Mini-Review

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Versatility of pneumococcal surface proteins

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Surface-exposed proteins are key players during the infectious process of pathogenic bacteria. The cell surface of the Gram-positive human pathogen *Streptococcus pneumoniae* is decorated not only by typical Gram-positive surface proteins, but also by a family of proteins that recognizes the phosphorylcholine of the lipoteichoic and teichoic acids, namely the choline-binding proteins, and by non-classical surface proteins that lack a leader peptide and membrane-anchor motif. A comprehensive understanding of how microbial proteins subvert host immunity or host protein functions is a prerequisite for the development of novel therapeutic strategies to combat pneumococcal infections. This article reviews recent progress in the investigation of the versatility and sophistication of the virulence functions of surface-exposed pneumococcal proteins.

The power of combinatorial diversity

Streptococcus pneumoniae (the pneumococcus) is a commensal of the nasopharyngeal cavity, but under appropriate conditions, pneumococci cause serious and life-threatening infections, including pneumonia, septicaemia and meningitis (Cartwright, 2002). Pneumococci are encased by a capsular polysaccharide which has been recognized as *a sine qua non* of virulence. On the other hand, several studies have indicated that high amounts of capsular polysaccharide prevent attachment to host cells, probably by masking underlying virulence determinants. Interestingly, the amount of capsule is substantially reduced upon contact with epithelial cells (Hammerschmidt *et al.*, 2005). The role of the capsule in immune evasion and the impact of the virulence factor pneumolysin, which represents a cholesterol-dependent cytolysin, on pathogenesis are reviewed by Paterson & Mitchell (2006) in this issue. The pneumococcal outer cell wall is composed of peptidoglycan, teichoic (TA) and lipoteichoic acids (LTA), which differ only in their attachment to the pneumococcal cell wall, and phosphorylcholine (PCho). PCho is not only targeted by the choline-binding domain (CBD) of choline-binding proteins (CBP), but functions itself as an adhesin by recognizing the platelet-activating factor receptor (PAFr) of host cells (Cundell *et al.*, 1995).

Bioinformatic analysis of the genome of *S. pneumoniae* R6, the rough derivative of serotype 2 strain D39, predicts the presence of 153 proteins with a leader peptide. The leader peptide is recognized by complex secretion machineries known as translocons, and is required for protein traversal across the membranes. In contrast to strain R6, analysis of the sequence of the serotype 4 Norwegian clinical isolate TIGR4 suggests the existence of 256 proteins with a leader peptide (Tettelin & Hollingshead, 2004). Interestingly, TIGR4

contains two *secA* genes (*secA1* and *secA2*), whereas R6 and probably many other pneumococci contain only *secA1*. SecA is the main factor of the general secretory pathway, and the ATPase activity of this protein is the molecular motor of protein translocation across the membranes. SecA2 is also present in other Gram-positive bacteria, and has been characterized in *Streptococcus gordonii* strain M99, in which it is thought to be part of a specialized system for the transport of large serine-rich repeat proteins (Bensing & Sullam, 2002; Takamatsu *et al.*, 2004).

Three clusters of surface proteins can be distinguished by genome analysis: the lipoproteins (42 in R6 and 47 in TIGR4), the CBP family (10 in R6 and 15 in TIGR4), and proteins with an LPxTG motif (13 in R6 and 19 in TIGR4) that are covalently anchored in the cell wall after cleavage of the LPxTG sequence by a transpeptidase, designated a sortase (Fig. 1). Bioinformatic analysis of the pneumococcal genomes also indicates the presence of incomplete biosynthetic pathways, which is consistent with the inability of this pathogen to carry out respiratory metabolism, and also explains the high number of ATP binding cassette (ABC) transporters produced by *S. pneumoniae*. In addition to these predicted surface proteins, non-classical surface proteins that lack a classical leader peptide and membrane-anchoring motifs have been identified on the pneumococcal surface (Fig. 2). These proteins have received considerable attention for their contribution to the virulence of pneumococci and other pathogenic bacteria. Proteome analysis of the secretome and outer-membrane proteins of pneumococci is required to reveal the total composition of these protein fractions and the number of non-classical proteins translocated across the cell wall. To date, the mechanism of secretion and anchoring of these proteins, sometimes known as 'moonlighting' proteins, remains unknown for pneumococci and many other Gram-positive pathogens.

