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REVIEW ARTICLE

Complement evasion by the human respiratory tract pathogens *Haemophilus influenzae* and *Moraxella catarrhalis*

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All infective bacterial species need to conquer the innate immune system in order to colonize and survive in their hosts. The human respiratory pathogens *Haemophilus influenzae* and *Moraxella catarrhalis* are no exceptions and have developed sophisticated mechanisms to evade complement-mediated killing. Both bacterial species carry lipooligosaccharides preventing complement attacks and attract and utilize host complement regulators C4b binding protein and factor H to inhibit the classical and alternative pathways of complement activation, respectively. In addition, the regulator of the terminal pathway of complement activation, vitronectin, is hijacked by both bacteria. An array of different outer membrane proteins (OMP) in *H. influenzae* and *M. catarrhalis* simultaneously binds complement regulators, but also plasminogen. Several of the bacterial complement-binding proteins are important adhesins and contain highly conserved regions for interactions with the host. Thus, some of the OMP are viable targets for new therapeutics, including vaccines aimed at preventing respiratory tract diseases such as otitis media in children and exacerbations in patients suffering from chronic obstructive pulmonary disease.

Keywords: complement; evasion; *Haemophilus influenzae*; *Moraxella catarrhalis*; serum resistance

Haemophilus influenzae and *Moraxella catarrhalis* are together with *Streptococcus pneumoniae* the most common pathogens found in the human respiratory tract. Preschool children carry these pathogens as commensals, and occasionally, also upon a simultaneous viral infection compromising the host's epithelial barrier, the polymicrobial dynamics will change and result in bacterial respiratory tract infections as exemplified by acute otitis media, rhinosinusitis, tracheitis or bronchitis/pneumonia [1,2]. In contrast to children, adults are less often colonized by *H. influenzae* and *M. catarrhalis* and, consequently, may be infected upon exposure, for example after contact with grandchildren carrying pathogens. In particular, patients suffering from chronic obstructive pulmonary disease (COPD) are often infected with *M. catarrhalis* and/or *H. influenzae*, which are associated with exacerbations of COPD [3–5]. This review will focus upon how the

Abbreviations
C4BP; C4b binding protein; COMP, cartilage oligomeric matrix protein; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; ECM, extracellular matrix; FH, factor H; HBD, heparin-binding domain; Hib, *Haemophilus influenzae* type b; Hsf, *Haemophilus* surface fibril; LPS, lipopolysaccharide; LOS, lipooligosaccharide; MAC, membrane attack complex; MBL, mannose-binding lectin; NTHi, nontypeable *H. influenzae*; OMP, outer membrane proteins; OMV, outer membrane vesicle; PCho, phosphorylcholine; SBP, solute-binding protein; SCR, short consensus repeat; TAA, trimeric autotransporter adhesin; uPa, urokinase plasminogen activator; Usp, ubiquitous surface protein; vtn, vitronectin.

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Gram-negative bacteria *H. influenzae* and *M. catarrhalis* survive in the host by conquering the innate immunity by utilizing, interfering with or merely inhibiting the different components of the complement system. More data exist on *H. influenzae* and its interactions with the human host (Table 1) as compared to *M. catarrhalis* (Table 2), and this is also reflected by the numbers of publications on each pathogen.

Haemophilus influenzae

The *H. influenzae* polysaccharide capsule

Prior to introduction of the *H. influenzae* type b (Hib) conjugated capsular polysaccharide vaccine in the 1990s [6,7], the encapsulated Hib was by far the most common bacterial *Haemophilus* species causing invasive disease in children. Encapsulated microbes have significant survival gains, and the capsule as such prevents both complement-mediated killing of bacteria and opsonization resulting in protection against phagocytosis [8]. The Hib capsule consists of a polymer of ribose and ribitol-5-phosphate (polyribosylribitol phosphate – PRP), and the amount of PRP is related to complement resistance (Table 1). If a Hib strain carries several copies of the capsule locus a thicker capsule will be manufactured and consequently the bacterium becomes more resistant against complement. [9]. Capsule type a, b and e transformants having the same genetical background do not differ in complement resistance, further supporting the importance of the capsule [10]. Despite that Hib has significantly decreased in incidence, non-typeable *H. influenzae* (NTHi) in addition to other serotypes, for example *H. influenzae* type a (Hia) and f (Hif), are slowly emerging and are common in certain geographical areas [11,12].

