

# Molecular mechanisms of complement evasion: learning from staphylococci and meningococci

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**Abstract** | The complement system is a crucial component of the innate immune response in humans. Recent studies in *Staphylococcus aureus* and *Neisseria meningitidis* have revealed how these bacteria escape complement-mediated killing. In addition, new structural data have provided detailed insights into the molecular mechanisms of host defence mediated by the complement system and how bacterial proteins interfere with this process. This information is fundamental to our understanding of bacterial pathogenesis and may facilitate the design of better vaccines.

To survive in the human host, bacteria have developed many different strategies to escape the innate immune response, including the expression of an extracellular capsule and 'hiding' within host cells, either in a vacuole or in the cytoplasm. Over the past few years it has become clear that, in addition, bacteria can escape recognition by the complement system through the actions of cell-surface or secreted proteins<sup>1,2</sup>.

Complement is one of the most important components of the innate immune system and is composed of more than 30 proteins that are present in human serum and tissue fluid as well as on cell surfaces<sup>3,4</sup>. There are three main complement pathways, each of which can distinguish self from non-self targets. The classical pathway is initiated by antibody recognition of a microbial target and subsequent binding of complement component C1 to the antigen-antibody complex. The lectin pathway recognizes specific patterns composed of saccharides and related molecules on microbial surfaces<sup>5,6</sup>. The alternative pathway, which is probably the oldest in evolutionary terms, does not involve a specific mechanism to distinguish non-self from self, relying instead on protection of self tissues using host cell surface molecules<sup>7-9</sup> (FIG. 1).

After their initial activation by different targets, all three pathways converge on

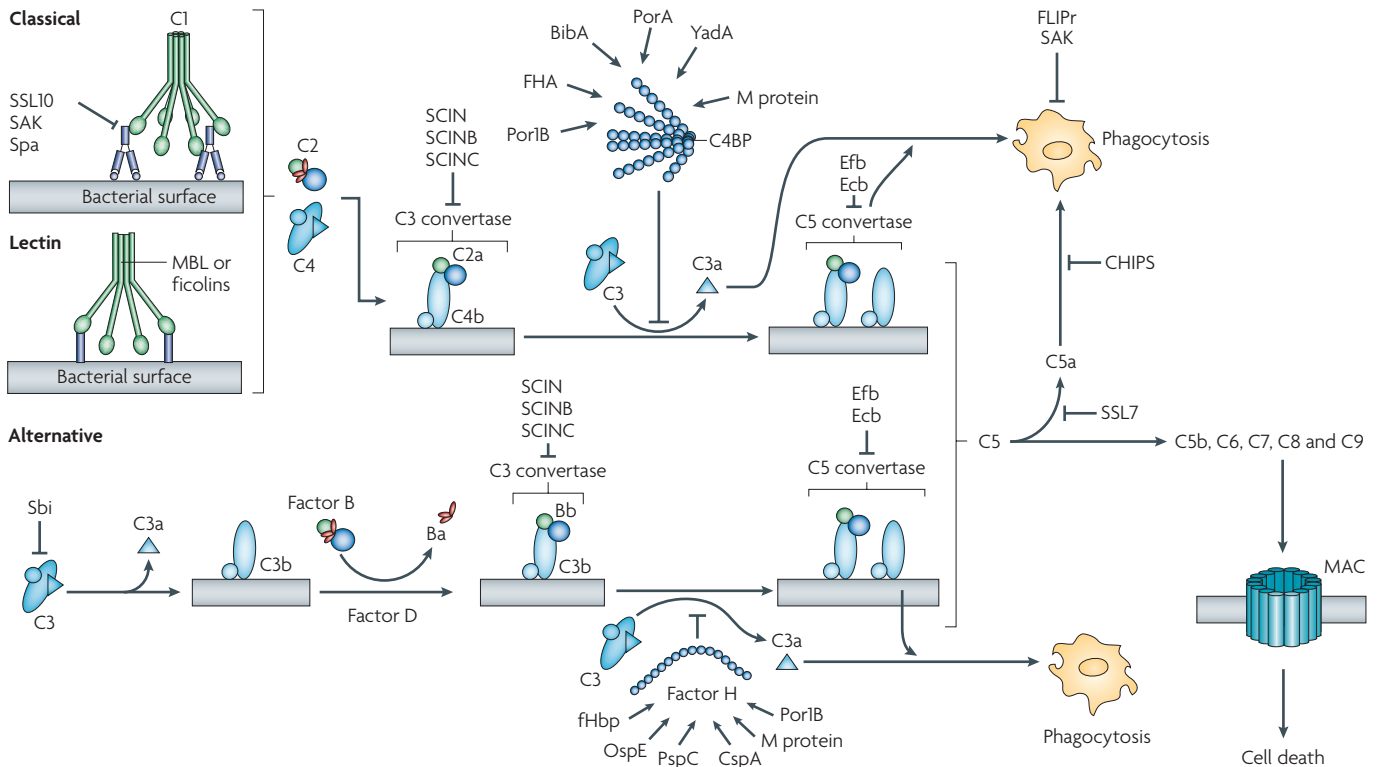
cleavage of complement component C3, which leads to the covalent attachment of fragment C3b to the target surface, and they end with the generation of the anaphylatoxin C5a and, in the case of Gram-negative bacteria, direct killing by the formation of pores in the target cell membranes (by formation of the membrane attack complex (MAC), composed of C5b, C6, C7, C8 and C9) (FIG. 1). In this context, the distinction between Gram-positive bacteria (for example, staphylococci) and Gram-negative bacteria (for example, *Neisseria meningitidis*, also known as the meningococcus) becomes evident. Gram-positive bacteria have a thick peptidoglycan layer that prevents incorporation of the MAC into the membrane, whereas Gram-negative bacteria are surrounded by two membranes, the inner and outer membranes, both of which can be attacked by the MAC; however, how MAC proteins reach the inner membrane is poorly understood. Fragment C3b tags bacteria for phagocytosis and subsequent killing by phagocytes and promotes antigen processing by antigen-presenting cells, thereby bridging the innate and adaptive immune responses. The generation of C5a is essential for attracting phagocytes to the site of infection and also for activating these cells for optimal phagocytosis. Thus, the removal of Gram-negative bacteria is mediated by phagocytes through C3b and