Fig. 1. Proportion of classical pneumococcal surface proteins based on the genome data for strain R6 (the nonencapsulated derivative of serotype 2 strain D39) (right-hand panel) and serotype 4 strain TIGR4 (Tettelin & Hollingshead, 2004) (left-hand panel). Surface-exposed proteins are distinguished according to their binding mechanism to the cell wall (lipoproteins and LPxTG proteins) or their non-covalent attachment to the cell wall via PCho. Bioinformatic analysis of TIGR4 predicts the presence of 256 proteins carrying a signal peptide for secretion, of which 81 contain classical anchoring motifs. In pneumococcal strain R6, 153 proteins are thought to be secreted, and of these, 65 exhibit classical anchoring motifs. Non-classical surface-exposed proteins are not considered. The numbers in parentheses show the number of proteins in each class.

Biological activities of unusually cell-wall-anchored choline-binding proteins

PCho is an unusual and physiologically important component of the pneumococcal cell wall. The pneumococcal PCho is recognized by components of the host innate immune system, such as C-reactive protein and the PAFr (Fig. 3; Cundell *et al.*, 1995). In addition, the amino alcohol PCho anchors a special class of pneumococcal proteins, the CBPs, non-covalently to the cell wall. CBPs have a modular organization and consist in general of a leader peptide, a biologically active N-terminal domain, and the conserved CBD that targets PCho. Pneumococci can produce 13 to 16 different CBPs, including four cell wall hydrolases that are important for virulence: the major autolysin LytA (*N*-acetylmuramoyl-L-alanine amidase), a β -*N*-acetylglucosamidase (LytB), a β -*N*-acetylmuramidase (LytC; lysozyme), and a

phosphorylcholine esterase. LytA has been characterized in detail, and recently the structural analysis of the CBD of LytA demonstrated that this module adopts a peculiar solenoid structure (López & García, 2004). LytB is highly expressed in the early exponential growth phase and has been shown to be important for cell separation of pneumococci. Loss of function of LytB or LytC significantly reduces the nasopharyngeal colonization of rats (Gosink *et al.*, 2000). In contrast to other CBPs, LytB and LytC possess the CBD as an N-terminal domain (López & García, 2004).

The phosphorylcholine esterase activity of the Pce protein (also referred to as CbpE) removes PCho from the cell wall and causes changes in colony phenotype (de las Rivas *et al.*, 2001; Vollmer & Tomasz, 2001). Recently, structural information for the catalytic domain (Garau *et al.*, 2005)

Fig. 2. Schematic model of the pneumococcal outer cell wall and surface-exposed proteins. The pneumococcal cell wall consists of a phospholipid membrane (LM), peptidoglycan (PG), and teichoic and lipoteichoic acids. An unusual component of the cell wall is phosphorylcholine (PCho), which anchors the choline-binding proteins (CBPs) non-covalently on the cell wall. Virulence proteins of the different classes of pneumococcal surface proteins are depicted.

