and C3d fragments under physiological conditions (205),

CR1, factor H (FH), C4b-binding protein (C4BP), membrane cofactor protein (MCP) and decay accelerating factor (DAF) consist of short consensus repeat (SCR) domains. SCRs are also called complement control protein domains or Sushi repeats. They are encoded by genes in one cluster in chromosome 1, called the regulators of the C activation gene cluster (RCA) (141). Each globular SCR has approximately 60 amino acids held together by two disulphide bonds (169).

CR1 has not only a role in the regulation of C but also in the transport and removal of IC and opsonized targets. CR1 on blood cell surfaces binds C3b and C4b mediating transport of particles coated with C3b and C4b on blood cell surfaces to liver or spleen for degradation. (278). Binding of CR1 to the collagenous tail of C1q might also play a role in the clearance of ICs (171).

2.4.2 Decay-accelerating factor (CD55)

DAF is a 70 kD transmembrane protein widely expressed on various tissues and cells (206). It consists of four SCRs and is attached to a cell surface with a glycophosphatidylinositol-anchor (GPI-anchor). DAF was first isolated from erythrocytes as a protein capable of accelerating the decay of the CP C3-convertase (219). It inhibits both AP and CP C3-convertases by displacing Bb from the C3bBb complex and C2a from the C4b2a complex (110). The functional site has been located to SCRs 2-4 for the AP inactivation and to SCRs 2 and 3 for the CP inactivation (45, 65). Soluble forms of DAF have been found in different body fluids (206).

Microbes can exploit DAF in pathogenesis, as DAF has been found to be a receptor for many viruses (echoviruses, coxsackieviruses) as well as for uropathogenic *E. coli* strains (192).

2.4.3 Membrane cofactor protein (CD46)

First after its discovery MCP was called glycoprotein (gp) 45-70 due to its broad size variation in SDS-PAGE (60). It was soon found that MCP has cofactor-activity for FI-mediated cleavage of C3b and C4b (283). Unlike DAF, it does not have decay-accelerating activity. MCP is expressed on a vast variety of tissues and circulating cells, but not on red blood cells. It is expressed as isoforms due to alternative splicing of the gene (249). Structurally the protein has a cytoplasmic domain, a transmembrane part and four SCRs (195). SCRs 3 and 4 are essential for the function (3). There are small amounts of functionally active soluble MCP in serum and other body fluids (282).

Some microbes, like measles virus, use MCP as a receptor to facilitate entry to nucleated cells. *Neisseria gonorrhoeae* and *N. meningitidis* attach by their pili to MCP expressed on epithelial cells (192). Some M-proteins of group A *Streptococcus* also seem to mediate adhesion to keratinocytes by binding to MCP (115).

Table 3. Properties of the membrane bound inhibitors of C.

Regulator	Binds to	Structure/size	Function in C
CR1 (CD35)	C3b, C4b, C1q	30 SCRs 200 kD	DAA[1], CFA[2]
DAF (CD55)	C3b, C4b	4 SCRs, GPI-anchor 65 kD	DAA
MCP (CD46)	C3b, C4b	4 SCRs 60 kD	CFA
CD59	C5b-8, C5b-9	Single domain, GPI-anchor 20 kD	Prevents binding of C9 to C5b-8

[1] DAA=decay accelerating activity
[2] CFA=cofactor activity

2.4.4 Protectin (CD59)

Protectin is the most abundant cell surface regulator of C. It is expressed by all circulating cells, endothelial and epithelial cells and in many tissues (80, 209). Functionally protectin can bind to the C5b-8 complex and

prevent binding of C9 to the complex and block formation of MAC (207, 271)

CD59 and DAF are both GPI-anchored proteins. Paroxysmal nocturnal hemogloninuria (PNH) is a disease caused by a somatic mutation in hematopoietic stem cells. The mutation affects synthesis of the GPI-anchor and as a result cells without the respective C regulators are found in the circulation. This leads to C-mediated lysis of unprotected red blood cells and symptoms of PNH (anemia, hemoglobinuria) (220, 232).

2.5 Fluid phase regulators

2.5.1 C1-inhibitor

C1-inhibitor (C1INH) is a fluid phase regulator of the CP (Table 4). It belongs to the serine protease inhibitor -family called serpins (116). C1INH inhibits C1 by two mechanisms. First, it binds to the activated C1r and C1s molecules and removes them from the C1-complex (330). Secondly, C1INH prevents autoactivation of C1 that could occur in the absence of activating antibodies (329). C1INH is the only regulator of the C1-complex, but it acts also in the kinin-kallikreinin system (42).

The importance of C1INH is demonstrated by patients who are deficient in C1INH. The deficiency leads to a disease called hereditary angioedema (HAE). The main symptom of HAE is edema in the gastrointestinal tract, airways and in the skin. Decreased amounts of C1INH or mutations at the functionally critical sites cause uncontrolled CP activation and also activation of the kinin system. The symptoms are believed to be largely due to the release of bradykinin (81).

2.5.2 Factor I

FI is a very important serine protease enzyme in the C cascade. It was originally named as the C3b inactivator (C3bINA) because it participates in the inactivation of C3b. By that time it was already known that FI needs a cofactor for its enzymatic function (234, 323). FI cleaves C3b when a cofactor FH, CR1 or MCP is present. For the inactivation of C4b FI needs

C4b-binding protein (C4BP), CR1 or MCP as a cofactor (215). C3b gets cleaved into iC3b by FI at two sites in its α'-chain releasing a small fragment called C3f (118). Further FI-mediated cleavage of iC3b occurs only when CR1 acts as a cofactor (272). C4b α-chain is cleaved by FI and a cofactor at two sites releasing a C4c fragment and leaving C4d to the surface.

2.5.3 Factor H

FH is the most important fluid phase regulator of the AP. Initially it was described as β1H -globulin (221) capable of regulating C3 activation (234). FH acts at the C3-level by three different mechanisms (323). It is a cofactor for FI in the cleavage of C3b, resulting in formation of inactivated iC3b (127, 234). Two cleavages of C3b by FI and FH can occur *in vivo,* and *in vitro* further cleavages are possible under nonphysiological conditions (272). FH also accelerates a decay of the AP C3-convertase by dissociating Bb from the convertase and by competing with FB for binding to C3b (320, 323). These functions make FH a very efficient regulator of the AP amplification phase. New suggested functions for FH outside the C system include mediation of cell adhesion (15, 87)

Structurally FH consists of 20 SCRs (287) (Fig.1). The regulatory site of FH for C3b has been located to SRCs 1-4 (119, 184, 186). Other binding sites to C3b have been localized to SCRs 12-14 and 19-20 (159, 161, 285). FH interacts with heparin via three binding sites; one in SCR7 a second in SCR 20 and third in SCR9 (32, 33, 227, 231).

Together with C3b FH has an important role in determining whether a surface acts as an activator of the AP or not. FH can bind to polyanions (like sialic acids and glycosaminoglycans such as heparan sulphate) on surfaces. When bound to these negatively charged structures FH has a higher affinity for the target-bound C3b (208). Sialic acids have been shown to protect a surface and their removal makes the surface an activator by inhibiting the decay-accelerating activity of FH (99). On the other hand, C3b bound to an activating surface has a decreased affinity for FH, which makes the target more susceptible to the AP activation.

FH, like its truncated form factor H-like protein (FHL-1) (see below), has been shown to be secreted by some tumour cells, such as human glioblastoma cells. The secretion and/or binding of FH/FHL-1 increases the C resistance of these cells (163).

Fig. 1. Schematic structure of FH and FHL-1.

A very interesting aspect of FH is its newly discovered role in three diseases, like atypical hemolytic syndrome (aHUS), membranoproliferative glomerulonephritis (MPGNII) and age-related macular degeneration (AMD).

Atypical HUS is not followed by infection with enterohemorragic *E.coli* like the typical HUS but is a s.c. familial or sporadic disease usually with a genetic background. C3 deposition in tissues and decreased amounts of C3 in the patient sera were found in atypical HUS patients as early as in 1977 (24). Several different mutations in the FH-coding gene have been discovered from aHUS patients. A premature stop-codon in the reading frame affects the amount of FH and point mutations alter its function (273). Point mutations are clustered in the C-terminus and especially in the SCR20 of FH (51, 238, 270). SCR20 is an important interaction site for FH, as it contains partially overlapping binding sites for C3b, C3d and heparin (132). Thus it is not a surprise that point mutations in FH SCR20 affect its ability to bind to a surface-bound C3b (196, 276) or surface polyanions (230, 240).

Another disease associated with FH deficiency or dysfunction is type II MPGNII. In this disease FH in unable to protect the basement membranes in

the kidney glomeruli. As a result, extensive C activation leads to C3b and terminal C component depositions that lead to obstructions of the filtering function of the basal membranes.

FH deficiency has been described in approximately 12 families. Individuals without FH are more prone to MPGNII and bacterial infections, especially by *Neisseria* (213). Pigs deficient in FH have a single nucleotide change, which causes a framework shift and blockes secretion of FH (130). FH-deficient knock-out mice suffer from MPGNII and extensive deposits of C3b are found in the kidney glomeruli (243).

Recently several groups reported that a polymorphism in the gene encoding FH is a risk factor for AMD (92, 124), the most common cause of blindness in industrialized countries. Increased amounts of deposited C5b-9 are found in the maculae of the patients with AMD (170). In the risk allele a tyrosine at position 402 is SCR7 is converted to histidine.

2.5.4 Factor-H like protein

FHL-1 is a 42 kD alternatively spliced product of the FH-coding gene (97). The protein consists of seven SCRs identical to the N-terminal SCRs of FH and an unique tail of four amino acids at the C-terminus (334). FHL-1 has one binding site for C3b and can act as a cofactor in FI-mediated cleavage of C3b similarily to FH (184). Decay accelerating activity for C3- and C5-convertases has also been described (186). In addition, FHL-1 has been suggested to mediate cell adhesion to a fibronectin matrix (134).

2.5.5 Factor-H related proteins

Recently several proteins consisting of FH-like SCRs have been described, as reviewed in (332). These proteins are transcripts of different genes in a same chromosome (1q32) as FH and FHL-1. They share structural similarity with FH (Fig. 2), and therefore they are considered to belong to one family and are called FH-related proteins (FHRs) (333). Five human FHR proteins have been identified in a family and they are named FHR-1 through FHR-5.

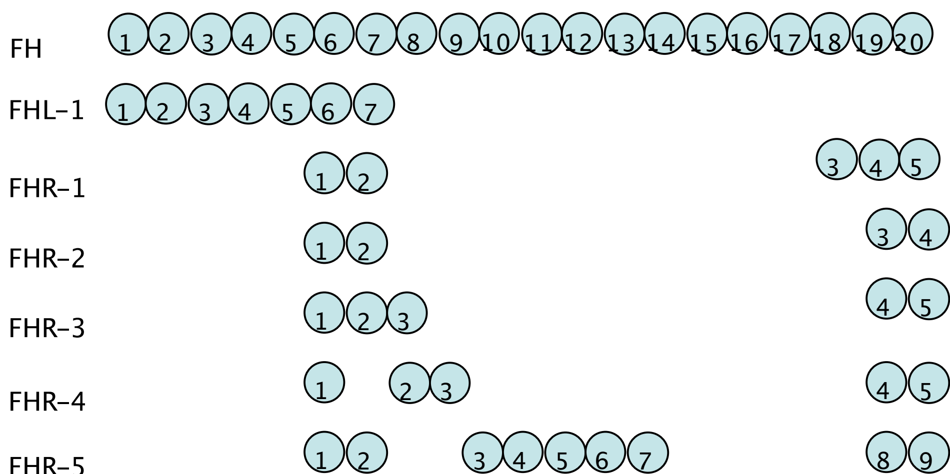


Fig. 2. The FH protein family. Numbered circles represent SCR domains and the alignment is shown according to highest homology between SCRs.

FHR-1 consists of 5 SCRs and FHR-2 of 4 SCRs. Both are found in two differently glycosylated forms in plasma. The two C-terminal SCRs in all FHRs share high homology with the C-terminal SCRs of FH (96, 290, 292). FHR-3 and FHR-4 are structurally very similar (289, 291) and they bind to C3b, C3d and opsonized pneumococci (133, 135). Physiological functions of these proteins are still not known (335). Recombinant FHR-5 binds to C3b *in vitro* (203) and has been detected in glomerular immune complexes (214). In rat, FHR-5 homologue inhibits the AP of rat C (268).

Yet another FHR of 63 kD, named FHR4A, was recently described. Structurally it is slightly different from the other FHRs, as it has an internal duplication of four SCR domains (162). FHR-4B (previous FHR-4) is an alternatively spliced product of the FHR-4A gene.

2.5.6 C4b-binding protein

The major fluid phase regulator of the CP and LP pathways is C4BP (23). It was isolated from mouse and human serum in the 1970's (102, 277).

The appearance of C4BP has been described as spider-like (Fig. 3). It consists of 7 or 8 α-chains and one β-chain (76, 77), which are held

together by disulphide bonds and hydrophobic interactions (166). Like other C regulatory proteins of the RCA gene cluster, C4BP consists of SCRs. There are eight SCRs in each α-chain and three in the β-chain (57, 58, 139). C4BP circulates in plasma in a complex with protein S (73). Vitamin K-dependent protein S is bound to the β-chain via SCR1 (74, 75, 125). A reason for complex formation between protein S and C4BP is not well understood. C4BP-bound protein S is not active in its anticoagulant function. Localization of C4BP via protein S to negatively charged surfaces (281) or to apoptotic cells (319) has been proposed as a physiological function. Interestingly, C4BP bound to apoptotic cells in complex with protein S inhibits phagocytosis of these cells (167).

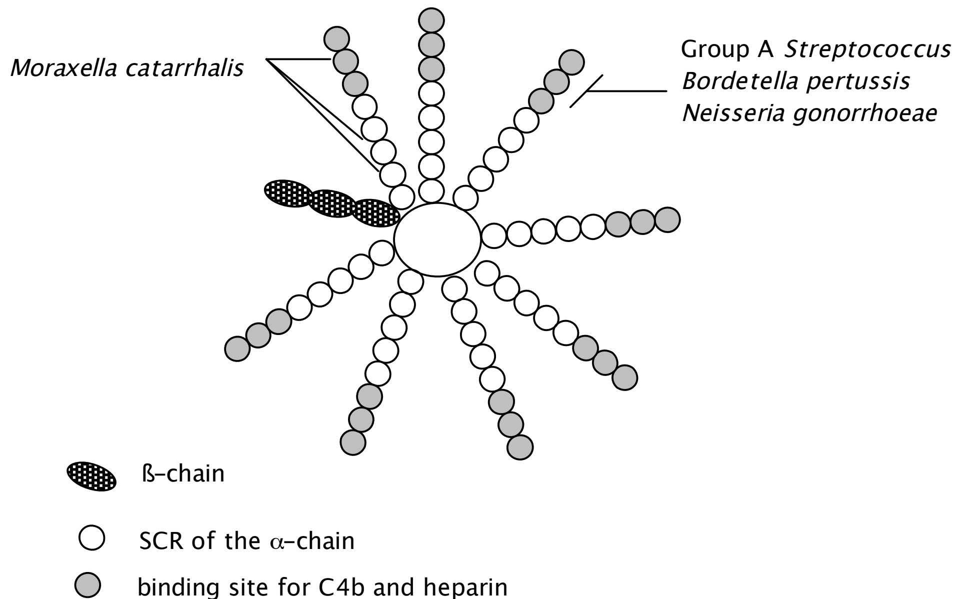


Fig. 3. Schematic structure and microbial interaction sites in C4BP

C4BP binds to its major ligand, C4b, via α-chains (137). Only four α-chains can bind simultaneously to C4b molecules on one surface, probably because of sterical hindrance (331). N-terminal SCRs 1-2 and a cluster of positively charged amino-acids mediate binding of C4BP to C4b (39, 40). The binding site for C4BP in C4b is in the α-chain (136). When bound to C4b, C4BP acts as a cofactor for FI-mediated cleavage of C4b (111, 112)

and accelerates the decay of the CP C3-convertase C4b2a (109). C4BP can also participate in regulation of the AP. At high concentrations (approximately 1000x higher when compared to FH) C4BP can accelerate the decay of the AP C-convertase C3bBb (36).

2.5.7 S-protein (vitronectin)

Fluid phase regulators of the terminal pathway prevent the attachment of C5b67 to the target membrane or prevent the binding of C9 to the C5b-8 complex. The short life-time of the C5b67 complex is a control mechanism in itself. If the complex does not bind first the membrane and then C8 within a few hundreds of milliseconds the C5b67 complex is unable to bind C9 and the TP activation stops (218).

Vitronectin and S-protein were described by two different groups and thus originally named differently (22, 248). Later structural and functional studies showed that the two proteins were the same (251). S-protein is a fluid-phase protein in plasma and in a variety of body fluids (284).

In the C system S-protein acts as an inhibitor of MAC formation by binding to the soluble C5b67-complex and preventing the attachment of the complex to a membrane (248). Binding of S-protein to C5b-7 does not inhibit subsequent binding of C8 and C9, but the end result is a soluble SC5b-9, which is not able to cause lytic holes to the membrane. Other, not C-related, functions for S-protein are multiple. It has been shown, for example, to mediate of cell adhesion to tissues (129) and regulate heparin-mediated anticoagulation (252).

2.5.8 Clusterin

Clusterin (SP-40,40) is a protein with many names and proposed functions. It is heavily glycosylated protein and has two chains, α and β, held together by disulphide bonds (313). Multiple functions of clusterin include clearing of cell debris and lipid transport (152). Clusterin is present in serum and other body fluids.

In the C system clusterin, similarily to S-protein, binds to the C5b-7 complex and prevents its attachment to cell surfaces (56). It can bind to C7, C8 and C9 (311), but the role of clusterin in the regulation of MAC is still uncertain.

Table 4. Properties of the fluid phase C inhibitors.

Regulator	Binds to	Structure/size (kD)	Function	Serum conc. (μg/ml)
C1 inhibitor (C1INH)	C1r C1s	serine protease inhibitor/71	dissociation of C1r, C1s from C1q inhibition of C1r, C1s	200-250
Factor I (FI)	C3b C4b	Serine protease /90	Cleaves C3b and C4b; needs a cofactor	30-40
Factor H (FH)	C3b	Single chain with 20 SCRs/150	DAA[1], CFA[2]	450
C4b-binding protein (C4BP)	C4b	7-8 α-chains of 8 SCRs, 1 β-chain with 4 SCRs /540-590	DAA, CFA	150-170
S-protein (vitronectin)	TCC[3]	n x 77	Inhibits binding of C5b-7 to membrane	250-540
Clusterin	C5b7	Dimer with α and β chains/70	Inhibits binding of C5b-7 to membrane	250-420

[1] DAA=decay accelerating activity
[2] CFA=cofactor activity
[3] TCC=terminal C complexes (C5b-7, C5b-8, C5b-9)

2.5.9 Properdin

Properdin is the only known positive regulator of C. It binds to the AP C3-convertase C3bBb and increases its life span and activity (101, 204). Properdin is a fluid phase protein with a serum concentration of approximately 25 μg/ml. Properdin-deficient individuals suffer from bacterial, especially meningococcal, infections (61).

2.6 Functions of the complement system

One major function of the C system is protection against invading micro-organisms and there are several mechanisms to achieve this. First, opsonization is an important consequence of the C activation. C4b, C3b

and its cleavage fragment iC3b attached to a surface are recognized by specific C-receptors (CR1 and CR3) on phagocytic cells. Binding of the target to phagocytes is a prerequisite for its phagocytosis and killing. Second, MAC-complexes formed during the TP activation can directly lyse the target cells such as invading microbes. Third, anaphylatoxins C3a and C5a are cleaved from C3 and C5 during C activation and have two roles in enhancing the inflammatory reaction. Both C3a and C5a act as anaphylatoxins. For example, binding of C3a to its receptor on mast cells and basophils triggers degranulation of the cells and subsequently increases vascular permeability and facilitates cell trafficking (72). Phagocytes are attracted to the site by chemoattractive C5a and are activated via binding of C5a and C3a to their receptors (321).

Fourthly, C3a and its carboxypeptidase cleaved inactivated form, C3a-desArg, have recently been shown to act as antimicrobial peptides against Gram-negative and Gram-positive bacteria by breaking bacterial membranes (223). The fifth antimicrobial role for C is actually a link between the C system and the adaptive immunity. C3d is able to act as a potent molecular adjuvant in antibody production. When attached to an antigen C3d and especially multiple C3d molecules enhance the acquired immune response by B cells (82) and affect also the amount and repertoire of IgM (53).

Clearing of immune complexes (IC) and debris from the human body is another important function of the C system (278). ICs are formed when antigens and antibodies are aggregated together in the human body. These complexes activate the C system, which leads to opsonization. CR1-receptors on erythrocytes regocnize C3b and C4b and transfer the complexes to liver or spleen for degradation.

3 MECHANISMS OF MICROBIAL COMPLEMENT EVASION

The C system can be activated efficiently on a foreign or nonprotected target and the main results of a successful activation are opsonization for phagocytosis and lysis of the target cell. In a general, microbes are susceptible to C activation. There are numerous microbes which are able to multiply in the human host and cause diseases despite of the activities of the C system. Many mechanisms have evolved in microbes for C evasion and probably their ensure survival by using more than one. As an example, the blood trematode *Schistosoma mansoni* has been described to possess nine different C evasion strategies (326). Microbial C evasion is a very complex subject, and new observations will eventually increase our understanding of the ingenious mechanisms the pathogenic microbes have for their survival. Excellent reviews of the subject have recently been published (34, 160, 326).