C5a and by direct killing by the complement system itself, whereas the removal of Gram-positive bacteria is solely dependent on C3b, C5a and subsequent phagocytic killing.

Convertase molecules are crucial for all of these steps. On the surface of the bacterial target, C3 convertases are formed — either C4b2a or C3bBb, the classical and alternative pathway convertases, respectively (C3bBb is discussed in detail below) — and these, in turn, deposit more and more C3b molecules on the surface of the target bacterium. These C3 convertases also bind additional C3b molecules to form C5 convertases. The C5 convertases cleave C5, resulting in the release of C5a, the sequential association of C5b, C6, C7, C8 and C9, and the deposition of the assembled MAC.

To avoid destruction of host tissue, complement activation is inhibited on the surface of host cells, mainly at the level of C3, which is at the heart of the complement system. To mediate this inhibition, host cells express membrane-bound complement regulators such as complement decay-accelerating factor (DAF; also known as CD55), membrane cofactor protein (MCP; also known as CD46), complement receptor type 1 (CR1; also known as CD35) and complement receptor of the immunoglobulin superfamily (CRIg; also known as VSIG4), or markers (often polyanionic structures) that attract soluble complement regulators such as factor H (which downregulates activation of the alternative pathway) and the soluble negative regulator C4b-binding protein (C4BP) (which inhibits the classical and lectin pathways). Recent structural data have elucidated the molecular mechanisms that are responsible for the activation and regulation of C3 molecules.

Bacterial pathogens have evolved diverse strategies to avoid complement activation by mimicking host surfaces, attracting host complement regulators through surface-exposed proteins, or targeting complement components with secreted proteins. The list of microbial molecules that have been reported to bind complement regulators is constantly growing (FIG. 1). Examples include: factor H-binding protein (fHbp) from *N. meningitidis*<sup>10</sup>, outer surface protein E (OspE) and CspA from *Borrelia burgdorferi*<sup>11,12</sup>, staphylococcal binder of



**Figure 1 | The complement system and selected bacterial inhibitors.** The three pathways of the human complement system and the bacterial factors that target the complement regulators factor H and C4-binding protein (C4BP). Ba, fragment Ba of factor B; Bb, fragment Bb of factor B; CHIPS, chemotaxis inhibitory protein of staphylococci; Ecb, extracellular complement-binding protein; Efb, extracellular fibrinogen-binding protein; FHA, filamentous haemagglutinin; fHbp, factor H-binding protein; FLIPr, formyl peptide receptor-like 1 inhibitory protein; MAC, membrane attack complex; MBL, mannose-binding lectin; OspE, outer surface protein E; PspC, pneumococcal surface protein C; SAK, staphylokinase; Sbi, staphylococcal binder of immunoglobulin; SCIN, staphylococcal complement inhibitor; Spa, staphylococcal protein A; SSL, staphylococcal superantigen-like; YadA, *Yersinia* adhesin A.

immunoglobulins (Sbi) from *Staphylococcus aureus*<sup>13</sup> and pneumococcal surface protein C (PspC) from *Streptococcus pneumoniae*<sup>14</sup>, which all bind factor H; BibA from group B *Streptococcus* (also known as *Streptococcus agalactiae*)<sup>15</sup>, filamentous haemagglutinin (FHA) from *Bordetella pertussis*<sup>16</sup>, PorA from *N. meningitidis*<sup>17</sup> and *Yersinia* adhesin A (YadA) from *Yersinia enterocolitica*<sup>18</sup>, which all bind C4BP; and M protein from group A *Streptococcus* (also known as *Streptococcus pyogenes*), which binds both factor H and C4BP<sup>19,20</sup>. Additionally, clumping factor A (ClfA) of *S. aureus* binds to factor I<sup>21</sup>. Once a given pathogen has recruited complement regulators to its surface, it is recognized as self and is protected from complement activation and amplification through the alternative pathway. In addition to expressing cell surface inhibitors, bacteria also use secreted molecules to inhibit complement deposition on their surface. *S. aureus*, for example, secretes several proteins that target different steps of the complement pathway. Both strategies allow bacteria to survive and successfully multiply in the host.

