Catch Me if You Can: *Streptococcus pyogenes* Complement Evasion Strategies

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Keywords

*Streptococcus pyogenes* · Complement · M protein · Immune evasion · Innate immunity · Pathogenicity

Abstract

The human host has evolved elaborate protection mechanisms to prevent infection from the billions of microorganisms to which it is exposed and is home. One of these systems, complement, is an evolutionary ancient arm of innate immunity essential for combatting bacterial infection. Complement permits the efficient labelling of bacteria with opsonins, supports phagocytosis, and facilitates phagocyte recruitment to the site of infection through the production of chemoattractants. However, it is by no means perfect, and certain organisms engage in an evolutionary arms race with the host where complement has become a major target to promote immune evasion. *Streptococcus pyogenes* is a major human pathogen that causes significant morbidity and mortality globally. *S. pyogenes* primarily colonises the epithelial surface of the nasopharynx and skin and, in most cases, results in asymptomatic carriage. Under certain conditions and observed more frequently in specific serotypes, *S. pyogenes* can cause a suite of diseases, from superficial to life-threatening infections, as well as post-infection immune-related diseases [2]. Worryingly, *S. pyogenes* invasive infection has a high mortality ranging between 10 and 30%, which results in approximately 600,000 deaths globally, mostly occurring in resource-limited regions [1, 2]. *S. pyogenes* isolates are commonly typed according to the variable 5′ region of the *emm* gene coding for the M protein, a major virulence determinant [3]. Epidemiological studies have illustrated that GAS M type is intimately correlated with both clinical disease presentations and geographical location where M type diversity is significantly reduced among invasive isolates, in comparison to those causing superficial, self-limiting diseases [4].

*Streptococcus pyogenes* is an excellently equipped pathogen harbouring a multitude of virulence determinants and genetic regulators that facilitate its associated diverse infection profile [2]. *S. pyogenes* pathogenesis occurs by step-
wise progression and can be divided into 2 distinct stages: (1) the initial attachment facilitating colonisation that requires multiple surface proteins, including the M protein family and pili, which interact with a myriad of host extracellular proteins and cell receptors; (2) resistance to host immunity, whereby *S. pyogenes* employs mechanisms to evade or inhibit complement and/or antibody-mediated opsonisation, phagocytosis, neutrophil killing, and destruction by antimicrobial peptides (Fig. 1, 2; online suppl. Table 1; for all online suppl. material, see www.karger.com/doi/10.1159/000492944). Importantly, not all virulence factors are conserved in all *S. pyogenes* serotypes, and intricate regulatory bodies coordinate virulence factor expression under specific conditions [2]. However, a large degree of functional redundancy exists in the GAS virulome, with many virulence determinants cooperating to mediate a specific task, such as evading complement which will be the focus of this review.

Complement consists of > 35 soluble and immobilised proteins, receptors, and regulators, playing central roles in immune surveillance, clearance of toxic materials, activation of the adaptive immune response, and defence against infection [5]. Bacterial activation of complement proceeds in a well-defined, tightly regulated cascade of enzymatic reactions originating from 3 different pathways: the classical pathway (CP), the lectin pathway (LP), and the alternative pathway (AP) [5]. Activation of the CP begins with the recognition and binding of microbial surfaces by the immunoglobulins, IgM and/or IgG, followed by recognition and binding by C1q via Ig Fc regions [6]. Additionally, members of the pentaxin family, which interact with specific structures on bacterial surfaces, also...
provide binding sites for C1q and activate the CP [6]. The LP is activated through recognition of carbohydrate ligands displayed on the microbial surface via mannose-binding lectins (MBLs), ficolins (1–3), and collectin-11 [5]. Both C1q and LP recognition molecules form complexes with a set of distinct serine proteases, C1r/s, and MBL-associated serine proteases (MASPs), respectively. Binding of CP and LP recognition molecules to their respective ligands results in the activation of serine proteases which cleave C4 and C2 to generate the C3 convertase enzyme, C4bC2a [7]. In contrast to the CP and LP, the AP represents a constitutively active system. Spontaneous hydrolysis of a thioester bond within C3 generates a conformationally altered C3 molecule called C3(H2O), capable of interaction with factor B (CFB). Binding of CFB to C3(H2O) alters the CFB structure, resulting in the cleavage of CFB by the serine protease factor D (CFD) into the products Ba and Bb. Bb interacts with C3(H2O) which can cleave C3 into cleavage products C3a and C3b in a similar fashion to the classical C3 convertase. Importantly, deposited C3b generated from any of the 3 pathways can interact with CFB and be subsequently processed by CFD, resulting in the formation of the AP C3 convertase, C3bBb, which rapidly converts more C3 molecules into C3b and C3a, serving as an amplification loop for the whole complement system [6]. Labeling of bacteria with C3b and the release of anaphylatoxin, C3a, are required for efficient phagocytosis and bacterial eradication. The alternative C3 convertase in amplifying C3b opsonisation also leads to the generation of the C5 convertases, which cleave soluble C5 into C5a, a potent leukocyte chemoattractant, and C5b, an integral component of the membrane attack complex (MAC) [5]. Following interaction with the complement components C6–9, the MAC may then insert into the cell membrane of susceptible bacteria, resulting in cell lysis and death [5].

