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INVITED REVIEW

Impact of pneumococcal microbial surface components recognizing adhesive matrix molecules on colonization

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SUMMARY

Microorganisms have evolved elaborate strategies to adhere to host cells and to evade the host complement and immune attack, ensuring survival in various host niches and dissemination into sterile parts of the human body. *Streptococcus pneumoniae* (the pneumococcus) is not only a commensal of the human respiratory tract but also the etiological agent of severe and life-threatening diseases. Pneumococcal attachment to mucosal surfaces is a highly dynamic process requiring the contact of pneumococcal surface-exposed proteins with soluble or immobilized host factors. These avid interactions may trigger proteolytic cascades or result in engagement of cell surface receptors and intracellularly associated signaling machineries for subsequent uptake of pneumococci into host cells. In the present review, the intimate communication of *S. pneumoniae* molecules recognizing adhesive matrix molecules (microbial surface components recognizing adhesive matrix molecules) with their host counterparts and their individual role in pneumococcal colonization is discussed.

INTRODUCTION

The interplay between pathogenic bacteria and their host structures is a complex scenario and involves a multitude of interactions of molecules secreted by bacteria or displayed on the bacterial surface with soluble host factors or host cellular receptors. Apparently the exploration of the dynamic interplay between the pathogen and the host is crucial to provide insights into the infectious process and to combat bacterial infections. *Streptococcus pneumoniae* (the pneumococcus) asymptomatically colonizes the human upper and lower respiratory tract, but can also cause severe local infections, e.g. otitis media and pneumonia, or even systemic diseases such as sepsis and meningitis, both associated with high

mortality rates (Bogaert *et al.*, 2004; Gámez & Hammerschmidt, 2012). However, the repertoire of pneumococcal factors involved in pneumococcal colonization, lung infections, survival in the blood or transmigration of the blood–brain barrier represents an extensively investigated area of research. The recruitment of host proteins or the intimate contact between pneumococci and host surface structures is critical to withstand host innate defense mechanisms and also to allow successful competition with resident commensal microorganisms for the limited nutritional resources and space. Pneumococci predominantly reside on mucosal surfaces of the human respiratory system either by binding directly to cellular receptors of epithelial cells or by targeting secreted polysaccharides and proteins that subsequently mediate pneumococcal binding to host cell receptors. The main factors determining whether bacteria remain extracellular or undergo internalization after adhesion include (i) the type of host cell encountered by the pathogen, (ii) the type of host cell receptor recognizing a particular bacterial ligand, (iii) the strength of the physical interaction between the bacterial ligand and the host cell receptor, and (iv) whether the microbe has the capability to trigger host cell signaling cascades, so modulating the host cytoskeleton and allowing bacteria to gain access to secured intracellular niches (Isberg & Tran Van Nhieu, 1994). Similar to other human pathogens *S. pneumoniae* exposes a variety of surface proteins that bind to cellular and extracellular host factors, i.e. human serum proteins for immune evasion and survival in the blood, host cell receptors for colonization and internalization, and host proteolytic enzymes for pneumococcal penetration through the extracellular matrix (ECM) and dissemination into deeper tissues. These bacterial molecules are collectively referred to as microbial surface components recognizing adhesive matrix molecules (MSCRAMMs). In this review we provide an overview of pneumococcal strategies to colonize the human respiratory airways with a focus on the extended repertoire of pneumococcal MSCRAMMs directly targeting receptors, soluble host proteins or proteins of the ECM, which then trigger activation of integrin receptors and subvert the host cell homeostasis. In addition, we discuss pneumococcal mechanisms to overcome epithelial and endothelial barriers to access underlying human tissues.

HUMAN RESPIRATORY TRACT COLONIZATION BY *S. PNEUMONIAE*

The commensal flora of the human upper respiratory tract is composed of both gram-negative bacteria (including *Moraxella catarrhalis*, *Haemophilus influenzae*, *Neisseria meningitidis*) and gram-positive bacteria (such as *Staphylococcus aureus*, *Streptococcus pneumoniae* and other hemolytic streptococci). At least once during life, every individual is likely to be colonized asymptomatically by these microorganisms, a phenomenon considered as carriage. Children younger than 5 years are at particular risk for colonization with *S. pneumoniae* (37%) and carriage rates may rise up to 58% in crowded situations, e.g. schools, hospitals, and day-care centers. Typically, pneumococci are transmitted by aerosols and enter the human body via the nasopharynx, where they easily encounter mucosal secretions and reach the respiratory epithelium. Pneumococcal carriage is supposed to be a key source of horizontal spread of *S. pneumoniae* within the community and importantly, pneumococcal infections are mostly preceded by nasopharyngeal colonization with a homologous strain (Bogaert *et al.*, 2004).