This Progress article takes advantage of the recent determination of the three-dimensional structures of several complement components and their complexes to discuss advances in our understanding of the molecular mechanisms used by bacteria to evade complement activation. To aid our understanding of complement evasion at a molecular level, we first discuss recent insights into the molecular details of the amplification of complement activation by the alternative pathway and into complement regulation by the so-called regulators of complement activation (RCA) family. Next, we discuss the diverse evasion mechanisms deployed by bacterial pathogens, using as examples two human bacterial pathogens, *S. aureus* and *N. meningitidis*, which avoid complement mainly by using secreted and surface-exposed proteins, respectively.

**Insights into activation and regulation**

In recent years, detailed insights into complement activation and regulation have been provided by the determination of the structures of several key complement

proteins<sup>22,23</sup> and, more recently, protein complexes involved in the alternative pathway. Moreover, structures of immune evasion molecules in complex with their target complement proteins have revealed how pathogens thwart the cascade of molecular events that normally form the complement defence system. Here, we consider the recent structural data obtained about complement amplification by C3 and factor B and about complement regulation by factor H, which downregulates this amplification.

The structure of C3, the central molecule of the complement cascade, shows that this large, 187 kDa protein is composed of 13 domains and that the reactive thioester is buried inside the core and thus protected from interaction with the environment<sup>24,25</sup> (FIG. 2). Proteolysis of C3 removes the anaphylatoxin domain (the C3a fragment) to generate C3b<sup>26</sup>. The structures of C3 and C3b indicate that there are marked conformational changes on activation of native C3 into the reactive C3b species<sup>27,28</sup>. On removal of the C3a fragment, the remaining twelve domains of C3b rearrange to relocate the

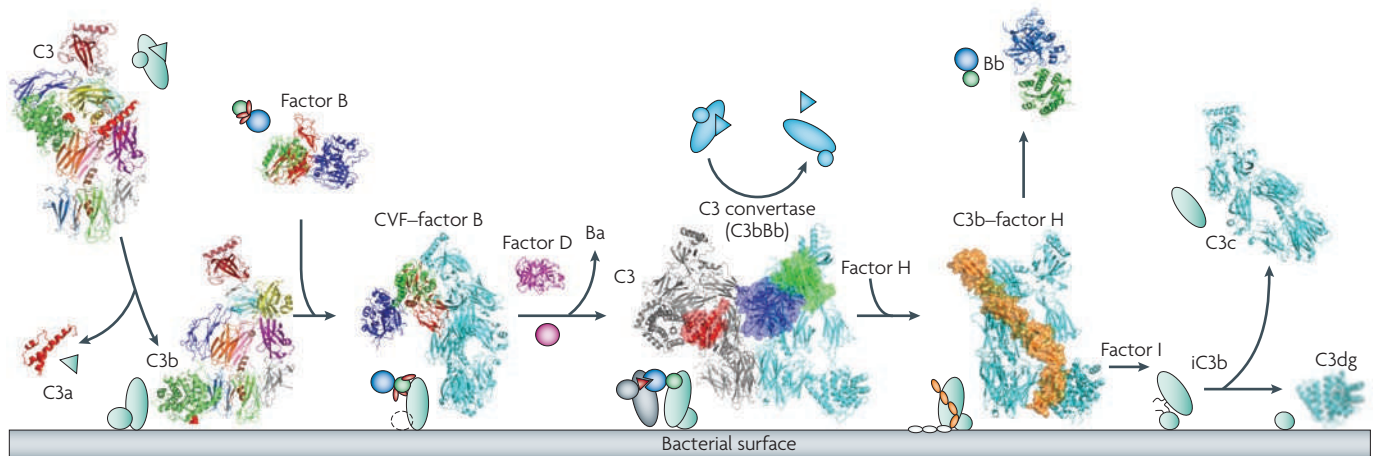


Figure 2 | **The available structural data for the alternative pathway.**

The central molecule C3 consists of 13 domains, shown by the different colours in the left-most structure. C3 is cleaved by the C3 convertase (generated either through the classical and lectin pathways or through the alternative pathway). Cleavage results in release of the small anaphylatoxin C3a and the large opsonin C3b. The activated C3b exposes its thioester and covalently binds to hydroxyls on adjacent surfaces. C3b binds pro-enzyme factor B, which consists of five domains: the Von Willebrand factor A-type domain (green), the serine protease domain (dark blue) and the domains comprising fragment Ba (red). Binding of factor B to C3b is indicated by the complex formed by factor B and the C3b homologue cobra venom factor (CVF). Next,