Some general C evasion mechanisms of microbes are discussed in this chapter. Utilization of fluid phase regulators FH, FHL-1 and C4BP are discussed in more detail.

3.1 Microbial surface structures

Physical barriers, like capsule and outer membrane of bacteria or tegument of parasites, like *S. mansoni*, protect the microbes from a damage caused by antimicrobial proteins of the host. In *Staphylococcus aureus* the expression of capsular polysaccharides enhances survival from opsonophagocytosis (307). Long surface molecules and dense capsular structures can sterically hinder the attachment of C proteins to the surface (325) or participate in the release of TCC (158). *Salmonella montevideo* binds preferentially C3b via long polysaccharide side chains of LPS. As a consequence, C becomes activated more remotely from the cell membrane disabling membrane insertion of the C5b-9-complex (157).

Sialic acids or glycoproteins on the microbial surface can impede the recognition and activation of C. *Trypanosoma cruzi* has a very active trans-sialidase enzyme on its surface. Sialylation of the surface is rapid and

protects *T. cruzi* from C attack. This is probably mediated by acquisition of the host FH to the sialylated surface (310).

3.2 Shedding and degradation of C components

The removal of surface bound C components can prevent harmful effects of C activation. Promastigotes of *Leishmania major* prevent formation of MAC by releasing C5b–9–complexes to the surroundings (254), and promastigotes of *L. donovani* can release 50% of bound C3b within 30 minutes by a proteolytic cleavage (255). Secreted proteolytic enzymes can degrade C components. For example, secreted proteases of *S. mansoni* schistosomula can cleave C3b (197) and other surface-attached host proteins and make the parasites resistant to the AP attack (198). *Streptococcus pyogenes* inactivates chemotactic activity of the anaphylatoxin C5a by an enzymatic cleavage (322). *Candida albicans* proteinases have been suggested to degrade C3b and thus disable AP activation (164).

3.3 Microbial C inhibitors

Microbial C inhibiting proteins have been described in bacteria, parasites and viruses. Glycoprotein C (gC) of herpes simplex virus 1 is expressed on the surface of the virus-infected cells and can bind C3b and iC3b. After binding to C3b gC blocks the interaction between C3b and properdin and accelerates the decay of C3bBb. These functions have also been detected with purified gC (108, 175). In a murine model of herpes infection the viruses that lacked the functional domain of gC were less infective (194).

Molecular mimicry of C proteins is a very special mechanisms for C evasion by microbes. *Trypanosoma cruzi* gp160 protein is anchored to the surface of *T. cruzi* trypomastigotes via a GPI-anchor and is able to accelerate decay of the AP C3-convertase. At the DNA-level the sequence coding for gp160 is 60% homologous to a gene encoding DAF (225, 236). Cells infected with vaccinia-viruses secrete vaccinia virus C-control protein (VCP), which is capable of inhibiting the CP. VCP contains four SCRs and the sequence similarity to the SCRs 1-4 of the C4BP α-chain is 38% (178). Mutated vaccinia viruses without VCP are less infective in an animal model

(146). Herpesvirus saimiri has a homologue of CD59, which can bind to MAC and inhibit C9 polymerization and lytic effects of MAC (8, 9).

Candida albicans hyphal forms have been reported to have two proteins that are functionally similar to human CR2-like and a CR3 -like proteins. These surface proteins are suggested to bind C3d or iC3b in a manner that inhibits opsonization and phagocytosis (50, 117, 131).

Paramyosin of *S. mansoni* binds C1q, the first component of the CP. As a result the activity of C1q in CP activation is inhibited (188). Paramyosin in schistosomes is a multifunctional regulator of the C, as it can also bind to C8 and C9 in such a manner that TP activation is stopped (83). This inhibitory protein of *S. mansoni* has been suggested to resemble human CD59 both structurally and functionally (235).

3.4 Utilization of host cell surface C regulators

Microbes, especially viruses, can alter the expression of host proteins. Cytomegalovirus-infected cells express higher amounts of the C regulators MCP and DAF on their surfaces, when compared to noninfected cells (294). Viruses can also acquire C regulators to their surfaces when budding out from the host cell (316).

Some encapsulated strains of *E. coli* and *Helicobacter pylori* are able to acquire GPI-anchored CD59 from host cells. "Hijacked" CD59 inhibits formation of C5b-9 on the surface of the bacteria and increases their survival in serum (265, 266). DAF, another GPI-anchored regulator on the cell surfaces, has been shown to be acquired by *S. mansoni.* DAF on the microbial surface can regulate AP activation (236).

3.5 Utilization of fluid phase regulators

3.5.1 FH and FHL-1

S. pyogenes (group A streptococcus; GAS) is a Gram-positive bacterium. It can cause, for example, throat infections, scarlet fever and severe invasive infections, such as cellulitis and septicaemia. The major virulence factors of

GAS are hyaluronic capsule and M-proteins. M-proteins are fibrillar proteins with alpha-helical coiled-coil structures (242). There are more than 100 different M-serotypes recognized by antigenicity or sequence of the N-terminal hypervariable region of the M-proteins (103). GAS was the first microbe that was shown to bind FH. The ligand for FH was identified as M-protein. M-protein had been reported to be important in preventing opsonization and phagocytosis of GAS (241). Later it was shown that strains which bind FH are in general more resistant to opsonophagocytosis.

The first FH-binding site in M-protein (serotype 6) was mapped to the conserved C-repeat region at the C-terminus of the protein (31, 105), while later the binding site turned out to be in the N-terminal hypervariable region (155). The binding site of FH for M-proteins was first located to SCRs 6-10 (286). Later, the binding site was narrowed to SCR7 (177) and inhibition of the interaction by heparin was shown (31). Thus, it seemed that the binding site of FH for M-protein is near to or overlapping with the heparin-binding site in the SCR7, and the binding sites for M-protein and heparin were localized to the same positively charged region in SCR7 (114).

M5-protein of GAS binds also another AP regulator, FHL-1, and even with a higher affinity than FH (177). In two different M-proteins studied, M5 and M6, the hypervariable regions were responsible for binding of FHL-1 (155).

The existence of a second ligand or binding region in M-protein for FH was suggested, when a GAS strain expressing a mutant M6-protein lacking the FH-binding domain was resistant to phagocytosis (239). The importance of M-proteins in the resistance of GAS to phagocytosis was further questioned, when a strain expressing a M5-mutant protein incapable of binding FH and FHL-1 was as resistant to phagocytosis as a strain carrying a full length M-protein (176). However, a second ligand for FH and FHL-1 in GAS was identified and turned out to be a fibronectin-binding protein (Fba) (228). FH and FHL-1 bind to Fba via SCR7. Interestingly, binding of FHL-1 to the Fba -protein increases invasion of *S. pyogenes* to epithelial cells (229).

Pneumococci (*Streptococcus pneumoniae*) are common human pathogens. They can cause invasive infections like septicaemia, meningitis and pneumonia, especially in children and elderly people. The major virulence factor of pneumococci is a capsule; the lethal dose of encapsulated strains can be up to 50% higher when compared to strains without the capsule (13). Serotyping (up to 80 different types) of pneumococci is based on polysaccharides of the capsule. Evasion of C is important for pneumococci to prevent opsonization. As they are Gram-positive bacteria, MAC is unable to penetrate the peptidoglycan and lyse the bacteria. One mechanism for C evasion in pneumococci is binding of FH. Neeleman showed that FH binds to encapsulated type 3 pneumococci, possibly via a surface-exposed protein (217). Later, *PspC* locus encoded proteins Hic and PspC were shown to bind FH via homologous N-terminal regions (78, 148). SCRs 13-15 (91) and 8-11 (150) of FH were reported to bind to PspC of a serotype 2 and Hic of serotype 3, respectively. Later, the first binding site was located to SCRs 12-14 with the serotype 3 Hic -protein (149). Thus, a new microbial binding site in FH was discovered, as GAS bound FH only via SCR7. Recently, another binding site on FH for PspC (serotype 2) was mapped to SCRs 6-10 (79). Further evidence of the importance of C avoidance for streptococci was obtained when group B streptococci were reported to bind FH via β-protein (14). Two different binding sites on FH were found (149).

Neisseria gonorrhoeae (gonococcus) is a Gram-negative noncapsulated bacterium that causes the sexually transmitted disease gonorrhoea. Another bacterium from the same family, *N. meningitidis* (meningococcus), causes meningitis and septicemia. (269). Gonococci can be lysed by MAC. Thus, it is very important for pathogenic strains to avoid C. Nonsialylated strains of gonococci are serum-sensitive while sialylation of lipo-oligosaccharides (LOS) makes gonococci resistant to serum. One reason for the detected serum resistance is binding of FH. Sialic acids of LOS or the loop 5 of the outer surface porin 1A can bind FH (260, 261). *N. meningitidis*, in contrast to *N. gonorrhoeae*, is usually capsulated, and the capsulated strains are more virulent. It has been suggested that the capsular polysaccharides inhibit formation of MAC and in the noncapsulated strains sialylation might participate in serum resistance by binding of FH (259).

Parasites are multicellular organisms and obviously the pathogens that live for a long time in contact with plasma and are resistant to C mediated cell lysis and also well protected against phagocytosis. *Echinococcus granulosus* was the first parasite shown to bind FH as an immune evasion mechanism. *E. granulosus* is a cestode parasite, which forms slowly growing hydatid cysts to organs of the host. Cysts are surrounded by a two-layer wall and filled with hydatid cyst fluid. When cofactor activity of the cyst fluid for the cleavage of bovine C3b was analyzed, it was found that the cofactor activity was due to the host FH inside the cyst fluid (84), which may have been transported there.

Viruses can obtain membrane C regulators from the host cell surfaces upon budding, but they can also benefit from the host fluid phase regulators. The human immunodeficiency virus (HIV) has been suggested to bind FH by a gp41 protein (301). The binding of FH to virions or virus-infected cells could increase the cleavage of C3b to opsonizing fragments iC3b and C3d, which might lead to improved uptake of viruses into the C-receptor carrying CD4+ T-cells (300).

3.5.2 C4BP

GAS is thus far the best example of how bacteria need multiple mechanisms to evade C. In addition to FH acquisition they can bind C4BP. The multiple mechanisms are a further argument for the importance of C evasion. Structurally homologous proteins of the M-protein family mediate binding of C4BP (308) through the highly variable N-terminal region (156, 212). In C4BP, the binding sites for GAS and C4b are overlapping but not identical (2, 35). Binding of C4BP is beneficial for GAS, as it significantly increases the resistance of bacteria to phagocytosis (27, 52). In the M18 serotype of *S. pyogenes* another molecule resembling M-protein, Enn18, has recently been shown to be a ligand for C4BP (237).

Bordetella pertussis, the causative agent of whooping cough, and other members of the family *Bordetella*, were the second type of bacteria shown to bind human C4BP. Mutant strains of *B. pertussis* and comparison of the outer surface proteins of different strains showed that one potential ligand

for C4BP is a filamentous hemagglutinin (FHA). FHA is encoded by a single gene locus *bvg*, and mutants lacking the whole locus were negative for binding of C4BP. However, FHA-negative mutants still bound some C4BP. These findings could be explained by an existence of another ligand for C4BP on *B. pertussis* (26). SCRs 1-2 in C4BP, the binding site for C4b, are involved in binding of C4BP to *B. pertussis* (28).

Gonococci and meningococci are able to acquire both FH and C4BP for protection against C. Porins, which bind FH (260), can also bind C4BP (151, 258). Identification of another C4BP-binding structure on *N. gonorrhoeae,* type IV pili, indicates the importance of C evasion for gonococci (38). When binding of C4BP to gonococci (serotypes Por1A and Por1B) was inhibited, the bacteria changed their serotypes from serum resistant to serum sensitive in 30 minutes (257).

Escherichia coli is the most common commensal in the intestine. In addition, it can cause infections, for example, in the urinary tract. Enteropathogenic strains of *E. coli* cause severe diarrhea and enteritis. Septicemias caused by *E. coli* are found mostly in neonates and usually the serotype causing the disease is K1. *E. coli* has several virulence mechanisms. One is the capsular polysaccharide (like K1) and the other, the expression of outer surface protein A (OmpA). OmpA-negative bacteria are serum-sensitive and OmpA-positive are resistant to serum. Binding of C4BP to OmpA of *E. coli* K1 has been shown to contribute to its serum resistance (250).

Moraxella catarrhalis is a Gram-negative coccus. It can be part of the normal upper respiratory tract flora, but it is also a causative agent of otitis media and sinusitis. *M. catarrhalis* binds C4BP via two different ligands, ubiquitous surface proteins A1 (UspA1) and A2 (UspA2)(224). Unlike with the other microbes studied, multiple SCRs (2, 5, 7) of C4BP are involved in the interaction.

4 OVERVIEW OF MICROBES EXAMINED IN THIS STUDY

4.1 *Borrelia burgdorferi* sensu lato

There are two different groups of human pathogens in the genus of *Borrelia*; borrelia causing Lyme disease and those causing relapsing fever (will be discussed later). The Lyme disease borreliae belong to the complex *B. burgdorferi* sensu lato, which contains 11 different genospecies. Human pathogens in the complex are *B. garinii*, *B. afzelii* and *B. burgdorferi* sensu stricto. Lyme disease and its causative agent were first described as late as 1982 (46). Lyme disease borreliae are transmitted by hard-shelled ticks from the *Ixodes*-family. *B. burgdorferi* s.s. is the only human pathogenic genospecies found in North-America, while all three exist in Europe (94). Human beings are not the only hosts for borrelia, as other mammals, birds and reptiles can also be reservoir hosts. Ticks are infected by borrelia during a blood meal from an infected host. First, the spirochetes reside inside the midgut of the tick. Later they move to salivary glands from where they are transferred to the next "lunch restaurant" of the tick.

The outer membrane of spirochetes is unique. There is no LPS and the number and amount of transmembrane proteins are low. On the other hand, the amount of lipoproteins (proteins attached to the membrane via a lipid-tail) is high (29, 66). Between the outer membrane and the cytoplasmic membrane is a periplasmic space, where flagellas of borreliae are attached to either pole of the inner membrane. The expression of the outer surface proteins is dependent on temperature, pH, nutritional status and several other factors (262, 298). By being able to change the composition of its outer membrane borreliae can adapt to the different hosts in its life cycle. Some outer membrane proteins are important virulence factors. Decorin and fibronectin binding proteins have been suggested to mediate adherence to tissues (66).

Lyme disease, called also Lyme borreliosis, is a systemic inflammatory disease. Symptoms of Lyme disease vary according to the causative agent and geography. Thus, symptoms caused by *B. burgdorferi* are different in North-America and Europe. Infection usually starts as an erythema migrans

(EM) lesion (296), which is the most common clinical manifestation of borreliosis. EM develops to a site of the tick bite in about 50% of cases and is a typical ring-like rash. All three genospecies can cause EM. In a chronic phase the skin involvement is called acrodermatits chronica atrophicans (ACA). The majority of the ACA cases in Europe are caused by *B. afzelii*. After initial exposure the spirochetes first reside in the skin but are later transferred via circulation to different organs. In the late stages of infection the most affected sites are the nervous system and joints. Arthritis is the major manifestation of borreliosis in North America (295).

Major differences in the serum sensitivity between the strains of *Borrelia* have been reported (43, 172). *B. garinii* is serum sensitive, while *B. burgdorferi s. s.* and *B. afzelii* are partially or totally serum resistant. Serum sensitive strains are killed in normal human serum (NHS) mostly by the AP of C (44, 187, 315).

4.2 Relapsing fever borreliae

Relapsing fever (RF) *Borreliae* are divided into two groups of spirochetes on the basis on vectors. *B. recurrentis* is the only known borrelia transmitted via body or head lice. It causes louse-borne relapsing fever (LBRF), also called epidemic relapsing fever. As the name of the disease implies, LBRF has the potential to cause epidemics. For example during the World War II millions of people were infected in Southern Europe. Nowadays LBRF is found in various parts of Africa, for example in Ethiopia (302), and in China and Peru. It was only recently when *in vitro* cultivation of *B. recurrentis* and characterization of the species was accomplished (69, 70). Tick-borne relapsing fever (TBRF) is caused by at least 15 species of *Borrelia*, and the disease has a scattered world-wide distribution. Vectors for TBRF-borrelia are soft-bodied ticks from the genus *Ornithodorus*.

Transmission of RF occurs when spirochetes enter blood circulation from the site of a tick or louse bite wound. A massive spirochetemia follows. The first septic episode ends upon development of antibodies, but because the spirochetes are able to change their variable outer surface proteins, the spirochetemia returns causing one or more fever relapses. Relapsing fever is clinically a severe disease, and its mortality is 30-70% without antimicrobial treatment (68).

One of the major mechanisms enabling survival of relapsing fever *Borrelia* in a human host is antigenic variation. *B. hermsii* is the best studied example of the antigenic variation. By changing its main antigens on the outer membrane a single strain of *B. hermsii* can form up to 24 different serotypes *in vivo* (299). Two plasmid-encoded major proteins, variable large protein (Vlp) and small protein (Vsp), are responsible for the antigenic variation (17-19, 21). At the genome level there are four mechanisms behind the antigenic variation: (i) modification of transcript levels, (ii) gene conversion, (iii) DNA rearrangement and (iv) multiple point mutations (for review see reference (20)). In *B. turicatae* four different serotypes have been recognized to exist due to antigenic variation (264). It is probable that all relapsing fever borreliae share the same antigenic variation scheme as the two species described above.

Relapsing fever borreliae are able to persist blood for long periods. Structurally they are probably fairly similar to Lyme disease borrelia. Protection against AP and CP is obviously essential for the pathogenicity of relapsing fever borreliae.

4.3 *Candida albicans*

Candida albicans is the yeast that is most often found in human infections (25). It belongs to the genera *Candidaea*, which contains more than 100 species. Other important *Candida* species found in human infections are, for example, *C. tropicalis, C. glabrata*, *C. parapsilosis* and *C. kruzei*. The majority of the members of the genus are plant or animal pathogens. *C. albicans* is the one exceptional species of the genus that is able to grow as a cellular, pseudohyphal and hyphal form. This phenomenon, called morphogenesis, is reversible and has been considered important for the pathogenesis of *C. albicans* (67). Transformation from a cellular to pseudohyphal or hyphal form is dependent on the temperature, pH and nutritional conditions on the surroundings.

Most often *C. albicans* is a harmless commensal on the skin, oral cavity or urogenital and gastrointestinal tracts in healthy human beings. In immunocompromised patients, like granulocytopenic or lymphopenic

patients, *C. albicans* tends to cause local or systemic infections (104). Disseminated *C. albicans* infections are often lethal due to problems in early diagnosis and worsening condition of immunocompromised patients (93).

In addition to morphogenesis *C. albicans* has several other virulence factors. It expresses adhesion molecules on its surface. Adhesion of the yeast to proteins of extracellular matrix of the host, such as fibronectin and laminin, enhances its pathogenicity (49). Several secreted aspartyl proteinases (216) and phospholipases have been recognized from *C. albicans* (41), and these enzymes can degrade many host molecules.

The C system has been shown to participate in immune defence against *C. albicans*. Complement deficient guinea pigs have severe invasive *C. albicans* infections with higher mortality than normal animals (113). In addition, an efficient C opsonization on the fungal surface is needed for effective clearance of *Candida* by antibodies (54). *C. albicans* activates to some extent all three pathways of C (179). AP is activated via attachment of C3b molecules directly to the surface antigens (180). Mannan is one of the major component on the outer surface of candida and acts as a ligand for MBL, which activates LP (303). Mannan-specific IgG antibodies are found in approximately 70% of healthy individuals (1), and by binding to the yeast they can activate the CP of complement.

4.3 *Onchocerca volvulus*

Man is the only known host for the filarial parasite *O. volvulus*, which is transmitted by *Simulium* black flies. During a blood meal of an infected fly, larvae of *O. volvulus* are transmitted to a subcutaneous tissue of the future host. The larvae develop to adult worms in approximately 30 days. The adult worms (males measuring 2-4 cm, females 70 cm) live in subcutaneous nodules encapsulated by the host for up to 15 years. Females release thousands of live microfilariae (mf) daily. Mf reside mainly in the subepidermis of the skin and in the eyes (165). Eventually mf are transmitted back to a *Simulium* -fly and the life cycle of the parasite continues.

Onchocerciasis (also called river blindness) is found in Africa, Latin America and the Arabian Peninsula (48). Almost 18 million people are infected with the parasite (324). The disease is mainly caused by the immunological responses of the host to the wandering mf. Inflammatory reactions lead to blindness, skin alterations and muscoskeletal disorders. Ivermectin is an effective drug against microfilariae, but not against the adult worms. Patients treated once a year with ivermectin are symptomless. Several ivermectin-treatment trials have been successful in eliminating the symptoms of the river blindness in the endemic areas, but eradication of onchocerciasis is still far in the future (47, 71).