Fig. 3. Interactions of pneumococcal surface proteins with host components of the mucosal cavity (A) and cellular receptors (B). (A) The ECM represents the first mechanical barrier for pneumococcal colonization. Colonization is facilitated by cleavage of bound IgA1 by the IgA1-protease and by the removal of terminal sialic acids by NanA. The glycolytic enzymes enolase and GAPDH bind human plasminogen (PLG), which is converted to the protease plasmin (PA) and promotes degradation of various ECM compounds. ZmpC activates MMP-9, which has collagenase activity and degrades the ECM. The non-classical surface-associated protein PavA has been shown to bind to immobilized fibronectin (Fn) and to modulate pneumococcal adherence. (B) Degradation of ECM is possibly a prerequisite for the intimate contact of pneumococci with cellular receptors. The major adhesin of pneumococci identified is PspC, which interacts with the ectodomain of the human polymeric immunoglobulin receptor (pIgR). PCho mediates pneumococcal adherence to stimulated cells via an interaction with upregulated PAFr. It is thought that the lipoprotein PsaA may also function as an adhesin, but the cellular receptor is not yet defined.

and for the complete Pce lacking only the C-terminal 85-residue tail (Hermoso *et al.*, 2005) has been reported. Pce belongs to the metallo- β -lactamase family, and structural information provides evidence that only PCho residues that are located at the end of the teichoic acid chains are accessible to the catalytic centre. The ability of Pce to modify the amount of PCho on the cell wall has been shown to be relevant for pneumococcal adherence to human cells and for nasopharyngeal colonization of rats (Gosink *et al.*, 2000). Strikingly, loss of function has also been shown to increase the virulence of pneumococci when inoculated into the peritoneum of mice (Vollmer & Tomasz, 2001). It is possible that the increased number of choline residues promotes interaction with the PAFr during infection. On the other hand, PCho binds the C-reactive protein, and it has been shown that higher amounts of PCho favour binding of the host protein. This might lead to enhanced phagocytosis of pneumococci. Moreover, Pce is involved in hydrolysis of the choline-containing platelet-activating factor. In conclusion, Pce may have a dual function and may favour both

colonization and invasive infection by modulating the amount of PCho on the pneumococcal cell wall.

A role in colonization has been suggested for CbpD and CbpG, which are thought to be serine proteases (Gosink *et al.*, 2000). Recent reports indicate that CbpD is a competence-stimulating-peptide-inducible protein, and a function as a murein hydrolase has been proposed. CbpD has been demonstrated to assist LytA in competence-induced cell lysis (Kausmally *et al.*, 2005). A further study has provided experimental evidence that the biological activity of CbpD is involved in the ability of competent bacteria to trigger the release of virulence factors from non-competent *S. pneumoniae* (Guiral *et al.*, 2005).

The highly variable pneumococcal surface protein A (PspA) is expressed by virtually all important clinical serotypes and has significant immune protective potential. Loss of function attenuates virulence and increases complement-receptor-mediated clearance of pneumococci (Ren *et al.*, 2004). PspA also binds the iron transporter lactoferrin

(Hammerschmidt *et al.*, 1999), but pneumococci are not able to utilize lactoferrin as an iron source. Lactoferrin plays an important role in innate immunity, and the iron-depleted form, apolactoferrin, is bacteriostatic or bactericidal for bacteria. Shaper *et al.* (2004) demonstrated that expression of PspA protects against the bactericidal effect of apolactoferrin. In the presence of antibodies recognizing PspA, this effect is abrogated, suggesting that binding of apolactoferrin to PspA blocks the bactericidal activity of apolactoferrin. Recently it has been shown that the phosphorylated VicR (YycF) response regulator of the two-component regulatory system (TCS) VicRK (YycF) binds to a region upstream of *pspA* and regulates expression of *pspA* (Ng *et al.*, 2005).

