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Review for Molecular Immunology

Human Complement Control and Complement Evasion by Pathogenic Microbes - Tipping the Balance

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Abstract

Complement is a central homeostatic system of mammals and represents the first defense line of innate immunity. The human complement system is aimed to maintain homeostasis by recognizing and removing damaged or modified self material, as well as infectious foreign microbes. However, pathogenic microbes also control and escape the host complement and immune attack. The increasing resistance of microbial pathogens to either antibiotics or antifungal drugs is a major health problem and is of global interest. Therefore the topic how pathogenic microbes escape human complement and immune control is of high and of central interest. Identifying and defining the action of proteins involved in this intense immune interaction and understanding how these molecules interact is of relevance to design new control strategies. In this review we summarize the complement system of the human host and how this cascade drives effector functions. In addition, we summarize how diverse pathogenic microbes control, modulate and block the complement response of their host. The characterization of pathogen derived virulence factors and complement escape proteins reveals patterns of multiplicity, diversity and redundancy among pathogen encoded proteins. Sequence variability of immune and also complement escape proteins is largely driven by antigenic diversity and adaptive immunity. However common complement escape principles are, emerging in terms of conserved binding repertoire for host regulators and evasion among the large variety of infectious microbes. These conserved and common escape features are relevant and provide challenging options for new therapeutic approaches.

1. Human Complement: Homeotic Immune Protection and Immune Defense

The complement system serves as the first line of human immune defense and this central homeotic system of a vertebrate organism acts all the time, continuously and by default and at all sites of the body.

Complement was initially identified by Paul Ehrlich 1890 and Jules Bordet 1898 as a compartment of blood, which enhances opsonophagocytosis, lyses target cells and assists, -what is today known- as antibody dependent effector functions (Schmalstieg and Goldman, 2010). In modern words, the two authors already described innate and adaptive immune reactions and the cooperation between these two central immune defense systems. Today it is well established, and it is becoming more and more evident that complement is the central homeotic system of all vertebrates. Complement controls cellular homeostasis, maintains tissue integrity and in addition interacts with many other cascade effector systems of the vertebrate organism, including the coagulation cascade (Amara et al., 2008; Oikonomopoulou et al., 2012; Zipfel, 2009). Thus, complement efficiently recognizes, targets and eliminates unwanted self material and immune complexes, but also foreign, infectious microbes (Zipfel and Skerka, 2009). Unwanted self material includes the billions of cells that die each day in the vertebrate organism, e.g., by necrosis or apoptosis.

In its standard default setting, complement recognition and elimination is highly efficient and the cascade acts in a tightly regulated and non-inflammatory manner. However, if these processes and reactions are not properly acting or not properly regulated, cellular self debris or cellular material persists longer as needed. This material generates danger, which results in inflammation and ultimately also in the

production of autoantibodies towards self structures. Complement serves several additional functions, the system links innate and adaptive immunity and is tightly connected with other cascade systems (Amara et al., 2008; Dunkelberger and Song, 2010). Several of the newly generated effector fragments of the activated cascade have anaphylactic as well as chemotactic activity, can act as growth and differentiation factors and, finally, as immunomodulatory agents.

The complement system consist of about 30 to 50 germline encoded genes, and the corresponding proteins either circulate in plasma and body fluids or are present on the surface of endogenous cells, biological and tissue membranes. Broadly speaking, complement components are grouped in inactive precursors, activation fragments that serve effector function, as well as regulators and inhibitors. The regulators ensure that the cascade is activated properly in a controlled manner, and that effector actions occur at the right time and at the right site (Zipfel and Skerka, 2009). Regulators also efficiently block activation and cascade progression on self surfaces.

Based on the cascade type manner, normally inactive precursor proteins are proteolytically cleaved, conformational as well as structural changes are induced and the modified proteins are activated. The activated proteins then expose new binding and interaction sites. Newly generated cleavage products serve important biological functions and trigger cascade amplification and cascade progression. Complement activation triggers a powerful amplification loop allowing formation of more effector

compounds and peptides that enhance complement effector functions. For example, this amplification loop can deposit 10^6 to 10^8 C3b molecules onto an activating surface within 5 to 15 min (Pangburn et al., 1983).