Studies on *O. volvulus* are difficult because there is no animal model available and the parasites can not be grown *in vitro*. Therefore the only material is from infected individuals. Studies with mf from other species have shown, that opsonization by C together with antibodies are needed for phagocytosis (4, 244, 263). *In vitro* studies with *O. volvulus* have shown, that NHS increases eosinophil-mediated killing of the mf (121). How the mf evade C attack in blood is not known.

5 AIMS OF THIS STUDY

The general aim of this study was to analyze if different pathogenic microbes can utilize the host fluid phase C regulators FH, FHL-1 and C4BP for C evasion.

The more detailed aims were:

- To assay if different genospecies of the complex *Borrelia burgdorferi* sensu lato can bind the AP regulator FH and to identify the ligand for FH
- To study if the relapsing fever *Borrelia* acquire host FH and C4BP
- To study if nonbacterial human pathogens, pathogenic yeast and even a multicellular nematode *O. volvulus*, can interact with soluble host C regulators
- To test functional activity of host regulators on different microbial surfaces and therefore to assess the importance of the interaction for pathogenicity of the microbes

6 MATERIAL AND METHODS

6.1 Materials

6.1.1 Sera and cells

Normal human serum (NHS) samples were obtained from healthy laboratory personnel (I, II, V) or from a blood bank (University Clinics of Friedrich Schiller University, Jena, Germany) (III, IV). Sera were stored at –70°C prior to use. C was inactivated from the serum either by adding ethylenediaminetetraacetic acid (EDTA) to a final concentration of 10 mM (NHS-EDTA) or by heat inactivation at +56°C for 30 min for (NHS-HI). NHS was depleted of C4BP (NHS-C4BP) by absorption with anti-C4BP Sepharose (319). Normal mouse serum was obtained from BALB/c laboratory mice and stored at –20°C. Human umbilical vein endothelial cells (HUVEC) were purchased from ATCC (Vanassas, VA, USA) and cultured according to standard cell culture procedures.

6.1.2 Chemicals, plastic materials and buffers

Bovine serum albumin (BSA), trypsin, soybean trypsin inhibitor, inulin, Calcofluor-stain, BSK-H and BSK-H Complete media were from Sigma, St. Louis, MO, USA. RPMI1640 medium, DMEM medium, L-glutamine, HEPES, penicillin and streptomycin were from Gibco, Karlsruhe, Germany. Saboraud agar was from Biokar, Diffchamb, Gothenburg, Sweden and heparin from Lövens Kemiske Fabrik, Ballerup, Denmark. MaxiSorp microtiter plates were manufactured by Nalge Nunc, New York, NY, USA and the C3a Enzyme Immunoassay Kit by Quidel Corp., San Diego, CA, USA. The reagents for the surface plasmon resonance technique, 0.2 M N-ethyl-N'-(dimethyl-aminopropyl)-carbodiimide and 0.05 M N-hydroxysuccinimide were from Biacore AB, Uppsala, Sweden. The BCA Protein Assay was from Pierce Chemical Company, Rockford, IL, USA.

The buffers used were: phosphate buffered saline (PBS; 0.135 M NaCl, 15 mM phosphate, pH 7.4), PBS with 0.05% Tween, veronal buffered saline (VBS; 135 mM NaCl, 3.3 mM diethyl barbiturate, pH 7.3-7.5), GVBS (VBS

containing 0.1% gelatin) and a coating buffer (50 mM carbonate, pH 9.6) for ELISA.

6.1.3 Equipment

The microscopes used were an Olympus BX10 fluorescence microscope (Olympus Optical, Tokyo, Japan) and a laser scanning microscope LSM 510 META (Zeiss, Jena, Germany). Flow cytometry was performed by a FACScan (Becton Dickinson, Heidelberg, Germany). The ELISA-reader used was from Spectramax, Molecular Devices, Munich, Germany. The Biacore 2000 instrument and sensor chips were from Biacore AB, Uppsala, Sweden. Gammacounter was from Wallac, Turku, Finland.

6.1.4 Purified and recombinant proteins

Plasma proteins FH and C3 were either purified and C3b generated as described (173, 234) or purchased from Calbiochem, La Jolla, CA, USA. C4b, C5 and factor I were from Calbiochem. Recombinant proteins FHL-1, FHR-3, FHR-4 and deletion mutants of FH were cloned, expressed in the baculovirus system and purified as described (185). C4BP was purified from plasma as described (74) or expressed using recombinant technology (39). Deletion mutants of C4BP were constructed and expressed as described and purified by affinity chromatography (37, 39).

The expression and purification of the recombinant *Borrelia burgdorferi* sensu stricto surface proteins is decribed in detail elsewhere (5, 122, 256).

6.1.5 Antibodies

Primary antibodies used in the analyses are listed in Table 5. Secondary antibodies used were: Fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG (I, II) and FITC-conjugated rabbit anti-mouse IgG antibodies (Alexa 488) (I, II, IV) from Molecular Probes, Eugene, OR, USA. FITC-conjugated goat anti-rabbit IgG (III), FITC-conjugated rabbit anti-mouse IgG (IV), horseradish peroxidase (HRP) -conjugated donkey anti-goat IgG antibodies (II) were from Santa Cruz Biotechnology, Santa Cruz, CA, USA.

Table 5. The primary antibodies used in the assays. The original publication where the antibody was used is marked on the right column. Details of the manufacturers: Behring and Calbiochem, La Jolla, CA, USA; Dako, Glostrup, Denmark ; DiaSorin, Stillwater, MN, USA; Quidel, San Diego, CA, USA; Sigma Chemical Co., St. Louis, MO, USA; The Binding Site, Birmingham, UK. Abbreviations used: mAb, monoclonal antibody; pAb, polyclonal antibody.

Antibody	Description	Reference or source	Used in
196X	mouse mAb against FH	(161)/J. Tamerius, LaJolla, CA	II
AF1	mouse mAb against HF1 B-cell lymphoma	M. Kaartinen, Helsinki, Finland	I
Anti-βIH	goat pAb against βIH (FH)	DiaSorin	V
Anti-C1q	rabbit pAb against C1q	Dako	II
Anti-C4BP	sheep pAb against C4BP	The Binding Site	V
Anti-C5	goat pAb against C5	Quidel	II
Anti-C6	goat pAb against C6	Quidel	II
Anti-CD11b	mouse mAb against CD11b	Sigma	IV
Anti-Crasp1	mouse mAb against CRASP-1	R. Wallich, Frankfurt, Germany	II
Anti-FH	goat pAb against FH	Calbiochem, La Jolla, CA	II, III
Anti-iC3b	mouse mAb against iC3b neoepitope	Quidel	I, II
Anti-SC5b-9	mouse mAb against C5b-9	Quidel	II
Anti-SCR1-4	rabbit pAb against FH SCR1-4	J. Hellwage, Jena, Germany	III
Anti-SCR19-20	rabbit pAb against FH SCR19-20	J. Hellwage	III
mAb140	mouse mAb against C4BP CCP1	(126)/B. Dahlbäck, Malmö, Sweden	IV
mAb70	mouse mAb against C4BP CCP1	(126)/ B. Dahlbäck	IV
VIG8	mouse mAb against FH SCR19-20	(253)/ W. Prodinger, Innsbruck, Austria	I
WU13/15	mouse mAb against C5b-9 neoepitope	R. Würzner, Innsbruck, Austria	II

FITC-conjugated goat anti-rabbit IgG (II), HRP-conjugated rabbit anti-goat IgG (III), HRP-conjugated rabbit anti-mouse IgG (IV), HRP-conjugated donkey anti-goat IgG antibodies from Dako, Glostrup, Denmark. HRP-conjugated rabbit anti-sheep IgG (V) was from Jackson Immunoresearch Laboratories, Cambridgeshire, UK.

6.2 Microbiological methods

6.2.1 *Borrelia burgdorferi* sensu lato strains

B. burgdorferi sensu stricto (strain Bbia) was isolated from the cerebrospinal fluid of a Finnish neuroborreliosis patient. *B. afzelii* (strains BaA91 and 1082) and *B. garinii* (strains 13, 28, 40, 46, 50) were isolated from skin biopsies of Finnish patients with erythema migrans. They were kind gifts from prof. Matti Viljanen (National Public Health Institute, Turku, Finland). The strains were identified to the species level by sequencing the polymerase chain reaction-amplified fragment of a flagellin gene (293). All strains were grown in BSK-H Complete medium at 33°C.

6.2.2 *Onchocerca volvulus* microfilariae

Nodules containing adult *O. volvulus* worms were prepared from patients in Guinea, West Africa, as described (7), and adult worms were isolated from the freshly collected nodules (280). Microfilariae (MF) were isolated directly from the female worms, transferred into RPMI1640 medium supplemented with L-glutamine, penicillin and streptomycin and immediately frozen with liquid nitrogen. For measuring C activation by the MF (C3a and TCC ELISA-assays) they were pretreated with trypsin (3 mg/ml at +37°C for 45 min). The activity of trypsin was stopped by soybean trypsin inhibitor (6 mg/ml). MF were washed 5 times with RPMI before incubation in 50% NHS for 15 minutes at +37°C. C activation was stopped by adding EDTA to a final concentration of 10 mM. All samples were centrifuged and the supernatants were frozen to -20°C.

6.2.3 Strains of *Candidae* and *Saccharomyces*

C. albicans (strains SC5314 (106), ATCC18804 and EBP), *C. glabrata*, *C. krusei*, *C. tropicalis*, *C. parapsilosis* and *Saccharomyces cerevisiae* (strain SEY6210) were grown at +28°C on yeast-peptone-dextrose medium (2% glucose, 2% peptone, 1% yeast extract) or on Saboraud agar. Hyphal growth was induced by a further incubation for 90 min at 37 ^{0}C in RPMI supplemented with 25 mM HEPES and L-glutamine or by incubating the cells at +37°C for 120 min.

6.2.4 *Borrelia recurrentis* and *B. duttonii* strains

Strains of *Borrelia recurrentis* (A5, A6, A11, A17) and *Borrelia duttonii* (La, Ku, Kw) were isolated from relapsing fever patients in Ethiopia and Tanzania as described (69). Strains were grown at +33°C in BSK-H Complete medium.

6.3 Immunological methods

Immunological methods used in each original publication are listed in Table 6 and described here on a general basis.

6.3.1 Cell absorption assay

Microbes were incubated in NHS, NHS-EDTA or with purified proteins. After washing the microbes five times the proteins bound to their surfaces were eluted with an acidic buffer (0.2 M glycine-HCl, pH 2.0) and the supernatants were collected. Samples of the wash and elute fractions were subjected to sodium-dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto nitrocellulose membranes. Bound proteins were detected by a primary antibody and a HRP-conjugated secondary antibody, which was detected by enhanced chemiluminescence (ECL).

Table 6. Methods used in individual studies.

Method	Publication
Cell absorption assay	III, IV, V
Cell adhesion assay	IV
Cofactor-assay	II, III, IV, V
Enzyme-linked immunosorbent assay	II, IV
Flow cytometry	III, IV
Immunofluorescence microscopy	I, II, III, IV
Protein binding assay	I, II, III, IV, V
Serum sensitivity assay	I, V
Surface plasmon resonance (Biacore)	I

6.3.2 Cell adhesion assay

Approximately 10^5 HUVEC cells were seeded onto microtiter plates and cultivated for 48 h at 37^0C in a humidified 5% CO_2 atmosphere in RPMI1640-medium. After washing with PBS, cells were cultivated in DMEM medium for an additional 24 h. *C. albicans* cells were added on top of the HUVEC-cells with or without purified C4BP. After incubation for 3 h at 37^0C *C. albicans* cells were stained with Calcofluor-stain (30 min at 37 ^{0}C), washed and the amount of the HUVEC-bound *C. albicans*-cells were determined using a fluorescence reader.

6.3.3 Cofactor assay

Cofactor activities for C3b or C4b inactivation of surface attached C regulators FH or C4BP were measured after preincubating the microbes with NHS-EDTA or with purified C regulators for 120 min at +37°C. Bacteria were washed for three times and FI (50 ng/reaction) together with either ^{125}I-C3b, ^{125}I-C4b (approximately 50,000 cpm/assay) or biotin-labeled C4b were added. The samples were further incubated for 60 min at +37°C. The samples were centrifuged and the supernatants were subjected to SDS-PAGE under reducing conditions to detect the cleavage of ^{125}I-C3b or ^{125}I-C4b. The gels were fixed with 5% acetic acid for 10 minutes and dried prior to autoradiography. The cleavage of the biotin-labeled C4b was detected by Western blotting.

6.3.4 ELISA for detecting C3a and TCC

For the C3a enzyme immunoassay samples were diluted into the manufacturers sample buffer and subjected to the ELISA assay according to the manufacturer's instructions.

For the detection of TCC, MaxiSorp microtiter plates were coated overnight with a monoclonal antibody specific for the SC5b-9 neoepitope (5 µg/ml) in an ELISA coating buffer at +4°C for 12 hours. The wells were washed five times with PBS-T. The samples were diluted 1:200 and 1:1000 and aliquots of 100 µl were placed into the wells. After a 60 min incubation at +22°C the wells were washed for five times with PBS-T. A mixture of polyclonal goat anti-human C5 and anti-human C6 antibodies, diluted 1:1000 in PBS-T, was added (100 µl/well) and the plates were incubated for 1 hour at +22°C. After washing the wells a peroxidase-conjugated secondary donkey anti-goat IgG antibody, diluted in PBS-T and supplemented with 1% normal mouse serum (100 µl/well), was incubated in the wells for 60 min at +22°C. After further washings the substrate, o-phenyl-diamine diluted in H_20 and supplemented with 0.04% H_2O_2, was added. After a 15 min incubation at +22°C the reaction was stopped by adding 50 µl of 2M H_2SO_4 per well. The absorbances were read with an ELISA-reader using a 492 nm filter. Purified SC5b-9 was used to set up the standard curve.

6.3.5 ELISA for the binding assays with *C. albicans*

Microtiter plates were coated for four hours with cells of *C. albicans* in RPMI1640 medium at +37°C to obtain the hyphal growth. Nonspecific binding sites were blocked with 2% BSA in PBS and proteins (C4BP, recombinant deletion mutants of C4BP) (10 µg/ml) in 1% BSA/PBS were added. In inhibition assays antibodies or proteins (10 µg/ml) were added to the reaction mixture. The proteins were allowed to bind at +37°C for 120 min. After five washes with 1% BSA/PBS a monoclonal antibody (0.5 µg/ml in 1% BSA/PBS;100 µl/well) was added and the plates were incubated for 60 min at +22°C. After five washes a peroxidase-conjugated secondary antibody (diluted 1:4000, 100 µl/well) was added. After 60 min

incubation in RT the substrate, o-phenyl-diamine diluted in H_20 and supplemented with 0.04% H_2O_2, was added. The reaction was stopped and the result read as described above.

6.3.6 Flow cytometry and immunofluorescence microscopy

The microbes were incubated in NHS, NHS-EDTA or with the purified C regulator using optimized incubation times and temperatures according to the microbe used. After washing the samples for three times, nonspecific binding sites were blocked (*Candida albicans*) by incubating the samples with 1% fat free milk or 1% BSA. Primary antibodies were added and the mixtures were incubated for variable times (30 min with *Borrelia* 12 hours with *C. albicans*) and washed for three times. Secondary FITC-labeled antibodies (diluted 1:200) were incubated with the samples for 30 min (*Borrelia*), 45 min (MF) or 60 min (*C. albicans*) at +22°C. After washing the samples for three times the microbes were mounted with mounting media and microscoped using a fluorescence microscope or subjected to flow cytometry.

6.3.7 Protein binding assay

The C regulators, their recombinant fragments or BSA were radiolabeled with ^{125}Iodine using the Iodogen-method as described (275). The indicated amounts of microbes were incubated with the labeled proteins (approximately 40,000 – 60,000 cpm/tube) in GVB or 1/3 GVB for 30°C at +37°C. The bound protein was separated from the unbound by centrifuging the samples through 20% sucrose. The supernatant and the pellet were subjected to γ-counting and the amount of bound protein was calculated as a percentage of pellet vs. total radioactivity in the pellet plus supernatant. Inhibitory effects of the same or other unlabeled proteins were analyzed by adding the indicated amounts of the unlabeled proteins to the reaction mixtures before the radioactive one.

6.3.8 Serum sensitivity assay

Freshly harvested *Borreliae* ($1x10^6$) were washed for three times with VBS. Cells were incubated with 70% NHS in serum free BSK-H medium in a total

volume of 600 µl at +37°C on a shaker (500 rpm). As a control, the cells were incubated in the absence of NHS. After 1, 2, 3, 4, 5 and 24 hours aliquots of 20 µl were subcultured to 150 µl of BSK-H Complete medium and cultured at +33°C for three days. At each timepoint fresh serum (2% of the total reaction volume) was added. After cultivation the viability of the cells was evaluated microscopically under dark field.

The effect of soluble OspE on the survival of *B. burgdorferi* in NHS was assayed with freshly harvested or frozen bacteria. The bacteria were washed with GVB and incubated for 2 hours at 37°C with either NHS-HI, NHS or NHS supplemented with 0, 20 or 200 µg/ml (final concentration) of soluble OspE protein. Serum was added after 30 and 60 min to avoid depletion of the C components caused by blocking of FH. The final serum concentration shifted from 20 to 33% during the experiment. The surviving bacteria were counted microscopically after 2 hours, and the percentage of alive spirochetes was calculated.

6.3.9 Surface plasmon resonance assay

Protein-protein interactions were analyzed in real time by the surface plasmon resonance technique using the Biacore 2000 instrument. Proteins were immobilized via a standard amine-coupling procedure to flowcells of a sensor chip. Flowcells were activated with the mixture of 35 µl NHS-EDC. The protein to be immobilized was dialyzed against 20 mM acetate buffer (pH 4.8 – 5.5) and 20 µg portions (>150 µg/ml) of the proteins were injected into one of the flowcells. A flowcell without any protein was used as a control. Unreacted groups in the flowcells were inactivated by a ethanolamine-HCl injection (35 µl). After the coupling procedure, the flowcells were washed thoroughly with sequential injections of 1/3 VBS (pH 7.4), and 3 M NaCl in 10 mM acetate buffer (pH 4.6). Binding proteins were dialyzed against 1/3 VBS and protein concentrations were measured usingthe BCA protein assay. Each ligand was injected separately to a blank control flowcell and to a coated flowcell using a flow rate of 5 µl/min at +22°C. The final concentrations of the fluid-phase ligands in the binding assay ranged from 125 to 200 µg/ml.

7 RESULTS

7.1 *Borrelia burgdorferi* sensu lato (I)

Three different genospecies in the group *B. burgdorferi* sensu lato are human pathogens: *B. burgdorferi* sensu stricto, *B. afzelii* and *B. garinii*. It has previously been shown that *B. burgdorferi* sensu stricto and *B. afzelii* are more resistant to human C than *B. garinii* (44, 187). We wanted to examine, if differences in the C-mediated serum sensitivity among borrelial genospecies could be due to the utilization of the AP fluid phase regulators.

First we tested binding of FH from non-immune NHS and NHS-EDTA to *Borreliae* by immunofluorescence (IFL)-microscopy. We saw clear deposition of FH on the surfaces of the C-resistant *B. burgdorferi* sensu stricto and *B. afzelii,* and a weaker deposition on the C-sensitive *B. garinii*. When C activation in NHS was blocked with EDTA, the result was the same, indicating that binding of FH to the borrelia occurred also independently of C3b deposition on the cells. Binding of FH and FHL-1 to the bacteria in the absence of serum was tested further using a direct binding assay with purified and ^{125}I-labeled FH and FHL. Binding of FH to the *B. burdorferi* sensu stricto strain was strong (up to 8% of the total radioactivity) and to the strains of the serum sensitive *B. garinii* it varied between 1-2%. ^{125}I-FHL bound to the *B. burgdorferi* sensu stricto (5% binding), but not to *B. garinii* (approximately 1%). Binding of FH and FHL-1 to different genospecies of borrelia correlated with the sensitivity to C-mediated killing; the resistant strain bound the regulators and the sensitive did not.

Next we aimed at identifying a ligand for FH by testing a selection of *B. burgdorferi* sensu stricto major outer surface proteins (P35/BBK32, OspA (outer surface protein A), OspC, OspD, OspE and DbpA) using the surface plasmon resonance. FH was coupled to the sensor chip by the standard amine coupling method and the borrelial proteins were applied to the fluid phase. The only protein that bound to FH was OspE. The same result was obtained on a turned setup where OspE was coupled to the sensor chip and FH was in the fluid phase. No direct interaction between OspE and C3b

or C5 was observed. The specificity of the interaction was confirmed in a binding assay where soluble OspE inhibited the binding of ^{125}I-FH to the bacteria in a dose-dependent manner. According to the these results OspE was recognized as the first specific ligand for FH in *B. burgdorferi* sensu stricto.

To evaluate the role of FH acquisition in the serum resistance of borreliae we analyzed the effect of soluble OspE on the survival of the three genospecies in NHS in the presence of OspE. Introduction of OspE (200 µg/ml) resulted in a significantly decreased survival of *B. burgdorferi* sensu stricto (90% vs. 5%) and *B. garinii* (20% vs. 1%). *B. afzelii* was not affected by NHS or the addition of OspE. We concluded, that OspE-mediated binding of FH to the *B. burgdorferi* sensu stricto is a very important mediator of serum resistance and therefore a clear survival factor.