Lipooligosaccharide contains phosphorylcholine and is decorated with sialic acid

Non-Hib strains, including unencapsulated and hence NTHi, are at present the most common *Haemophilus* isolated from patients suffering from respiratory or invasive disease. Both encapsulated *H. influenzae* and NTHi are, however, equipped with lipooligosaccharide (LOS) that lacks the repetitive O-chain carbohydrate extension, as compared to lipopolysaccharide (LPS). This major glycolipid displays a large variation between different strains, depending on phase-variable gene expression of LOS biosynthetic genes. For example, mutation of a galactosyltransferase encoded by the *lgtC* gene results in enhanced levels of C4b deposition, and consequently, bacterial survival in blood is diminished [13]. Similarly, in a recent study on Australian NTHi isolates obtained from patients having sepsis it was shown that the LOS biosynthetic genes *lic2A* and *oafA* were found more commonly in OFF and ON states, respectively, in invasive NTHi isolates compared to carriage isolates [14].

Already more than 20 years ago, Weiser *et al.* [15] demonstrated that LOS decorated with phosphorylcholine (PCho) contributed to bacterial pathogenesis and sensitivity to serum-mediated killing related to C-reactive protein (CRP). The *lic1* operon encodes four proteins that synthesize and transfer PCho to NTHi LOS. CRP binds to PCho and in turn activates the classical pathway of complement activation by attracting C1q. Intriguingly, PCho also mimics the platelet-activating factor (PAF) and binds to the PAF-receptor on epithelial cells [16]. It has been shown that colonizing NTHi have more PCho for efficient adherence to the upper respiratory tract, whereas invasive NTHi are more likely to be devoid of PCho, a result of classical phase variation (bacteria turning off genes). This is one explanation of many as to how NTHi can invade the blood stream of certain individuals, particularly immunocompromised hosts and the elderly [17,18]. An increased serum resistance linked to invasive NTHi isolates seems to be merely related to the PCho, and not to binding of complement regulators, as discussed more in detail below [19]. Moreover, the decreased complement-mediated killing is associated with lower binding of IgM to invasive isolates compared to colonizing NTHi isolated from the oropharynx [20,21].

Eukaryotic cells are decorated with sialic acid [generic name for a class of 9-carbon sugar acids; *N*-Acetylneuraminic acid (Neu5Ac)] at their surfaces in order to attract factor H (FH) and hereby prevent spontaneous activation of the alternative pathway (C3b) of complement activation [22]. In order to evade the host complement-mediated attack by attracting FH, and to escape recognition by the host immune system, pathogens build in sialic acid in their LPS. Nontypeable *H. influenzae* cannot, however, synthesize sialic acid (Neu5Ac) itself. In contrast, most virulent NTHi incorporate sialic acid into LOS as a terminal nonreducing sugar from the environment using an uptake mechanism depending on a solute-binding protein (SBP) system, which belongs to the tripartite ATP-independent periplasmic (TRAP) transporter family [23]. The SiaPQM sialic acid uptake system in *H. influenzae* is one of first SBPs described [24]. In parallel, four sialyltransferases encoded by the genes *lic3a*, *siaA*, *lsgB* and *lic3B* are also produced by NTHi. Lic3A is considered to be one of the most important

Table 1. *Haemophilus influenzae* and examples of molecular structures and proteins that interact with the innate immune system of the human host. Only a few studies are selected for illustration. PAF, platelet-activating factor