factor B is cleaved by factor D, yielding C3bBb, which is the active C3 convertase of the alternative pathway. In the scheme, the putative binding of C3 to C3bBb is indicated (here, C3 is in grey, with the C3a domain in red; C3b is shown in light blue); the factor B protease fragment Bb (dark blue and green) is associated with the carboxyl terminus of the C3 substrate. The C3 convertase cleaves additional C3 molecules into C3a and C3b, thereby amplifying complement activation. Host cells are protected by complement regulators such as factor H. Factor H dissociates the C3bBb complex by binding to C3b (shown is the complex of C3b and factor H domains CCP1–CCP4). Subsequent binding of protease factor I cleaves C3b into inactive C3b (iC3b) and the further degradation products C3dg and C3c.

thioester-containing domain (TED) and expose and chemically modify the reactive thioester moiety for covalent attachment to hydroxyl groups on the target surface<sup>26–28</sup> (FIG. 2). The proteolysis of C3 is achieved by short-lived protease complexes, called C3 convertases, that form on target surfaces<sup>29,30</sup>. The classical and lectin pathways involve a C3 convertase that is formed by C4b and C2 (yielding a C4b2a complex), whereas the alternative pathway involves a homologous complex that is formed by C3b and factor B. The C3 convertase of the alternative pathway yields a positive feedback loop that results in the amplification of C3b production and the deposition of many C3b molecules on target surfaces, causing opsonization<sup>24</sup>.

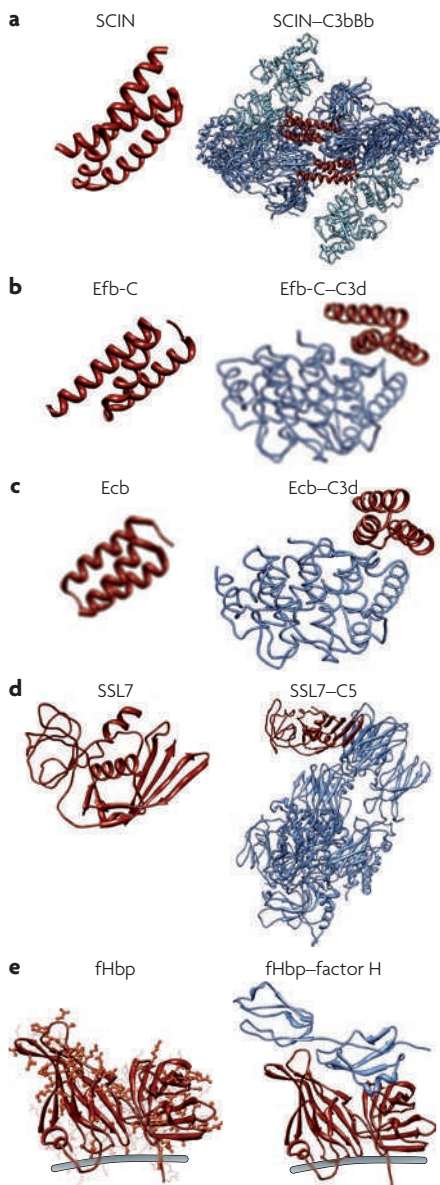
Structural data are now available for: the isolated inactive protease fragment of factor B, known as the Bb fragment<sup>31</sup> (and its C2a homologue<sup>32,33</sup>); the native pro-enzyme form of factor B<sup>34</sup>; native C3 and its proteolytic fragments C3a, C3b, C3c and C3d<sup>25,27,28,35,36</sup>; the pro-convertase complex formed by C3b and factor B (both low-resolution data from negative-stained electron microscopy<sup>37,38</sup> and high-resolution data from X-ray diffraction of the C3b homologue cobra venom factor<sup>39</sup>); the protease factor D<sup>40</sup>, which is required to proteolytically activate C3bB into C3bBb; and the convertase complex C3bBb<sup>41</sup> (FIG. 2). Briefly, the pro-enzyme factor B is first loaded onto C3b, making contact

through both the pro-peptide segment Ba and the protease segment Bb. Factor B then changes from a 'loading' state to an 'activation' state, which probably exposes the scissile loop for cleavage by factor D. Cleavage by factor D removes the Ba segment (formed by the three amino-terminal domains and a linker), which results in the formation of the active, short-lived C3 convertase consisting of C3b and the two-domain protease, fragment Bb. In this C3bBb complex, the protease fragment is bound to the carboxyl terminus of C3b through a critical Mg<sup>2+</sup>-dependent adhesion site that is formed by a Von Willebrand factor A-type domain. The serine protease domain with the catalytic centre is covalently attached to this domain and is oriented outwards.