During the last century complement activation was mainly considered a self amplifying cascade that generates the major anaphylactic peptides C3a and C5a and that cascade activation leads to formation of the terminal complement complex (TCC), which generates C5b-9 (also termed MAC, membrane attack complex). C5b-9 insert in the membrane of a target cell, cause membrane damage and forms a lytic pore. Additional functions have been proposed for soluble TCC, such as a cytokine like activity (Kilgore et al., 1996; Tedesco et al., 1997). In the present century it is becoming more and more clear that complement has many additional functions, and links central cascade systems of a vertebrate organism.

1.1 Complement Activation and Effector Functions

Complement acts on four central levels which represent one activation- and three separate effector levels (Figure 1). Each effector level acts as a more or less independent circuit, displays unique biological functions and is separately regulated.

The four levels are:

1.1.1 Activation level. Complement is activated via three major and separate pathways. The ***alternative pathway*** is spontaneously activated by default and is kept under control by efficient regulators (Thurman and Holers, 2006; Zipfel and Skerka,

2009). The **classical pathway** is induced by immunoglobulins bound to the corresponding antigen, but also by endogenous self molecules including CRP (C reactive protein), Serum Amyloid P and Pentraxin 3, and the (iii) **lectin pathway** is induced by specific carbohydrate structures that are present on the surface of foreign cells and bacteria.

1.1.2 Effector Level I - C3 convertase circuit. This first complement effector level forms an active enzyme, the C3 convertase. The alternative pathway and the classical/lectin pathway form separate C3 convertases. Once assembled, the C3 convertase of either the alternative (C3bBb) or the classical pathway (C4bC2b) cleave C3, the central complement component. In the absence of regulators, the reaction progresses and forms a powerful amplification loop that significantly amplifies C3 convertase formation and C3a generation as well as C3b surface deposition. Newly generated C3a is an inflammatory and chemotactic mediator that aids in the recruitment and effector function of C3a receptor (C3aR) bearing cells including neutrophils and macrophages (Klos et al., 2013). C3a also possesses antimicrobial activity against Gram-negative and Gram-positive bacteria as well as fungi (Malmsten and Schmidtchen, 2007; Reuter and Zipfel, 2009). Activated C3b deposits onto any surface, including surfaces of self cells, damaged self cells but also on foreign surfaces. Next, the type of surface and in particular the attached and surface expressed regulators define the fate of C3b. Amplification of the reaction results in a high density of surface deposited C3b that is central for opsonophagocytosis. On the surface of intact self cells, regulators determine the fate of surface deposited C3b and block amplification and cascade progression (Zipfel and Skerka, 2009). The coordinated action of these inhibitors cause ordered

processing and inactivation of C3b and the generation of new C3 isoforms, such as iC3b, C3dg and C3d. The next steps and effector functions are influenced by the density of surface deposited C3b, as well as the type of deposited and processed C3 activation fragment. The number and type of C3 molecules on the surface in combination with self structures like heparin and glycoaminoglycans, determine further interaction and fate of a C3 decorated cell or particle. For this, a series of human cells equipped with specific receptors for the C3 activation fragments exist. The receptors that recognize C3b or C3 variants include complement receptor (CR) 1, CR2, CR3, CR4 and CR of the Ig superfamily (CRIg) which are expressed by various cell types, including monocytes, macrophages, neutrophils, endothelial cells as well as T and B lymphocytes (Zipfel and Skerka, 2009).

1.1.3 Effector Level II - C5 convertases. The C5 convertase, which is either formed by the alternative pathway (C3bBbC3b) or the classical pathway (C4bC2bC3b) cleave C5 into C5a and C5b. The activation peptide C5a is a powerful inflammatory mediator and anaphylatoxin and acts via two receptors, i.e., C5aR and C5aL2 (Klos et al., 2013; Ward, 2009). The role of the second C5a Receptor C5aL2 is still unclear and some reports argue for a specific effector role and other discuss a function as a decoy receptor (Klos et al., 2009; Klos et al., 2013). C5a directs inflammation, cell recruitment, as well as chemotactic activity. Newly generated C5b has a short half live and exposes a metastable binding site for C6. When propagated and not inactivated, C5b induces the next effector level, the terminal pathway.