Bacterium	Bacterial component	Ligand/molecule affected (human host)	Function in serum resistance	Other functions (examples)	Reference
Encapsulated *H. influenzae*	**Capsule**, polymers of ribose and ribitol-5-phosphate (polyribosylribitol phosphate – PRP)		Mediates serum resistance	Protects against phagocytosis	[9,10,13,14]
All *H. influenzae*	**LOS**				
	PCho	CRP	CRP binds to PCho -> increased killing (C1q binding)	Increased adhesion *via* the PAF receptor, phase variation when invading the blood stream	[15,16]
H. influenzae type d and NTHi	SiaPQM sialic uptake system (ATP-dependent periplasmic transporter family) SiaB (CMP-Neu5AC synthetase) Lic3A (sialyltransferase) etc.	Sialic acid (Neu5Ac)	Inhibition of the classical pathway (by blocking IgM) and the alternative pathway		[24,25,28,32]
	OMP				
Encapsulated *H. influenzae*	Protein H	FH, FHL-1	Inhibition of the alternative pathway	Adhesion	[47,49]
	Protein H	Vitronectin	Inhibition of the terminal pathway and MAC	Adhesion	[61]
H. influenzae type b	*Haemophilus* surface fibrils (Hsf)	Vitronectin	Inhibition of the terminal pathway and MAC	Adhesion to epithelial cells and vitronectin in the ECM	[36,57–60]
NTHi	Unknown receptor	C4BP	Inhibition of the classical pathway		[38]
	P4	Vitronectin	Inhibition of the terminal pathway and MAC	Adhesion	[67]
	P5	FH	Inhibition of the alternative pathway	Adhesion	[50,51]
	Protein E	Vitronectin	Inhibition of the terminal pathway and MAC	Adhesion to laminin and epithelial cells	[62,63]
	Protein E	Plasminogen	C3b cleavage upon activation	Fibrinogen cleavage	[62,71]
	Protein F	Vitronectin	Inhibition of the terminal pathway and MAC	Adhesion to epithelial cells and laminin	[66,100]
	OMV	Several ligands	Increased vitronectin production leads to bacterial binding and inhibition of MAC formation		[69]

sialyltransferases since it adds the terminal Neu5Ac after activation by SiaB, an CMP-Neu5Ac synthetase. The importance of these enzymes has also been proven in an acute otitis media chinchilla model, where both NTHi *lic3a* and *siaB* mutants were attenuated [25].

Interestingly, > 90% of clinical NTHi isolates have a highly prevalent molecular pattern including *lic3A* (in addition to the genes *lgtF*+, *lic2A*+, *lic3B*+, *siaA*−, *lic2C*+, *ompP5*+ and *opaA*+) further proving the importance for sialylation of LOS [26]. Post *et al.* [27] also demonstrated that sialylation is an important feature for the decoration of the LOS of NTHi as compared to *Haemophilus haemolyticus*, a subspecies that is considerably less virulent; the majority of *H. haemolyticus* clinical isolates lacked the genes *lic3A*, *lic3B* and *siaA*. An investigation of mutants having spontaneous decreased serum resistance revealed that a phase variation in the N′-terminal region of the *lpsA*

gene results in a truncated version of the glycosyltransferase (LpsA) resulting in decreased decoration with sialic acid of the LOS [28].