Host cells are protected from complement by a set of membrane-bound or soluble inhibitors [6, 8]. Membrane-bound complement regulators include decay-accelerating factor (DAF/CD55), membrane cofactor protein (MCP/CD46), and complement C3b/C4b receptor 1 (CR1/CD35) [7, 8]. DAF disrupts both the classical/lectin and alternative C3 convertases by promoting subunit dissociation of generated C3 convertases and inhibiting the formation of new convertases. MCP serves as a cofactor for C3b and C4b cleavage by the plasma serine protease, factor I (CFI), C4b-binding protein (C4BP) and factor H (FH) are soluble, negative regulates targeting the classi-
A total of 200 different groups have been identified [11]. These proteins are commonly used to classify GAS strains; to date, responsible for this complement evasion strategy. M proteins (e.g., protein H, H also presents a coiled-coil dimer structure with a heptad repeat pattern [21]. It can bind to a variety of human serum proteins, such as albumin, the complement inhibitors FH and C4BP, and IgG, via their Fc portion [12, 14, 16]. Complement activators, utilising both surface-bound and secreted complement evasion factors.

Surface-Bound Virulence Factors

*S. pyogenes* expresses a broad variety of surface-bound virulence factors (online suppl. Table 1; Fig. 1), allowing it to efficiently escape immune recognition and prevent phagocytic uptake. One of the most prominent and well-studied virulence factors of *S. pyogenes* are the M proteins and M-related proteins.

**M Protein Family**

Several bacteria, including *S. pyogenes*, have evolved methods to recruit C4BP and FH, the 2 major soluble complement regulatory proteins, to their surface. Once bound, both C4BP and FH serve as cofactors to the serum protease (CFI) which inactivates deposited C4b and C3b, respectively. Additionally, C4BP accelerates the decay of the AP convertase, whereas FH is responsible for the decay of the AP convertase. As a result, *S. pyogenes* covered with complement inhibitors has less C3b deposited on its surface than strains that cannot bind these inhibitors [10].

The members of the M protein family, i.e., M protein itself, M-like, and M-related proteins (e.g., protein H, Enn, Arp, or Sir), are amongst the surface proteins responsible for this complement evasion strategy. M proteins are commonly used to classify GAS strains; to date, >200 different groups have been identified [11]. These proteins possess direct antiphagocytic properties and play a major role in mediating the adherence and invasion of host cells (Fig. 1 (1)) [12, 13]. Furthermore, and probably as important, these virulence factors can bind a broad variety of host proteins, namely C4BP and FH, fibrinogen, fibrin, plasmin(-ogen), neural-cell adhesion molecule (NCAM), and different immunoglobulins [14–17].