The binding site of OspE for FH was located to the C-terminal SCRs 15-20 using recombinant constructs of FH (SCR1-7, 8-20 and 15-20) and the Biacore method.

7.2 *Onchocerca volvulus* (II)

Microfilariae of *O. volvulus*, the causative agent of river blindness, can survive in tissues of the human host for up to two years. Opsonization by C has been shown to be important for the phagocytosis of mf of other species of parasitic nematode species. We were interested in seeing whether mf of *O. volvulus* can utilize the AP regulator FH for their protection against C attack and evasion from phagocytosis.

As there were no published studies available about the C sensitivity of *O. volvulus*, we first tested, whether mf activate the C system or not. When mf of *O. volvulus* were incubated in non-immune NHS detectable, although minor, C activation took place. In this experiment some amount of C3a, the anaphylatoxin which is cleaved from C3 during the C activation, was formed. When trypsin was used to remove polypeptides from the surface of mf, formation of C3a reduced. When the amount of TCC was measured after the exposure to NHS no TCC generation could be observed indicating

that C activation was restricted on the mf surface. Trypsin treatment, however, increased the generation of TCC about two-fold.

Next we analyzed depositions of iC3b and C5b-9 on the mf after their exposure to non-immune NHS. iC3b, but no deposition of the terminal complex C5b-9, could be detected. We concluded that on the surface of mf AP was activated to some extent, but the formed C3b was quickly inactivated to iC3b and no TP activation nor formation of TCC took place.

Using IFL microscopy we observed that mf bound FH. Binding was verified using purified ^{125}I-labeled FH and recombinant proteins in direct binding assays and the mediator site was located to C-terminal SCRs 8-20.

The functional activity of the serum acquired host C regulator was tested in a cofactor assay. Mf were preincubated in non-immune NHS, washed and incubated with ^{125}I-C3b together with factor I. As a result, FH became bound to the mf and had maintained its cofactor-activity for inactivation C3b. We concluded that acquisition of soluble FH from serum contributed to the C resistance and survival of mf in human tissues.

7.3 *Candida albicans* (III, IV)

In further comparison between different types of microbes we next tested *C. albicans*. *C. albicans* activates all three pathways of C to some extent (179) and causes high numbers of invasive infections, especially in patients with deficiencies in acquired cell-mediated immunity. This suggests that the pathogenic strains have the ability to escape from the opsonizing effects of C.

Binding of FH, FHL-1 and C4BP to the cells and hyphal forms of *C. albicans* was assayed in a serum-bathing assay, by IFL-staining, flow cytometry, direct binding assays and ELISA. In a serum bathing assay FH and FHL-1 were detected on the cellular forms, while C4BP bound both to the cellular forms and hyphae. The localization of C regulators on the surface on *C. albicans* was further assayed using IFL-staining and confocal microscopy. FH and FHL-1 bound evenly to the cell surface, while C4BP had a patchy distribution and bound more clearly to the tips of the hyphae. The bound

regulators were quantified using flow cytometry and binding was shown to be dose-dependent.

The specificities of FH and C4BP binding were assayed in direct binding assays with ^{125}I-FH or ^{125}I-C4BP, where increasing amounts of unlabeled protein were added. The binding of both labeled proteins were found to be inhibited in a dose-dependent manner. Dose-dependency of the binding of C4BP to the hyphal forms was confirmed by ELISA.

Using recombinantly expressed constructs we identified two sites on FH that mediate binding to *C. albicans*. The first is in SCR7, which is also the binding site in FHL-1, and the second is in the C-terminal SCRs 19-20. The binding sites were confirmed, when we observed that heparin, which binds to FH via the same SCRs, inhibited the binding of FH to *C. albicans*. The binding site on C4BP for *Candida* was localized to the SCRs 1-2 of the α-chain using deletion mutants of C4BP in the ELISA assay.

7.4 Relapsing fever borreliae (V)

Relapsing fever borreliae can obviously survive in direct contact with the human plasma for long periods, as they cause a septic disease with relapses. We compared their serum-sensitivity with *B. burgdorferi* sensu stricto and *B. garinii* strains. Both tested relapsing fever borreliae, *B. recurrentis* and *B. duttonii*, survived the 24-hour NHS exposure, while *B. garinii* was killed within 4 hours and *B. burgdorferi* sensu stricto within 5 hours.

Next we analyzed the binding of the C regulators FH and C4BP from non-immune serum to six different strains of relapsing fever borrelia. Clear binding to all the strains was detected. The results were confirmed with the purified ^{125}I-labeled proteins in direct binding assays. The amount of C4BP binding varied three-fold between the different strains and FH bound to all the studied strains with a lower intensity. Binding of C4BP to the relapsing fever borreliae was specific, as shown by using ^{125}I-labeled C4BP and increasing concentrations of unlabeled C4BP in a binding assay. We concluded that the studied relapsing fever borreliae are able to bind C4BP specifically and binding occurs in the absence of serum.

Finally, we tested whether the *Borrelia*-bound C4BP and FH are still functional in downregulating the C activation. *Borrelia* that had been pre-incubated with NHS possessed cofactor activity for FI-mediated cleavage of ^{125}I-labeled C4b and C3b, while the spirochetes that had been pre-incubated in buffer did not possess any cofactor activity. Similar results were obtained when the borreliae were pre-incubated with purified C4BP or FH instead of NHS. Thus, the C regulators are bound to the relapsing fever borreliae in such a way that they are able to contribute and even be responsible for the C resistance of the relapsing fever borreliae.

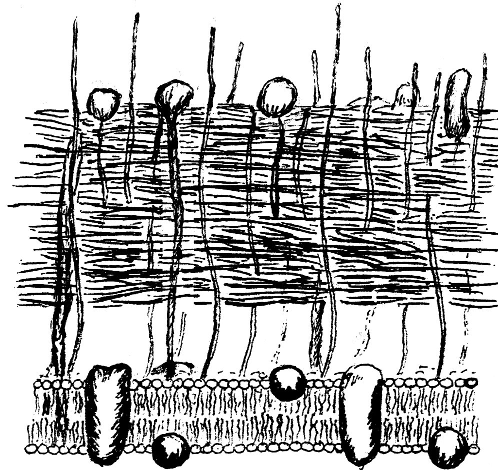
8 DISCUSSION

8.1 Serum sensitivity

Complement can lyse susceptible target cells by forming MAC-complexes on the cell membranes. Many microbes, especially pathogenic ones, are more or less well protected against the MAC-mediated lysis. Gram-positive bacteria are generally not susceptible to lysis due to the thick peptidoglycan layer around the cell membrane (Fig. 4). The same is true for smooth strains of Gram negative bacteria with long O-antigens, while the rough strains that have less or no O-antigens at all on LPS are usually more susceptible to serum-mediated killing (306). Yeasts and most multicellular parasites have a chitinous or tegumental surface structure, which can prevent lysis by MAC. For pathogenic microbes specific C evasion mechanisms are mainly needed for protection from C-mediated opsonization and phagocytosis. Some microbes, on the other hand, are susceptible to C-mediated lysis. For these microbes C is a double-edged sword since activation can lead to lysis as well as to opsonophagocytosis. Microbes, which are able to prevent C activation, are generally considered to be more virulent and cause more severe and invasive infections. As an example, patient-derived strains of *Neisseria gonorrhoeae*, which are able to cause disseminated infections, are usually serum-resistant, and strains causing localized symptomatic infections are serum-sensitive, when exposed to NHS *in vitro* (269).

Complement is the only clearly microbicidal system in non-immune human plasma, although some other plasma constituents such as antimicrobial peptides can be found in patient sera during invasive infections. When non-immune serum is used for analysis of serum sensitivity the analysis actually measures C-sensitivity. Serum sensitivities of the microbes examined in this thesis are different.

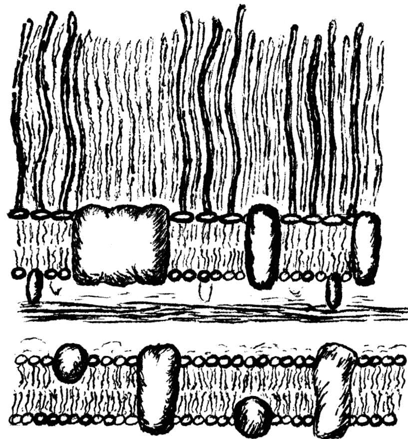
Gram-positive



peptidoglycan

inner membrane

Gram-negative



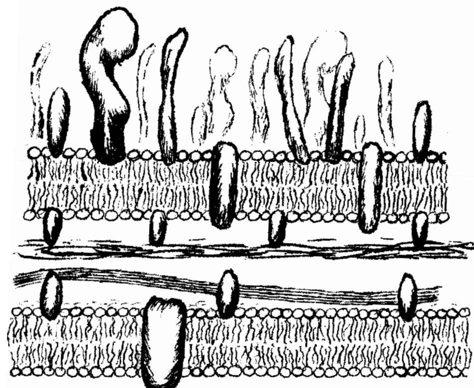
lipopolysaccharide

outer membrane

peptidoglycan

inner membrane

Borrelia



outer membrane

peptidoglycan

periplasmic flagella

inner membrane

AL -05

Fig. 4. Schematic structures of the cell walls of Gram positive bacteria, Gram negative bacteria and *Borrelia.*

It is well documented that two genospecies of *B. burgdorferi* sensu lato, *B. burgdorferi* sensu stricto and *B. afzelii*, are serum resistant, while *B. garinii* is serum sensitive. These differences are known to be C-dependent (12, 44, 187). For relapsing fever borreliae no such data existed. Therefore, we compared the serum-sensitivities of these borreliae with *B. burgdorferi* sensu stricto and *B. garinii* (I). Relapsing fever agents are able to persist in the human circulation for weeks. Thus, it was not a surprise that they were even more resistant to serum C than *B. burgdorferi* sensu stricto. By adding fresh serum we prevented depletion of C activity during the prolonged incubation, but still both of the tested relapsing fever borreliae tolerated non-immune NHS for at least 24 hours (V). Serum C can kill the serum-sensitive genospecies *B. garinii* by the lytic activity of the MAC, but it was not known, if the relapsing fever borreliae could be lysed. In general due to structural similarities between the different spirochetes it is likely that MAC complexes would lyse also the relapsing fever borreliae if C was not regulated on their surfaces.

Onchocerca volvulus mf circulate in the host for up to two years, but mainly in the subcutaneous tissues, skin and eyes. At least the extracellular fluid in subcutaneous tissue contains all the essential components for C activation and MAC-formation. We observed that mf activate C to the C3-level, as during the incubation in serum C3 was cleaved to C3a and C3b. This formed C3b was, however, quickly turned into iC3b, and no detectable TCC formation took place. We thus concluded that mf are obviously serum resistant (II).

Candida albicans is known to efficiently activate C, even via all the three pathways (180, 181). It is also known that *C. albicans* is resistant to the lytic effect of serum, as cells can be in direct contact with serum for many hours and still be viable. The TP of C is ineffective in lysing *C. albicans*, probably because of the rigid chitinous cell wall structure. But the opsonizing effect of C affects the survival of *C. albicans in vivo,* and thus C evasion is supposed to be important for the virulence of *C. albicans*.

8.2 Acquisition of FH and FHL-1

In this work FH was shown to bind all the microbes studied. On the basis of the current and several other studies it can be concluded that FH is bound directly and specifically to the microbial surfaces, and no other serum component mediates the interaction. FHL-1 was found to bind to *Candida albicans*, *Borrelia burgdorferi* sensu stricto and to two relapsing fever borrelia (I, III, IV).

From the microbes studied, we were able to identify one ligand for FH. This was the outer surface protein E (OspE) of *B. burgdorferi* sensu stricto. None of the other tested surface proteins of borrelia (OspA, OspC, OspD, fibronectin binding protein P35 and decorin-binding protein DbpA) bound FH. OspE belongs to a group OspE paralogs, which are encoded by a gene in a circular plasmid and coexpressed with OspF (189). These proteins are also called Erps (OspEF-related proteins) (189). Expression of OspE is upregulated by a temperature shift from 23° to 35°C, i.e. in circumstances that the spirochetes meet while entering the human host (297). After our original discovery more data on the interaction between OspE and FH has been published. The C-terminus of OspE and its three-dimensional coiled-coil structure are important in binding of FH (10, 11, 202, 211). Another FH -binding protein from this family was called BbCRASP-3 (183). Later another type of ligand for FH, BbCRASP-1 (also named as Bba68) was cloned (107, 182). The crystal structure of BbCRASP-1/Bba68 revealed a protein with several α-helixes (63, 64). Serum resistant *Borrelia afzelii* also expresses a FH-binding protein (317). According to our own findings (Alitalo et. al, unpublished data) patient-derived strains of *B. garinii* bind FH too, but the binding is lost upon cultivation *in vitro*. In the Lyme disease borreliae binding of FH and/or FHL-1 has been shown to be a very important determinant of pathogenicity. Furthermore, multiple ligands for the C regulators highlight the importance of C evasion for the bacteria.

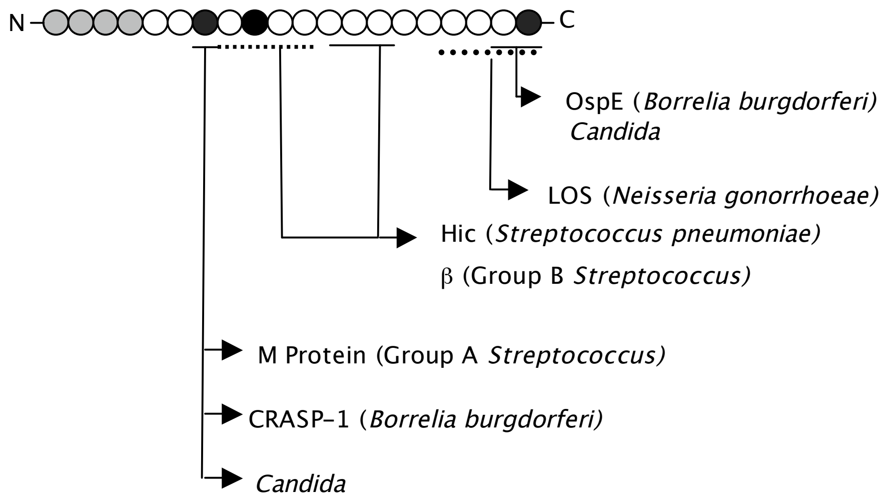


Fig. 5. Some microbial interactions sites in FH

Relapsing fever borreliae are also able to bind FH, which has been shown by us (210), in V and by others (201). McDowell et. al detected a 20 kD FH-binding protein in *B. hermsii* and *B. parkerii* by an affinity ligand test (201). Hovis et. al identified the ligand in *B. hermsii* for FH as a linear-plasmid encoced protein, which is not homologous to any other FH -binding protein of borreliae known to date. Interestingly, the sequence of this protein was not found from the genome of *B. parkeri*. (142). We used several strains of the only species causing LBRF, *B. recurrentis,* and several strains of species causing TBRF, *B. duttonii*, in direct binding assays and serum-bathing assays and saw binding of FH to these borreliae too.

Interestingly many microbes bind FH via the same SCRs, that are important in target recognition by FH (Fig. 5). SCR7 and SCR20 contain heparin binding sites (32, 33) and the C-terminal SCRs 19-20 mediated binding of FH to *C. albicans* and to OspE of *Borrelia burgdorferi* sensu stricto. Another binding site on FH and FHL-1 to *C. albicans* was localized to SCR7, which has previously been shown to mediate binding of FH to M-proteins of group A streptococci (31, 177). Furthermore, the binding sites on SCR7 the for heparin and M protein seem to be overlapping (114). Since SCR7 is common for FH and FHL-1 a microbe, that binds to SCR7, is able to acquire both the regulators to its surface, like CRASP-1 of *B. burgdorferi* sensu stricto does (182). When binding is mediated via SCR7 or more C-terminal region, the functional SCRs 1-4 are free for exhibiting the C regulatory activity. During evolution it might have been more beneficial for a microbe

Table 7. Microbes that have been reported to bind the C regulator FH. The main ligand, if known, and the key references are given.

Microbe	Ligand for FH	Main reference
Gram-positive cocci		
Streptococcus pyogenes	M-protein	(140)
	Fba	(228)
Streptococcus pneumoniae	PspC	(217)
	Hic	(148)
Staphylococcus aureus	?	(153)
Gram-negative cocci		
Neisseria gonorrhoeae	Porin	(260)
	LOS	(261)
N. meningitidis	LOS	(259)
Gram-negative bacilli		
Yersinia enterocolitica	YadA	(55)
Pseudomonas aeruginosa	?	(143)
Spirochetes		
Borrelia burgdorferi s.l.	OspE/CRASP-3	(I)/(183)
	CRASP1/Bba68	(182)
Relapsing fever borrelia	?	(V)
Leptospires	?	Meri et. al, unpublished
Yeasts		
Candida albicans	?	(III)
Cryptococcus neoformans	?	(274)
Helminths		
Echinococcus granulosus	?	(84)
Onchocerca volvulus	?	(II)
Nippostrg. brasiliensis	?	(16)
Loa Loa	?	Haapasalo et. al, unpublished
Viruses		
HIV	gp41, gp120	(245,246)

to use such a host structure, that is very important also for the host and therefore less likely to be mutated.

C. albicans has been reported to have complement receptor-like surface molecules which were suggested to bind C3d or iC3b (6, 131) and be virulence factors (226). The human complement receptor 3 (CR3;CD11b/18) expressed on neutrophils has been suggested to be a receptor for factor H (87). It might be speculated that the *C. albicans* CR3-like protein may actually be the ligand for FH and FHL-1, but this hypothesis remains to be tested.

Microbes, which have been suggested to bind FH, are listed in Table 7. Many interactions have been well characterized, and mutations made on the ligands have been shown to affect the binding and survival of the microbe. On the other hand, some interactions might be nonspecific and due to general adhesion of FH to the microbes.

8.3 Acquisition of C4BP

Microbes that have been previously shown to bind C4BP, are listed in the Table 8. They are Gram negative or positive cocci or Gram negative bacilli. In this thesis two new groups of microbes, spirochetes and yeasts, are reported to able to acquire C4BP (IV, V).

Evasion of the classical pathway is beneficial for both the microbes we have shown to bind C4BP. *C. albicans* can cause severe invasive infections, but it is also a harmless commensal found frequently on mucous membranes. Thus the naturally occuring antibodies against *C. albicans* are common and those antibodies can usually activate the CP (327). Mannan on the outer surface of the yeast activates the lectin pathway and binding of C4BP might protect *C. albicans* also from the lectin pathway.

Relapsing fever borreliae are able to persists in human blood from several days to weeks. The best known immune evasion mechanism demonstrated for these borreliae is antigenic variation, i.e. ability to change the major outer surface proteins in cycles, leading to a persistence as a new “serotype” in the human host. The antigenic variation leads to changing of

only one type of a surface protein, while the other surface structures are not be changed. Therefore, the acquisition of C4BP onto the bacterial surface could reduce the antibody-dependent CP activation below a rate that is required for opsonization or propagation of the C activation to MAC-formation. Evasion of adaptive immunity by a combination of antigenic variation and C4BP acquisition might be needed for the long bacteremic and highly lethal course of the disease.

Also other microbes causing persistent infections, like Lyme disease and river blindness, have to protect themselves against antibodies, which are raised during the long existence in the human host. For these microbes evasion from CP might also be essential for survival and pathogenicity and it would be interesting to know if they can acquire C4BP too.

Most of the studied microbes, *Streptococcus pyogenes* (35), *Bordetella pertussis* (28) and *C. albicans* (IV), interact with C4BP via the N-terminal SCRs 1-2 of the α-chain. C4b and heparin have also binding sites in the SCR1-2 (138), but due to its multimeric nature, binding of C4BP via these SCR domains does not arrest the functional activity of the microbe-bound regulator.

Localization of C4BP to the tips of the hyphae in *C. albicans* was an interesting finding, as the morphogensis is one of the major virulence factors of *C. albicans* and moreover, hyphal tips are considered very important in the tissue penetration. *C. albicans* is able to change its outer surface composition during morphogenesis and simultaneous upregulation of C4BP-binding structures might be beneficial.

Acquisition of all three fluid phase regulators has previosly been shown occur only on *S. pyogenes,* that binds C4BP, FHL-1 and FH via the M-proteins (177, 308). *N. gonorrhoeae* binds C4BP and FH via surface expressed porins 1A and 1B (258, 259). *C. albicans* and relapsing fever borrelia are able to acquire both FH and C4BP. *C. albicans* binds also FHL-1 and some strains of relapsing fever borreliae seen to acquire also FHL-1 or FHR-1 from human serum. The ligands for FH, FHL-1 and C4BP binding on *C. albicans* and relapsing fever borrelia remain to be discovered. But in the competition assay FHL-1, but not FH, inhibited binding of C4BP to the

cellular forms of *C. albicans*. This might imply that both the regulators are bound to the same structure on the surface of *C. albicans*. This seems possible and logical, as the regulators are structurally related, but unlikely, as simultaneous evasion from both AP and CP should be beneficial for the microbe. For example, in group A streptococcus, FHL-1 and C4BP have been shown to use different ligands on the same strain (237).

Table 8. Microbes reported to bind the C regulator C4BP. The main ligand, if known, and the key reference are given.