Determination of the structure of the short-lived C3bBb complex was facilitated by the use of staphylococcal complement inhibitor (SCIN) from *S. aureus*<sup>41</sup>. This secreted protein stabilizes and inhibits the convertase<sup>42,43</sup>. The structure of the C3bBb–SCIN complex provided insights into both the activity and specificity of the convertase, as well as the inhibitory mechanism of SCIN (FIG. 3a). The observed dimeric C3bBb–C3bBb complex, which is stabilized by SCIN, suggests that the substrate specificity of the convertase is determined by C3 binding to C3b and forming a C3–C3bBb complex, with C3–C3b in a heterodimeric

arrangement. Positioning C3 in place of one of the C3b molecules brings the scissile bond of C3 in front of the catalytic site of the serine protease domain of the opposing C3bBb complex. Three inhibitors that block substrate binding to the convertase are known. The structures of these inhibitors bound to C3b (or the breakdown product C3c)<sup>28,44,45</sup> show, indeed, that they sterically block the formation of the C3–C3bBb complex. Obviously, SCIN-mediated stabilization of the C3bBb–C3bBb complex also results in the blocking of C3 substrate binding and, thus, in inhibition of the convertase. However, a chimeric variant of SCIN inhibited convertase activity but did not induce dimerization. In this case, SCIN probably obstructs the ability of the serine protease domain to bind the scissile loop of the substrate.

Structural information has also contributed to our understanding of the regulation of complement activity. Proteins of the RCA family consist of strings of complement control protein (CCP) domains and function through two possible mechanisms. Most regulators, such as MCP, DAF and CR1, are surface bound. By contrast, factor H is an abundant soluble regulator that consists of 20 CCP domains. Recognition of host cells and surfaces by factor H can be attributed to the binding of polyanions such as glycosaminoglycans by domains



**Figure 3 | Structures of bacterial proteins that bind complement components and of their complexes with complement proteins.** **a** | Staphylococcal complement inhibitor (SCIN) (Protein Data Bank (PDB) entry [2QFF](#)) and its dimeric complex with the C3 convertase of the alternative pathway, C3bBb (PDB entry [2WIN](#)). **b** | The staphylococcal extracellular fibrinogen-binding protein (Efb; also known as Fib) carboxy-terminal domain (PDB entry [2GOM](#)), and Efb-C in complex with C3d (PDB entry [2GOX](#)). **c** | The staphylococcal extracellular complement-binding protein (Ecb; also known as Ehp) and the complex with C3d (PDB entry [2NOJ](#)). **d** | Staphylococcal superantigen-like protein 7 (SSL7) and the complex with C5 (PDB entry [3KLS](#)). **e** | Factor H-binding protein (fHbp) of *Neisseria meningitidis* (PDB entry [2KC0](#)) and its complex with factor H (PDB entry [2W80](#)). Complement proteins are in blue, and bacterial proteins are in red. Variable residues of fHbp are rendered as ball-and-stick drawings.

CCP6–CCP8 and by the C-terminal domains CCP19–CCP20 (these C-terminal domains form a ternary complex with both surface glycans and the TED domain of C3b)<sup>46,47</sup>. Domains CCP1–CCP4 harbour the functional activity of factor H<sup>48</sup>. The structure of C3b in complex with CCP1–CCP4 of factor H shows that the CCP domains bind in an extended fashion to C3b<sup>49</sup> (FIG. 2). This arrangement indicates that domains CCP1–CCP2 are directly responsible for dissociating the C3bBb complex, giving rise to the so-called decay-accelerating activity and stopping C3b production. As an alternative mechanism, C3b–factor H binds the protease factor I, which cleaves the CUB domain of C3b. A putative binding site for factor I is formed by the C-terminal domain of C3b and domains CCP2–CCP3 of factor H, which lie adjacent to the CUB domain in the C3b–factor H complex. Cleavage of C3b by factor I results in iC3b, which does not support formation of the convertase complex and thereby stops the local production of C3b. These host protection molecules have been adopted by vaccinia and variola viruses, which express proteins consisting of four CCP domains on their surface, mimicking the mechanisms of host factor H. Bacterial pathogens have also evolved proteins that bind the soluble regulators, as is the case for meningococcal fHbp.

**Soluble complement inhibitors**

*S. aureus* is a major pathogen that is responsible for both nosocomial and community-acquired infections and that has developed sophisticated mechanisms to adapt, persist and cause infection in different host environments<sup>50</sup>. Numerous secreted proteins that block the cellular receptors and serum proteins involved in innate immunity have been identified and characterized in *S. aureus* and, interestingly, many of these are complement inhibitors.

