Pneumococcal virulence factors in community-acquired pneumonia

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Purpose of review
This manuscript reviews the recent literature related to new developments in the understanding of existing and novel virulence factors of the pneumococcus that are of potential importance in the development of novel preventive and therapeutic strategies.

Recent findings
The pneumococcal capsule and pneumolysin have long been recognized as being two of the most prominent virulence factors, with much recent research having revealed previously unrecognized mechanisms by which they contribute to the pathogenesis of infection. Although the pneumococcal capsule has been considered a *sine qua non* for virulence, the emergence of pathogenic nonencapsulated strains with newly recognized virulence determinants has also been described. Not unexpectedly, but of concern, nonencapsulated strains are unaffected by current pneumococcal vaccines. This, together with the finding of novel virulence factors, as well as new mechanisms of pathogenicity of established virulence determinants, underscores the resilience of the pneumococcus in confronting challenges in its environment, most importantly those posed by antibiotics and vaccines.

Summary
Recent advances in the understanding of pneumococcal virulence factors provide potential opportunities for the development of novel putative therapeutic or preventive strategies.

Keywords
capsule, nonencapsulated pneumococci, pneumococcus, pneumolysin, virulence factors

INTRODUCTION

The Global Burden of Disease Study [1] estimated the global, regional, and national morbidity, mortality, and etiology of lower respiratory tract infections (LRTIs) for 195 countries. It confirmed the high mortality from LRTIs (predominantly pneumonia and bronchiolitis) overall [2 377 697 (214 584–2 512 809) deaths, globally, during 2016], particularly affecting children younger than 5 years of age and the elderly over 70 years of age. It also confirmed that *Streptococcus pneumoniae* (pneumococcus) was the leading cause of LRTI morbidity and mortality globally, causing more deaths than those attributable to all of the other studied causes combined (*Haemophilus influenzae* type b, influenza, respiratory syncytial virus).

Much recent literature has focused on pneumococcal virulence factors and their roles in transmission, colonization, immune evasion, and invasion, all of which are key factors in the pathogenesis of infection [2▪,3▪]. The pneumococcus has a myriad of virulence factors, the most prominent of which are shown in Table 1. A better understanding of these virulence factors, their exact roles, and their mechanisms of interaction with the human host may allow the development of novel treatment or prevention strategies for this 'truly resilient foe' [2▪,3▪].

Successful bacterial pathogens are reliant on ongoing genotypic and phenotypic adaptability to outmaneuver and ensure survival within the host. Given this scenario, it is hardly surprising that novel pneumococcal virulence factors continue to be discovered, as well as ancillary roles for a number of those already known to be involved in disease pathogenesis.

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Curr Opin Pulm Med 2020, 26:222–231
DOI:10.1097/MCP.0000000000000674

KEY POINTS

- Globally, *Streptococcus pneumoniae* (pneumococcus) remains a common, formidable and adaptable human respiratory pathogen, because of its myriad of highly effective virulence factors.
- The ongoing discovery of novel virulence determinants, as well as previously unrecognized activities of established virulence factors, underscore the resilience of the pneumococcus.
- Notwithstanding the recognition of novel mechanisms of pathogenicity associated with the pneumococcal capsule, long considered a *sine qua non* for virulence of the microorganism, the emergence of pathogenic nonencapsulated strains is of concern.
- As with the capsule, current research continues to reveal novel mechanisms of pathogenicity associated with the pneumococcal pore-forming toxin, pneumolysin, itself recognized as a key virulence factor

Together with an update on the two most prominent pneumococcal virulence factors, namely the polysaccharide capsule and the pore-forming protein toxin, pneumolysin (PLY), it is these issues, which represent the primary thrusts of the current review.

NOVEL PNEUMOCOCCAL VIRULENCE FACTORS

PynA [4▪], UbK [5], and SpFakB3 are examples of novel, pneumococcal protein virulence factors [6▪].

PynA

PynA, known until recently as Spr1057, to which no known key function had been assigned, was recently reported by Ulrych *et al.*, to possess pyrimidine nucleotidase activity, hence the name PynA [4▪]. PynA enzymatically inactivates potentially mutagenic pyrimidine derivatives, such as 5-fluoro-2′-deoxyuridine [4▪]. The protective role of PynA is evident from observations that deletion of this enzyme increases the mutation rate 30-fold, thereby confirming the significant survival benefit of PynA on the pneumococcus [4▪].