However, preventing complement activation is not only achieved through bound complement inhibitors. It has been shown that the recruitment of fibrinogen and its cleavage products also reduce complement activation. The mechanism here is still not entirely clear, but it is proposed that the binding of fibrinogen may prevent phagocytes from recognising deposited C3b due to sterical hindrance [16]. Additionally, fibrinogen inhibits the formation of the classical/lectin C3 convertase [18]. Thus, preventing complement activation via the CP seems to be of importance to *S. pyogenes*. This is achieved by binding C4BP or fibrinogen, which both inhibit the CP. Interestingly, M proteins binding C4BP usually do not bind fibrinogen and vice versa, suggesting that the 2 proteins amend each other in the protection of *S. pyogenes*.

Another way to counteract opsonisation is by the recruitment of the host serum protease plasmin, to directly inactivate deposited C3b. *S. pyogenes* binds and recruits plasminogen directly at the cell surface, which subsequently becomes activated by the host activators urokinase and tissue plasminogen activator. Interestingly, it has been shown that the recruitment of fibrinogen and its cleavage products also reduce complement activation.

The M1T1 subclone of M1 serotype is a highly virulent, globally disseminated strain, correlated with severe invasive GAS diseases such as necrotising fasciitis and streptococcal toxic shock-like syndrome (STTS). One of the key features of this strain is the co-expression of M protein and another member of the M-protein family, protein H. More than 30% of all M1 strains are supposed to express protein H. The gene for protein H is encoded adjacent to the M protein and, presumably, has evolved by gene duplication [20]. Similar to M proteins, protein H also presents a coiled-coil dimer structure with a heptad repeat pattern [21]. It can bind to a variety of human serum proteins, such as albumin, the complement inhibitors FH and C4BP, and IgG, via their Fc portion [12, 14, 16].
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Interestingly, certain ligands such as IgG or albumin as well as temperature can influence the structure of protein H and the M proteins [21, 23]. For example, the binding of serum proteins is dependent on the dimeric state of protein H. Recently, we showed that C4BP binding to S. pyogenes is enhanced by human IgG [24]. Monomeric protein H does not bind C4BP and other ligands, but as soon as the protein forms a homodimer, ligands can bind. The formation of homodimers is supported by temperatures < 37 °C. Although IgG cannot induce dimerization, at high temperatures, it can stabilize already-formed dimers and prevent monomerisation, even at temperatures as high as 41 °C. The increased C4BP binding results directly in decreased opsonophagocytosis and a dramatic increase in mortality in experimental streptococcal infections in mice.

FH-Binding Proteins: FbaA and Scl1

In addition to the M protein family, other cell-surface-expressed proteins participate in complement evasion. The fibronectin-binding protein FbaA (Fig. 1 (2)) and streptococcal collagen-like protein 1 (Scl1) (Fig. 1 (3)) also mediate the recruitment of FH [25, 26]. The 40.5-kDa protein FbaA was initially identified as a fibronectin-binding protein and was shown to be important in adhesion and pathogenesis. An fbaA mutant showed significantly less lethality in mice, most likely due to the lack of fibrinogen binding causing reduced adherence and increased complement activation [25].

Scl1, a 44.5-kDa protein, binds FH, FH-related protein 1 (CFHR1), a2β1 integrins, low-density lipoprotein (LDL), fibronectin, laminin, and the thrombin-activatable fibrinolysis inhibitor [26]. Interestingly, Scl1 binds FH via the conserved C-terminal domains 18–20, while all other FH-binding proteins recruit FH via the more N-terminal domains 5–7 [27]. The protective effect for complement evasion is mediated through FH and FHL1 or CFHR1, similar to the M protein family. As a result, there is no direct interaction between the 2 virulence factors and the complement effectors.

Sfb1/PrtF1 and the Capsule: Virulence Factors Directly Affecting Complement Deposition

Not all virulence factors require the acquisition of a host regulator to affect complement activation. For example, fibronectin-binding protein Sfb1/PrtF1 seems to directly reduce C3b deposition on S. pyogenes (Fig. 1 (4)) [28]. Sfb1/PrtF1 causes increased invasion of epithelial cells and confers resistance to phagocytosis. The mechanism of how the protein interferes with C3b deposition is not completely clear, but it has been speculated that the protein itself, or when bound to fibronectin, results in steric hindrance, preventing efficient C3b deposition.