1.1.4 Effector Level III - The terminal pathway. The third effector level of complement, the terminal pathway is initiated when C5b is formed. Assembly of the biologically active component, the C5b-9 complex occurs in a non-enzymatic manner by sequential addition of the soluble components C6, C7, C8 and C9 and subsequent formation of membrane binding intermediates (Hadders et al., 2012). The terminal complex C5b-9 inserts into the target membrane, causes cell membrane damage and target cell lysis. In addition soluble TCC (sC5b-9) exists in plasma, which lacks lytic activity but may have cytokine like activities, induces cell signaling as well as proinflammatory effects (Kilgore et al., 1996; Tedesco et al., 1997). Such cellular stimulation can result in release of interleukin-1 (IL-1), prostaglandins, tumor necrosis factor α (TNF α) from mesangial cells, oxygen radical generation from leukocytes and monocytes and release of proinflammatory IL-8, as well as chemotactic MCP-1 from endothelial cells (Hallett et al., 1981; Hansch et al., 1987; Kilgore et al., 1996; Lovett et al., 1987). In addition, sC5b-9 induces P-selectin expression, increases the synthesis of inflammatory leukotrienes and prostaglandins and upregulates the production of oxygen-derived free radicals (Hansch et al., 1987; Suttorp et al., 1987; Vakeva et al., 1993). sC5b-9 acts synergistically with TNF α to increase ICAM-1 and E-selectin expression (Vakeva et al., 1993). The unambiguous identification and characterization of these additional effects of sC5b-9 will provide new ground for TCC action in the vertebrate organism.

One additional, relevant aspect on the difference of the three complement effector levels is the concentration of their major components C3, C5 as well as C6 to C9 in human plasma. C3 has a plasma concentration of 1.5 mg/ml and thus C3 levels

exceeds the plasma levels of C5 (0.075 mg/ml), C6, C7, C8 and C9 (0.045; 0.055; 0.08 and 0.06 mg/ml, respectively) by a factor 20 (Hobart et al., 2000). This reflects also the level of effector compounds that are formed in each effector circuit. Thus, C3 effector compounds can be generated at significantly higher levels, as compared to effector compounds generated in complement activation levels II and III. This further highlights the essential role of the amplification loop of the complement cascade.

Complement activation is tightly regulated in terms of time and space to ensure and guarantee that the individual, newly generated, highly toxic complement effector compounds are properly directed to their target site, i.e., sites where they are needed and wanted. The site of activation needs to be directed to the target surface, damaged and modified self cells, or foreign microbes and particles. At the same time any action on intact self surfaces, like cells, biological membranes or tissue surfaces needs to be controlled, restricted and properly modulated. This precise control is performed and ensured by a series of inhibitors and regulators which are distributed in the plasma and body fluids or which are attached to the surface of biomembranes.

2. Infectious or Pathogenic Microbes - what is the difference?

Infectious microbes are generally recognized and eliminated by complement and the immune system of their human host. However, pathogenic microbes survive in an immunocompetent human organism, as they efficiently control and block complement and the immune reactions. Thus, *'immunologically visible'* microbes are recognized, attacked and ultimately eliminated by the efficient complement and

immune defense. In contrast '*immunologically invisible*' microbes survive and propagate as pathogens. Thus, pathogenic microbes use numerous and highly sophisticated strategies to control, modulate and block the complement response of their host, and they also control innate as well as adaptive immune reactions (Blom et al., 2009; Lambris et al., 2008; Rooijackers and van Strijp, 2007; Zipfel et al., 2007).

Upon infection, a microbe aims to spread and propagate within a vertebrate host and within the human organism. Complement, as the first line of host immune defense acts in combination with the innate immune response and forms a constant and specific attack. Thus, a pathogenic infectious microbe which survives and propagates in a vertebrate host inactivates and controls each of the specific response of complement, and also the effector reactions of innate and of adaptive immunity. Over the last years a large number of pathogen encoded immune evasion proteins have been identified, their corresponding host ligands have been defined and their precise mode of action or mechanism of immune inactivation has been delineated.