MOLECULAR OVERVIEW OF THE FIRST CONTACT BETWEEN *S. PNEUMONIAE* AND THE HOST: PNEUMOCOCCAL PROTEINS INVOLVED IN UNMASKING RECEPTORS AND CYTOTOXICITY

To ensure its success as a colonizer reaching the respiratory epithelium, *S. pneumoniae* has evolved diverse molecular strategies for preventing mucus entrapment and subsequent clearance. Initially, the electrostatic repulsion between the pneumococcal polysaccharide capsule and the mucosal polysaccharides prevents pneumococci from being entrapped in the mucus and engulfed by host immune cells (Nelson *et al.*, 2007b). Nevertheless, the same thick capsule covers important pneumococcal molecules recognizing host epithelial cells. Hence, *S. pneumoniae* spontaneously undergoes phase variation to transparent variants, exhibiting a lower expression of capsule polysaccharides and a higher expression of certain surface-associated proteins and carbohydrate-containing cell wall structures (Weiser & Kapoor, 1999). Moreover, the intimate contact of

pneumococci with host cells is associated with a reduction of capsular material, thereby unmasking surface-exposed adhesins and intensifying the host–pathogen interaction (Hammerschmidt *et al.*, 2005). Another molecular strategy used by *S. pneumoniae* to encounter its host is the sequential removal of terminal sugars by surface-associated exoglycosidases. The main exoglycosidases removing carbohydrate structures are the surface-exposed neuraminidase NanA, the β-galactosidase BgaA, and the exo-β-D-*N*-acetylglucosaminidase StrH (King, 2010). In a concerted action, these enzymes reduce the mucus viscosity, promote pneumococcal resistance to opsonophagocytic killing by neutrophils, unmask host receptors for adherence, and provide nutrients to colonizers (Dalia *et al.*, 2010). This initial step of pneumococcal colonization is most likely mediated by a low-affinity binding of *S. pneumoniae* to host cell surface carbohydrates, including low-molecular-weight oligosaccharides and glycosaminoglycans, such as heparin sulfate or chondroitin sulfate, which are constituents of the ECM (Tonnaer *et al.*, 2006). However, the pneumococcal surface molecules involved in binding to human glycoconjugates remain elusive. Remarkably, and independently of its β-galactosidase activity, BgaA has been shown to act as an adhesin mediating attachment of pneumococcal clinical isolates to human respiratory epithelial cells *in vitro* (Limoli *et al.*, 2011). Moreover, choline-binding proteins (CBPs), a class of surface-exposed proteins non-covalently attached to the pneumococcal cell wall, are also proposed to facilitate adherence of pneumococci to glycoconjugates, partially through non-specific, physicochemical interactions (Swiatlo *et al.*, 2002). In addition, several pneumococcal proteins, such as the pyruvate oxidase SpxB and the peptide permeases pneumoniae-like protein A (PlpA) and oligopeptide-binding protein AmiA, respectively, seem to be involved in the interaction with host carbohydrates (Cundell *et al.*, 1995; Spellerberg *et al.*, 1996). However, it remains to be determined whether these proteins bind directly to human glycoconjugates or indirectly modulate the functional properties of other pneumococcal proteins, mediating binding to carbohydrates, as shown for the intracellular pneumococcal repair enzyme methionine sulfoxide reductase A (MsrA) (Wizemann *et al.*, 1996). Finally, by secretion of the pore-forming pneumococcal cytotoxin (the Pneumolysin, Ply), the pneumococcus inhibits ciliary beating of respiratory epithelial cells and enables itself to bind to the cell surface without being eliminated with the mucus (Marriott *et al.*, 2008).