The pneumococcal surface protein C (PspC; also known as SpsA or CbpA) is a multifunctional CBP of pneumococci; PspC promotes uptake of pneumococci into nasopharyngeal epithelial cells and interferes with components of the innate immune system. PspC promotes pneumococcal adherence via a human-specific interaction with the ectodomain of the polymeric Ig receptor (Hammerschmidt *et al.*, 2000; Zhang *et al.*, 2000). The ectodomain of pIgR is also known as the secretory component (SC), and independent reports have indicated that the human specificity of the PspC–SC interaction is determined by amino acid differences in ectodomains D3 and D4 of the SC (Elm *et al.*, 2004; Lu *et al.*, 2003). Despite such human specificity, loss of function in PspC has been shown to reduce colonization of infant rats (Rosenow *et al.*, 1997) and pIgR knockout mice (Zhang *et al.*, 2000). The hexameric SC-binding site in PspC is located in N-terminal repeated domains (Hammerschmidt *et al.*, 2000), recently designated R1 and R2. Structural analysis of R1 (amino acids 175–285) and R2 (amino acids 327–442) of PspC derived from TIGR4 has demonstrated that the R domains adopt an unusual and simple structure composed of three α -helices. Bundling of the helices through α -helix– α -helix interactions results in a flat, raft-like structure in which the residues YPT of the minimal SC-binding motif are located in a loop between helix 1 and helix 2 and form a ‘tyrosine fork’ structure (Luo *et al.*, 2005).

PspC molecules are divided into different groups. Interestingly, both the classical PspC proteins containing a CBD and the PspC-like Hic (PspC 11.4) bind the complement factor H (Janulczyk *et al.*, 2000). Hic is produced predominantly by serotype 3 pneumococcal strains, negative for SC binding, and contains an LPxTG motif that anchors the protein in a sortase-dependent manner to the peptidoglycan backbone of the cell wall. Factor H is a fluid-phase regulator of the alternative complement pathway, and consists of 20 short consensus repeats (SCRs). Hic interacts with the SCRs 8–11 and 12–14 of factor H (Jarva *et al.*, 2004), whereas a role for the SCRs 6–10 and 13–15 of factor H has been suggested in the interaction with PspC (Dave *et al.*, 2004; Duthy *et al.*, 2002). Recruitment of factor H by Hic has been shown to efficiently prevent activation of C3b and complement-mediated opsonophagocytosis of

pneumococci (Jarva *et al.*, 2004). The improved survival of pneumococci expressing PspC or Hic in a systemic mouse infection model provides further evidence for the versatility and importance of PspC in different host niches (Iannelli *et al.*, 2004; Quin *et al.*, 2005).

The ubiquitous mechanism of Gram-positive protein anchoring to the cell wall peptidoglycan

Sortase is a transpeptidase that recognizes the LPxTG motif of classical Gram-positive surface proteins and anchors these proteins covalently to the cell wall peptidoglycan. In strain R6 (the nonencapsulated derivative of serotype 2 strain D39), sequence analysis has revealed a single gene that codes for a sortase, whereas four genes (*srtA*, *srtB*, *srtC* and *srtD*) are predicted for the TIGR4 strain. The *sortase* of R6 and *srtA* of TIGR4 show 99 % identity, and both genes are located downstream of the DNA gyrase-encoding gene (*spr1099* and *SP1219*, respectively). The genes *srtB*, *srtC* and *srtD* are sequentially organized at a different gene locus. The presence of up to four genes that code for a transpeptidase is interesting, because only a minority of pneumococcal surface proteins are processed by a sortase (Fig. 2). Kharat & Tomasz (2003) have provided evidence that the sortase A processes functionally different surface-exposed pneumococcal proteins, such as the β -galactosidase and neuraminidase A. Loss of function in *SrtA* in a type 3 encapsulated derivative of R36A (R6 is a derivative of R36A) has not been associated with impaired virulence in a mouse intraperitoneal model of infection, but has been shown to decrease significantly adherence of R36A or R6 to nasopharyngeal cells (Kharat & Tomasz, 2003). In serotype 4 strain TIGR4, the *srtD* knockout significantly impairs pneumococcal pneumonia (Hava & Camilli, 2002). Recently, Paterson & Mitchell (2005) have indicated in *in vivo* studies the contribution of *SrtA* in pneumococcal pneumonia and bacteraemia. In conclusion, it seems that different sortases process functionally different surface-exposed proteins and have, therefore, different impacts on pneumococcal adherence and virulence.