Staphylococcal protein A (Spa), staphylococcal binder of immunoglobulins (Sbi) and staphylococcal superantigen-like protein 10 (SSL10) bind immunoglobulin G (IgG) that is deposited on the surface of the bacterium and interfere with activation of the classical pathway<sup>50–52</sup>. Staphylokinase (SAK) removes IgG from the surface of bacteria indirectly, by cleaving it<sup>50</sup>. These proteins also influence IgG-mediated phagocytosis at a later stage of the immune response. Other proteins act at the next level of complement activation and inhibit the C3 convertases. SCIN and its homologues SCINB and SCINC bind and inhibit both the classical pathway convertase, C4b2a, and the alternative pathway

convertase, C3bBb<sup>41,42</sup>. As discussed above, SCIN blocks, stabilizes and dimerizes C3bBb (a detail that was used to resolve the three-dimensional structure of this convertase). The formation of C3bBb dimers inhibits complement-mediated phagocytosis by blocking the receptor sites on C3b<sup>53</sup>. In addition, other proteins from *S. aureus* act on C3 and its proteolytic fragments. Sbi inhibits complement activation by binding factor H when it is in combination with another complement protein (C3, C3b or C3d) adjacent to binding immunoglobulins<sup>13</sup>. Extracellular fibrinogen-binding protein (Efb; also known as Fib) and the homologous extracellular complement-binding protein (Ecb; also known as Ehp) bind to the TED domain present in C3, C3b and C3d<sup>54,55</sup> (FIG. 3b,c). Functionally, the binding of Efb and Ecb to the TED domain blocks the alternative pathway convertase and, thus, subsequent C3b deposition. Furthermore, these proteins down-modulate the function of both the alternative pathway and the classical pathway C5 convertases (presumably by binding the TED domain of the C3b molecule in these complexes), resulting in diminished C5a generation<sup>56</sup>. SSL7 binds to C5 and, through an alternative mechanism, inhibits the conversion of C5 into C5a<sup>57</sup>. These examples show that bacterial pathogens use various complement-binding proteins to block the complement cascade at different stages. This was also shown by the recent observation that SCINB and Efb are produced simultaneously, as they are located in a single operon, and act synergistically to inhibit all C3 and C5 convertases<sup>58</sup>.

The anaphylatoxin C5a stimulates a strong inflammatory response. The expression of P-selectin in the local endothelium is increased under the influence of C5a, formylated peptides and other secondary chemokines, paving the way for neutrophil rolling, adherence and transmigration. In this way, neutrophils can extravasate and move up the gradient of chemoattractants to the site of infection. *S. aureus* has evolved an armamentarium of weapons for this phase of infection. SSL5 blocks neutrophil extravasation at two crucial points: first, it inhibits a crucial interaction between neutrophils and the endothelium (the P-selectin glycoprotein ligand 1–P-selectin interaction), and second, it blocks the activation of G protein-coupled receptors (GPCRs) by chemokines<sup>59</sup>. Extracellular adherence protein (Eap) blocks the next step in neutrophil extravasation, blocking firm adhesion of neutrophils to the endothelium by interfering with the integrin–intercellular adhesion

molecule 1 interaction<sup>60</sup>. *S. aureus* also interferes with the next steps in neutrophil movement: the chemotaxis, priming and activation events that are mediated mainly by GPCRs on the neutrophil surface. SSL10 blocks CXC-chemokine receptor 4 (CXCR4) and, thus, the action of CXC-chemokine ligand 12 (CXCL12)<sup>61</sup>. Chemotaxis inhibitory protein of staphylococci (CHIPS)<sup>62</sup> inhibits C5a receptor (C5AR1) as well as formyl peptide receptor (FPR1), thereby blocking the activation of formylated peptides as well as the potent anaphylatoxin C5a. Thus, staphylococci have evolved SSL7 to bind the C5 molecule itself and prevent its cleavage<sup>63</sup>, can block both C5 convertases using Efb and Ecb<sup>56</sup>, and can also block the receptor for C5a using CHIPS. All of these factors are expressed simultaneously in most clinical isolates of staphylococci and provide a seemingly redundant but possibly synergistic inhibition. FPR-like 1 (FPRL1) inhibitory protein (FLIPr) inhibits FPRL1, a homologue of FPR1, and FLIPr-like protein inhibits the activation of neutrophils by both FPR1 and FPRL1 (also known as FPR2)<sup>64</sup>. Finally, *S. aureus* also secretes proteins that interact with the process of phagocytosis itself, such as SSL10, Spa and Sbi (which act on IgG-mediated phagocytosis), SAK (which cleaves C3b and IgG) and SSL7 (which binds IgA and C5 simultaneously)<sup>50–52,57</sup>. The recently obtained crystal structure of the C5–SSL7 complex confirms that binding to C5 occurs exclusively through the C-terminal  $\beta$ -grasp domain of SSL7, leaving the OB domain free to interact with IgA<sup>63</sup> (FIG. 3d). Once inside the neutrophil, various molecules protect the bacterium against the attack of proteases and oxygen radicals, but that is beyond the scope of this article.