Microbe	Ligand for C4BP	Main reference
Gram-positive cocci		
Streptococcus pyogenes	Sir, Arp	(308)
	Enn18	(237)
Gram-negative cocci		
N.gonorrhoeae	Porin	(258)
	type IV pili	(38)
N. meningitidis	PorA	(151)
Moraxella catarrhalis	UspA1, UspA2	(224)
Gram-negative bacilli		
E. coli	OmpA	(250)
Bordetella pertussis	FHA	(26)
Spirochetes		
B. recurrentis, B. duttonii	?	(V)
Yeasts		
Candida albicans	?	(IV)
Parasites		
Schistosoma mansoni	?	Meri et. al., unpublished
Loa Loa	?	Haapasalo et. al, unpublished

8.4 Functions of the surface-acquired regulators

Surface-bound FH was shown to be functionally active on the surface of *O. volvulus*, *C. albicans* and relapsing fever borrelia as it acted as a cofactor in FI-mediated cleavage of C3b. In the absence of FH no cleavage occurred.

C. albicans has been reported to have a protease able to cleave C3 (164), but in our assay *C. albicans* cells without a contact with serum or purified FH were unable to cleave C3b. Also C4BP was functionally active in C4b cleavage, when bound to *C. albicans* and relapsing fever borrelia.

It has been shown that in other bacteria, like in *Streptococcus pyogenes*, binding of FH and C4BP increases survival by directly interfering opsonophagocytosis (27, 140) and in *Neisseria gonorrhoeae* FH acquisition mediates serum sensitivity (260, 261).

The importance of OspE in survival of *B. burgdorferi* sensu stricto was obvious in our serum sensitivity assay; when we blocked binding of FH to the borrelia by soluble OspE, the serum-mediated lysis of the spirochetes increased notably. On the basis of our results (I) soluble OspE makes the *B. burgdorferi* sensu stricto and *B. garinii* serum-sensitive indicating that it is a major mediator of FH-acquisition onto these microbes. The same has not been shown with the other identified FH ligands of Lyme disease borrelia.

In addition to downregulation of C the attached C4BP seems to mediate or enhance adhesion of *C. albicans* to host endothelial cells. Adhesion is an important virulence factor of *Candida* during infections. Mediation of adherence is also a novel function for C4BP. As binding of C4BP was located to the tips of the hyphae, it might increase the virulence of *C. albicans* in two ways, in evasion of C and in adhesion.

8.5 Conclusions

Binding of FH and C4bp arrests C activation at the C3/C5 convertase level, thus preventing the attachment of opsonins to the microbial surface as well as progression of the C activation to the formation of MAC. Escape from opsonization is obviously important for microbial virulence. We have shown that very different microbes utilize fluid phase C regulators. Most probably the acquisition of the regulators increases survival of these microbes in blood or tissues and therefore contributes to the virulence

In these studies we show, that *B. burdorferi* sensu stricto binds FH via OspE. Binding of FH increases the survival of bacteria when in contact with

human serum. Relapsing fever borrelia are able to bind both FH and C4BP is a manner which enables the bound regulators to maintain their regulatory functions. *C. albicans* is able to bind FH, FHL-1 and C4BP in a functionally active form. Mf of *O. volvulus* bind FH enabling inactivation of surface-bound C3b.

8.6 Future prospects

On the basis of the reports from our laboratory and other groups it is evident that FH and/or C4BP acquisition is somewhat usual among pathogenic microbes. How binding of C regulators affects the virulence and survival of microbes is a very interesting question, which has been addressed with some microbes, but still not so many examples exist. For *in vitro* analysis we need to identify the ligands for binding of the C regulators and after inactivating them one by one to detect possible changes in serum sensitivity and/or susceptibility to phagocytosis. *In vivo* animal models, possibly with the available C regulator-deficient animals, is a prerequisite for assaying the real importance of the acquisition of FH and C4BP and necessary for the evaluation of the potential that the ligands might possess in vaccine development.

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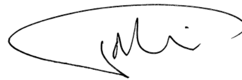

Taru Meri

11 REFERENCES

1. **Abrahamson, D. R., and D. T. Fearon.** 1983. Endocytosis of the C3b receptor of complement within coated pits in human polymorphonuclear leukocytes and monocytes. Lab Invest **48:**162-168.
2. **Accardo, P., P. Sanchez-Corral, O. Criado, E. Garcia, and S. Rodriguez de Cordoba.** 1996. Binding of human complement component C4b-binding protein (C4BP) to *Streptococcus pyogenes* involves the C4b-binding site. J Immunol **157:**4935-4939.
3. **Adams, E. M., M. C. Brown, M. Nunge, M. Krych, and J. P. Atkinson.** 1991. Contribution of the repeating domains of membrane cofactor protein (CD46) of the complement system to ligand binding and cofactor activity. J Immunol **147:**3005-3011.
4. **Aime, N., A. Haque, B. Bonnel, G. Torpier, and A. Capron.** 1984. Neutrophil-mediated killing of *Dipetalonema viteae* microfilariae: simultaneous presence of IgE, IgG antibodies and complement is required. Immunol **51:**585-594.
5. **Akins, D. R., S. F. Porcella, T. G. Popova, D. Shevchenko, S. I. Baker, M. Li, M. V. Norgard, and J. D. Radolf.** 1995. Evidence for in vivo but not in vitro expression of a *Borrelia burgdorferi* outer surface protein F (OspF) homologue. Mol Microbiol **18:**507-520.
6. **Alaei, S., C. Larcher, C. Ebenbichler, W. M. Prodinger, J. Janatova, and M. P. Dierich.** 1993. Isolation and biochemical characterization of the iC3b receptor of *Candida albicans*. Infect Immun **61:**1395-1399.
7. **Albiez, E., D. Büttner, and B. Duke.** 1988. Diagnosis and extirpation of nodules in human onchocerciasis. Trop Med Parasitol **39:**331-346.
8. **Albrecht, J. C., and B. Fleckenstein.** 1992. New member of the multigene family of complement control proteins in herpesvirus saimiri. J Virol **66:**3937-3940.
9. **Albrecht, J. C., J. Nicholas, K. R. Cameron, C. Newman, B. Fleckenstein, and R. W. Honess.** 1992. Herpesvirus saimiri has a gene specifying a homologue of the cellular membrane glycoprotein CD59. Virology **190:**527-530.
10. **Alitalo, A., T. Meri, T. Chen, H. Lankinen, Z. Z. Cheng, T. S. Jokiranta, I. J. Seppala, P. Lahdenne, P. S. Hefty, D. R. Akins, and S. Meri.** 2004. Lysine-dependent multipoint binding of the *Borrelia burgdorferi* virulence factor outer surface protein E to the C terminus of factor H. J Immunol **172:**6195-6201.
11. **Alitalo, A., T. Meri, H. Lankinen, I. Seppala, P. Lahdenne, P. S. Hefty, D. Akins, and S. Meri.** 2002. Complement inhibitor factor H binding to lyme disease spirochetes is mediated by inducible expression of multiple plasmid-encoded outer surface protein E paralogs. J Immunol **169:**3847-3853.
12. **Alitalo, A., T. Meri, L. Ramo, T. S. Jokiranta, T. Heikkila, I. J. Seppala, J. Oksi, M. Viljanen, and S. Meri.** 2001. Complement evasion by *Borrelia burgdorferi*: serum-resistant strains promote C3b inactivation. Infect Immun **69:**3685-3691.

13. **AlonsoDeVelasco, E., A. F. Verheul, J. Verhoef, and H. Snippe.** 1995. *Streptococcus pneumoniae*: virulence factors, pathogenesis, and vaccines. Microbiol Rev **59:**591-603.
14. **Areschoug, T., M. Stalhammar-Carlemalm, I. Karlsson, and G. Lindahl.** 2002. Streptococcal beta protein has separate binding sites for human factor H and IgA-Fc. J Biol Chem **277:**12642-12648.
15. **Avery, V. M., and D. L. Gordon.** 1993. Characterization of factor H binding to human polymorphonuclear leukocytes. J Immunol **151:**5545-5553.
16. **Baker, S., H. Wang, P. R. Giacomin, L. A. Dent, and D. L. Gordon.** 2004. Binding of complement factor H to the nematode *Nippostrongylus brasiliensis*. Congress abstract, XXth International Complement Workshop, Honolulu, Hawaii. Mol Immunol, vol 41
17. **Barbour, A. G., O. Barrera, and R. C. Judd.** 1983. Structural analysis of the variable major proteins of *Borrelia hermsii*. J Exp Med **158:**2127-2140.
18. **Barbour, A. G., C. J. Carter, and C. D. Sohaskey.** 2000. Surface protein variation by expression site switching in the relapsing fever agent *Borrelia hermsii*. Infect Immun **68:**7114-7121.
19. **Barbour, A. G., and C. F. Garon.** 1988. The genes encoding major surface proteins of *Borrelia burgdorferi* are located on a plasmid. Ann N Y Acad Sci **539:**144-153.
20. **Barbour, A. G., and B. I. Restrepo.** 2000. Antigenic variation in vector-borne pathogens. Emer Inf Dis **6:**449-457.
21. **Barbour, A. G., S. L. Tessier, and H. G. Stoenner.** 1982. Variable major proteins of *Borrellia hermsii*. J Exp Med **156:**1312-1324.
22. **Barnes, D. W., and J. Silnutzer.** 1983. Isolation of human serum spreading factor. J Biol Chem **258:**12548-12552.
23. **Barnum, S. R.** 1991. C4b-binding protein, a regulatory protein of complement. Immunol Res **10:**28-42.
24. **Barre, P., B. S. Kaplan, J. P. de Chadarevian, and K. N. Drummond.** 1977. Hemolytic uremic syndrome with hypocomplementemia, serum C3NeF, and glomerular deposits of C3. Arch Pathol Lab Med **101:**357-361.
25. **Beck-Sague, C., S. Banerjee, and W. R. Jarvis.** 1993. Infectious diseases and mortality among US nursing home residents. Am J Public Health **83:**1739-1742.
26. **Berggard, K., E. Johnsson, F. R. Mooi, and G. Lindahl.** 1997. *Bordetella pertussis* binds the human complement regulator C4BP: role of filamentous hemagglutinin. Infect Immun **65:**3638-3643.
27. **Berggard, K., E. Johnsson, E. Morfeldt, J. Persson, M. Stalhammar-Carlemalm, and G. Lindahl.** 2001. Binding of human C4BP to the hypervariable region of M protein: a molecular mechanism of phagocytosis resistance in *Streptococcus pyogenes*. Mol Microbiol **42:**539-551.
28. **Berggard, K., G. Lindahl, B. Dahlback, and A. M. Blom.** 2001. *Bordetella pertussis* binds to human C4b-binding protein (C4BP) at a site similar to that used by the natural ligand C4b. Eur J Immunol **31:**2771-2780.

29. **Bergström, S., L. Noppa, Å. Gylfe, and Y. Östberg.** 2002. Molecular and cellular biology of *Borrelia burgdorferi* sensu lato. CABI Publishing, Oxon.
30. **Bhakdi, S., and J. Tranum-Jensen.** 1991. Complement lysis: a hole is a hole. Immunol Today **12:**318-320; discussion 321.
31. **Blackmore, T. K., V. A. Fischetti, T. A. Sadlon, H. M. Ward, and D. L. Gordon.** 1998. M protein of the group A Streptococcus binds to the seventh short consensus repeat of human complement factor H. Infect Immun **66:**1427-1431.
32. **Blackmore, T. K., J. Hellwage, T. A. Sadlon, N. Higgs, P. F. Zipfel, H. M. Ward, and D. L. Gordon.** 1998. Identification of the second heparin-binding domain in human complement factor H. J Immunol **160:**3342-3348.
33. **Blackmore, T. K., T. A. Sadlon, H. M. Ward, D. M. Lublin, and D. L. Gordon.** 1996. Identification of a heparin binding domain in the seventh short consensus repeat of complement factor H. J Immunol **157:**5422-5427.
34. **Blom, A. M.** 2004. Strategies developed by bacteria and virus for protection from the human complement system. Scand J Clin Lab Invest **64:**479-496.
35. **Blom, A. M., K. Berggard, J. H. Webb, G. Lindahl, B. O. Villoutreix, and B. Dahlback.** 2000. Human C4b-binding protein has overlapping, but not identical, binding sites for C4b and streptococcal M proteins. J Immunol **164:**5328-5336.
36. **Blom, A. M., L. Kask, and B. Dahlback.** 2003. CCP1-4 of the C4b-binding protein alpha-chain are required for factor I mediated cleavage of complement factor C3b. Mol Immunol **39:**547-556.
37. **Blom, A. M., L. Kask, and B. Dahlback.** 2001. Structural requirements for the complement regulatory activities of C4BP. J Biol Chem **276:**27136-27144.
38. **Blom, A. M., A. Rytkonen, P. Vasquez, G. Lindahl, B. Dahlback, and A. B. Jonsson.** 2001. A novel interaction between type IV pili of *Neisseria gonorrhoeae* and the human complement regulator C4B-binding protein. J Immunol **166:**6764-6770.
39. **Blom, A. M., J. Webb, B. O. Villoutreix, and B. Dahlback.** 1999. A cluster of positively charged amino acids in the C4BP alpha-chain is crucial for C4b binding and factor I cofactor function. J Biol Chem **274:**19237-19245.
40. **Blom, A. M., A. F. Zadura, B. O. Villoutreix, and B. Dahlback.** 2000. Positively charged amino acids at the interface between alpha-chain CCP1 and CCP2 of C4BP are required for regulation of the classical C3- convertase. Mol Immunol **37:**445-453.
41. **Borg, M., and R. Ruchel.** 1988. Expression of extracellular acid proteinase by proteolytic *Candida* spp. during experimental infection of oral mucosa. Infect Immun **56:**626-631.
42. **Bos, I. G., C. E. Hack, and J. P. Abrahams.** 2002. Structural and functional aspects of C1-inhibitor. Immunobiol **205:**518-533.
43. **Brade, V., I. Kleber, and G. Acker.** 1992. Differences of two *Borrelia burgdorferi* strains in complement activation and serum resistance. Immunobiol **185:**453-465.
44. **Breitner-Ruddock, S., R. Wurzner, J. Schulze, and V. Brade.** 1997. Heterogeneity in the complement-dependent

bacteriolysis within the species of *Borrelia burgdorferi.* Med Microbiol Immunol **185:**253-260.
45. **Brodbeck, W. G., D. Liu, J. Sperry, C. Mold, and M. E. Medof.** 1996. Localization of classical and alternative pathway regulatory activity within the decay-accelerating factor. J Immunol **156:**2528-2533.
46. **Burgdorfer, W., A. G. Barbour, S. F. Hayes, J. L. Benach, E. Grunwaldt, and J. P. Davis.** 1982. Lyme disease-a tick-borne spirochetosis? Science **216:**1317-1319.
47. **Burnham, G.** 1995. Ivermectin treatment of onchocercal skin lesions: observations from a placebo-controlled, double-blind trial in Malawi. Am J Trop Med Hyg **52:**270-276.
48. **Burnham, G.** 1998. Onchocerciasis. Lancet **351:**1341-1346.
49. **Calderone, R. A., and P. C. Braun.** 1991. Adherence and receptor relationships of *Candida albicans*. Microbiol Rev **55:**1-20.
50. **Calderone, R. A., L. Linehan, E. Wadsworth, and A. L. Sandberg.** 1988. Identification of C3d receptors on Candida albicans. Infect Immun **56:**252-258.
51. **Caprioli, J., P. Bettinaglio, P. F. Zipfel, B. Amadei, E. Daina, S. Gamba, C. Skerka, N. Marziliano, G. Remuzzi, and M. Noris.** 2001. The molecular basis of familial hemolytic uremic syndrome: mutation analysis of factor H gene reveals a hot spot in short consensus repeat 20. J Am Soc Nephrol **12:**297-307.
52. **Carlsson, F., K. Berggard, M. Stalhammar-Carlemalm, and G. Lindahl.** 2003. Evasion of phagocytosis through cooperation between two ligand-binding regions in *Streptococcus pyogenes* M protein. J Exp Med **198:**1057-1068.
53. **Carroll, M. C., and A. P. Prodeus.** 1998. Linkages of innate and adaptive immunity. Curr Opin Immunol **10:**36-40.
54. **Chan, A. C., K. R. Mitchell, T. W. Munns, D. R. Karp, and J. P. Atkinson.** 1983. Identification and partial characterization of the secreted form of the fourth component of human complement: evidence that it is different from major plasma form. Proc Natl Acad Sci USA **80:**268-272.
55. **China, B., M. P. Sory, B. T. N'Guyen, M. De Bruyere, and G. R. Cornelis.** 1993. Role of the YadA protein in prevention of opsonization of *Yersinia enterocolitica* by C3b molecules. Infect Immun **61:**3129-3136.
56. **Choi, N. H., T. Mazda, and M. Tomita.** 1989. A serum protein SP40,40 modulates the formation of membrane attack complex of complement on erythrocytes. Mol Immunol **26:**835-840.
57. **Chung, L. P., D. R. Bentley, and K. B. Reid.** 1985. Molecular cloning and characterization of the cDNA coding for C4b- binding protein, a regulatory protein of the classical pathway of the human complement system. Biochem J **230:**133-141.
58. **Chung, L. P., J. Gagnon, and K. B. Reid.** 1985. Amino acid sequence studies of human C4b-binding protein: N-terminal sequence analysis and alignment of the fragments produced by limited proteolysis with chymotrypsin and the peptides produced by cyanogen bromide treatment. Mol Immunol **22:**427-435.
59. **Cochrane, C. G., and H. J. Muller-Eberhard.** 1968. The derivation of two distinct anaphylatoxin activities from the third and fifth components of human complement. J Exp Med **127:**371-386.

60. **Cole, J. L., G. A. Housley, Jr., T. R. Dykman, R. P. MacDermott, and J. P. Atkinson.** 1985. Identification of an additional class of C3-binding membrane proteins of human peripheral blood leukocytes and cell lines. Proc Natl Acad Sci USA **82:**859-863.
61. **Colten, H. R., and F. S. Rosen.** 1992. Complement deficiencies. Annu Rev Immunol **10:**809-834.
62. **Cooper, N. R., and H. J. Muller-Eberhard.** 1970. The reaction mechanism of human C5 in immune hemolysis. J Exp Med **132:**775-793.
63. **Cordes, F. S., P. Kraiczy, P. Roversi, C. Skerka, M. Kirschfink, M. M. Simon, V. Brade, E. D. Lowe, P. Zipfel, R. Wallich, and S. M. Lea.** 2004. Crystallization and preliminary crystallographic analysis of BbCRASP-1, a complement regulator-acquiring surface protein of *Borrelia burgdorferi*. Acta Crystallogr D Biol Crystallogr **60:**929-932.
64. **Cordes, F. S., P. Roversi, P. Kraiczy, M. M. Simon, V. Brade, O. Jahraus, R. Wallis, C. Skerka, P. F. Zipfel, R. Wallich, and S. M. Lea.** 2005. A novel fold for the factor H-binding protein BbCRASP-1 of Borrelia burgdorferi. Nat Struct Mol Biol **12:**276-277.
65. **Coyne, K. E., S. E. Hall, S. Thompson, M. A. Arce, T. Kinoshita, T. Fujita, D. J. Anstee, W. Rosse, and D. M. Lublin.** 1992. Mapping of epitopes, glycosylation sites, and complement regulatory domains in human decay accelerating factor. J Immunol **149:**2906-2913.
66. **Cullen, P. A., D. A. Haake, and B. Adler.** 2004. Outer membrane proteins of pathogenic spirochetes. FEMS Microbiol Rev **28:**291-318.
67. **Cutler, J. E., and T. Kanbe.** 1993. Antigenic variability of *Candida albicans* cell surface. Curr Top Med Mycol **5:**27-47.
68. **Cutler, S. J.** 2001. in Molecular biology of the relapsing fever borrelia, in Molecular Medical Microbiology. ed. M. Sussman. Chapter 98:2093-2113.
69. **Cutler, S. J., D. Fekade, K. Hussein, K. A. Knox, A. Melka, K. Cann, A. R. Emilianus, D. A. Warrell, and D. J. Wright.** 1994. Successful in-vitro cultivation of *Borrelia recurrentis*. Lancet **343:**242.
70. **Cutler, S. J., J. Moss, M. Fukunaga, D. J. Wright, D. Fekade, and D. Warrell.** 1997. *Borrelia recurrentis* characterization and comparison with relapsing- fever, Lyme-associated, and other Borrelia spp. Int J Syst Bacteriol **47:**958-968.
71. **Dadzie, K. Y., J. Remme, G. De Sole, B. Boatin, E. S. Alley, O. Ba, and E. M. Samba.** 1991. Onchocerciasis control by large-scale ivermectin treatment. Lancet **337:**1358-1359.
72. **Daffern, P. J., P. H. Pfeifer, J. A. Ember, and T. E. Hugli.** 1995. C3a is a chemotaxin for human eosinophils but not for neutrophils. I. C3a stimulation of neutrophils is secondary to eosinophil activation. J Exp Med **181:**2119-2127.
73. **Dahlback, B.** 1983. Purification of human C4b-binding protein and formation of its complex with vitamin K-dependent protein S. Biochem J **209:**847-856.