One central aspect of modern infection biology is to define in molecular and mechanistic terms the difference between infectious microbes that are recognized, attacked and eliminated by the activated complement system and by innate immunity and pathogenic microbes, which persist, efficiently control and inhibit the damaging effect of the activated complement system. This topic is of further relevance as such microbial pathogens are diverse and as Gram-negative and Gram-positive bacteria, fungi, protozoa, multicellular parasites or viruses use common and similar evasion

features to control host complement attacks. Defining the common features of these emerging common complement escape principles is of interest not only to understand pathogenicity and virulence of disease causing microbes but also to exploit the conserved mechanism to interfere with the infection process. In addition, this may further identify new target structures that allow to interfere with pathogenic immune control.

After crossing mechanical barriers like the skin or entering the vertebrate host via damaged surfaces, infectious microbes, which come in direct contact with host plasma or body fluids immediately trigger the complement system. This first immunological defense line is activated immediately, amplified within seconds, generates and targets a whole battery of toxic effector compounds to the surface of the invading microbe. After surviving the complement battle, infectious pathogenic microbes aim to spread into blood vessels and deeper organs. Thus, in order to colonize a human organism they must adhere to and form intimate contact with the host cells in particular epithelial and endothelial cells and tissue surfaces, and cross tissue or cell barriers. For this steps adhesion and interaction with the extracellular matrix (ECM) and ECM components like fibronectin, laminin, vitronectin and fibrinogen are relevant.

2.1 Pathogenic infectious microbes are diverse in shape, surface composition

Pathogenic microbes are specialized to propagate in an immunocompetent host, to spread and to disseminate into unique host compartments and niches. The surface of

these diverse pathogens varies to a large extent but also includes common structural components, as well as unique proteins.

2.1.1 The surface of pathogenic microbes. Pathogenic microbes are diverse in shape, surface composition and are specialized to spread and distribute into unique host compartments and niches. The cell membranes of Gram-negative and Gram-positive bacteria differ (Figure 2). The inner membrane is shielded by either a single layer of peptidoglycans in combination with a second outer membrane into which LPS is integrated (Gram-negative bacteria) or by multiple layers of peptidoglycans into which teichoic acid is integrated (Gram-positive bacteria) (Johnson et al., 2013; Sukhithasri et al., 2013). Many bacteria use an additional protective shield, and they are thus encased by a capsule which consists of loosely attached polysaccharides (Corbett and Roberts, 2009). In addition to these structural, mechanical surfaces, such cell walls, peptidoglycan layers, and capsules, microbial pathogens have specific surface proteins that are facing to the environment and that make contact with host plasma and cell proteins. The long outward facing M protein of Gram-positive *S. pyogenes* is an example for a pleiotropic and well characterized pathogen encoded complement and immune evasion protein (Smeesters et al., 2010). The M protein has a highly polymorphic region and individual proteins bind many human complement proteins, like Factor H, Factor H like protein 1 (FHL1), complement Factor H related protein 1 (CFHR1), C4b binding protein (C4BP), plasminogen, and also components of the extra-cellular matrix (ECM) and binds to the surface of human immune cells (Haapasalo et al., 2008; Oehmcke et al., 2010; Perez-Caballero et al., 2004; Smeesters et al., 2010). Human pathogenic yeasts have a cell envelope

consisting of a plasma membrane, periplasmic space and a cell wall made of a variety of glucans, chitin and chitosan, mannans and glycoproteins (Free, 2013). Similar to bacteria, human pathogenic yeast also exploit outward facing proteins. These proteins are cross-linked into the fungal cell wall matrix and can bind and interact with human proteins. Based on the common features among the diverse group of pathogenic microbes here we define emerging general features of complement escape strategies for pathogenic microbes including different phyla, like Gram-negative, Gram-positive bacteria as well as fungal pathogens.

2.2 General Escape Principles

Pathogenic microbes control and modulate host complement reactions and evade host immune defenses (Blom et al., 2009; Lambris et al., 2008; Rooijackers and van Strijp, 2007; Zipfel et al., 2007). To this end each pathogenic microbe interferes and inhibits the damaging complement and immune effector reactions. Pathogens express numerous escape proteins that are diverse in sequence, have pleiotropic functions and control different immune reactions of their hosts.