Neu5Ac and Neu5Gc are the two most prominent forms of sialic acids in mammals. Intriguingly, humans do not express Neu5Gc on cells but have antibodies directed against it. Recently, it was revealed that NTHi SiaB (CMP-Neu5AC synthetase) has a ≈ 4000-fold higher catalytic activity for Neu5Ac compared to Neu5Gc proving a specific adaptation of NTHi for the human host [29]. More recently, it has been shown that a multifunctional enzyme (LsgB) that mainly functions when NTHi is cultured on a solid surface can add either a ketodeoxyoctanoate or Neu5Ac moiety to a terminal *N*-acetyllactosamine structure of the LOS [30]. Uptake of sialic acid by NTHi has also been suggested to increase complement resistance *via* a diminished IgM-dependent complement activation [31]. More recently, Jackson *et al*. identified the LOS targets of bactericidal IgM blocked by sialic acid. Evidence that sialic acid protects *via* blocking IgM was obtained *via* adsorption of IgM specific to LgtC- and Lic2A-dependent LOS glycans, which decreased the sensitivity of nonsialylated relative to sialylated NTHi [32]. Taken together, addition of sialic acid (Neu5Ac) as a terminal sugar on NTHi LOS is extraordinary important for serum resistance and consequently increases virulence for optimal survival in the human host.

Haemophilus-dependent interference with the classical and lectin pathways of complement activation

Bacteria use several different approaches to combat the host immune response [33]. A major strategy employed by pathogens to avoid complement-mediated attacks by the adaptive and innate immune system is to vary outer membrane proteins (OMP) and their expression in order to reduce specific antibody binding and consequently complement fixation. NTHi is not an exception and expresses several OMP with a high degree of heterogeneity in addition to regulation by phase variation that collectively are responsible for *H. influenzae* serum resistance [34]. This fact partly hampered vaccine development against NTHi until highly conserved surface-exposed proteins were defined [6]. Whole-genome sequencing and bioinformatics have been used to identify and assess NTHi population structure and find proteins that would be conserved in a majority of strains, and hence play a role in adaptation to the host in addition to virulence [6,35].

A highly efficient strategy by bacteria to avoid the classical pathway of complement activation is to bind C4b binding protein (C4BP) to the surface [36] and thereby inhibit the formation of C3-convertase (C4bC2b) in addition to accelerating the decay of the enzyme. Moreover, C4BP is a cofactor for factor I in the proteolytic degradation of C4b [37]. NTHi binds C4BP to its surface and significantly contributes to serum resistance [38]. The major targets for *H. influenzae* on the C4BP molecule seem to be control complement protein domains 2 and 7 as revealed by using different truncated recombinant proteins. It remains, however, to define the specific protein responsible for the NTHi-dependent binding of C4BP. In contrast to NTHi, encapsulated *H. influenzae* including type b and f do not significantly attract C4BP.

The lectin pathway of complement activation is induced by pattern recognition molecules mannose-binding lectin (MBL), ficolins (ficolin-1 to 3) or collectin-10/-11 that all sense carbohydrate ligands at microbial surfaces [39]. The importance of MBLs in killing of NTHi *via* the lectin pathway has not been fully evaluated, but a series of clinical reports exist. In contrast to the Gram-positive *Staphylococcus aureus* and *Streptococcus pyogenes*, heterogenous binding patterns of MBL have been observed with *Escherichia coli* and the encapsulated *H. influenzae* type b (Hib) [40]. From a clinical point of view, there are, however, no differences in MBL genotype frequencies between patients having community-acquired pneumonia caused by *S. pneumoniae* or *H. influenzae* compared to the control population [41]. In parallel, no difference in disease severity and *H. influenzae* carriage is seen in patients with cystic fibrosis having MBL deficiency compared to controls having normal MBL levels [42]. We recently encountered a patient with severe sepsis with *H. influenzae* type f [43]. This particular patient suffered from MBL deficiency in addition to IgG3 deficiency and is a vivid example of that MBL deficiency may play a partial role in severe outcomes of infectious diseases. MBL deficiency is common in the general population (~ 10%), but is not, however, related to an increased incidence of infection in otherwise immunocompetent individuals due to redundancy [44]. Intriguingly, patients with COPD and MBL deficiency have a more diverse lung microbiota and are colonized with less *Haemophilus* spp. [45].