Another example is the hyaluronic acid capsule, which also reduces C3b deposition and confers resistance to phagocytosis [29] (Fig. 1 (5)). It has been speculated that the hyaluronate capsule may serve as a physical barrier, which would prevent the phagocyte receptor from interacting with the underlying C3b deposited directly on the cell wall.

ScpA and GAPDH: Anaphylatoxin-Inactivating Evasins

A completely different but equally important target is the anaphylatoxin C5a, which recruits and activates professional phagocytes to the site of infection. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a pivotal enzyme of glycolysis but has been recently described as a complement evasion. It is classically found as a cytosolic enzyme but was recently shown to have alternative moonlighting functions. It has been observed both on the bacterial surface and in culture supernatant, where it binds and sequesters C5a, preventing neutrophil influx (Fig. 1 (6)) [30]. The classical C5a peptidase, ScpA (Fig. 1 (7)), inactivates C5a and suppresses neutrophil activation. ScpA is a member of the subtilisin-like serine protease family and is known to promote bacterial persistence and dissemination in mouse infection models. Recently, ScpA was also found to inactivate both C3 and C3a [31]. After cleavage, abnormally sized C3a and C3b molecules are formed, which cannot attract neutrophils or be deposited on the bacterial surface, respectively. Importantly, both GAPDH and ScpA work in concert for the efficient cleavage of surface-bound C5a [30].

Secreted Complement Evasins of GAS

The S. pyogenes secretome consists of a multitude of proteins which disrupt innate immune responses. For complement evasion, these factors can target both complement components and/or immunoglobulins for degradation or bind and interfere with Fc recognition by professional phagocytes. In both cases, complement and antibody-mediated phagocytosis are impaired.

Cysteine Proteinase

The cysteine proteinase, SpeB, is classically considered as the major secreted virulence factor of S. pyogenes. The gene, speB, is chromosomally encoded in virtually all se-
quenced strains, and codes for an inactive 40-kDa protein which is autocatalytically processed into a 28-kDa broad spectrum cysteine proteinase [32].

Several lines of evidence point to the importance of SpeB in GAS pathogenicity. Mutagenesis of speB results in decreased skin lesions, tissue destruction, dissemination, and lethality in murine models of infection [33]. Moreover, speB is expressed during infection, humans generate anti-SpeB antibodies, and acute-phase sera from patients suffering from GAS bacteremia have lower SpeB antibodies than patients with uncomplicated tonsilitis [2, 34]. Furthermore, a recent study analysing >9,000 GAS strains highlighted that the overwhelming majority of isolates derived from serotypes associated with invasive disease secrete SpeB [35].

At the heart of SpeB activity is the non-restrictive substrate specificity permitting the hydrolysis of proteins (Fig. 2 (A)) [32]. SpeB importance as a virulence factor is primarily based on its ability to target and inactivate many different host and bacterial proteins, thereby modulating both innate and adaptive immunity and virulence regulation. The proteolytic activity of SpeB on complement was shown in 2 studies [36, 37], both illustrating that SpeB targets the central C3 molecule and results in degradation. SpeB-treated serum diminishes CP, LP, and AP activation, and prevents activation and killing by human neutrophils by interfering with complement deposition. Complementing these observations, in vitro human neutrophil and blood survival models and mouse models of infection have shown that an speB mutant has reduced survival [36, 37]. Interestingly, the inhibition of iC3b interaction with CD11b/CD18 in human immune sera permitted GAS growth compared to Fc blocking, confirming the importance of iC3b deposition for controlling GAS infection [38].

SpeB also targets several other complement components, i.e., C1 inhibitor (C1-INH), C2, C4, C5a, C6, C7, C8, and C9 (Fig. 2 (A)) [39]. Degradation of C1-INH prevents the inhibition of serine proteases of the CP (C1s and C1r) and LP (MASPs) which may result in C4 and C2 cleavage away from the bacterial surface, analogous to the Vag8-mediated complement evasion of Bordetella pertussis [40]. Proteolytic inactivation of the above complement components will also likely impact on C3 convertase and membrane attack formation and chemotaxis, thereby disrupting innate immunity.