Having described the four central circuits of the human complement system (see [1.1](#)), we here summaries how a pathogenic microbe respond and controls the complement attack of their host. To reveal common strategies, we provide central examples of how pathogenic microbes target complement effector proteins and how they modulate the complement and immune reactions of their human host. The pathogenic microbe target complement at each of the four complement circuits and thereby blocks and influences host response (Figure 3).

2.2.1 Interference at the Complement Activation level.

Pathogenic microbes influence complement activation and already interfere at the activation level. Pathogen encoded proteins bind to intact C3, and likely freeze C3 in the native state or conformation, and block activation and processing.

In addition, pathogenic microbes express and secrete endogenous proteases that cleave and degrade human C3 and other complement components. Aureolysin is a metalloprotease of *Staphylococcus aureus* that is an example of a pathogen encoded protease that cleaves C3 and inhibits complement activation (Laarman et al., 2011). Aureolysin inhibits C3b deposition, opsonophagocytosis, bacterial killing by human neutrophils and release of the chemoattractant inflammatory activation product C5a.

Pathogenic microbes also bind and acquire proteases or protease precursors from human plasma. Many pathogenic microbes bind the human pre-protease plasminogen to their surface and they use either pathogen encoded or host activators to generate the proteolytically active plasmin, like streptokinase from *S. pyogenes* or they utilize host activators like uPA to generate active plasmin (Lahteenmaki et al., 2001). Plasmin cleaves and degrades C3 thereby blocking C3 effector functions (Barthel et al., 2012; Koch et al., 2012). Many pathogenic microbes express several plasminogen binding proteins (Bhattacharya et al., 2012). The Gram-negative bacterium *Borrelia burgdorferii* expresses at least seven plasminogen binding proteins (Brissette et al., 2009; Fuchs et al., 1994; Hallstrom et al., 2010; Lagal et al., 2006), the Gram-positive bacterium *S. pyogenes* at least five (Berge and Sjobring, 1993; Pancholi and Fischetti, 1998; Ringdahl and Sjobring, 2000; Sanderson-Smith et al., 2007) and for the human pathogenic yeast *Candida albicans*

a total of eleven different fungal plasminogen binding proteins are identified (Crowe et al., 2003; Luo et al., 2013; Luo et al., 2009; Poltermann et al., 2007). Thus interference at the early level of complement activation is an efficient evasion strategy leading to a block of downstream effects. In addition, pathogenic microbes use surface proteins that bind IgG via the conserved C terminal domain and binding proteins, like the M protein of *Streptococcus pyogenes* (group A streptococci) and Protein A of *S. aureus*, thus these species block activation of the classical pathway (Carlsson et al., 2003; Schalen et al., 1985).

2.2.2 Interference at the C3 convertase Level

Proteins derived from pathogenic microbes affect the action of the C3 convertase and influence the C3 convertases in multiple manners. They either block the enzyme directly, adjust enzyme activity, or modulate the strength of the amplification loop (Laarman et al., 2010; Rooijackers et al., 2005). Thus, pathogens adjust the activity, as well as the type and the number of the effector components that are generated. In addition, microbial escape proteins influence the action of the effector molecules. The density and type of surface attached C3 activation fragments determines host response and effector functions. The pathogen evasion proteins influence two major aspects; the number and density of surface deposited C3b or of the other activation fragments thereby influencing opsonophagocytosis and microbial elimination. Similarly C3b processing, i.e., the type of the surface attached C3 activation fragment modulates processing and host effector function. The immune evasion protein Sbi of *S. aureus* forms a tripartite complex with C3b and Factor H, and allows for tripartite

complexed C3b processing and inactivation by the complement protease Factor I (Haupt et al., 2008).