The alternative pathway and *H. influenzae*

Most bacterial species can bind factor H (FH) to the surface and hereby inhibit activation of the alternative pathway of complement activation [46]. *H. influenzae*

is not an exception, and early studies have shown that both NTHi and Hib bind FH and factor H-like protein 1 (FHL-1) [47], the latter a product of alternative splicing of the *FH* gene. These complement regulatory proteins coordinate the alternative pathway *via* binding to C3b and hereby accelerate the decay of the C3-convertase (C3bBb), in addition to being a cofactor for the factor I (FI)-assisted cleavage of C3b [48]. In parallel to C4BP, FH consists of repeated domains, short consensus repeats (SCRs), and FH binds to Hib with SCRs 1–7 and 15–20 [47].

When comparing different *H. influenzae* including NTHi and Hib, *H. influenzae* type f (Hif) was observed to be the best FH-binding *Haemophilus* [47]. This later resulted in the discovery of protein H, a lipoprotein that exists in both Hib and Hif, but not the NTHi that are by definition unencapsulated. Despite sharing only 56% identical amino acids, the FH-binding proteins from Hib and Hif similarly interacted with the complement regulator FH SCRs 7 and 18–20 [49]. Protein H from both Hib and Hib were successfully expressed in *E. coli* and the non-FH-binding NTHi strain 3655. In addition to specific *H. influenzae* mutants devoid of Protein H, these two FH-expressing transformants (*E. coli* and NTHi 3655) proved that Protein H-dependent binding of FH plays a significant role in protecting *H. influenzae* from factor C3 deposition and further down-stream events in the complement cascade resulting in decreased killing by the membrane attack complex (MAC). Moreover, it has been shown that the *H. influenzae* adhesin P5 is a FH-binding protein contributing to evasion of the alternative pathway [50,51]. Differences in FH binding are observed between strains, and this fact might be associated with sequence heterogeneity of the surface-exposed loops. Importantly, these loops mediate interactions of P5 with the host extracellular milieu. Differences in FH-binding may explain why certain *H. influenzae* strains are more virulent. In experiments with factor H (FH) domains 6 and 7 fused with IgG Fc, it has also been shown that mutants devoid of P5 can bind to an NTHi molecule distinct from P5 [52].

Haemophilus influenzae prevents formation of the membrane attack complex by interfering with vitronectin

The three complement pathways converge to the common C3 convertases (from the classical and lectin pathways respectively alternative pathway) that result in formation of two C5 convertases that subsequently build up the pore in the MAC [53]. All parts of the MAC (except for C5b) belong to the MAC/perforin/cholesterol-dependent cytolysin protein superfamily, and recent structural insights by using flow cytometry and atomic force microscopy have shown how the MAC ruptures lipid membranes by local assembly of C5b6 [54].

Clusterin and vitronectin (vtn) can inactivate MAC precursors preventing lysis (of both host cells and bacteria) [55]. Vitronectin has a multifunctional role as a soluble plasma complement regulator by preventing C5b-C7 complex formation and C9 polymerization, but also as a component of the extracellular matrix (ECM) having additional antimicrobial properties when degraded by proteases [56]. Intriguingly, it has been shown that vtn is hijacked by most bacterial species, including *H. influenzae*, in order to prevent MAC formation [36].

Haemophilus surface fibrils (Hsf) expressed by the encapsulated Hib strongly bind both soluble and immobilized vtn, and this complement regulator remains biologically active, thus efficiently preventing MAC formation and consequently bacterial killing [57]. Hsf is a large trimeric autotransporter (245 kDa, 100 nm) protruding from the bacterial surface and bind vtn residues 352–374 [located within heparin-binding domain (HBD) 3] at its tip. Studies on this unique structure revealed that it resembles a twisted hairpin at the bacterial surface [58,59]. By binding vtn, Hsf significantly inhibits complement-mediated killing of Hib [60]. In contrast to Hib, *H. influenzae* type f (Hif) employs FH-binding Protein H to efficiently attract vtn [61].