UbK

Pelletier *et al.* [5], recently reported that the pneumococcus expresses the ubiquitous bacterial kinase, UbK. This is a tyrosine kinase, which, as the name implies, is commonly found in bacteria, though its role may vary between genera and species. In the pneumococcus, UbK possesses a 'tyrosine-kinase activity and autophosphorylates on a unique tyrosine *in vivo*' [5]. Although the precise function of pneumococcal UbK remains to be established, disabling this enzyme resulted in severe defects of both cell growth and morphology, consistent with a possible role in cell-wall assembly [5].

SpFakB3

The fatty acid-binding protein known as SpFAKB3 is another recently documented pneumococcal protein with virulence/survival potential [6▪]. This protein, which is not found in *Staphylococcus aureus,* enables uptake of host polyunsaturated fatty acids by the pneumococcus. Together with two other fatty acid-binding proteins, namely, SpFakB1 and SpFakB2 (both present in *S. aureus*), which promote selective uptake of saturated and monounsaturated fatty acids, respectively, complementation by SpFakB3 enables the pneumococcus to utilize the entire range of host fatty acids for membrane synthesis at sites of infection, while conserving energy necessary for fatty acid synthesis [6▪].

A summary of the activities of these novel pneumococcal virulence factors is shown in Table 2.

NOVEL ACTIVITIES OF ESTABLISHED PNEUMOCOCCAL VIRULENCE FACTORS

Relative to the small group of novel pneumococcal virulence factors, those recognized virulence factors to which ancillary, potentially pathogenic activities have recently been assigned constitutes a somewhat larger group. These include pro-adhesive, pro-inflammatory, and immune-evasive activities.

Pro-adhesive activity

This group includes the iron-binding lipoprotein SPD_1609 [7▪] and the pilus-1 structural backbone protein, RrgB [8].

SPD_1609

Originally described as a conserved, iron-binding protein expressed in a range of Gram-positive bacteria, including the pneumococcus [7▪], recent studies have identified that deletion of the *spd_1609* gene in strain D39 of the pneumococcus resulted in decreased adherence to, and invasion of, the A549 human alveolar epithelial cell line [8]. Deletion of the *spd_1609* gene also resulted in decreased virulence of the pneumococcus in a murine bacteremia infection model [8]. These pro-adhesive activities of SPD_1609 appeared, however, to be achieved by an unidentified, indirect mechanism involving

Table 1. Major pneumococcal virulence factors

Virulence factor	Description	Function pathogenesis
CPS	Major surface antigen 98 structurally distinct serotypes	Prevents entrapment by mucus during colonization Inhibits opsonophagocytosis by preventing the interaction of iC3b and the Fc fragment of IgG bound to deeper bacterial surface structures with receptors on phagocytic cells
ChoP on teichoic acid	PAFR ligand	Binds PAFR on surface of epithelial and endothelial cells, facilitating adherence and invasion
lipopeptides, lipoteichoic acid and peptidoglycan fragments	Pathogen-associated molecular patterns	Promote inflammation
Ply	Pore-forming toxin TLR4 ligand	Cytotoxic and pro-apoptotic for a wide variety of host cells Activates classical complement pathway and depletes serum opsonic activity Highly pro-inflammatory at sub-lytic levels Activates TLR4, NLRP3 inflammasome, and p38-MAPK pathways
PspA	CBP	Limits C3 deposition on pneumococcal surface Protects against bactericidal effects of free lactoferrin
CbpA (also known as PspC)	CBP	Binds C3 and factor H and limits C3b deposition on pneumococcal surface Binds PIGR and laminin receptor through separate domains Facilitates adherence and invasion of respiratory epithelium and blood–brain barrier
LytA	CBP Autolysin	Digests cell wall Releases Ply and pro-inflammatory cell wall fragments Mediates capsule shedding during cellular invasion
CbpD	CBP Murein hydrolase	Mediates fratricide and release of extracellular DNA Promotes biofilm formation
CbpE (also known as Pce)	CBP Phosphorylcholine esterase	Decreases neutrophil activity by inactivation of host PAF Binds plasminogen
CbpG	CBP serine protease	Cell-attached form promotes adherence Extracellular form degrades fibronectin Important for mucosal and Invasive disease
CpbL	CBP	Binds collagen, elastin, and C-reactive protein Promotes dissemination from nasopharynx to lungs and blood by inhibiting phagocytosis
NanA	Neuraminidase A LPXTG	Cleaves terminal sialic acid from host mucin and cell surface glycoconjugates Unmasks receptors for adhesins Important role in otitis media Triggers TGF-β signalling to facilitate endothelial invasion
BgaA	β-Galactosidase LPXTG	Sequentially cleaves sugars from host glycoconjugates
StrH	β-*N*-acetylglucosaminidase LPXTG	Sequentially cleaves sugars from host glycoconjugates
EndoD	Endo-*N*-acetylglucosaminidase LPXTG	Sequentially cleaves sugars from host glycoconjugates
Hyl	Hyaluronate lyase LPXTG	Degrades extracellular matrix Facilitates tissue penetration
PrtA	Cell wall-associated serine protease LPXTG	Cleaves lactoferrin Possible adhesin
ZmpA (also known as IgA1 protease)	Zinc metalloprotease LPXTG	Cleaves human IgA1
ZmpB	Zinc metalloprotease LPXTG	Possible adhesin
ZmpC	Zinc metalloprotease LPXTG	Cleaves human matrix metalloproteinase 9
PepO	Endopeptide	Binds fibronectin and plasminogen Facilitates adherence and invasion Binds C1q to inhibit classical complement pathway