Additionally, SpeB targets the alternative pathway by degrading the positive regulator, properdin [41]. Properdin acts as a local initiator for the AP, by stabilising the alternative C3 convertase, and it is thus critical for AP activation and C3b amplification on the bacterial surface [5]. Disruption of properdin destabilises this complex, disrupting efficient phagocytosis and phagocyte recruitment that permit bacterial dissemination.

Classically, SpeB was thought to be an important IgG-degrading enzyme; however, recent data suggests that SpeB only cleaves immunoglobulins when they are in a reduced state, i.e. not under physiological conditions [42]. Importantly, GAS also secretes 2 dedicated IgG-degrading enzymes, discussed in the following section.

It is important to note that the broad substrate specificity of SpeB permits the degradation of a whole suite of host proteins, not restricted to the complement system, and the cumulative effect of this activity is most likely the essential feature of GAS pathogenicity.

**IdeS/Mac-1, Mac-2, and EndoS IgG Degradation and Interference**

IgG molecules are the most abundant class of antibodies in human serum and represent a critical element in the fight against infection by activating the complement system through IgG-Fc-C1q-binding and mediating efficient opsonophagocytosis via interaction with Fcγ receptors on phagocytes.

To combat this host response, *S. pyogenes* secretes 3 main immunoglobulin-degrading enzymes, known as IdeS/Mac-1, Mac-2, and EndoS. Two study groups independently identified a non-SpeB protease with efficient IgG-degrading activity and a secreted protein with homology to the α-subunit of human Mac-1 (CD11b/CD18, CR3), resulting in the IdeS/Mac-1 nomenclature [43, 44]. IdeS/Mac-1 is a 35-kDa cysteine endopeptidase with strict specificity to IgG and is present in all strains in 1 of 2 allelic variants [45]. IdeS/Mac-1 interaction with IgG results in proteolytic cleavage at a specific site present in the lower region of the heavy chain between 2 glycine residues (G236 and G237), efficiently inhibiting complement-binding and Fc recognition (Fig. 2 (B)) [44]. Hydrolysis of streptococcal IgG antibodies by IdeS/Mac-1 produces high amounts of circulating F(ab′)2 fragments, which can rebind to the bacterial surface but cannot mediate complement activation or immune cell signaling, thus exerting a protective effect [45]. IdeS/Mac-1 also contains an Arg-Gly-Asp (RGD) motif, a sequence commonly associated with integrin binding. IdeS/Mac-1 produced by serotype 1 *S. pyogenes* can inhibit neutrophil activation, phagocytosis, and the release of bactericidal reactive oxygen species (ROS) by binding the Fc receptor CD16/FcγRIIIB [43].

Mac-2 (Fig. 2 (C)), an allelic variant of IdeS/Mac-1, differs by approximately 50% of amino acids localised to
the middle one-third of the protein [46]. Mac-2 was originally believed to display weak endopeptidase activity, instead exerting its biological function primarily through binding to Fcγ receptors (FcγRI/CD32 and FcγRIII/CD16) [46]. However, this weak enzymatic activity was shown to be an exception, specific to serotype M28 (Mac-2_M28) [47]. Using the enzymatically inert Mac-2_M28, it was shown that the ability to inhibit ROS production, as a measure of phagocyte activation, was independent of the IgG endopeptidase activity [47]. However, the ROS inhibitory function of IdeS/Mac-1 and Mac-2 did not confer protection in a whole human immune blood infection model, while impairment of the IgG endopeptidase significantly reduced bacterial survival [47].