74. **Dahlback, B.** 1983. Purification of human vitamin K-dependent protein S and its limited proteolysis by thrombin. Biochem J **209:**837-846.
75. **Dahlback, B., B. Frohm, and G. Nelsestuen.** 1990. High affinity interaction between C4b-binding protein and vitamin K-dependent protein S in the presence of calcium. Suggestion of a third component in blood regulating the interaction. J Biol Chem **265:**16082-16087.
76. **Dahlback, B., and H. J. Muller-Eberhard.** 1984. Ultrastructure of C4b-binding protein fragments formed by limited proteolysis using chymotrypsin. J Biol Chem **259:**11631-11634.
77. **Dahlback, B., C. A. Smith, and H. J. Muller-Eberhard.** 1983. Visualization of human C4b-binding protein and its complexes with vitamin K-dependent protein S and complement protein C4b. Proc Natl Acad Sci USA **80:**3461-3465.
78. **Dave, S., A. Brooks-Walter, M. K. Pangburn, and L. S. McDaniel.** 2001. PspC, a pneumococcal surface protein, binds human factor H. Infect Immun **69:**3435-3437.
79. **Dave, S., S. Carmicle, S. Hammerschmidt, M. K. Pangburn, and L. S. McDaniel.** 2004. Dual roles of PspC, a surface protein of Streptococcus pneumoniae, in binding human secretory IgA and factor H. J Immunol **173:**471-477.
80. **Davies, A., D. L. Simmons, G. Hale, R. A. Harrison, H. Tighe, P. J. Lachmann, and H. Waldmann.** 1989. CD59, an LY-6-like protein expressed in human lymphoid cells, regulates the action of the complement membrane attack complex on homologous cells. J Exp Med **170:**637-654.
81. **Davis, A. E., 3rd.** 2005. The pathophysiology of hereditary angioedema. Clin Immunol **114:**3-9.
82. **Dempsey, P. W., M. E. Allison, S. Akkaraju, C. C. Goodnow, and D. T. Fearon.** 1996. C3d of complement as a molecular adjuvant: bridging innate and acquired immunity. Science **271:**348-350.
83. **Deng, J., D. Gold, P. T. LoVerde, and Z. Fishelson.** 2003. Inhibition of the complement membrane attack complex by *Schistosoma mansoni* paramyosin. Infect Immun **71:**6402-6410.
84. **Diaz, A., A. Ferreira, and R. B. Sim.** 1997. Complement evasion by *Echinococcus granulosus*: sequestration of host factor H in the hydatid cyst wall. J Immunol **158:**3779-3786.
85. **DiScipio, R. G.** 1992. Formation and structure of the C5b-7 complex of the lytic pathway of complement. J Biol Chem **267:**17087-17094.
86. **DiScipio, R. G., D. N. Chakravarti, H. J. Muller-Eberhard, and G. H. Fey.** 1988. The structure of human complement component C7 and the C5b-7 complex. J Biol Chem **263:**549-560.
87. **DiScipio, R. G., P. J. Daffern, I. U. Schraufstatter, and P. Sriramarao.** 1998. Human polymorphonuclear leukocytes adhere to complement factor H through an interaction that involves alphaMbeta2 (CD11b/CD18). J Immunol **160:**4057-4066.
88. **Discipio, R. G., and J. Gagnon.** 1982. Characterization of human complement components C6 and C7. Mol Immunol **19:**1425-1431.

89. **DiScipio, R. G., and T. E. Hugli.** 1989. The molecular architecture of human complement component C6. J Biol Chem **264:**16197-16206.
90. **DiScipio, R. G., C. A. Smith, H. J. Muller-Eberhard, and T. E. Hugli.** 1983. The activation of human complement component C5 by a fluid phase C5 convertase. J Biol Chem **258:**10629-10636.
91. **Duthy, T. G., R. J. Ormsby, E. Giannakis, A. D. Ogunniyi, U. H. Stroeher, J. C. Paton, and D. L. Gordon.** 2002. The human complement regulator factor H binds pneumococcal surface protein PspC via short consensus repeats 13 to 15. Infect Immun **70:**5604-5611.
92. **Edwards, A. O., R. Ritter Iii, K. J. Abel, A. Manning, C. Panhuysen, and L. A. Farrer.** 2005. Complement Factor H Polymorphism and Age-Related Macular Degeneration. Science.
93. **Edwards, J. E., Jr.** 1991. Invasive candida infections--evolution of a fungal pathogen. N Engl J Med **324:**1060-1062.
94. **Eisen, L., and R. S. Lane.** 2002. Vectors of *Borrelia burgdorferi* sensu lato. in Lyme borreliosis. Biology, epidemiology and control. CABI Publishing, Oxon.
95. **Esser, A. F.** 1991. Big MAC attack: complement proteins cause leaky patches. Immunol Today **12:**316-318; discussion 321.
96. **Estaller, C., V. Koistinen, W. Schwaeble, M. P. Dierich, and E. H. Weiss.** 1991. Cloning of the 1.4-kb mRNA species of human complement factor H reveals a novel member of the short consensus repeat family related to the carboxy terminal of the classical 150-kDa molecule. J Immunol **146:**3190-3196.
97. **Estaller, C., W. Schwaeble, M. Dierich, and E. H. Weiss.** 1991. Human complement factor H: two factor H proteins are derived from alternatively spliced transcripts. Eur J Immunol **21:**799-802.
98. **Fearon, D. T.** 1980. Identification of the membrane glycoprotein that is the C3b receptor of the human erythrocyte, polymorphonuclear leukocyte, B lymphocyte, and monocyte. J Exp Med **152:**20-30.
99. **Fearon, D. T.** 1978. Regulation by membrane sialic acid of beta1H-dependent decay-dissociation of amplification C3 convertase of the alternative complement pathway. Proc Natl Acad Sci USA **75:**1971-1975.
100. **Fearon, D. T.** 1979. Regulation of the amplification C3 convertase of human complement by an inhibitory protein isolated from human erythrocyte membrane. Proc Natl Acad Sci USA **76:**5867-5871.
101. **Fearon, D. T., and K. F. Austen.** 1975. Properdin: initiation of alternative complement pathway. Proc Natl Acad Sci USA **72:**3220-3224.
102. **Ferreira, A., M. Takahashi, and V. Nussenzweig.** 1977. Purificaiton and characterization of mouse serum protein with specific binding affinity for C4 (Ss protein). J Exp Med **146:**1001-1008.
103. **Fischetti, V. A.** 1989. Streptococcal M protein: molecular design and biological behavior. Clin Microbiol Rev **2:**285-314.
104. **Fisher-Hoch, S. P., and L. Hutwagner.** 1995. Opportunistic candidiasis: an epidemic of the 1980s. Clin Inf Dis **21:**897-904.

105. **Fishetti, V., R. Horstmann, and V. Pancholi.** 1995. Location of the complement factor H binding site on Streptococcal M6 protein. Infect Immun **63:**149-153.
106. **Fonzi, W. A., and M. Y. Irwin.** 1993. Isogenic strain construction and gene mapping in Candida albicans. Genetics **134:**717-728.
107. **Fraser, C. M., S. Casjens, W. M. Huang, G. G. Sutton, R. Clayton, R. Lathigra, O. White, K. A. Ketchum, R. Dodson, E. K. Hickey, M. Gwinn, B. Dougherty, J. F. Tomb, R. D. Fleischmann, D. Richardson, J. Peterson, A. R. Kerlavage, J. Quackenbush, S. Salzberg, M. Hanson, R. van Vugt, N. Palmer, M. D. Adams, J. Gocayne, J. C. Venter, and et al.** 1997. Genomic sequence of a Lyme disease spirochaete, *Borrelia burgdorferi*. Nature **390:**580-586.
108. **Fries, L. F., H. M. Friedman, G. H. Cohen, R. J. Eisenberg, C. H. Hammer, and M. M. Frank.** 1986. Glycoprotein C of herpes simplex virus 1 is an inhibitor of the complement cascade. J Immunol **137:**1636-1641.
109. **Fujita, T., I. Gigli, and V. Nussenzweig.** 1978. Human C4-binding protein. II. Role in proteolysis of C4b by C3b- inactivator. J Exp Med **148:**1044-1051.
110. **Fujita, T., T. Inoue, K. Ogawa, K. Iida, and N. Tamura.** 1987. The mechanism of action of decay-accelerating factor (DAF). DAF inhibits the assembly of C3 convertases by dissociating C2a and Bb. J Exp Med **166:**1221-1228.
111. **Fujita, T., and V. Nussenzweig.** 1979. The role of C4-binding protein and beta 1H in proteolysis of C4b and C3b. J Exp Med **150:**267-276.
112. **Fujita, T., and N. Tamura.** 1983. Interaction of C4-binding protein with cell-bound C4b. A quantitative analysis of binding and the role of C4-binding protein in proteolysis of cell-bound C4b. J Exp Med **157:**1239-1251.
113. **Gelfand, J. A., D. L. Hurley, A. S. Fauci, and M. M. Frank.** 1978. Role of complement in host defense against experimental disseminated candidiasis. J Infect Dis **138:**9-16.
114. **Giannakis, E., T. S. Jokiranta, D. A. Male, S. Ranganathan, R. J. Ormsby, V. A. Fischetti, C. Mold, and D. L. Gordon.** 2003. A common site within factor H SCR 7 responsible for binding heparin, C-reactive protein and streptococcal M protein. Eur J Immunol **33:**962-969.
115. **Giannakis, E., T. S. Jokiranta, R. J. Ormsby, T. G. Duthy, D. A. Male, D. Christiansen, V. A. Fischetti, C. Bagley, B. E. Loveland, and D. L. Gordon.** 2002. Identification of the streptococcal M protein binding site on membrane cofactor protein (CD46). J Immunol **168:**4585-4592.
116. **Gigli, I., S. Ruddy, and K. F. Austen.** 1968. The stoichiometric measurement of the serum inhibition of the first component of complement by the inhibition of immune hemolysis. J Immunol **100:**1154-1164.
117. **Gilmore, B. J., E. M. Retsinas, J. S. Lorenz, and M. K. Hostetter.** 1988. An iC3b receptor on *Candida albicans*: structure, function, and correlates for pathogenicity. J Infect Dis **157:**38-46.
118. **Gitlin, J. D., F. S. Rosen, and P. J. Lachmann.** 1975. The mechanism of action of the C3b inactivator (conglutinogen-

activating factor) on its naturally occurring substrate, the major fragment of the third component of complement (C3b). J Exp Med **141:**1221-1226.

119. **Gordon, D. L., R. M. Kaufman, T. K. Blackmore, J. Kwong, and D. M. Lublin.** 1995. Identification of complement regulatory domains in human factor H. J Immunol **155:**348-356.
120. **Gorski, J. P., T. E. Hugli, and H. J. Muller-Eberhard.** 1979. C4a: the third anaphylatoxin of the human complement system. Proc Natl Acad Sci USA **76:**5299-5302.
121. **Greene, B. M., H. R. Taylor, and M. Aikawa.** 1981. Cellular killing of microfilariae of *Onchocerca volvulus*: eosinophil and neutrophil-mediated immune serum-dependent destruction. J Immunol **127:**1611-1618.
122. **Hagman, K. E., X. Yang, S. K. Wikel, G. B. Schoeler, M. J. Caimano, J. D. Radolf, and M. V. Norgard.** 2000. Decorin-binding protein A (DbpA) of *Borrelia burgdorferi* is not protective when immunized mice are challenged via tick infestation and correlates with the lack of DbpA expression by B. burgdorferi in ticks. Infect Immun **68:**4759-4764.
123. **Haines, A. L., and I. H. Lepow.** 1964. Studies On Human C'1-Esterase. Ii. Function Of Purified C'1-Esterase In The Human Complement System. J Immunol **92:**468-478.
124. **Haines, J. L., M. A. Hauser, S. Schmidt, W. K. Scott, L. M. Olson, P. Gallins, K. L. Spencer, S. Y. Kwan, M. Noureddine, J. R. Gilbert, N. Schnetz-Boutaud, A. Agarwal, E. A. Postel, and M. A. Pericak-Vance.** 2005. Complement Factor H Variant Increases the Risk of Age-Related Macular Degeneration. Science.
125. **Hardig, Y., and B. Dahlback.** 1996. The amino-terminal module of the C4b-binding protein beta-chain contains the protein S-binding site. J Biol Chem **271:**20861-20867.
126. **Hardig, Y., A. Hillarp, and B. Dahlback.** 1997. The amino-terminal module of the C4b-binding protein alpha-chain is crucial for C4b binding and factor I-cofactor function. Biochem J **323:**469-475.
127. **Harrison, R. A., and P. J. Lachmann.** 1980. The physiological breakdown of the third component of human complement. Mol Immunol **17:**9-20.
128. **Haviland, D. L., J. C. Haviland, D. T. Fleischer, A. Hunt, and R. A. Wetsel.** 1991. Complete cDNA sequence of human complement pro-C5. Evidence of truncated transcripts derived from a single copy gene. J Immunol **146:**362-368.
129. **Hayman, E. G., M. D. Pierschbacher, S. Suzuki, and E. Ruoslahti.** 1985. Vitronectin-a major cell attachment-promoting protein in fetal bovine serum. Exp Cell Res **160:**245-258.
130. **Hegasy, G. A., T. Manuelian, K. Hogasen, J. H. Jansen, and P. F. Zipfel.** 2002. The molecular basis for hereditary porcine membranoproliferative glomerulonephritis type II: point mutations in the factor H coding sequence block protein secretion. Am J Pathol **161:**2027-2034.
131. **Heidenreich, F., and M. P. Dierich.** 1985. *Candida albicans* and *Candida stellatoidea*, in contrast to other Candida species, bind iC3b and C3d but not C3b. Infect Immun **50:**598-600.
132. **Hellwage, J., T. S. Jokiranta, M. A. Friese, T. U. Wolk, E. Kampen, P. F. Zipfel, and S. Meri.** 2002. Complement

C3b/C3d and cell surface polyanions are recognized by overlapping binding sites on the most carboxyl-terminal domain of complement factor H. J Immunol **169:**6935-6944.
133. **Hellwage, J., T. S. Jokiranta, V. Koistinen, O. Vaarala, S. Meri, and P. F. Zipfel.** 1999. Functional properties of complement factor H-related proteins FHR-3 and FHR-4: binding to the C3d region of C3b and differential regulation by heparin. FEBS Lett **462:**345-352.
134. **Hellwage, J., S. Kuhn, and P. F. Zipfel.** 1997. The human complement regulatory factor-H-like protein 1, which represents a truncated form of factor H, displays cell-attachment activity. Biochem J **326:**321-327.
135. **Hellwage, J., C. Skerka, and P. F. Zipfel.** 1997. Biochemical and functional characterization of the factor-H-related protein 4 (FHR-4). Immunopharmacology **38:**149-157.
136. **Hessing, M., C. van 't Veer, T. M. Hackeng, B. N. Bouma, and S. Iwanaga.** 1990. Importance of the alpha 3-fragment of complement C4 for the binding with C4b-binding protein. FEBS Lett **271:**131-136.
137. **Hessing, M., T. V. C. van, and B. N. Bouma.** 1990. The binding site of human C4b-binding protein on complement C4 is localized in the alpha'-chain. J Immunol **144:**2632-2637.
138. **Hessing, M., R. A. Vlooswijk, T. M. Hackeng, D. Kanters, and B. N. Bouma.** 1990. The localization of heparin-binding fragments on human C4b-binding protein. J Immunol **144:**204-208.
139. **Hillarp, A., and B. Dahlbäck.** 1990. Cloning cDNA coding for the beeta-chain of human complement component C4b-binding protein:sequence homology with the alpha chain. Proc Natl Acad Sci USA **87:**1183.
140. **Horstmann, R. D., H. J. Sievertsen, J. Knobloch, and V. A. Fischetti.** 1988. Antiphagocytic activity of streptococcal M protein: selective binding of complement control protein factor H. Proc Natl Acad Sci USA **85:**1657-1661.
141. **Hourcade, D., V. M. Holers, and J. P. Atkinson.** 1989. The regulators of complement activation (RCA) gene cluster. Adv Immunol **45:**381-416.
142. **Hovis, K. M., J. V. McDowell, L. Griffin, and R. T. Marconi.** 2004. Identification and characterization of a linear-plasmid-encoded factor H-binding protein (FhbA) of the relapsing fever spirochete *Borrelia hermsii*. J Bacteriol **186:**2612-2618.
143. **Huehn, M., K. Kändler, T. S. Jokiranta, H. Seeberger, U. Möllman, P. Zipfel, and J. Hellwage.** 2004. Binding of factor H and C3 cleavage by intrinsic proteases are two independent mechanisms in serum resistance of *Pseudomonas aeruginosa*, Congress abstract, XXth International Complement Workshop, Honolulu, Hawaii. Mol Immunol, vol. 41
144. **Hugli, T. E.** 1975. Human anaphylatoxin (C3a) from the third component of complement. Primary structure. J Biol Chem **250:**8293-8301.
145. **Iida, K., and V. Nussenzweig.** 1981. Complement receptor is an inhibitor of the complement cascade. J Exp Med **153:**1138-1150.
146. **Isaacs, S. N., G. J. Kotwal, and B. Moss.** 1992. Vaccinia virus complement-control protein prevents antibody-dependent

complement-enhanced neutralization of infectivity and contributes to virulence. Proc Natl Acad Sci USA **89:**628-632.

147. **Ishizaka, T., K. Ishizaka, T. Borsos, and H. Rapp.** 1966. C'-1 fixation by human isoagglutinins: fixation of C'-1 by gamma-G and gamma-M but not by gamma-A antibody. J Immunol **97:**716-726.
148. **Janulczyk, R., F. Iannelli, A. G. Sjoholm, G. Pozzi, and L. Bjorck.** 2000. Hic, a novel surface protein of *Streptococcus pneumoniae* that interferes with complement function. J Biol Chem **275:**37257-37263.
149. **Jarva, H., J. Hellwage, T. S. Jokiranta, M. J. Lehtinen, P. F. Zipfel, and S. Meri.** 2004. The group B streptococcal beta and pneumococcal Hic proteins are structurally related immune evasion molecules that bind the complement inhibitor factor H in an analogous fashion. J Immunol **172:**3111-3118.
150. **Jarva, H., R. Janulczyk, J. Hellwage, P. F. Zipfel, L. Bjorck, and S. Meri.** 2002. *Streptococcus pneumoniae* evades complement attack and opsonophagocytosis by expressing the pspC locus-encoded Hic protein that binds to short consensus repeats 8-11 of factor H. J Immunol **168:**1886-1894.
151. **Jarva, H., S. Ram, U. Vogel, A. M. Blom, and S. Meri.** 2005. Binding of complement inhibitor C4bp to serogroup B *Neisseria meningitidis*. J Immunol in press.
152. **Jenne, D. E., and J. Tschopp.** 1992. Clusterin: the intriguing guises of a widely expressed glycoprotein. Trends Biochem Sci **17:**154-159.
153. **Jiang, H., and M. M. Frank.** 2004. Staphylococcus binds to factor H and H-related proteins. Congress abstract, XXth International Complement Workshop, Honolulu, Hawaii. Mol Immunol, vol. 41
154. **Jiang, H., F. A. Robey, and H. Gewurz.** 1992. Localization of sites through which C-reactive protein binds and activates complement to residues 14-26 and 76-92 of the human C1q A chain. J Exp Med **175:**1373-1379.
155. **Johnsson, E., K. Berggard, H. Kotarsky, J. Hellwage, P. F. Zipfel, U. Sjobring, and G. Lindahl.** 1998. Role of the hypervariable region in streptococcal M proteins: binding of a human complement inhibitor. J Immunol **161:**4894-4901.
156. **Johnsson, E., A. Thern, B. Dahlback, L. O. Heden, M. Wikstrom, and G. Lindahl.** 1996. A highly variable region in members of the streptococcal M protein family binds the human complement regulator C4BP. J Immunol **157:**3021-3029.
157. **Joiner, K. A., N. Grossman, M. Schmetz, and L. Leive.** 1986. C3 binds preferentially to long-chain lipopolysaccharide during alternative pathway activation by Salmonella montevideo. J Immunol **136:**710-715.
158. **Joiner, K. A., C. H. Hammer, E. J. Brown, R. J. Cole, and M. M. Frank.** 1982. Studies on the mechanism of bacterial resistance to complement-mediated killing. I. Terminal complement components are deposited and released from Salmonella minnesota S218 without causing bacterial death. J Exp Med **155:**797-808.
159. **Jokiranta, T. S., J. Hellwage, V. Koistinen, P. F. Zipfel, and S. Meri.** 2000. Each of the three binding sites on

complement factor H interacts with a distinct site on C3b. J Biol Chem **275:**27657-27662.

160. **Jokiranta, T. S., L. Jokipii, and S. Meri.** 1995. Complement resistance of parasites [editorial]. Scand J Immunol **42:**9-20.

161. **Jokiranta, T. S., P. F. Zipfel, J. Hakulinen, S. Kuhn, M. K. Pangburn, J. D. Tamerius, and S. Meri.** 1996. Analysis of the recognition mechanism of the alternative pathway of complement by monoclonal anti-factor H antibodies: evidence for multiple interactions between H and surface bound C3b. FEBS Lett **393:**297-302.

162. **Jozsi, M., H. Richter, I. Loschmann, C. Skerka, F. Buck, U. Beisiegel, A. Erdei, and P. F. Zipfel.** 2005. FHR-4A: a new factor H-related protein is encoded by the human FHR-4 gene. Eur J Hum Genet **13:**321-329.