PNEUMOCCOCAL MSCRAMMs: STRONGER INTERACTIONS BETWEEN *S. PNEUMONIAE* AND THE HOST

As mentioned above, the initial contact of *S. pneumoniae* with the host, after acquisition, is mediated by weak interactions with surface glycoconjugates. However, to ensure an effective colonization of the human respiratory tract, pneumococci require stronger protein–protein interactions with the host. To do so, this human pathogen expresses the so-called MSCRAMMs, which are able to bind to constituents of the human ECM with high levels of specificity and affinity, and redundancy in interaction modalities (Patti *et al.*, 1994). That means a single bacterial MSCRAMM is able to recognize a specific or several ECM proteins and conversely, various MSCRAMMs are able to bind the same ECM protein. Pneumococcal MSCRAMMs seem to be represented by all classes of proteins identified in the surfaceome of *S. pneumoniae* (Table 1). The pneumococcal surfaceome, i.e. proteins decorating the cell wall, is usually divided into three main kinds of classical surface-exposed proteins. The first class comprises pneumococcal surface proteins that are covalently linked to the bacterial peptidoglycan via a transpeptidation reaction catalysed by sortases recognizing a conserved C-terminal pentapeptide motif, LPxTG or LPxTG-like (as for pilin proteins). The second class comprises the family of non-covalently anchored choline-binding proteins, which bind to phosphorylcholine ChoP (a pneumococcal cell wall constituent) and to lipid-anchored teichoic acids. The third class is represented by lipoproteins that are embedded in the phospholipid bilayer by linkage of a diacylglycerol moiety to an indispensable cysteine residue within the lipoprotein. But in addition to the classical surfaceome, the cell wall of *S. pneumoniae* is also decorated with an unknown number of proteins that do not possess classical features of bacterial surface proteins. These are, for example, the pneumococcal adherence and virulence factor A (PavA), and at least two glycolytic enzymes, namely the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and the α-enolase (Berg-

mann & Hammerschmidt, 2006; Hammerschmidt, 2007).

The majority of these pneumococcal surface-associated proteins and MSCRAMMs are encoded by genes belonging to the core genome. Nevertheless, distinct clonal lineages of *S. pneumoniae* acquired additional genes organized in so-called pathogenicity islets or islands (PIs) (Dagerhamn *et al.*, 2008; Blomberg *et al.*, 2009). The genes of this flexible and dispensable genome encode also virulence determinants in a subpopulation of pneumococcal clinical isolates. The precise distribution and their impact on colonization and invasive pneumococcal disease remain elusive. The *pit2* locus, encoding for an iron uptake ABC transporter implicated in pulmonary and systemic infection, was most likely the first PI described in *S. pneumoniae* (Brown *et al.*, 2001). Consecutive studies have revealed further PI-encoded genes involved in transcriptional regulation, pilus formation (PI-1 and PI-2), protein secretion, translocation and surface-anchoring, capsule biosynthesis, and virulence (Nobbs *et al.*, 2009; Gámez & Hammerschmidt, 2012).

Interestingly, and depending on the molecular players, pneumococci are able to adhere to human respiratory cells, either directly by interacting with surface receptors or indirectly by binding ECM components (Figs 1 and 2). The interaction of pathogens with components of the ECM is a typical feature of gram-positive pathogens and has been studied in detail for group A streptococci and staphylococci (Talay, 2005; Nobbs *et al.*, 2009). However, gram-negative bacteria that also reside in the human respiratory system (such as *H. influenzae* and *N. meningitidis*) engage the ECM to facilitate adherence, strikingly, in a similar way to pneumococci (Singh *et al.*, 2010; Hallstrom *et al.*, 2011). In specific cases, *S. pneumoniae* exploits ECM proteins as molecular colonization bridges to link itself to integrins and, hence, to manipulate the host cell signal transduction cascades for internalization.

PNEUMOCOCCAL MSCRAMMs RECOGNIZING CELLULAR RECEPTORS FOR DIRECT ADHESION TO RESPIRATORY EPITHELIAL CELLS

Colonization of the respiratory tract by pneumococci is in particular mediated by the unique interaction of the pneumococcal surface protein C, PspC (also