Given the importance of vtn for controlling the terminal pathway of complement activation and the formation of the MAC, also unencapsulated NTHi has developed sophisticated strategies to recruit this complement regulator to its surface. In addition to using vtn as a target for adhesion to the vtn-containing ECM and epithelial cells coated with vtn, NTHi can bind soluble biologically active vtn and hereby prevent killing by complement. As with most strategies employed by bacteria to evade the host, NTHi has several OMP that simultaneously bind vtn, that is the vtn 'interactome'. The highly conserved Protein E having a molecular weight of 16 kDa existing in virtually all *H. influenzae* is one of the most studied outer membrane structures [62] and significantly binds to HBD3 of vtn [63]. In addition to vtn, the extraordinary small Protein E binds laminin, another ECM protein found in the respiratory tract, particularly in COPD patients with a medical history of smoking. The two binding sites are located at different regions on the Protein E molecule, and simultaneous binding to the two ECM components is explained by a dimeric appearance of

Protein E at the bacterial cell surface. The affinity for vtn is, however, stronger as compared to binding to laminin [64,65]. Protein F and P4 are two other important NTHi surface proteins that function as adhesins, but also mediate binding of vtn thereby conferring resistance against the terminal pathway and MAC formation [66,67].

Another way for microbes to conquer the host innate immune defence is to shed extracellular vesicles (nanoparticles). All Gram-negative bacteria release outer membrane vesicles (OMVs) that consist of the outer membrane, proteins from the periplasm, and, to a lower extent, also from the cytoplasm [68]. OMVs are also produced by NTHi, and they specifically interact with host components, resulting in induction of biologically active vtn in the lung, which protects the bacteria, as revealed by bronchoalveolar lavage fluid and experiments in the mouse [6,69]. Taken together, NTHi has an array of different proteins to evade the terminal pathway of complement activation, but the majority of those proteins has also other functions such as mediating adherence to epithelial cells and different ECM proteins.

Haemophilus influenzae binds plasminogen conferring proteolytic cleavage of complement

Another strategy to evade the innate immune system and invade the host is to degrade complement proteins and the ECM by proteases. NTHi has a fairly small genome, and the only characterized secreted protease is IgA protease [6]. This problem has, however, in parallel with many other microorganisms been solved by binding of the inactive proenzyme plasminogen [70]. Plasminogen can in turn be converted and enzymatically activated to plasmin at the bacterial surface upon exposure of, for example, urokinase plasminogen activator (uPa). NTHi Protein E is one such example of a plasminogen-binding receptor [71]. This particular protein hence plays an important role in virulence by interacting with both vtn and plasminogen [62]. When bound plasminogen is converted to active plasmin, the natural substrates fibrinogen and C3b are cleaved and inactivated, respectively.

Moraxella catarrhalis

Moraxella catarrhalis LOS and outer membrane proteins important for serum resistance

It was early shown that serum-resistant *M. catarrhalis* have diverged from a common ancestor [72], and further comparative genomic analyses have verified the occurrence of an independent evolution of serum-sensitive and serum-resistant lineages [73] (Table 2). As expected and in parallel with other Gram-negative bacteria, *M. catarrhalis* LOS is important for serum resistance [74]. Mutant *M. catarrhalis* strains lacking the lipid A or the core oligosaccharides in LOS render bacteria significantly more susceptible to the bactericidal activity of serum [75]. When *M. catarrhalis* is exposed to human serum, 84 and 134 genes are upregulated or reduced, respectively [76]. The most upregulated genes encode for ABC transporter systems and

Table 2. *Moraxella catarrhalis* and interactions with human host innate immune system. Only a few studies are selected for illustration.