Although the majority of proteins that inhibit complement and neutrophils are secreted, some cell wall-anchored proteins on staphylococci also act in this way. Recently, ClfA was shown to inhibit phagocytosis by binding to factor I<sup>21,65</sup>; furthermore, adenosine synthetase inhibits general neutrophil function<sup>66</sup> and iron-regulated surface determinant protein H (IsdH) inhibits complement activation by enhancing C3b degradation<sup>67</sup>. Sbi and Spa are also present on the bacterial surface and can fulfil their functions at this location as well as functioning as soluble proteins<sup>50,51</sup>.

Thus, staphylococci have evolved an arsenal of molecules to counteract the complement system. Using a plethora of small secreted proteins is still a highly energy-efficient way to evade the innate immune response compared with, for example, capsule formation.

### Complement evasion by meningococci

*N. meningitidis* is a Gram-negative, capsulated bacterium that causes meningococcal meningitis and septicaemia. The bacterium is adapted to the human host and is carried asymptotically in the nasopharynx of approximately 5–10% of the human population<sup>68</sup>. *N. meningitidis* uses the surface-exposed proteins PorA and fHbp to bind C4BP<sup>69</sup> and human factor H<sup>70,71</sup>, respectively. The general mechanisms of *N. meningitidis* resistance to host immunity have been recently reviewed elsewhere<sup>72</sup>; here, we focus on fHbp-mediated regulation of the alternative pathway, as the three-dimensional structure of fHbp has now been solved.

fHbp is a surface-exposed lipoprotein that was first identified by genomic screening as a protein antigen able to induce bactericidal antibodies<sup>73</sup>. It was initially termed genome-derived *Neisseria* antigen 1870 (GNA1870). The same protein was also identified through a biochemical approach and named LP2086 (REF. 74). Later, GNA1870 was found to bind factor H and was renamed fHbp. By recruiting factor H to its surface, *N. meningitidis* resembles self tissue and is no longer attacked by complement, so can survive and grow in the bloodstream<sup>71,75</sup>. When it was later found that fHbp binds only human factor H and not factor H from mouse, rat or lower primates, it became clear why *N. meningitidis* is a human-specific pathogen and why we have so far been unable to develop reliable animal models of *N. meningitidis* infection. Whereas fHbp allows survival of the bacterium in human blood, in animal blood it does not recruit factor H and so the bacterium is killed by complement<sup>76</sup>. This suggests that mice that are transgenic for human factor H might serve as good animal models for meningococcal disease. Finally, binding of factor H by fHbp might also explain, in part, the devastating haemorrhagic syndrome that is associated with meningococcal septicaemia: recruitment of factor H by bacteria that have grown to high density in the bloodstream could deplete factor H in the surrounding tissue, so this self tissue becomes prone to attack by complement.

The three-dimensional structure of fHbp, which was solved by nuclear magnetic resonance (NMR), revealed that this protein consists of two domains that are both rich in  $\beta$ -strands and are arranged in a butterfly-like structure<sup>77–79</sup>. A flexible N-terminal tail anchors the protein to the bacterial cell through a lipid chain that is covalently attached to the first cysteine residue. The sequence variability to which fHbp is prone among the meningococcal population is

concentrated mostly on one side of the molecule. Such a distribution indirectly suggests the orientation of fHbp with respect to the bacterial outer membrane, with the groove that separates the N- and C-terminal domains oriented towards the external environment (FIG. 3e).

This orientation is also favourable for the interaction with domains CCP6 and CCP7 of human factor H, as depicted by the recently published crystal structure of this complex<sup>80</sup> (FIG. 3e). Although the interaction between CCP7 and fHbp is limited and ascribed to just two loops of the N-terminal domain of the bacterial protein, CCP6 accommodates itself on the fHbp groove owing to good shape complementarity and a strong electrostatic interaction between His371 and Lys351 of CCP6 and Glu218 and Glu239 of fHbp, respectively. These two residue pairs determine the efficacy of the interaction. Glu218Ala and Glu239Ala mutations dramatically reduce human factor H binding. Further, CCP6 is in the region of factor H that is responsible for the recognition of host cell polyanions. This suggests that meningococci evolved the ability to recruit human factor H to their surface using a surface-exposed protein that mimics the function of polyanions in host carbohydrates. The crystal structure of the fHbp–factor H complex also reveals that the surface area of factor H that interacts with fHbp consists of residues that are not conserved between humans and lower vertebrates. This provides a structural rationale for the strict human specificity of fHbp and the bacterium itself<sup>76</sup>. Taken together, this suggests that binding of fHbp to factor H domains CCP6 and CCP7 localizes factor H to the meningococcal surface, where it then provides protection against complement activation by binding to deposited C3b molecules (through the factor H domains CCP1–CCP4) and, thus, blocks amplification of the complement response.