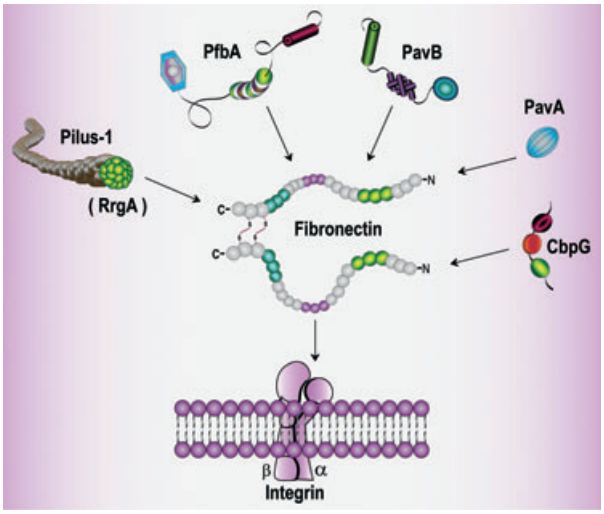

Figure 1 Pneumococcal microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) binding to fibronectin for adhesion. Five pneumococcal fibronectin-binding proteins encoded by genes of the core genome (PavA, PavB, PfbA and CbpG) or accessory genome (RrgA of Pilus-1) are shown here. Shapes and colors are just illustrative, but modular structures are depicted for each pneumococcal protein: PavA is represented as a whole soluble protein, for PavB and PfbA, the transmembrane domain (including the LPxTG motif), the fibronectin-binding region (SSURE domain for PavB) and the N-terminal region are shown, for CbpG, the choline-binding domain, the flexible linker region and the individual biological active domain are also shown, and for Pilus-1, a backbone structure made of RrgB and a tip of RrgA, which is the adhesin of Pilus-1, are depicted. Fibronectin is represented as a dimer made of two polypeptide chains cross-linked by disulfide bonds. Binding sites of fibronectin for collagen and glycosaminoglycans are shown in yellow and green, respectively. Purple depicts a different site on the fibronectin molecule, which is recognized by cell surface receptors (integrins) and therefore responsible for attachment to the host cells.

referred to as CbpA and SpsA), with the secretory component of the human polymeric immunoglobulin receptor (hpIgR). The secretory component represents the ectodomain of hpIgR which is expressed on respiratory epithelial cells. A highly conserved hexapeptide motif, which occurs once in each R domain of the mature N-terminal part of PspC, binds in a human-specific manner to the ectodomains D3 and D4 of the pIgR. Usually, pIgR transports IgA-bound immune complexes and probably also antigens and pathogens from the basolateral to the apical surface of an epithelial layer for safe disposal. Strikingly, pneumococci engage unloaded human pIgR for adherence to and transcytosis across mucosal epithelial cells (Hammerschmidt, 2006).

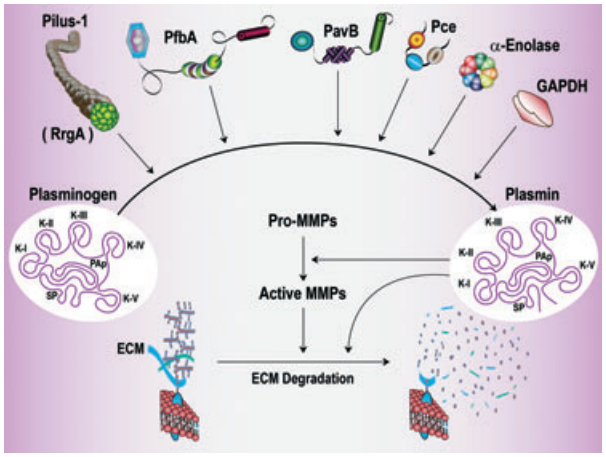

Figure 2 Pneumococcal microbial surface components recognizing adhesive matrix molecules MSCRAMMs as plasminogen activators for extracellular matrix (ECM) degradation. Six plasmin(ogen) binding proteins of *Streptococcus pneumoniae* recruiting host proteolytic activity to the bacterial cell surface and so facilitating degradation of ECM and dissolution of fibrin clots are shown here. Shapes and colors are just illustrative for each pneumococcal protein. GAPDH is represented as a soluble dimer and α-enolase is depicted as an octamer, composed of four homodimers. For Pce, the choline-binding domain, the flexible linker region and its globular N-terminal module are shown. Circulating plasmin(ogen) consists of five kringle (K-I to K-V) domains, a serine protease (SP) domain, and a Pan-apple (PAp) domain. Plasminogen is activated into plasmin upon proteolytic cleavage of the peptide bond between amino acid residues R561 and V562. Plasmin is then able to activate pro-matrix metalloproteinases (Pro-MMPs) and also to cause ECM degradation by itself.