Acquisition and binding of soluble human complement regulators to the surface of the microbial pathogen is a common complement evasion strategy and has been shown for many microbial pathogens. The major human proteins that are bound by Gram-negative and Gram-positive bacteria as well as human pathogenic fungi and *Plasmodium falciparum* include Factor H and FHL-1, human regulators of the alternative pathway, as well as C4BP a regulator of the classical pathway (Blom et al., 2009; Lambris et al., 2008; Simon et al., 2013; Zipfel et al., 2007). The human regulators when attached to the microbial surface inhibit and control the action of either the alternative or the classical C3 convertase, the amplification loop, and they further influence C3 processing and inactivation (Zipfel et al., 2011; Zipfel et al., 2007).

Several pathogenic microbes express and secrete proteins that target and bind to the complement effector receptors CR1, CR2, CR3, CR4 or CR1g. These pathogen encoded proteins, when bound to the human effector receptors modulate cytoplasmic signaling and in consequence receptor mediated effector functions. One example is Pra1 from *C. albicans*, which binds CR3 and thereby inhibits uptake of *S. pneumoniae* by lung epithelial cells (Agarwal et al., 2010). Another example is Efb of *S. aureus* as a pathogen encoded complement effector function controlling protein. Efb binds to C3d and influences the effector function mediated by CR2-C3d interaction (Ricklin et al., 2008).

2.2.3 Interference at the C5 convertase Level

Pathogens use proteases that cleave and degrade host C5. Similar to the control at the C3 convertase level, such proteases are either encoded in the genome of the pathogen or they represent host regulators and plasma proteins. The staphylococcal SSL7 (staphylococcal superantigen-like protein 7) binds C5 and thereby blocks cleavage by the C5 convertase (Langley et al., 2005; Laursen et al., 2010). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) from *S. pyogenes* binds to C5a and the inhibitory action is assisted by ScpA, the streptococcal C5a peptidase which degrades C5a (Terao et al., 2006).

Pathogenic microbes also acquire C5 convertase inhibitors, like human CFHR1, which blocks C5 convertase activity but also TCC assembly (Heinen et al., 2009) CFHR1 binding has been reported for *B. burgdorferi*, *Leptospira interrogans*, *S. pyogenes*, *P. aeruginosa* and for *C. albicans* and *A. fumigatus* (Behnsen et al., 2008; Hallstrom et al., 2012; Luo et al., 2013; Reuter et al., 2010; Verma et al., 2006).

2.2.4 Interference at the C5b-9 Level

Pathogens also control and influence the action of the terminal pathway. For a long time it was considered that the terminal pathway of complement is relevant for elimination and killing of Gram-negative bacteria, and that Gram-positive bacteria and fungi with their thick cell wall are protected from the lytic action of the terminal pathway. In these days evidence is increasing that more pathogens control and exploit the terminal pathway of complement. Two terminal pathway inhibitors were identified from the Gram-negative spirochete, *B. burgdorferi*, a CD59-like protein

(Pausa et al., 2003) and CspA/CRASP-1, a C7 binding and TCC formation inhibiting /controlling protein (Hallström et al, *submitted*). The Gram-positive bacterium *S. pyogenes* expresses SIC (Streptococcal inhibitor of complement) which also binds C7, *Entamoeba histolytica* expresses a Galactose-specific adhesin and the multicellular parasites *Schistosoma mansoni* and *Trichinella spiralis*, use Paramyosin for TCC control (Braga et al., 1992; Deng et al., 2003; Fernie-King et al., 2001; Zhang et al., 2011). *S. mansoni* uses SCIP-1 (shistosoma C inhibitory protein type 1) as a second TCC inhibiting protein (Parizade et al., 1994).

A large number of pathogenic microbes, again Gram-negative and Gram-positive bacteria, as well as human pathogenic yeast bind vitronectin, the soluble human TCC inhibitor. Vitronectin is a human plasma protein that binds to and blocks the membrane binding site of C5b-7 and the polymerization of C9 and thereby blocks TCC action (Podack et al., 1984; Preissner et al., 1985; Singh et al., 2010). However, vitronectin controls additional biological processes, including adhesion, cell migration, angiogenesis as well as plasminogen activation (Singh et al., 2010). Pathogens bind human vitronectin, e.g. the Gram-negative bacteria *Haemophilus influenzae*, *Moraxella catarrhalis* and *P. aeruginosa*, the Gram-positive bacteria *S aureus*, *S. pneumoniae* and *S. pyogenes* and similarly the human pathogenic yeast *C. albicans* (Singh et al., 2010). A specific pathogen generally uses several vitronectin-binding proteins as exemplified with non-typable *H. influenzae* that attracts vitronectin with both protein E and F, albeit at different affinities (Hallstrom et al., 2009; Singh et al., 2011; Su et al., 2013).