163. **Junnikkala, S., T. S. Jokiranta, M. A. Friese, H. Jarva, P. F. Zipfel, and S. Meri.** 2000. Exceptional resistance of human H2 glioblastoma cells to complement-mediated killing by expression and utilization of factor H and factor H-like protein 1. J Immunol **164:**6075-6081.

164. **Kaminishi, H., H. Miyaguchi, T. Tamaki, N. Suenaga, M. Hisamatsu, I. Mihashi, H. Matsumoto, H. Maeda, and Y. Hagihara.** 1995. Degradation of humoral host defense by Candida albicans proteinase. Infect Immun **63:**984-988.

165. **Karam, M., H. Schulz-Key, and J. Remme.** 1987. Population dynamics of *Onchocerca volvulus* after 7 to 8 years of vector control in West Africa. Acta Trop **44:**445-457.

166. **Kask, L., A. Hillarp, B. Ramesh, B. Dahlback, and A. M. Blom.** 2002. Structural requirements for the intracellular subunit polymerization of the complement inhibitor C4b-binding protein. Biochemistry **41:**9349-9357.

167. **Kask, L., L. A. Trouw, B. Dahlback, and A. M. Blom.** 2004. The C4b-binding protein-protein S complex inhibits the phagocytosis of apoptotic cells. J Biol Chem **279:**23869-23873.

168. **Kerr, M. A.** 1980. The human complement system: assembly of the classical pathway C3 convertase. Biochem J **189:**173-181.

169. **Kirkitadze, M. D., and P. N. Barlow.** 2001. Structure and flexibility of the multiple domain proteins that regulate complement activation. Immunol Rev **180:**146-161.

170. **Klein, R. J., C. Zeiss, E. Y. Chew, J. Y. Tsai, R. S. Sackler, C. Haynes, A. K. Henning, J. P. Sangiovanni, S. M. Mane, S. T. Mayne, M. B. Bracken, F. L. Ferris, J. Ott, C. Barnstable, and J. Hoh.** 2005. Complement Factor H Polymorphism in Age-Related Macular Degeneration. Science.

171. **Klickstein, L. B., S. F. Barbashov, T. Liu, R. M. Jack, and A. Nicholson-Weller.** 1997. Complement receptor type 1 (CR1, CD35) is a receptor for C1q. Immunity **7:**345-355.

172. **Kochi, S. K., R. C. Johnson, and A. P. Dalmasso.** 1991. Complement-mediated killing of the Lyme disease spirochete *Borrelia burgdorferi*. Role of antibody in formation of an effective membrane attack complex. J Immunol **146:**3964-3970.

173. **Koistinen, V., S. Wessberg, and J. Leikola.** 1989. Common binding region of complement factors B, H and CR1 on C3b revealed by monoclonal anti-C3d. Complement Inflamm **6:**270-280.

174. **Kolb, W. P., and H. J. Muller-Eberhard.** 1973. The membrane attack mechanism of complement. Verification of a stable C5-9 complex in free solution. J Exp Med **138:**438-451.
175. **Kostavasili, I., A. Sahu, H. M. Friedman, R. J. Eisenberg, G. H. Cohen, and J. D. Lambris.** 1997. Mechanism of complement inactivation by glycoprotein C of herpes simplex virus. J Immunol **158:**1763-1771.
176. **Kotarsky, H., M. Gustafsson, H. G. Svensson, P. F. Zipfel, L. Truedsson, and U. Sjobring.** 2001. Group A streptococcal phagocytosis resistance is independent of complement factor H and factor H-like protein 1 binding. Mol Microbiol **41:**817-826.
177. **Kotarsky, H., J. Hellwage, E. Johnsson, C. Skerka, H. G. Svensson, G. Lindahl, U. Sjobring, and P. F. Zipfel.** 1998. Identification of a domain in human factor H and factor H-like protein- 1 required for the interaction with streptococcal M proteins. J Immunol **160:**3349-3354.
178. **Kotwal, G. J., and B. Moss.** 1988. Vaccinia virus encodes a secretory polypeptide structurally related to complement control proteins. Nature **335:**176-178.
179. **Kozel, T. R.** 1996. Activation of the complement system by pathogenic fungi. Clin Microbiol Rev **9:**34-46.
180. **Kozel, T. R., R. R. Brown, and G. S. Pfrommer.** 1987. Activation and binding of C3 by *Candida albicans*. Infect Immun **55:**1890-1894.
181. **Kozel, T. R., L. C. Weinhold, and D. M. Lupan.** 1996. Distinct characteristics of initiation of the classical and alternative complement pathways by *Candida albicans*. Infect Immun **64:**3360-3368.
182. **Kraiczy, P., J. Hellwage, C. Skerka, H. Becker, M. Kirschfink, M. M. Simon, V. Brade, P. F. Zipfel, and R. Wallich.** 2004. Complement resistance of *Borrelia burgdorferi* correlates with the expression of BbCRASP-1, a novel linear plasmid-encoded surface protein that interacts with human factor H and FHL-1 and is unrelated to Erp proteins. J Biol Chem **279:**2421-2429.
183. **Kraiczy, P., J. Hellwage, C. Skerka, M. Kirschfink, V. Brade, P. F. Zipfel, and R. Wallich.** 2003. Immune evasion of *Borrelia burgdorferi*: mapping of a complement-inhibitor factor H-binding site of BbCRASP-3, a novel member of the Erp protein family. European Journal of Immunology. **33:**697-707.
184. **Kuhn, S., C. Skerka, and P. F. Zipfel.** 1995. Mapping of the complement regulatory domains in the human factor H-like protein 1 and in factor H1. J Immunol **155:**5663-5670.
185. **Kuhn, S., and P. F. Zipfel.** 1995. The baculovirus expression vector pBSV-8His directs secretion of histidine-tagged proteins. Gene **162:**225-229.
186. **Kuhn, S., and P. F. Zipfel.** 1996. Mapping of the domains required for decay acceleration activity of the human factor H-like protein 1 and factor H. Eur J Immunol **26:**2383-2387.
187. **Kurtenbach, K., H. S. Sewell, N. H. Ogden, S. E. Randolph, and P. A. Nuttall.** 1998. Serum complement sensitivity as a key factor in Lyme disease ecology. Infect Immun **66:**1248-1251.
188. **Laclette, J. P., C. B. Shoemaker, D. Richter, L. Arcos, N. Pante, C. Cohen, D. Bing, and A. Nicholson-Weller.** 1992. Paramyosin inhibits complement C1. J Immunol **148:**124-128.

189. **Lam, T. T., T. P. Nguyen, R. R. Montgomery, F. S. Kantor, E. Fikrig, and R. A. Flavell.** 1994. Outer surface proteins E and F of *Borrelia burgdorferi*, the agent of Lyme disease. Infect Immun **62:**290-298.
190. **Lambris, J. D.** 1988. The multifunctional role of C3, the third component of complement. Immunol Today **9:**387-393.
191. **Law, S. K., and R. P. Levine.** 1977. Interaction between the third complement protein and cell surface macromolecules. Proc Natl Acad Sci USA **74:**2701-2705.
192. **Lindahl, G., U. Sjobring, and E. Johnsson.** 2000. Human complement regulators: a major target for pathogenic microorganisms. Curr Opin Immunol **12:**44-51.
193. **Loos, M.** 1982. Antibody-independent activation of C1, the first component of complement. Ann Immunol (Paris) **133C:**165-179.
194. **Lubinski, J., L. Wang, D. Mastellos, A. Sahu, J. D. Lambris, and H. M. Friedman.** 1999. In vivo role of complement-interacting domains of herpes simplex virus type 1 glycoprotein gC. J Exp Med **190:**1637-1646.
195. **Lublin, D. M., M. K. Liszewski, T. W. Post, M. A. Arce, M. M. Le Beau, M. B. Rebentisch, L. S. Lemons, T. Seya, and J. P. Atkinson.** 1988. Molecular cloning and chromosomal localization of human membrane cofactor protein (MCP). Evidence for inclusion in the multigene family of complement-regulatory proteins. J Exp Med **168:**181-194.
196. **Manuelian, T., J. Hellwage, S. Meri, J. Caprioli, M. Noris, S. Heinen, M. Jozsi, H. P. Neumann, G. Remuzzi, and P. F. Zipfel.** 2003. Mutations in factor H reduce binding affinity to C3b and heparin and surface attachment to endothelial cells in hemolytic uremic syndrome. J Clin Invest **111:**1181-1190.
197. **Marikovsky, M., R. Arnon, and Z. Fishelson.** 1988. Proteases secreted by transforming schistosomula of *Schistosoma mansoni* promote resistance to killing by complement. J Immunol **141:**273-278.
198. **Marikovsky, M., F. Levi-Schaffer, R. Arnon, and Z. Fishelson.** 1986. *Schistosoma mansoni*: killing of transformed schistosomula by the alternative pathway of human complement. Exp Parasitol **61:**86-94.
199. **Matsushita, M., and T. Fujita.** 1992. Activation of the classical complement pathway by mannose-binding protein in association with a novel C1s-like serine protease. J Exp Med **176:**1497-1502.
200. **Mayer, M. M.** 1984. Complement. Historical perspectives and some current issues. Complement **1:**2-26.
201. **McDowell, J. V., E. Tran, D. Hamilton, J. Wolfgang, K. Miller, and R. T. Marconi.** 2003. Analysis of the ability of spirochete species associated with relapsing fever, avian borreliosis, and epizootic bovine abortion to bind factor H and cleave c3b. J Clin Microbiol **41:**3905-3910.
202. **McDowell, J. V., J. Wolfgang, L. Senty, C. M. Sundy, M. J. Noto, and R. T. Marconi.** 2004. Demonstration of the involvement of outer surface protein E coiled coil structural domains and higher order structural elements in the binding of infection-induced antibody and the complement-regulatory protein, factor H. J Immunol **173:**7471-7480.
203. **McRae, J. L., P. J. Cowan, D. A. Power, K. I. Mitchelhill, B. E. Kemp, B. P. Morgan, and B. F. Murphy.** 2001. Human

factor H-related protein 5 (FHR-5). A new complement-associated protein. J Biol Chem **276:**6747-6754.

204. **Medicus, R. G., O. Gotze, and H. J. Muller-Eberhard.** 1976. Alternative pathway of complement: recruitment of precursor properdin by the labile C3/C5 convertase and the potentiation of the pathway. J Exp Med **144:**1076-1093.

205. **Medof, M. E., K. Iida, C. Mold, and V. Nussenzweig.** 1982. Unique role of the complement receptor CR1 in the degradation of C3b associated with immune complexes. J Exp Med **156:**1739-1754.

206. **Medof, M. E., E. I. Walter, J. L. Rutgers, D. M. Knowles, and V. Nussenzweig.** 1987. Identification of the complement decay-accelerating factor (DAF) on epithelium and glandular cells and in body fluids. J Exp Med **165:**848-864.

207. **Meri, S., B. P. Morgan, A. Davies, R. H. Daniels, M. G. Olavesen, H. Waldmann, and P. J. Lachmann.** 1990. Human protectin (CD59), an 18,000-20,000 MW complement lysis restricting factor, inhibits C5b-8 catalysed insertion of C9 into lipid bilayers. Immunol **71:**1-9.

208. **Meri, S., and M. K. Pangburn.** 1990. Discrimination between activators and nonactivators of the alternative pathway of complement: regulation via a sialic acid/polyanion binding site on factor H. Proc Natl Acad Sci U S A **87:**3982-3986.

209. **Meri, S., H. Waldmann, and P. J. Lachmann.** 1991. Distribution of protectin (CD59), a complement membrane attack inhibitor, in normal human tissues. Lab Invest **65:**532-537.

210. **Meri, T., S. J. Cutler, J. Hellwage, P. F. Zipfel, S. Meri, and T. S. Jokiranta.** 2003. Congress abstract. Presented at the The Ninth European Complement Workshop, Trieste, Italy.

211. **Metts, M. S., J. V. McDowell, M. Theisen, P. R. Hansen, and R. T. Marconi.** 2003. Analysis of the OspE determinants involved in binding of factor H and OspE-targeting antibodies elicited during Borrelia burgdorferi infection in mice. Infect Immun **71:**3587-3596.

212. **Morfeldt, E., K. Berggard, J. Persson, T. Drakenberg, E. Johnsson, E. Lindahl, S. Linse, and G. Lindahl.** 2001. Isolated hypervariable regions derived from streptococcal M proteins specifically bind human C4b-binding protein: implications for antigenic variation. J Immunol **167:**3870-3877.

213. **Morgan, B. P., and M. J. Walport.** 1991. Complement deficiency and disease. Immunol Today **12:**301-306.

214. **Murphy, B., T. Georgiou, D. Machet, P. Hill, and J. McRae.** 2002. Factor H-related protein-5: a novel component of human glomerular immune deposits. Am J Kidney Dis **39:**24-27.

215. **Nagasawa, S., and R. M. Stroud.** 1977. Mechanism of action of the C3b inactivator: requirement for a high molecular weight cofactor (C3b-C4bINA cofactor) and production of a new C3b derivative (C3b'). Immunochemistry **14:**749-756.

216. **Naglik, J., A. Albrecht, O. Bader, and B. Hube.** 2004. *Candida albicans* proteinases and host/pathogen interactions. Cell Microbiol **6:**915-926.

217. **Neeleman, C., S. P. Geelen, P. C. Aerts, M. R. Daha, T. E. Mollnes, J. J. Roord, G. Posthuma, H. van Dijk, and A. Fleer.** 1999. Resistance to both complement activation and phagocytosis in type 3 pneumococci is mediated by the binding of

complement regulatory protein factor H. Infect Immun **67:**4517-4524.

218. **Nemerow, G. R., K. I. Yamamoto, and T. F. Lint.** 1979. Restriction of complement-mediated membrane damage by the eighth component of complement: a dual role for C8 in the complement attack sequence. J Immunol **123:**1245-1252.

219. **Nicholson-Weller, A., J. Burge, and K. F. Austen.** 1981. Purification from guinea pig erythrocyte stroma of a decay-accelerating factor for the classical c3 convertase, C4b,2a. J Immunol **127:**2035-2039.

220. **Nicholson-Weller, A., J. P. March, S. I. Rosenfeld, and K. F. Austen.** 1983. Affected erythrocytes of patients with paroxysmal nocturnal hemoglobinuria are deficient in the complement regulatory protein, decay accelerating factor. Proc Natl Acad Sci USA **80:**5066-5070.

221. **Nilsson, U. R., and H. J. Mueller-Eberhard.** 1965. Isolation Of Beta If-Globulin From Human Serum And Its Characterization As The Fifth Component Of Complement. J Exp Med **122:**277-298.

222. **Nonaka, M.** 2001. Evolution of the complement system. Curr Opin Immunol **13:**69-73.

223. **Nordahl, E. A., V. Rydengard, P. Nyberg, D. P. Nitsche, M. Morgelin, M. Malmsten, L. Bjorck, and A. Schmidtchen.** 2004. Activation of the complement system generates antibacterial peptides. Proc Natl Acad Sci USA **101:**16879-16884.

224. **Nordstrom, T., A. M. Blom, A. Forsgren, and K. Riesbeck.** 2004. The emerging pathogen *Moraxella catarrhalis* interacts with complement inhibitor C4b binding protein through ubiquitous surface proteins A1 and A2. J Immunol **173:**4598-4606.

225. **Norris, K. A., B. Bradt, N. R. Cooper, and M. So.** 1991. Characterization of a *Trypanosoma cruzi* C3 binding protein with functional and genetic similarities to the human complement regulatory protein, decay-accelerating factor. J Immunol **147:**2240-2247.

226. **Ollert, M. W., E. Wadsworth, and R. A. Calderone.** 1990. Reduced expression of the functionally active complement receptor for iC3b but not for C3d on an avirulent mutant of *Candida albicans*. Infect Immun **58:**909-913.

227. **Ormsby, R. J., T. S. Jokiranta, K. Kriggs, E. Giannakis, T. Sadlon, and D. L. Gordon.** 2004. Localisation of the third heparin-binding domain in human complement factor H. Congress abstract, XXth International Complement Workshop, Honolulu, Hawaii. Mol Immunol, vol. 41

228. **Pandiripally, V., E. Gregory, and D. Cue.** 2002. Acquisition of regulators of complement activation by *Streptococcus pyogenes* serotype M1. Infect Immun **70:**6206-6214.

229. **Pandiripally, V., L. Wei, C. Skerka, P. F. Zipfel, and D. Cue.** 2003. Recruitment of complement factor H-like protein 1 promotes intracellular invasion by group A streptococci. Infect Immun **71:**7119-7128.

230. **Pangburn, M. K.** 2002. Cutting edge: localization of the host recognition functions of complement factor H at the carboxyl-terminal: implications for hemolytic uremic syndrome. J Immunol **169:**4702-4706.

231. **Pangburn, M. K., M. A. Atkinson, and S. Meri.** 1991. Localization of the heparin-binding site on complement factor H. J Biol Chem **266:**16847-16853.
232. **Pangburn, M. K., R. D. Schreiber, and H. J. Muller-Eberhard.** 1983. Deficiency of an erythrocyte membrane protein with complement regulatory activity in paroxysmal nocturnal hemoglobinuria. Proc Natl Acad Sci USA **80:**5430-5434.
233. **Pangburn, M. K., R. D. Schreiber, and H. J. Muller-Eberhard.** 1981. Formation of the initial C3 convertase of the alternative complement pathway. Acquisition of C3b-like activities by spontaneous hydrolysis of the putative thioester in native C3. J Exp Med **154:**856-867.
234. **Pangburn, M. K., R. D. Schreiber, and H. J. Muller-Eberhard.** 1977. Human complement C3b inactivator: isolation, characterization, and demonstration of an absolute requirement for the serum protein beta1H for cleavage of C3b and C4b in solution. J Exp Med **146:**257-270.
235. **Parizade, M., R. Arnon, P. J. Lachmann, and Z. Fishelson.** 1994. Functional and antigenic similarities between a 94-kD protein of Schistosoma mansoni (SCIP-1) and human CD59. J Exp Med **179:**1625-1636.
236. **Pearce, E. J., B. F. Hall, and A. Sher.** 1990. Host-specific evasion of the alternative complement pathway by schistosomes correlates with the presence of a phospholipase C-sensitive surface molecule resembling human decay accelerating factor. J Immunol **144:**2751-2756.
237. **Perez-Caballero, D., I. Garcia-Laorden, G. Cortes, M. R. Wessels, S. R. de Cordoba, and S. Alberti.** 2004. Interaction between complement regulators and *Streptococcus pyogenes*: binding of C4b-binding protein and factor H/factor H-like protein 1 to M18 strains involves two different cell surface molecules. J Immunol **173:**6899-6904.
238. **Perez-Caballero, D., C. Gonzalez-Rubio, M. E. Gallardo, M. Vera, M. Lopez-Trascasa, S. Rodriguez de Cordoba, and P. Sanchez-Corral.** 2001. Clustering of missense mutations in the C-terminal region of factor H in atypical hemolytic uremic syndrome. Am J Hum Genet **68:**478-484.
239. **Perez-Casal, J., N. Okada, M. G. Caparon, and J. R. Scott.** 1995. Role of the conserved C-repeat region of the M protein of *Streptococcus pyogenes*. Mol Microbiol **15:**907-916.
240. **Perkins, S. J., and T. H. Goodship.** 2002. Molecular modelling of the C-terminal domains of factor H of human complement: a correlation between haemolytic uraemic syndrome and a predicted heparin binding site. J Mol Biol **316:**217-224.
241. **Peterson, P. K., D. Schmeling, P. P. Cleary, B. J. Wilkinson, Y. Kim, and P. G. Quie.** 1979. Inhibition of alternative complement pathway opsonization by group A streptococcal M protein. J Infect Dis **139:**575-585.
242. **Phillips, G. N., Jr., P. F. Flicker, C. Cohen, B. N. Manjula, and V. A. Fischetti.** 1981. Streptococcal M protein: alpha-helical coiled-coil structure and arrangement on the cell surface. Proc Natl Acad Sci USA **78:**4689-4693.
243. **Pickering, M. C., H. T. Cook, J. Warren, A. E. Bygrave, J. Moss, M. J. Walport, and M. Botto.** 2002. Uncontrolled C3

activation causes membranoproliferative glomerulonephritis in mice deficient in complement factor H. Nat Genet **31:**424-428.