Interestingly, PspC directly binds to an integrin receptor for laminin, present on the vascular endothelium of the blood–brain barrier, contributing to the development of pneumococcal meningitis, and probably other invasive infections due to the ubiquitous expression of the laminin receptor (Orihuela *et al.*, 2009). In addition to PspC, the substrate-binding lipoprotein for an ABC manganese transporter, PsaA, has been shown to interact with the transmembrane glycoprotein E-cadherin of epithelial cells. In this regard it remains open how pneumococci exploit the E-cadherin for colonization or invasive pneumococcal disease, although the PsaA–E-cadherin interaction induces downstream signaling effects (Anderton *et al.*, 2007). Recently, pneumococcal serine-rich protein PsrP was shown to mediate attachment of *S. pneumoniae* to keratin-10 on human lung epithelium, to be required for pneumococcal persistence in the lungs and proved to be relevant to pneumococcal aggregation *in vivo* and biofilm formation (Shivshankar *et al.*, 2009). However, PsrP is dispensable for pneumococcal colonization of the nasopharynx and replication in the blood during bacteremia, which accounts for the tissue tropism of PsrP (Sanchez *et al.*, 2010). Finally, RlrA-regulated gene A (RrgA), the tip protein of the pilus-1 whose subunits are all encoded by accessory genes located in the pilus-islet-1, was discovered as a pneumococcal adhesin involved in the first steps of nasopharyngeal carriage (Nelson *et al.*, 2007a).

PNEUMOCOCCAL MSCRAMMs RECRUITING ECM PROTEINS AS MOLECULAR BRIDGES FOR INDIRECT INTERACTIONS WITH HOST EPITHELIAL AND ENDOTHELIAL CELLS

The ECM is composed of collagens and non-collagenous glycoproteins showing a tremendous diversity because of multiple isoforms generated by the expression of homologous genes or alternative splicing of their respective pre-mRNA, proteoglycans, associated proteins and (pro-)enzymes, e.g. plasminogen (PLG) and matrix-metalloproteases. Fibronectins (FNs) and the 75-kDa glycoprotein vitronectin (VN) are prominent ECM proteins. A key feature of pneumococci seems to be the engagement of components of the extracellular matrix to achieve a stable and efficient colonization of the human respiratory tract. Through binding to ECM proteins pneumococci indirectly exploit integrins on human host cells. Many of the ECM molecules possess the tripeptide sequence arginine-glycine-aspartate (Arg-Gly-Asp, RGD) which is the binding site for integrins. Consequently, ECM proteins can function as a molecular bridge and link the pathogen with a cellular receptor, namely integrins. Integrins are highly conserved, ubiquitously expressed heterodimeric glycoproteins. In humans, 19 distinct α-subunits and eight different β-subunits are known, which associate non-covalently to form at least 25 heterodimer pairs with diverse but often redundant ligand-binding specificities (Hynes, 2002). Integrins provide a dynamic, physical link between the ECM and the intracellular actin cytoskeleton. Both VN and FNs were shown to bind to at least five or ten different integrin receptors, respectively, e.g. $\alpha_v\beta_3$-integrin and/or $\alpha_5\beta_1$-integrin (Podgorski & Sloane, 2006; Aziz-Seible & Casey, 2011). As integrins become activated upon ligand binding,

which may result in cytoskeleton rearrangements and modulation of multiple downstream signaling pathways, pneumococci use FN, VN and Factor H for effective adhesion to host epithelial cells and also endothelial cells, and for subsequent internalization (Gámez & Hammerschmidt, 2012). Similar to FN and VN, the soluble serum molecule Factor H, a regulatory component of the complement cascade and composed of 20 short-consensus repeats, has an RGD peptide in its short-consensus repeat 4 allowing it to bind to integrins on phagocytes and non-professional phagocytes (Zipfel *et al.*, 2008; Agarwal *et al.*, 2010; Asmat *et al.*, 2012).