Bacterium	Bacterial structure/protein	Ligand (human host)	Function in serum resistance	Other functions (examples)	Reference
Moraxella catarrhalis	**LOS**		Lack of lipid A and core oligosaccharides results in increased susceptibility to human serum		[74,75]
	OMP				
	OmpCD, OmpE and CopB		Mutants are more susceptible to human serum		[77–79]
	UspA1 and A2	C4BP	Inhibition of the classical pathway	Adhesins	[84]
	UspA1 and A2	C3	Inhibition of the classical and alternative pathway	Adhesins	[87]
	OlpA	Factor H	Inhibition of the alternative pathway	Adhesin	[90]
	UspA1 and A2	Vitronectin	Inhibition of the terminal pathway and MAC	Adhesins	[80,81]
	UspA2, A2H	COMP		Reduces bacterial uptake by epithelial cells and promotes bacterial survival	[91]
	UspA1, A2	Plasminogen	Plasminogen -> C3b cleavage upon activation	Adhesins and fibrinogen cleavage	[92]
	OMV	C3 binding (depletion)	Protect NTHi from being killed in polymicrobial cultures		[93]

ubiquitous surface protein (Usp) A1 and McaP. Interestingly, the disulfide bond formation (DSB) system, DsbB and DsbA, is required for complement resistance; the LOS structure and membrane stability were severely affected in *dsbA* mutants making bacteria devoid of DSBs significantly more susceptible to human serum.

In addition to LOS, an array of different outer membrane proteins (OmpCD, OmpE and CopB) plays a role in *Moraxella*-dependent serum resistance [77–79]. It has been demonstrated that mutants devoid of those proteins become serum-sensitive albeit the exact mechanisms are unknown. All *M. catarrhalis* isolates express trimeric autotransporter adhesin (TAA) (or coiled-coil adhesins) at their surface. Members of the UspA family are the most important TAAs for maintaining serum resistance. They consist of UspA1, A2, hybrid (UspA2H) and a UspA2 variant [80]. Since the multifunctional UspAs are crucial for *Moraxella* virulence, the antigen variation is extraordinary prominent, and UspA N termini are therefore highly variable in order to escape the humoral immunity but still retain biological function by binding ECM proteins, and promoting serum resistance by interactions with C4BP and vtn [81–84].

Moraxella catarrhalis and interference with the classical and lectin pathways of complement activation

C4BP is an attractive target molecule for *M. catarrhalis*, and UspA1 and A2 have both been shown to bind C4BP inhibiting the classical pathway of complement activation [84]. In contrast, it was recently shown that the ECM component proline/ arginine-rich end leucine-rich repeat protein (PRELP) can bind to the UspA2/A2H and competitively inhibit the interaction with C4BP rendering the bacteria more susceptible to human serum [85]. In parallel with this phenomenon, short leucine-rich proteoglycans fibromodulin, osteoadherin and biglycan also all competitively inhibit binding of C4BP to the *M. catarrhalis* cell surface [86]. This results in increased C3b/iC3b deposition, MAC formation and as a consequence decreased bacterial survival. Interestingly, *M. catarrhalis* has the capacity to directly bind C3d by both UspA1 and 2, and hereby inhibit both the alternative and classical pathways of complement activation [87]. The bacterium thus persists a very efficient strategy to block complement activation and inhibit C3a-mediated inflammation. In parallel, it has been shown that the lectin pathway is not activated in the presence of *M. catarrhalis*, but to date only one report exists, and this phenomenon has not been further investigated [88].

The alternative pathway and inhibition by *M. catarrhalis*

Moraxella catarrhalis outer membrane protein OlpA (Opa-like protein A; 24 kDa) [89] is related to the neisserial Opa adhesins that bind both carcinoembryonic antigen-related cell adhesion molecule (CAECAM) and heparan sulfate proteoglycan receptors. OlpA binds factor H to the bacterial surface of *Moraxella* and mediates resistance to the alternative pathway of complement activation [90]. Despite UspA1/A2-dependent binding of C4BP is extraordinary important for bacterial resistance against the classical pathway [84], OlpA also seems to play a relatively important role. A significantly better survival in serum was observed with OlpA-expressing *M. catarrhalis* compared to OlpA-deficient mutants when the alternative pathway was separately studied in the presence of Mg^{2+}-EGTA.