This overview shows that many pathogenic microbes interfere with complement and apparently they use a series of complement escape proteins to influence complement action. Apparently the repertoire of pathogenic complement evasion proteins is large and a single pathogen can influence complement at many levels and control several steps of complement action and function, likely also of each individual activation fragment.

3 Lessons learned- Common features Emerging

At present a large number of complement evasion proteins have been identified and characterized from the diverse group of pathogenic microbes including Gram-negative and Gram-positive bacteria, human pathogenic fungi, multicellular parasites and also from viruses. Although microbial complement and immune evasion is a rather complex topic several common and conserved evasion strategies are emerging. These features include diversity of complement and immune evasion proteins, multiplicity and redundancy.

Diversity: The individual escape proteins are diverse in sequence and eventually in structure. This antigenic variability is to some extent driven by the antigen specific response by the adaptive immunity. However, despite sequence variations in the hypervariability region, an M protein variant of *S. pyogenes* maintains conserved binding repertoire, e.g., for the human complement escape proteins Factor H and C4BP (Persson et al., 2006).

Multiplicity: A single pathogenic escape protein can bind several human complement proteins at the same time. The M protein of Gram-positive *S. pyogenes*

binds Factor H, FHL1, CFHR1, C4BP, plasminogen and fibrinogen (Margarit et al., 2009; Oliver et al., 2008). Also Pra1 from *C. albicans* binds Factor H, FHL1, C4BP, fibrinogen and also zinc (Citiulo et al., 2012; Zipfel et al., 2011). As a consequence they can control multiple steps of the complement cascade and immune reactions.

Redundancy. Most -likely all- pathogenic microbes express at the same time multiple immune evasion proteins to control host complement action. Examples include the Gram-negative bacterium *B. burgdorferi* which has five Factor H binding proteins i.e. CRASP-1/CspA; CRASP-2/CspZ; CRASP-3/ErpP; CRASP-4/ErpC and CRASP-5/ErpA) (Kraiczy and Stevenson, 2013). Each of the five CRASP proteins also binds plasminogen and borrelia has at least four additional plasminogen binding proteins, including enolase, OspA, OspC and FhbA (Brissette et al., 2009; Floden et al., 2011; Fuchs et al., 1994; Hallstrom et al., 2010; Hovis et al., 2008; Lagal et al., 2006). The Gram-positive bacterium *S. pyogenes* has three known Factor H- and FHL-1 binding proteins (M1 protein, Fba and Scl1.6), two C4BP binding proteins (M protein and Fib) and a total of 4 plasminogen binding proteins (M protein, α -enolase, GAPDH and Prp) (Margarit et al., 2009; Oliver et al., 2008; Pandiripally et al., 2002; Reuter et al., 2010; Sanderson-Smith et al., 2007; Walker et al., 2005). Similarly the pathogenic yeast *C. albicans* has four Factor H binding proteins, (Gpm1, Pra1, Hgt1p and Gpd2), two C4BP binding proteins (Pra1 and Hgt1p) and a total of eleven plasminogen binding proteins (Gpm1, enolase, Tsa1, Cta1, Tdh3, Tef1, Pgl1, Adh1, Fba1, Pra1 and Gpd2 (Crowe et al., 2003; Jong et al., 2003; Lesiak-Markowicz et al., 2011; Luo et al., 2013; Luo et al., 2009; Poltermann et al., 2007). Thus, a single pathogenic microbe expresses several proteins on its surface to acquire central host

complement and immune regulators. In summary pathogenic microbes are well equipped to control multiple steps of the host immune response.