MOLECULAR BRIDGES FOR CELL ADHESION AND INTERNALIZATION

Fibronectins are a family of high-molecular-weight multidomain glycoproteins composed of two structurally almost identical subunits linked by two C-terminal disulfide bonds. The structural and functional variety is attributed to the arrangement of repeating modules within each subunit. Soluble dimeric FNs circulate in the blood plasma (plasma-FN, pFN), whereas unsoluble, multimeric FN fibrils constitute the ECM when FNs cross-link via intermolecular disulfide bonds (cellular FN, cFN) (Ruoslahti, 1988). Pneumococci interact with soluble FN from human plasma as well as with its fibrillar multimeric form (Talay, 2005; Jensch *et al.*, 2010). Remarkably, and in contrast to other streptococci, pneumococci recognize the C-terminal heparin-binding domain of FN (Schwarz-Linek *et al.*, 2006). PavA was the first FN-binding protein identified in *S. pneumoniae* and shares about 70% sequence identity with the FN-binding proteins FBP54 of *Streptococcus pyogenes* and FbpA of *Streptococcus gordonii* (Christie *et al.*, 2002; Talay, 2005). Interestingly, PavA lacks the typical FN-binding repeats described, e.g. for the FN-binding proteins of *S. pyogenes* (SfbI) and *Staphylococcus aureus* (FNBPA) (Bingham *et al.*, 2008; Marjenberg *et al.*, 2011; Norris *et al.*, 2011). It was shown that anti-PavA generated against the N-terminal part of the protein did not impede binding of pneumococci to immobilized FN confirming that the C-terminal domain of PavA recognizes FN (Table 1) (Pracht *et al.*, 2005). As PavA-deficient pneumococci retained approximately 50% of their FN-binding activity, it was assumed that pneumococci produced additional FN-binding proteins. In addition to the choline-binding protein CbpG and the LPxTG-anchored proteins RrgA and plasmin- and FN-binding protein A (PfbA), the pneumococcal adherence and virulence factor B (PavB) was identified as a pneumococcal adhesin for FN (Table 1) (Mann *et al.*, 2006; Hilleringmann *et al.*, 2008; Yamaguchi *et al.*, 2008; Izore *et al.*, 2010; Jensch *et al.*, 2010; Moschioni *et al.*, 2010). PavB is highly conserved among pneumococci and orthologous sequences to PavB were confirmed in *Streptococcus agalactiae*, *S. gordonii* and *Streptococcus mitis*, but not found in *Streptococcus mutans*, *Staphylococcus aureus*, enterococci, or other relevant human pathogens. The core-domain of PavB consists of repetitive sequences, the streptococcal surface repeats (SSURE), whose number of repeat units, each of approximately 150 amino acid residues, is variable, strain-specific but serotype-independent (Jedrzejas, 2007; Jensch *et al.*, 2010). PavB has been demonstrated to interact via its SSURE domain with FN and PLG. Importantly, the efficiency of binding to immobilized FN increased with the number of SSUREs. This observation correlates with the interaction model established for other FN-binding proteins assuming that repetitive units interact independently with the type repeats of the FN molecule and that a high-affinity binding is only achieved via the repeating units while individual interactions between single repeats have a low-affinity binding (Fig. 1).

The interaction of the human pathogenic gram-negative bacteria *H. influenzae*, *N. meningitidis* and *M. catarrhalis* with VN has been studied in detail and revealed different strategies for the use of VN for serum resistance (Singh *et al.*, 2010). Alternatively VN is used to cross-link the bacteria to cell barriers to facilitate their entry into protected niches of the human body. In contrast, little is known about streptococcal binding to VN. Vitronectin is an abundant plasma glycoprotein that, in its monomeric form, exists either as a single-chain protein or as a two-chain polypeptide joined by an intramolecular disulfide bond and regulates the complement, fibrinolytic and coagulation systems. However, in its multimeric form, VN assembles the ECM of many different tissues. Vitronectin consists of an N-terminal somatomedin-B domain, an RGD motif for cell attachment, four hemopexin-like domains and three heparin-binding regions. *Streptococcus pneumoniae* was shown to attach to cell-associated multimeric VN by

recognizing heparin-binding domain(s) within the VN molecule. This intimate interaction exploits the $\alpha_v\beta_3$-integrin receptor, intracellularly associated signaling molecules of the focal adhesion complex, e.g. integrin-linked kinase, protein kinase B (Akt) and phosphatidylinositol-3-kinase, and the actin cytoskeleton. Interestingly, upon actin cytoskeleton reorganization pneumococci are entrapped into a microspike-like structure before they are internalized by endocytosis (Bergmann *et al.*, 2009).