EndoS is a secreted 108-kDa endoglycosidase that cleaves the chitobase core of the asparagine-linked glycan present on the heavy chain of native human IgG (Fig. 2 (D)) [48]. Glycosylation at asparagine-297 is crucial for the interaction with Fcγ receptors displayed by phagocytic cells and IgG-mediated complement activation [49]. Pretreatment of IgG with recombinant EndoS significantly increased GAS survival in a human opsonophagocytic killing assay [50]. In spite of the evolutionary conserved nature of the ideS/mac and ndoS genes and their bifunctional nature in targeting antibodies and phagocytes, the mutagenesis of ideS or ndoS in the highly virulent M1T1 clone did not impair phagocyte resistance or pathogenicity in a systemic mouse model of infection [51, 52]. It remains to be seen whether ideS or ndoS expression in vitro correlates with that in vivo, and whether IdeS/Mac-1 or EndoS is important in promoting virulence in other S. pyogenes serotypes and infection models.

**Endopeptidase O**

Recent work by Honda-Ogawa et al. [53] identified a novel secreted complement inhibitor, endopeptidase O (PepO). PepO is highly conserved in S. pyogenes and exhibits 68% amino acid sequence homology with Streptococcus pneumoniae PepO. In similar function to the pneumococcus PepO, GAS PepO binds to the CP activator, Clq (Fig. 2 (E)). PepO binds to Clq in an electrostatic-dependent fashion and inhibits the interaction of Clq with IgG under low pH conditions consistent with inflamed tissues. Although the authors did not show inhibition of the CP by PepO through conventional complement-activation assays, deletion of pepO did decrease bacterial growth in human serum at a low pH and resulted in reduced skin lesion size in murine infection models [53]. Given the importance of PepO in regulating SpeB expression [54], future studies will be required to fully elucidate the mechanism played by PepO alone in complement evasion.

**Inhibiting MAC Deposition**

The cellular architecture of Gram-positive bacteria, namely the thick outer wall of peptidoglycan, prevents MAC-mediated lysis [55]. In spite of this, S. pyogenes expresses proteins that can interfere with MAC assembly either directly, mediated by the streptococcal inhibitor of complement (SIC) [56], or indirectly, through the expression of vitronectin-binding proteins (VnBPs) [57]. SIC is a 31-kDa protein which can bind to C5b67 and C5b678 complexes (Fig. 2 (G)), preventing MAC formation and incorporation into susceptible membranes [56]. VnBPs interact with vitronectin [57] which, in turn, inhibits MAC assembly at the C9 polymerisation stage (Fig. 1 (8)). Additionally, both SIC and VnBPs have alternative roles that contribute to S. pyogenes pathogenicity, independent of MAC interference.

It is unclear if the inhibition of MAC formation is a by-product of a more important function of the 2 proteins or a pivotal mechanism to protect the bacteria. Although the MAC can deposit on the surface of S. pyogenes in distinct locations [58], the role this has in mediating an immune response is not understood and clearly calls for further investigation to elucidate if the MAC affects bacteria physiology or somehow serves as a signalling molecule for the cells of the immune system.

**Therapeutic Options**

Better than treating an infection is preventing it. Thus, the obvious choice for therapy against S. pyogenes infection would be to vaccinate. Since 1796, when Edward Jenner performed the first vaccination, uncountable infections have been prevented and lives been saved. Unfortunately, to date, S. pyogenes has consistently resisted all efforts to develop a vaccine (reviewed in [59]). The M protein has been considered a useful target since anti-M protein antibodies confer protection against streptococcal infections. However, due to molecular mimicry of the M protein, antibodies against this virulence determinant often react with host tissues and cause post-streptococcal acute glomerulonephritis and rheumatic fever [60]. Ideally, one vaccination should protect against as many different M serotypes of S. pyogenes as possible. However, due to its hypervariability at the N-terminus of the M protein, antibodies against M proteins are extremely serotype-specific and confer protection usually only against a single type [61]. This renders an
M protein vaccine very inefficient. Nevertheless, the M protein has great potential as a vaccine candidate if these above-mentioned were overcome.

Despite emerging resistance to antibiotics and the fear of entering a “post-antibiotic era”, *S. pyogenes* is susceptible to most antibiotic classes including penicillin. However, if bacteria would develop a β-lactam resistance, for example, we would face a very serious situation with dramatic infection rates and high mortality. Thus, alternative approaches beyond conventional antibiotic therapy must be developed to cope with increasing infections.