This outline provides insights in the complexity of complement and immune evasion mechanisms used by pathogenic microbes. At the same time these conserved patterns are emerging which indicate that pathogens during evolution has separately evolved related evasion strategies and mechanisms which ultimately have the same outcome; control of host complement attack and immune reactions. The increasing resistance of microbial pathogens to either antibiotics or antifungal drugs is a major health problem and is of global interest. Thus the identification and characterization of new targets is needed to develop novel control strategies. Complement evasion proteins of pathogenic microbes represent such interesting novel targets for drug and vaccine development. For example the Factor H binding protein of *Neisseria meningitidis* which has been identified by reverse vaccinology is included in new vaccine cocktails (Malito et al., 2013).

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Figure Legends

Figure 1: Complement is a central homeostatic and immune defense system of the human organism and related vertebrates. Complement consists of four major circuits: one activation step and three connected effector levels. **Activation Level:** Complement has three major activation pathways, the alternative pathway (AP), the classical pathway (CP) and the Lectin Pathway (LP). Activation of each pathway results in the assembly and generation of an active enzyme termed C3 convertase. **Effector Level I: C3 convertase.** Formation of the C3 convertase allows cleavage of C3 into the anaphylatoxin and antimicrobial peptide C3a as well as formation of C3b. C3b deposits onto surfaces and allows opsonization and subsequent opsonophagocytosis. The activation products generated by the C3 convertase also influence the activity of immune effector cells, including macrophages and neutrophils. **Effector Level II: C5 convertase.** Formation of the C5 convertase allows cleavage of C5 into the inflammatory and cell recruiting C5a peptide and into the metastable C5b. C5a is a potent inflammatory mediator that recruits and drives effector functions of a large number of immune cells. **Effector Level III: Terminal pathway.** C5b complexes the additional soluble complement proteins C6, C7, C8 and C9 and forms the terminal complement complex C5b-9. C5b-9 inserts into the membrane of the target cell, damages the membrane and forms a lytic pore. In addition a soluble form of sC5-9 exists for which additional cell activation roles have been proposed. Of note, the plasma concentration of C3 is 1.2 mg/ml and thus about 20 fold higher than the levels of C5 or C6-C9. Thus a fully active amplification loop in the C3 convertase circuit can generate 20 fold higher levels of C3a and C3b effector fragments.

Figure 2: Pathogenic Microbes have different surfaces.

Gram-negative and Gram-positive bacteria and fungal pathogens have a different surface composition. Bacteria have an inner membrane which is shielded by either a single layer of peptidoglycan in combination with a second outer membrane into which LPS is integrated (Gram-negative bacteria) or this inner membrane is overlaid by multiple layers of peptidoglycans into which teichoic acids are integrated (Gram-positive bacteria). Human pathogenic yeasts have a cell envelope consisting of a plasma membrane, periplasmic space and a cell wall made of a variety of glucans, chitin and chitosan, mannans and glycoproteins. Many bacteria and some fungi are encased by an additional protective shield, the capsule which consists of loosely attached polysaccharides. In addition microbes have unique proteins integrated into the membranes, peptidoglycan layers and cell wall matrixes that face outward and are exposed to the environment and that contact host plasma and cell components.

Figure 3: Pathogenic microbes control human complement effector functions

Complement Evasion proteins of pathogenic microbes control interfere cascade progression and amplification and interfere at each level. Complement activation is controlled already at the activation level, e.g. by proteases, C3 acquiring proteins, as well as host bound pre-proteases like plasminogen. Attached plasminogen is activated and converted to the protease plasmin either by pathogen encoded activators and proteases or by host acquired activators.

Complement evasion proteins bind to C3b, interfere with C3a function and block C3b CR3, CR4 interaction as well as C3d CR2 interaction and receptor binding. In

addition many pathogenic microbes bind host plasma proteins and complement regulators. like Factor H, FHL1 and C4BP, which influence C3 convertase activity and favor dissociation of the assembled cascade. Microbial proteins interfere with C5 circuit by degrading C5a, binding and blocking C5b. Similarly microbial pathogens acquire CFHR1 a human C5 convertase inhibitor. Microbial proteins interfere with terminal pathway by binding to components like C7 and by acquiring the human TCC inhibitors clusterin and CFHR1.

G⁻ - Gram-negative bacteria, G⁺ - Gram-positive bacteria, F - fungi.