The presence of a factor H-binding protein on the meningococcal surface outlines the relevance of an escape mechanism to avoid the killing mediated by the alternative pathway of the complement system. However, it was recently suggested that the alternative pathway acts mainly by amplifying the complement activation that is initially triggered by the classical pathway<sup>81</sup>.

### Implications for vaccine development

Surface-exposed molecules that can be targets for complement-activating antibodies are known to be excellent vaccine antigens. Examples include the capsular polysaccharides of pneumococcus and meningococcus

serogroups A, C, W135 and Y, for which the polysaccharides, delivered alone or as protein–polysaccharide conjugates, are part of effective licensed vaccines. More recently, genome-wide screening (also known as reverse vaccinology) has identified several novel vaccine candidate proteins that are good targets for complement-fixing antibodies. The most notable examples are fHbp, *Neisseria* adhesin A (NadA) and neisserial heparin-binding antigen (NHBA; also known as GNA2132) from *N. meningitidis*, which are now in phase III clinical trials, and pili from group A *Streptococcus*, group B *Streptococcus* and *S. pneumoniae*. In some cases, as well as being targets for complement-fixing antibodies, some antigens can interact directly with complement components. This is the case for fHbp of *N. meningitidis*, BibA of group B *Streptococcus*, PspC of *S. pneumoniae* and FHA of *B. pertussis*<sup>82</sup>.

fHbp can be considered a unique *N. meningitidis* vaccine antigen. Antibodies directed against fHbp have several modes of action. Those with serum bactericidal activity can mediate direct bacteriolysis through the classical complement pathway and also promote phagocytosis and subsequent intracellular killing. In parallel, fHbp-specific antibodies can block binding of factor H, increasing bacterial susceptibility to killing by the alternative pathway<sup>71,75,83</sup>. In addition, as complement activation would no longer be inhibited, more C5a would be generated, and phagocyte activation and subsequent phagocytosis and killing would be promoted. This suggests that using specific antibodies to block the activity of a complement inhibitor could be a potent strategy to design new vaccines for a large variety of bacteria. This is not a new strategy, if we consider the fact that the oldest known complement evasion strategy is the capsule, and we have been making effective capsule vaccines for decades. By understanding why antigens that bind to human complement components are good vaccine antigens, it might be possible to define the molecular basis of an ideal vaccine antigen. Understanding the molecular nature and the functional consequences of targeting certain candidate antigens will be instrumental in providing a scientific basis to guide the selection of vaccine targets and vaccine design. Furthermore, determining the three-dimensional structures of the complexes formed by pathogenic proteins and their complement targets will be advantageous for our understanding of the molecular mechanisms of complement evasion, as highlighted by SCIN, Efb, SSL7 and fHbp.

Although many factor H- and C4BP-binding proteins on the surface of various bacteria have been described, recent work on staphylococci indicates that most innate immune evasion molecules in these species are small secreted proteins. In part, this may reflect the difference between the surfaces of Gram-negative and Gram-positive species. In Gram-negative bacteria, membrane proteins or membrane-anchored proteins are free to move and can therefore cover the whole bacterial surface to prevent complement activation. On the surface of Gram-positive bacteria, however, cell wall-anchored proteins cannot reach and cover every part of the bacterial surface unless the density of the protein is extremely high. As indicated for staphylococci, secreted molecules can surround the bacterial environment and act on complement deposition on the surface by inhibiting various complement components at diverse stages of complement activation. So, for several aspects of immune attack, secreted molecules are an effective method of counter-attack by Gram-positive species. However, this does not rule out a function for some cell surface-bound molecules in immune evasion. Furthermore, Gram-positive organisms are insensitive to complement-mediated lysis, and removal of these pathogens is dependent only on phagocytes. Therefore, in developing evasion strategies, these pathogens have also evolved factors that specifically target complement receptors or other structures on phagocytes, and secreted proteins are again more suitable for such strategies than surface-associated proteins.

The complement system plays a crucial role in the host defence against systemic disease caused by *N. meningitidis*. This is proved by the fact that deficiencies of the complement system that affect the alternative pathway and the terminal complement components such as the MAC have been associated with increased susceptibility to meningococcal infections<sup>84</sup>. However, the same host deficiencies have not been associated with increased susceptibility to staphylococcal infections. This suggests that the complement system is the main innate host defence mechanism against *N. meningitidis*, but that a combination of complement and cellular innate immunity defends the host against *S. aureus*. This is in agreement with the observation that neutropenic patients are highly susceptible to staphylococcal infection.

In conclusion, focusing on the microbial molecules that interact with human complement has revealed important information about microbial pathogenesis. Elucidating the molecular mechanisms underlying

complement evasion will help to develop novel vaccine antigens and, in the meantime, improve our understanding of the molecular and functional basis of good vaccine antigens.

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#### Competing interests statement

The authors declare **competing financial interests**: see web version for details.

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