Complement inhibitors C4BP and FH play a crucial role during *S. pyogenes* infections [10]. The presence of both inhibitors reduces the necessary inoculum to cause systemic infections in an animal model by about $2 \log_{10}$. Such an obvious influence raises the question if complement inhibitors could be a suitable target for a medical intervention.

Shaughnessy et al. [62, 63] designed a chimeric FH-IgG protein, consisting of an IgG backbone fused to certain domains of FH known to bind to microbes. FH is a monomer with only 1 binding site, while fusing the FH domains to 1 heavy chain of IgG creates a molecule with 2 binding sites. This ensures a greater avidity of FH-IgG compared to serum FH. Consequently, they demonstrated the effectiveness of this human fusion protein of experimental bacterial infections of *Neisseria* sp. The same effect of FH-IgG was also observed by a different group using *H. influenzae* [64]. Since complement can lyse and kill Gram-negative bacteria through pore formation, removing the protection conveyed by complement inhibitors will eventually cause the death of the bacteria. As discussed above, on Gram-positive bacteria, this does not occur. Using *S. pyogenes* as an example, we investigated if FH-IgG would have an antibacterial effect on Gram-positive bacteria as well. Indeed, we observed binding of FH-IgG to the bacteria and a displacement of serum FH from the bacterial surface, leading to increased MAC deposition [22]. Increased killing, however, was not observed in serum sensitivity assays, but only when co-incubated in whole blood. The decrease of the bacterial blood burden was attributed to the presence of phagocytes. To our surprise, FH-IgG had nearly no effect on Fc-mediated phagocytosis but worked exclusively through enhanced opsonophagocytosis. This approach shows that complement-based therapeutics could play an important role in infection control in this era of dramatically increasing resistance to antibiotics.

Currently, there are several complement evasion molecules that hold promise as new vaccine candidates, displaying protection in mouse models of infection against important human pathogens. Recently, the *B. pertussis* autotransporter, Vag8, was used to immunise mice against subsequent respiratory infection [65]. Vag8 binds the complement regulatory C1 esterase inhibitor (C1-INH) [66], thereby promoting the interaction and inactivation of both CP and LP serine proteases (C1r/s and MASP) to prevent complement activation. Vaccination with recombinant Vag8 resulted in a significant reduction in lung bacterial load, most likely through inhibiting this important complement surface evasin, thereby highlighting the potential use of complement evasins as vaccine targets.

**Conclusions**

Complement is one of the human body’s first lines of defence and is crucial in preventing infections. Positive selection pushed bacteria to develop countermeasures to overcome complement attack. Looking at the different virulence factors, it is evident that all steps of complement activation are targets. This ensures that the bacteria can evade complement and even prevent further activation of immunity. There is evidence that recruiting human regulators could be a winning concept, especially C4BP and FH. About 2/3 of all serum protein-recruiting virulence factors bind FH and thus interfere with complement on the level of C3, preventing opsonisation and further immune activation. The amount of deposited C3b is a measure of complement activation and is proportional to the amount of released anaphylatoxins C3a and C5a, deposited membrane attack complex (MAC), and opsonophagocytosis. For the microbe, inhibiting complement early is an efficient evasive strategy, as, not only does it prevent C3b deposition (an “eat-me” signal for professional phagocytes), it also avoids anaphylatoxin release and subsequent immune activation and phagocyte recruitment to the infection site.

Besides the main goal of developing a protective vaccination, potential therapies could concentrate on complement and FH in particular. Not only one virulence factor would be addressed, but a key player for complement evasion in *S. pyogenes* would be approached. Up to now, *S. pyogenes* fortunately shows only few signs of antibiotic resistance. We should not feel safe, but rather immediately set to developing alternatives to prevent an ominous future where *S. pyogenes* infections would no longer be treatable. *S. pyogenes* is one of the most successful human pathogens, and clearly its vast arsenal of virulence factors has enabled it to cope with all immune defences of the human body.
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Acknowledgements

This work was supported by the Royal Physiographic Society, Lund, and the Lars Hierta Memorial, Tore Nilson, and Gyllenstierna Krapperup Foundations.

Disclosure Statement

The authors declare no conflict of interest.
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