The terminal pathway, binding of plasminogen and *M. catarrhalis* outer membrane vesicles

Moraxella catarrhalis has the capacity to resist the MAC by binding vtn to the UspA1/A2 [56,81]. The interaction with vtn is mainly depending on UspA2 amino acids 30–170, that is the N-terminal head of the molecule. Recent years, further studies have been done on *M. catarrhalis* UspAs and interactions with the human host. Cartilage oligomeric matrix protein (COMP) is found also in epithelial cells of the respiratory tract, and when bound to *M. catarrhalis* UspA2, the bactericidal activity of the MAC is inhibited in addition to reducing bacterial uptake by epithelial cells preventing intracellular killing [91]. In addition, the UspA2 and A2H can attract plasminogen to its surface [92]. This is an important observation since plasmin generated in the presence of uPa in turn degrades C3b and C5. Therefore, plasminogen binding to the bacterial surface and consequently plasmin generation potentially may contribute to reduced bacterial killing upon exposure to human serum.

The Gram-negative bacterium *M. catarrhalis* is more serum-resistant as compared to NTHi, and most likely, these two bacterial species cooperate in order to survive in the host mucosa. Intriguingly, OMVs generated by *M. catarrhalis* protect NTHi from being killed by complement and suggest that the two species collaborate in the upper respiratory tract, particularly in preschool children [93]. *M. catarrhalis* produces large numbers of OMVs, and these nanoparticles carry most proteins also seen in the parent cell [94]. By using an integrated bioinformatic and immunoproteomic approach, the protein content in OMVs has been

thoroughly analysed by Augustyniak *et al*. [95]. Their study revealed that *M*. *catarrhalis* has adapted to the human host by a distinct path of evolution as compared to other *Moraxella* subspecies.

Concluding remarks

The respiratory pathogens *H*. *influenzae* and *M*. *catarrhalis* have evolved similar strategies to resist the complement system of the innate immunity. Both bacteria express several multifunctional proteins to conquer the different stages and the pathways mediating complement activation. From a therapeutic point of view, the mechanisms and proteins responsible for intermolecular interactions are putative targets for intervention. An innovative direct treatment strategy to prevent NTHi infection is to target bacteria with factor H (FH) domains 6 and 7 fused with IgG Fc, as shown in a recent study [52]. The therapeutic fusion protein binds to NTHi P5 and mediates activation of the classical complement pathway resulting in increased killing of NTHi. Interestingly, the FH6,7-Fc fusion protein also functions in a mouse model upon intranasal delivery. In contrast, active immunization is in most cases very beneficial for protecting the population against infectious diseases. Development of a vaccine aiming at controlling infections, preferentially acute otitis media and exacerbations in COPD caused by NTHi and *M*. *catarrhalis,* is a final goal for many researches both in the academia and in the pharmaceutical industry. Importantly, GSK has developed a vaccine consisting of protein D, and a fusion protein of the major subunit of the NTHi type IV pilus (PilA) and Protein E [96]. This vaccine preparation is protective in the mouse and chinchilla [97] and has also been found safe in a phase 2 clinical trial giving a partial protection against exacerbations in COPD patients [98]. Moreover, an investigational NTHi-*M*. *catarrhalis* vaccine consisting of PilA-Protein E, Protein D and UspA2 has also recently been proven safe in a randomized, observer-blind, placebo-controlled study on COPD patients [99].

In conclusion, the current body of knowledge on the pathogenesis of the two bacterial species NTHi and *M*. *catarrhalis* has resulted in new promising therapeutic strategies. Since most bacteria are ever-evolving targets, more research is, however, required to fully elucidate bacterial virulence with respect to complement resistance in an era with emerging antimicrobial resistance.

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