However, the adhesin that mediates binding of *S. pneumoniae* to VN has not yet been identified. Given the redundancy in ECM-binding capability of pneumococcal surface-exposed proteins, it is likely that at least one of them will reveal VN-binding activity. Interestingly, *S. pyogenes* produces an extracellular cysteine protease (streptococcal pyrogenic exotoxin B, SpeB), which was proved to recognize and cleave VN, and its natural variant, mSpeB2, contains an RGD motif linking the pathogen directly to integrins on human host cells. In addition, *S. pyogenes* was shown to bind to a hydrophobic pentapeptide motif within the hemopexin-type repeats of VN; however, a direct link to the VN-binding activity of SpeB remains elusive (Liang *et al.*, 1997; Stockbauer *et al.*, 1999).

In the past three decades the intriguing interaction of human complement regulator Factor H with pathogenic bacteria has been elaborated. In contrast to gram-negative pathogens, which recruit Factor H to their surface mainly to prevent host complement-mediated lysis, *S. pneumoniae* deploys Factor H also to become internalized into human cells (Zipfel *et al.*, 2008; Agarwal *et al.*, 2010). This was emphasized by a recent work demonstrating the exclusive impact of PspC on the interplay with Factor H (and secretory component) using non-pathogenic, gram-positive *Lactococcus lactis* as heterologous expression system (Asmat *et al.*, 2012). Hence, Factor H bound to *S. pneumoniae* enables cell invasion in a two-step mechanism. An N-terminal 121-amino-acid-long stretch within PspC binds to the short consensus repeats 8–11 of Factor H, which orientates the Factor H molecule for the interaction with host cell glycosaminoglycans (Table 1). This facilitates the engagement of the CD11b/CD18-integrin on human polymorphonuclear cells and yet unidentified integrin receptor(s) expressed on tissue barrier cells, respectively, mediated via an RGD motif in short-consensus repeat 4. The subsequent invasion of Factor-H-coated pneumococci strongly depends on kinase activities of focal adhesion signaling molecules and reorganization of the host cell cytoskeleton.

MOLECULAR BRIDGES FOR MATRIX DEGRADATION AND BARRIER TRANSLOCATION

As an extracellular pathogen, the capacity of *S. pneumoniae* to invade tissues seems to be relatively poor. However the co-colonization with other microorganisms, activated innate immune responses, immune deficiencies caused by viral infections, and host barrier dysfunctions, respectively, may favor *S. pneumoniae* encountering normally sterile niches in the human body, e.g. the lungs, blood, or central nervous system. The ability of pneumococci to intervene in the host PLG/plasmin system by tethering GAPDH and α-enolase to the bacterial surface facilitates degradation of the ECM and basement membrane components (Table 1). The enzymatically active serine protease plasmin, either directly recruited or converted from PLG by host-derived PLG activators (PAs), tissue-type PA or urokinase PA, degrades important ECM components such as FN, VN, fibrinogen and laminin. To date, the mechanism and the structural basis for the interaction of pneumococcal GAPDH with plasmin(ogen) have not been investigated in detail, but binding of PLG to lysyl residues results in a conformational change of PLG, which renders the molecule more susceptible to activation. Indeed, GAPDH contains two C-terminal lysine residues separated by an isoleucine and alanine (KIAK) which have been suggested to be involved in the recognition of at least one of the lysine-binding sites in the Kringle domains of plasmin/PLG. The KIAK motif is supposed to be a common streptococcal binding pattern owing to the remarkable similarities between pneumococcal GAPDH and other streptococcal surface-exposed GAPDHs (Bergmann & Hammerschmidt, 2007). Interestingly, GapA of *Bacillus anthracis*, which shares 73% sequence similarity with streptococcal GAPDH, possesses only a single C-terminal lysyl residue but displays PLG-binding with comparable affinities (Matta *et al.*, 2010). α-Enolase of *S. pneumoniae* was identified and characterized as another surface-displayed protein, which binds to the Kringle domains of plasmin(ogen) and is implicated in pneumococcal virulence. Pneumococcal α-enolase is

Table 1 Pneumococcal microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) and their human extracellular matrix (ECM) targets