Pneumococci contain at least two genes, *nanA* and *nanB*, that encode neuraminidases, and virtually all strains produce a neuraminidase. A third putative neuraminidase, *nanC*, remains to be characterized. These exoglycosidases cleave terminal sialic acid (*N*-acetylneuraminic acid) from glycolipids, glycoproteins and oligosaccharides on host cell surfaces and in host body fluids. NanA and NanB are secreted via the general secretory pathway, but only the C-terminus of NanA contains the LPxTG anchoring motif. Loss of NanA has been shown to impair pneumococcal persistence in the nasopharynx and middle ear in a chinchilla infection model (Tong *et al.*, 2002). In contrast, the *nanA* knockout is not attenuated in an intraperitoneal infection model (Berry & Paton, 2000). The importance of NanA for colonization has been underlined by its ability to afford protection against nasopharyngeal colonization and otitis media in chinchilla models (Long *et al.*, 2004). Recently, two reports have demonstrated the precise

function of NanA during pneumococcal colonization and pathogenesis. Firstly, neuraminidase has been shown to cleave the terminal sialic acids of lipooligosaccharides from *Haemophilus influenzae* and *Neisseria meningitidis* (Shakhnovich *et al.*, 2002). Because terminal sialic acids protect these respiratory pathogens against complement-mediated phagocytosis, desialylation of competitors may provide an advantage during colonization of host niches. Secondly, NanA has been shown to be implicated in desialylation of human proteins exhibiting sialic acid, including the secretory component, lactoferrin and IgA2 (King *et al.*, 2004). These human proteins are pneumococcal host targets, and the removal of sialic acid may facilitate bacterial persistence in the respiratory tract. *nanA* gene diversity, which occurs by recombination events and is restricted to regions in *nanA* that are not required for enzymic activity, has been suggested to provide an important advantage in evading the adaptive immune response (King *et al.*, 2005).

The hyaluronate lyase (Hyl), which primarily hydrolyses hyaluronan, is a four-domain enzyme consisting of an N-terminal carbohydrate domain, followed by a spacer domain, the helical barrel-like catalytic domain, and finally the C-terminal domain (Rigden & Jedrzejak, 2003). *hyl* knockout mutants are attenuated in an intraperitoneal mouse infection model (Chapuy-Regaud *et al.*, 2003), but the precise function of Hyl during pathogenesis has yet to be clarified.

Armed with proteolytic activity

Pneumococci produce several proteases that are surface-exposed and implicated in pneumococcal virulence. The HtrA (high-temperature requirement A) protease functions in a temperature-dependent manner as a molecular chaperone or heat-shock-induced serine protease, and is regulated by the CiaRH TCS. HtrA has been shown to be implicated in resistance to oxidative stress, in the colonization of the nasopharynx of rats and in pneumococcal pneumonia. Moreover, *htrA* knockouts induce lower levels of inflammatory cytokines IL-6 and TNF- α in the lungs during pneumonia compared to the isogenic wild-type D39 (Sebert *et al.*, 2002; Mascher *et al.*, 2003; Ibrahim *et al.*, 2004).

S. pneumoniae strains produce up to four zinc metalloproteases, including IgA1-protease, ZmpB, ZmpC and ZmpD, which are anchored to the cell wall by an N-terminal LPxTG motif. The IgA1-protease is produced by virtually all pneumococci, and large-scale signature-tagged mutagenesis (STM) experiments have indicated the importance of the IgA-protease in pneumococcal lung infections and bacteraemia (Polissi *et al.*, 1998). Recently, Weiser *et al.* (2003) have demonstrated that cleavage of surface-bound serotype-specific IgA1 by the IgA1-protease markedly enhances adherence of pneumococci to host cells. It is assumed that bound Fab fragments neutralize the negatively charged capsule and negate the anti-adhesive effects of the capsule. ZmpC has been characterized in TIGR4 as a bacterial