244. **Piessens, W. F., and W. D. da Silva.** 1982. Complement-mediated adherence of cells to microfilariae of Brugia malayi. Am J Trop Med Hyg **31:**297-301.
245. **Pinter, C., A. G. Siccardi, R. Longhi, and A. Clivio.** 1995. Direct interaction of complement factor H with the C1 domain of HIV type 1 glycoprotein 120. AIDS Res Hum Retroviruses **11:**577-588.
246. **Pinter, C., A. G. Siccardi, L. Lopalco, R. Longhi, and A. Clivio.** 1995. HIV glycoprotein 41 and complement factor H interact with each other and share functional as well as antigenic homology. AIDS Res Hum Retroviruses **11:**971-980.
247. **Podack, E. R., G. Biesecker, and H. J. Muller-Eberhard.** 1979. Membrane attack complex of complement: generation of high-affinity phospholipid binding sites by fusion of five hydrophilic plasma proteins. Proc Natl Acad Sci USA **76:**897-901.
248. **Podack, E. R., and H. J. Muller-Eberhard.** 1979. Isolation of human S-protein, an inhibitor of the membrane attack complex of complement. J Biol Chem **254:**9808-9814.
249. **Post, T. W., M. K. Liszewski, E. M. Adams, I. Tedja, E. A. Miller, and J. P. Atkinson.** 1991. Membrane cofactor protein of the complement system: alternative splicing of serine/threonine/proline-rich exons and cytoplasmic tails produces multiple isoforms that correlate with protein phenotype. J Exp Med **174:**93-102.
250. **Prasadarao, N. V., A. M. Blom, B. O. Villoutreix, and L. C. Linsangan.** 2002. A novel interaction of outer membrane protein A with C4b binding protein mediates serum resistance of *Escherichia coli* K1. J Immunol **169:**6352-6360.
251. **Preissner, K. T., N. Heimburger, E. Anders, and G. Muller-Berghaus.** 1986. Physicochemical, immunochemical and functional comparison of human S-protein and vitronectin. Evidence for the identity of both plasma proteins. Biochem Biophys Res Commun **134:**951-956.
252. **Preissner, K. T., and G. Muller-Berghaus.** 1987. Neutralization and binding of heparin by S protein/vitronectin in the inhibition of factor Xa by antithrombin III. Involvement of an inducible heparin-binding domain of S protein/vitronectin. J Biol Chem **262:**12247-12253.
253. **Prodinger, W. M., J. Hellwage, M. Spruth, M. P. Dierich, and P. F. Zipfel.** 1998. The C-terminus of factor H: monoclonal antibodies inhibit heparin binding and identify epitopes common to factor H and factor H-related proteins. Biochem J **331:**41-47.
254. **Puentes, S. M., R. P. Da Silva, D. L. Sacks, C. H. Hammer, and K. A. Joiner.** 1990. Serum resistance of metacyclic stage *Leishmania major* promastigotes is due to release of C5b-9. J Immunol **145:**4311-4316.
255. **Puentes, S. M., D. M. Dwyer, P. A. Bates, and K. A. Joiner.** 1989. Binding and release of C3 from *Leishmania donovani* promastigotes during incubation in normal human serum. J Immunol **143:**3743-3749.
256. **Radolf, J. D., K. W. Bourell, D. R. Akins, J. S. Brusca, and M. V. Norgard.** 1994. Analysis of *Borrelia burgdorferi* membrane

architecture by freeze-fracture electron microscopy. J Bacteriol **176:**21-31.
257. **Ram, S., M. Cullinane, A. M. Blom, S. Gulati, D. P. McQuillen, R. Boden, B. G. Monks, C. O'Connell, C. Elkins, M. K. Pangburn, B. Dahlback, and P. A. Rice.** 2001. C4bp binding to porin mediates stable serum resistance of *Neisseria gonorrhoeae.* Int Immunopharmacol **1:**423-432.
258. **Ram, S., M. Cullinane, A. M. Blom, S. Gulati, D. P. McQuillen, B. G. Monks, C. O'Connell, R. Boden, C. Elkins, M. K. Pangburn, B. Dahlback, and P. A. Rice.** 2001. Binding of C4b-binding protein to porin: a molecular mechanism of serum resistance of *Neisseria gonorrhoeae*. J Exp Med **193:**281-295.
259. **Ram, S., F. G. Mackinnon, S. Gulati, D. P. McQuillen, U. Vogel, M. Frosch, C. Elkins, H. K. Guttormsen, L. M. Wetzler, M. Oppermann, M. K. Pangburn, and P. A. Rice.** 1999. The contrasting mechanisms of serum resistance of *Neisseria gonorrhoeae* and group B *Neisseria meningitidis*. Mol Immunol **36:**915-928.
260. **Ram, S., D. P. McQuillen, S. Gulati, C. Elkins, M. K. Pangburn, and P. A. Rice.** 1998. Binding of complement factor H to loop 5 of porin protein 1A: a molecular mechanism of serum resistance of nonsialylated *Neisseria gonorrhoeae*. J Exp Med **188:**671-680.
261. **Ram, S., A. K. Sharma, S. D. Simpson, S. Gulati, D. P. McQuillen, M. K. Pangburn, and P. A. Rice.** 1998. A novel sialic acid binding site on factor H mediates serum resistance of sialylated *Neisseria gonorrhoeae*. J Exp Med **187:**743-752.
262. **Ramamoorthy, R., and D. Scholl-Meeker.** 2001. *Borrelia burgdorferi* proteins whose expression is similarly affected by culture temperature and pH. Infect Immun **69:**2739-2742.
263. **Rao, U. R., R. Chandrashekar, and D. Subrahmanyam.** 1987. Complement activation by eggs and microfilariae of filarial parasites. Immunol Cell Biol **65:**365-370.
264. **Ras, N. M., D. Postic, P. Ave, M. Huerre, and G. Baranton.** 2000. Antigenic variation of *Borrelia turicatae* Vsp surface lipoproteins occurs in vitro and generates novel serotypes. Research in Microbiology. **151:**5-12.
265. **Rautemaa, R., G. A. Jarvis, P. Marnila, and S. Meri.** 1998. Acquired resistance of *Escherichia coli* to complement lysis by binding of glycophosphoinositol-anchored protectin (CD59). Infect Immun **66:**1928-1933.
266. **Rautemaa, R., H. Rautelin, P. Puolakkainen, A. Kokkola, P. Karkkainen, and S. Meri.** 2001. Survival of *Helicobacter pylori* From complement lysis by binding of GPI-anchored protectin (CD59). Gastroenterology **120:**470-479.
267. **Reid, K. B., and M. W. Turner.** 1994. Mammalian lectins in activation and clearance mechanisms involving the complement system. Springer Semin Immunopathol **15:**307-326.
268. **Ren, G., M. Doshi, B. K. Hack, J. J. Alexander, and R. J. Quigg.** 2002. Isolation and characterization of a novel rat factor H-related protein that is up-regulated in glomeruli under complement attack. J Biol Chem **277:**48351-48358.
269. **Rice, P. A.** 1989. Molecular basis for serum resistance in *Neisseria gonorrhoeae*. Clin Microbiol Rev **2 Suppl:**S112-117.

270. **Richards, A., M. R. Buddles, R. L. Donne, B. S. Kaplan, E. Kirk, M. C. Venning, C. L. Tielemans, J. A. Goodship, and T. H. Goodship.** 2001. Factor H mutations in hemolytic uremic syndrome cluster in exons 18-20, a domain important for host cell recognition. Am J Hum Genet **68:**485-490.
271. **Rollins, S. A., and P. J. Sims.** 1990. The complement-inhibitory activity of CD59 resides in its capacity to block incorporation of C9 into membrane C5b-9. J Immunol **144:**3478-3483.
272. **Ross, G. D., S. L. Newman, J. D. Lambris, J. E. Devery-Pocius, J. A. Cain, and P. J. Lachmann.** 1983. Generation of three different fragments of bound C3 with purified factor I or serum. II. Location of binding sites in the C3 fragments for factors B and H, complement receptors, and bovine conglutinin. J Exp Med **158:**334-352.
273. **Rougier, N., M. D. Kazatchkine, J. P. Rougier, V. Fremeaux-Bacchi, J. Blouin, G. Deschenes, B. Soto, V. Baudouin, B. Pautard, W. Proesmans, E. Weiss, and L. Weiss.** 1998. Human complement factor H deficiency associated with hemolytic uremic syndrome. J Am Soc Nephrol **9:**2318-2326.
274. **Sadlon, T., R. Ormsby, and D. L. Gordon.** 2004. *Cryptococcus neoformans* and *C. gattii* bind the complement regulatory protein factor H. Congress abstract, XXth International Complement Workshop, Honolulu, Hawaii. Mol Immunol, vol 41.
275. **Salacinski, P. R., C. McLean, J. E. Sykes, V. V. Clement-Jones, and P. J. Lowry.** 1981. Iodination of proteins, glycoproteins, and peptides using a solid-phase oxidizing agent, 1,3,4,6-tetrachloro-3 alpha,6 alpha-diphenyl glycoluril (Iodogen). Anal Biochem **117:**136-146.
276. **Sanchez-Corral, P., D. Perez-Caballero, O. Huarte, A. M. Simckes, E. Goicoechea, M. Lopez-Trascasa, and S. R. de Cordoba.** 2002. Structural and functional characterization of factor H mutations associated with atypical hemolytic uremic syndrome. Am J Hum Genet **71:**1285-1295.
277. **Scharfstein, J., A. Ferreira, I. Gigli, and V. Nussenzweig.** 1978. Human C4b-binding protein. I. Isolation and characterization. J Exp Med **148:**207-222.
278. **Schifferli, J. A., Y. C. Ng, and D. K. Peters.** 1986. The role of complement and its receptor in the elimination of immune complexes. N Engl J Med **315:**488-495.
279. **Schreiber, R. D., and H. J. Muller-Eberhard.** 1974. Fourth component of human complement: description of a three polypeptide chain structure. J Exp Med **140:**1324-1335.
280. **Schulz-Key, H., E. Albiez, and S. Golub.** 1977. Isolation of living adult *Onchocerca volvulus* from nodules. Trop Med Parasitol **28:**428-430.
281. **Schwalbe, R., B. Dahlback, A. Hillarp, and G. Nelsestuen.** 1990. Assembly of protein S and C4b-binding protein on membranes. J Biol Chem **265:**16074-16081.
282. **Seya, T., T. Hara, K. Iwata, S. Kuriyama, T. Hasegawa, Y. Nagase, S. Miyagawa, M. Matsumoto, M. Hatanaka, J. P. Atkinson, and et al.** 1995. Purification and functional properties of soluble forms of membrane cofactor protein (CD46) of complement: identification of forms increased in cancer patients' sera. Int Immunol **7:**727-736.

283. **Seya, T., J. R. Turner, and J. P. Atkinson.** 1986. Purification and characterization of a membrane protein (gp45-70) that is a cofactor for cleavage of C3b and C4b. J Exp Med **163:**837-855.
284. **Shaffer, M. C., T. P. Foley, and D. W. Barnes.** 1984. Quantitation of spreading factor in human biologic fluids. J Lab Clin Med **103:**783-791.
285. **Sharma, A. K., and M. K. Pangburn.** 1996. Identification of three physically and functionally distinct binding sites for C3b in human complement factor H by deletion mutagenesis. Proc Natl Acad Sci USA **93:**10996-11001.
286. **Sharma, A. K., and M. K. Pangburn.** 1997. Localization by site-directed mutagenesis of the site in human complement factor H that binds to *Streptococcus pyogenes* M protein. Infect Immun **65:**484-487.
287. **Sim, R. B., and R. G. DiScipio.** 1982. Purification and structural studies on the complement-system control protein beta 1H (Factor H). Biochem J **205:**285-293.
288. **Sim, R. B., T. M. Twose, D. S. Paterson, and E. Sim.** 1981. The covalent-binding reaction of complement component C3. Biochem J **193:**115-127.
289. **Skerka, C., J. Hellwage, W. Weber, A. Tilkorn, F. Buck, T. Marti, E. Kampen, U. Beisiegel, and P. F. Zipfel.** 1997. The human factor H-related protein 4 (FHR-4). A novel short consensus repeat-containing protein is associated with human triglyceride-rich lipoproteins. J Biol Chem **272:**5627-5634.
290. **Skerka, C., R. D. Horstmann, and P. F. Zipfel.** 1991. Molecular cloning of a human serum protein structurally related to complement factor H. J Biol Chem **266:**12015-12020.
291. **Skerka, C., S. Kuhn, K. Gunther, K. Lingelbach, and P. F. Zipfel.** 1993. A novel short consensus repeat-containing molecule is related to human complement factor H. J Biol Chem **268:**2904-2908.
292. **Skerka, C., C. Timmann, R. D. Horstmann, and P. F. Zipfel.** 1992. Two additional human serum proteins structurally related to complement factor H. Evidence for a family of factor H-related genes. J Immunol **148:**3313-3318.
293. **Junttila, J., Peltomaa, M., Soini, H., Marjamaki, M., and Viljanen, M.K.** 1999. Prevalence of *Borrelia burgdorferi* in Ixodes ricinus ticks in urban recreational areas of Helsinki. J Clin Microbiol **37**: 1361-1365.
294. **Spiller, O. B., B. P. Morgan, F. Tufaro, and D. V. w. Devine.** 1996. Altered expression of host-encoded complement regulators on human cytomegalovirus-infected cells. Eur J Immunol **26:**1532-1538.
295. **Stanek, G., S. F., J. S. Gray, and G. P. Wormser.** 2002. History and characteristics of Lyme borreliosis. CABI Publishing, Oxon.
296. **Steere, A. C.** 1989. Lyme disease. N Engl J Med **321:**586-596.
297. **Stevenson, B., J. L. Bono, T. G. Schwan, and P. Rosa.** 1998. *Borrelia burgdorferi* erp proteins are immunogenic in mammals infected by tick bite, and their synthesis is inducible in cultured bacteria. Infect Immun **66:**2648-2654.
298. **Stevenson, B., T. G. Schwan, and P. A. Rosa.** 1995. Temperature-related differential expression of antigens in the

Lyme disease spirochete, *Borrelia burgdorferi*. Infect Immun **63:**4535-4539.
299. **Stoenner, H. G., T. Dodd, and C. Larsen.** 1982. Antigenic variation of *Borrelia hermsii*. J Exp Med **156:**1297-1311.
300. **Stoiber, H., A. Clivio, and M. P. Dierich.** 1997. Role of complement in HIV infection. Annu Rev Immunol **15:**649-674.
301. **Stoiber, H., R. Schneider, J. Janatova, and M. P. Dierich.** 1995. Human complement proteins C3b, C4b, factor H and properdin react with specific sites in gp120 and gp41, the envelope proteins of HIV-1. Immunobiology **193:**98-113.
302. **Sundnes, K. O., and A. T. Haimanot.** 1993. Epidemic of louse-borne relapsing fever in Ethiopia.[comment][erratum appears in Lancet 1994 Jan 22;334(8891):244]. Lancet **342:**1213-1215.
303. **Tabona, P., A. Mellor, and J. A. Summerfield.** 1995. Mannose binding protein is involved in first-line host defence: evidence from transgenic mice. Immunol **85:**153-159.
304. **Tack, B. F., S. C. Morris, and J. W. Prahl.** 1979. Fifth component of human complement: purification from plasma and polypeptide chain structure. Biochemistry **18:**1490-1497.
305. **Takata, Y., T. Kinoshita, H. Kozono, J. Takeda, E. Tanaka, K. Hong, and K. Inoue.** 1987. Covalent association of C3b with C4b within C5 convertase of the classical complement pathway. J Exp Med **165:**1494-1507.
306. **Taylor, P. W.** 1983. Bactericidal and bacteriolytic activity of serum against gram-negative bacteria. Microbiol Rev **47:**46-83.
307. **Thakker, M., J. S. Park, V. Carey, and J. C. Lee.** 1998. *Staphylococcus aureus* serotype 5 capsular polysaccharide is antiphagocytic and enhances bacterial virulence in a murine bacteremia model. Infect Immun **66:**5183-5189.
308. **Thern, A., L. Stenberg, B. Dahlback, and G. Lindahl.** 1995. Ig-binding surface proteins of *Streptococcus pyogenes* also bind human C4b-binding protein (C4BP), a regulatory component of the complement system. J Immunol **154:**375-386.
309. **Thiel, S., T. Vorup-Jensen, C. M. Stover, W. Schwaeble, S. B. Laursen, K. Poulsen, A. C. Willis, P. Eggleton, S. Hansen, U. Holmskov, K. B. Reid, and J. C. Jensenius.** 1997. A second serine protease associated with mannan-binding lectin that activates complement. Nature **386:**506-510.
310. **Tomlinson, S., L. C. Pontes de Carvalho, F. Vandekerckhove, and V. Nussenzweig.** 1994. Role of sialic acid in the resistance of Trypanosoma cruzi trypomastigotes to complement. J Immunol **153:**3141-3147.
311. **Tschopp, J., A. Chonn, S. Hertig, and L. E. French.** 1993. Clusterin, the human apolipoprotein and complement inhibitor, binds to complement C7, C8 beta, and the b domain of C9. J Immunol **151:**2159-2165.
312. **Tschopp, J., E. R. Podack, and H. J. Muller-Eberhard.** 1982. Ultrastructure of the membrane attack complex of complement: detection of the tetramolecular C9-polymerizing complex C5b-8. Proc Natl Acad Sci USA **79:**7474-7478.
313. **Tsuruta, J. K., K. Wong, I. B. Fritz, and M. D. Griswold.** 1990. Structural analysis of sulphated glycoprotein 2 from amino acid sequence. Relationship to clusterin and serum protein 40,40. Biochem J **268:**571-578.

314. **Turner, M. W.** 1996. Mannose-binding lectin: the pluripotent molecule of the innate immune system. Immunol Today **17:**532-540.
315. **van Dam, A. P., A. Oei, R. Jaspars, C. Fijen, B. Wilske, L. Spanjaard, and J. Dankert.** 1997. Complement-mediated serum sensitivity among spirochetes that cause Lyme disease. Infect Immun **65:**1228-1236.
316. **Vanderplasschen, A., E. Mathew, M. Hollinshead, R. B. Sim, and G. L. Smith.** 1998. Extracellular enveloped vaccinia virus is resistant to complement because of incorporation of host complement control proteins into its envelope. Proc Natl Acad Sci USA **95:**7544-7549.
317. **Wallich, R., J. Pattathu, V. Kitiratschky, C. Brenner, P. F. Zipfel, V. Brade, M. M. Simon, and P. Kraiczy.** 2005. Identification and functional characterization of complement regulator-acquiring surface protein 1 of the Lyme disease spirochetes *Borrelia afzelii* and *Borrelia garinii*. Infect Immun **73:**2351-2359.
318. **Ward, P. A., C. G. Cochrane, and H. J. Muller-Eberhard.** 1966. Further studies on the chemotactic factor of complement and its formation in vivo. Immunol **11:**141-153.
319. **Webb, J. H., A. M. Blom, and B. Dahlback.** 2002. Vitamin K-dependent protein S localizing complement regulator C4b- binding protein to the surface of apoptotic cells. J Immunol **169:**2580-2586.
320. **Weiler, J. M., M. R. Daha, K. F. Austen, and D. T. Fearon.** 1976. Control of the amplification convertase of complement by the plasma protein beta1H. Proc Natl Acad Sci USA **73:**3268-3272.
321. **Wetsel, R. A.** 1995. Structure, function and cellular expression of complement anaphylatoxin receptors. Curr Opin Immunol **7:**48-53.
322. **Wexler, D. E., D. E. Chenoweth, and P. P. Cleary.** 1985. Mechanism of action of the group A streptococcal C5a inactivator. Proc Natl Acad Sci USA **82:**8144-8148.
323. **Whaley, K., and S. Ruddy.** 1976. Modulation of the alternative complement pathways by beta 1 H globulin. J Exp Med **144:**1147-1163.
324. **WHO.** 1995. Onchocerciasis and ots control. Report of a WHO Expert Committee on Onchocerciasis Control. WHO Tech Rep Ser **852:**1-104.
325. **Winkelstein, J. A., A. S. Abramovitz, and A. Tomasz.** 1980. Activation of C3 via the alternative complement pathway results in fixation of C3b to the pneumococcal cell wall. J Immunol **124:**2502-2506.
326. **Wurzner, R.** 1999. Evasion of pathogens by avoiding recognition or eradication by complement, in part via molecular mimicry. Mol Immunol **36:**249-260.
327. **Zhang, M. X., J. E. Cutler, Y. Han, and T. R. Kozel.** 1998. Contrasting roles of mannan-specific monoclonal immunoglobulin M antibodies in the activation of classical and alternative pathways by *Candida albicans*. Infect Immun **66:**6027-6029.
328. **Ziccardi, R. J.** 1983. The first component of human complement (C1): activation and control. Springer Semin Immunopathol **6:**213-230.

329. **Ziccardi, R. J.** 1982. A new role for C-1-inhibitor in homeostasis: control of activation of the first component of human complement. J Immunol **128:**2505-2508.
330. **Ziccardi, R. J., and N. R. Cooper.** 1979. Active disassembly of the first complement component, C-1, by C-1 inactivator. J Immunol **123:**788-792.
331. **Ziccardi, R. J., B. Dahlback, and H. J. Muller-Eberhard.** 1984. Characterization of the interaction of human C4b-binding protein with physiological ligands. J Biol Chem **259:**13674-13679.
332. **Zipfel, P. F., T. S. Jokiranta, J. Hellwage, V. Koistinen, and S. Meri.** 1999. The factor H protein family. Immunopharmacology **42:**53-60.
333. **Zipfel, P. F., and C. Skerka.** 1994. Complement factor H and related proteins: an expanding family of complement-regulatory proteins? Immunol Today **15:**121-126.
334. **Zipfel, P. F., and C. Skerka.** 1999. FHL-1/reconectin: a human complement and immune regulator with cell- adhesive function. Immunol Today **20:**135-140.
335. **Zipfel, P. F., C. Skerka, J. Hellwage, S. T. Jokiranta, S. Meri, V. Brade, P. Kraiczy, M. Noris, and G. Remuzzi.** 2002. Factor H family proteins: on complement, microbes and human diseases. Biochem Soc Trans **30:**971-978.