Pneumococcal MSCRAMM (description/key domain)	ECM target	Pathogenic role; target cells, tissue	Reference(s)
LPxTG/LPxTG-like proteins			
PavB (Pneumococcal adherence and virulence factor B/SSURE domain)	FN, Plasmin(ogen)	Adherence, internalization; nasopharynx, lung	Jensch *et al.* (2010)
PfbA (Plasmin- and fibronectin-binding protein A)	FN, Plasmin(ogen)	Adherence; lung	Yamaguchi *et al.* (2008)
RrgA (Rlr-regulated gene A)	FN, Collagen I, Laminin	Adherence; n.d.	Hilleringmann *et al.* (2008); Izore *et al.* (2010); Moschioni *et al.* (2010)
Choline-binding proteins			
PspC (Pneumococcal surface protein C, CbpA, SpsA/N-terminal 121-aa peptide)	Factor H	Adherence, internalization; nasopharynx, lung, vascular endothelium, PMNs	Agarwal *et al.* (2010)
Pce (Phosphorylcholine esterase, CbpE)	Plasmin(ogen)	ECM transmigration; n.d.	Attali *et al.* (2008a,b)
CbpG (Choline-binding protein G)	FN	ECM degradation; n.d.	Mann *et al.* (2006)
Non-classical surface proteins			
PavA (Pneumococcal adherence and virulence factor A/C-terminal 42kD peptide)	FN	Adherence; n.d.	Pracht *et al.* (2005)
α-Enolase (^{248}FYDKERKVY^{256} and ^{433}KK^{434})	Plasmin(ogen)	Adherence, ECM degradation, ECM transmigration; lung	Bergmann & Hammerschmidt (2007)
GAPDH (Glyceraldehyde-3-phosphate dehydrogenase/VRTLEYFA^{332}KIAK^{335})	Plasmin(ogen)	n.d.	Bergmann & Hammerschmidt (2007)

PMN, polymorphonuclear cells; n.d., not determined.

composed of a tetramer of dimers. Each monomer harbors two PLG-binding sites: two adjacent lysines in the C-terminus and an internal nine-residue motif (FYDKERKVY), the latter significantly more exposed to the surface and indispensable for PLG-binding (Bergmann & Hammerschmidt, 2007). In contrast, both lysine-rich motifs of group A streptococcal surface enolase are found in proximity to each other and play a concerted role in PLG-acquisition (Cork *et al.*, 2009). Strikingly, enolases with naturally occurring variations of the internal PLG-binding motif present in oral streptococci fully retain their ability to recruit PLG (Itzek *et al.*, 2010). Referring to the classical pneumococcal surface proteins, the LPxTG-anchored proteins PfbA and PavB, respectively, and the choline-binding protein phosphorylcholine esterase (Pce, also known as CbpE) were also identified as PLG-binding proteins (Table 1) (Attali *et al.*, 2008a,b; Yamaguchi *et al.*, 2008; Jensch *et al.*, 2010). Importantly, acquisition of plasmin(ogen) to the pneumococcal surface not only promotes pneumococcal adherence and ECM degradation, plasmin-coated pneumococci were further shown to disrupt intercellular junctions of epithelia and endothelia, facilitating migration of *S. pneumoniae* through cell barriers by a pericellular route. This finding points to the redundancy of functional attitudes of pneumococcal surface-exposed factors involved in the exploitation of the host fibrinolytic system (Fig. 2).

CONCLUSIONS

Despite significant advances in treatment of and vaccination against pneumococci, infectious diseases caused by *S. pneumoniae* remain associated with high morbidity and mortality, especially among young children, the elderly and immuno-compromised adults. Pneumococci have evolved elaborate strategies to successfully breach tissue barriers and to survive in secured host niches. Adhesion of pneumococci to the human respiratory epithelium is the critical initial step in colonization, which requires the expression of surface-exposed proteins. These adhesins either directly recognize host cell receptors or bind to ECM components to indirectly cross-link to tissue barriers. The MSCRAMMs of *S. pneumoniae* target the host ECM proteins, e.g. FN, VN and Factor H, which bind to integrin receptors to facilitate pneumococcal colonization and internalization, or plasmin(ogen), which promotes

pneumococcal transmigration through the ECM and cell barriers for spreading into deeper, protected niches of the host. Hence, it is clear that the pneumococcal MSCRAMMs are crucial for the virulence potential of pneumococci and that this repertoire of molecules has a strong impact on their dissemination by facilitating adherence, internalization, acquisition of host-derived proteolytic activity or evasion of the immune defense mechanisms of the host. Whereas one-two decades ago no pneumococcal MSCRAMMs had been deciphered, our current knowledge about pneumococcal MSCRAMM–ECM interactions is increasing, which not only helps to obtain a sophisticated view on pneumococcal colonization and pathogenesis, but may also provide new starting points for treatment and vaccination approaches (Gámez & Hammerschmidt, 2012).

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