protease cleaving human matrix metalloproteinase 9 (MMP-9), and inactivation of the *zmpC* gene in serotype 19F impairs virulence in a pneumonia mouse model (Oggioni *et al.*, 2003). MMP-9 is involved in matrix degradation and opening of the blood-brain barrier; therefore, cleavage of the MMP-9 proenzyme to active MMP-9 may promote disease. Intranasal infection experiments confirm the significant contribution of IgA1-protease and ZmpB to pneumococcal virulence (Blue *et al.*, 2003; Chiavolini *et al.*, 2003). PrtA is another surface-exposed serine protease, and *prtA* knockouts have been shown to be attenuated in an intraperitoneal mouse infection model (Bethe *et al.*, 2001). It seems obvious that the different proteases may elicit their function at different stages of the pneumococcal infection. To date, which of the proteases act in combination to enhance the virulence of *S. pneumoniae* remains unclear.

Lipoproteins: implications in substrate transport and virulence

Pneumococcal lipoproteins, including peptide permeases, have been shown to be essential for substrate transport and bacterial fitness. The pneumococcal surface adhesin A (PsaA) is the substrate-binding lipoprotein of an ABC-type manganese-transport system (Dintilhac *et al.*, 1997). Mutations in *psaA* cause pleiotropic effects, including reduced adherence of pneumococci to host cells, attenuation in an intranasal and intraperitoneal mouse infection model, and increased sensitivity to oxidative stress (Marra *et al.*, 2002; Tseng *et al.*, 2002). Since the detection of these pleiotropic effects, it has been assumed that PsaA does not itself function as an adhesin; however, a recent report has demonstrated that antibodies against PsaA reduce the adherence of pneumococci to nasopharyngeal epithelial cells (Romero-Steiner *et al.*, 2003). This is consistent with the finding that mucosal immunization of mice with PsaA is highly protective against pneumococcal carriage (Johnson *et al.*, 2002). Differential fluorescence induction (DIF) analysis has demonstrated the upregulation of the *psa* promoter of the *psaBCA* operon during lung infections, and microarray analysis has demonstrated that *psaA* is upregulated during attachment of pneumococci to nasopharyngeal cells (Marra *et al.*, 2002; Orihuela *et al.*, 2004).

PiaA and PiuA are lipoprotein components of two separate iron-uptake ABC transporters, and have been shown to be required for full pneumococcal virulence (Brown *et al.*, 2001). Immunization with PiaA and PiuA elicits protective antibodies that recognize these surface-exposed proteins, and recently these antibodies have been demonstrated to promote bacterial opsonophagocytosis rather than inhibit iron transport (Jomaa *et al.*, 2005).

Pneumococci produce two conserved surface-exposed lipoproteins belonging to a family of chaperones, the peptidyl-prolyl isomerases (PPIases), which are thought to be involved in secretion and activation of cell surface molecules. PpmA (putative proteinase maturation protein A) shares homology with members of the parvulin family,

whereas SlrA shares homology with members of the cyclophilin family. Both lipoproteins have been shown to be immunogenic (Adrian *et al.*, 2004). Involvement in virulence has been suggested for PpmA. Overweg *et al.* (2000) demonstrated that mutation of *ppmA* of strain D39 increased the survival rate of mice. *SlrA* knockout mutants have been shown to be less efficient in the nasopharyngeal colonization of mice, and this has been attributed to the decreased ability of the knockout mutants to adhere to non-professional cells (Hermans *et al.*, 2005). Further investigations are required to demonstrate the potential of PpmA and SlrA as vaccine targets.

Moonlighting proteins: keeping the secret

The surface of *S. pneumoniae* and other micro-organisms is further decorated by a number of proteins that do not possess classical features of bacterial surface proteins. The PavA protein (pneumococcal adherence and virulence factor A) has been identified as a pneumococcal adhesin for fibronectin and as a crucial virulence determinant in pneumococcal infections. In a systemic and experimental mouse meningitis model of infection, the pneumococcal *pavA* knockout of strain D39 is substantially attenuated (Holmes *et al.*, 2001; Pracht *et al.*, 2005). The impact of PavA on pathogenesis has been independently indicated by STM experiments using a mouse pneumonia model of infection (Lau *et al.*, 2001). In addition, PavA is most likely involved indirectly and in a fibronectin-independent manner in pneumococcal adherence to host cells. However, the precise function of PavA has yet to be clarified, because deficiency in PavA does not affect the expression and function of known virulence factors (Pracht *et al.*, 2005).

S. pneumoniae acquires host proteolytic activity by binding plasmin(ogen), and interestingly, the glycolytic enzymes enolase and GAPDH have been identified as plasmin(ogen)-binding proteins displayed on the cell wall (Bergmann *et al.*, 2004). The moonlighting function of the enolase has been shown to potentiate degradation of the extracellular matrix (ECM), dissolution of fibrin, and pneumococcal transmigration (Bergmann *et al.*, 2005). The key pneumococcal binding site in enolase responsible for plasmin-mediated ECM degradation has been attributed to the nonameric peptide FYDKERKYYD (Bergmann *et al.*, 2003). Crystal structure analysis has indicated an octameric composition of the pneumococcal enolase and has depicted the plasminogen-binding site on the surface of the protein. In contrast, the C-terminal lysine residues, which have previously been suggested to be involved in plasminogen binding, are placed in an interdimer groove and have been predicted to be important for correct folding and for the maintenance of oligomerization (Ehinger *et al.*, 2004). The importance of enolase in virulence has been demonstrated in intranasal infection of mice. Enolase mutants with functionally inactive plasminogen-binding sites are significantly attenuated compared to the isogenic D39 parental strain (Bergmann *et al.*, 2003).

Conclusions and perspectives

S. pneumoniae produces a broad variety of specialized surface proteins, and it is obvious that at least some of these are adapted to interact with host components during colonization or dissemination in the human host. In contrast to some Gram-negative pathogens, the virulence genes of *S. pneumoniae* are not generally clustered together. Whole-genome microarray analysis has depicted the differential expression of genes under invasive and non-invasive conditions, and cross-linking of the data with the results of STM and DIF screens provides an extended list of putative virulence factors (Ogunniyi *et al.*, 2002; Orihuela *et al.*, 2004; Marra *et al.*, 2002). These virulence factors can be regulated by one-component regulatory systems, including *luxS*, *rlrA*, *regM/R* and *mgrA*, or by one of the 14 TCSs that have been identified in *S. pneumoniae* (Hoskins *et al.*, 2001; Tettelin *et al.*, 2001). TCSs respond to environmental changes, and therefore mediate the adaptation of pneumococci to their different microenvironments. Throup *et al.* (2000) and other workers have demonstrated the impact of TCSs on pneumococcal virulence in a mouse infection model. Nevertheless, our current knowledge of the impact of surface-exposed proteins during specific stages of local and invasive infections caused by pneumococci is fragmentary. In this respect, the individual role of many surface and secreted proteins in pneumococcal virulence remains a mystery. The analysis of crystal structures of surface proteins, alone or in complex with host receptors, can provide detailed molecular insights into the function of these proteins. As virulence is a multifactorial process, a future challenge will be to further elucidate how virulence factors act in concert to determine disease outcome by, for example, sensing the host response. The results of such investigations may clarify the virulence potential of a particular strain and determine which minimal set of factors is required to cause disease.

ACKNOWLEDGEMENTS

We thank Mark J. Walker (School of Biological Sciences, University of Wollongong, Australia) for critical reading of the manuscript. The work of the group is supported by grants from the German Research Foundation (DFG-SFB 479 to S.H.) and the Federal Ministry of Education and Research (01KI0430 to S.H., Competence Network CAPNETZ). Our apologies in advance to the authors of primary articles whom we have failed to cite owing to space restrictions.

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