Table 1 (*Continued*)

Virulence factor	Description	Function pathogenesis
PsrP	Very large *O*-glycosylated serine-rich repeat protein LPXTG	Adhesin Binds to lung cells via keratin 10 Mediates bacterial aggregation and biofilm formation in lung tissue
RrgA, RrgB and RrgC	LPXTG proteins Structural components of pilus 1 Encoded by *rlr*A pathogenicity islet RrgA is tip adhesin	Adhesins Binds to a range of glycans Facilitate colonization and biofilm formation RrgA also binds PIGR and PECAM1 on endothelium of blood–brain barrier, which promotes brain invasion
PsaA	Lipoprotein Solute-binding component of Mn-specific ABC transporter	Mn uptake in host environment Essential for pneumococcal resistance to oxidative stress *in vivo*
AdcA and AdcAII	Lipoproteins Solute-binding component of a single Zn-specific ABC transporter	Zn acquisition *in vivo*
PiuA, PiaA, and PitA	Lipoproteins Solute-binding components of iron-specific ABC transporter	Fe acquisition *in vivo*
SlrA and PpmA	Lipoproteins Peptidyl-prolyl isomerases	Contribute to nasopharyngeal colonization
PhtA, PhtB, PhtD, and PhtE	Family of surface proteins with unusual His-triad motifs	May reduce C3 deposition on pneumococcal surface by binding factor H Putative adhesins Facilitate Zn acquisition together with AdcAII
PavA and PavB	Fibronectin-binding proteins NCSP	Adhere to host surfaces Important during sepsis and meningitis
Eno	Enolase NCSP	Binds and activates plasminogen Facilitates tissue invasion
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase NCSP	Binds and activates plasminogen Facilitates tissue invasion
SpxB	Pyruvate oxidase	Generates H_2O_2
GlpO	α-Glycerophosphate oxidase	Generates H_2O_2
SodA	Mn-dependent superoxide dismutase	Resistance to oxidative stress
Etrx1 and Etrx2	Surface-exposed thioredoxin-family lipoproteins	Resistance to oxidative stress
SpMsrAB2	Methionine sulfoxide reductase	Redox partner of Etrx1 and Etrx2

ABC, ATP-binding cassette; CBP, choline-binding protein; ChoP, phosphorylcholine; CPS, capsular polysaccharide; Etrx, surface-exposed thioredoxin family lipoprotein; Fe, iron; H_2O_2, hydrogen peroxide; iC3b, inactivated C3b; IgG, immunoglobulin G; LPXTG, sortase-anchored surface protein; MAPK, mitogen-activated protein kinase; Mn, manganese; NCSP, nonclassical surface protein lacking secretion signals or anchorage motifs; NLRP3, NACHT, LRR, and PYD domains-containing protein 3; PAF, platelet-activating factor; PAFR, PAF receptor; Pav, adherence and virulence protein; PECAM1, platelet endothelial cell adhesin molecule 1; PIGR, polymeric immunoglobulin receptor; Ply, pneumolysin; PpmA, foldase protein PrsA; PspA, pneumococcal surface protein A; Zn, zinc.
Reprinted with permission from Nature Reviews Microbiology (Wieser JN et al) [2▪].

increased expression of the pneumococcal adhesins, choline-binding protein A, and neuraminidase A [8].

RrgB

Although the pilus backbone protein of the pneumococcus, RrgB, has been proposed to function as a pneumococcal adhesin via interaction with components of the extracellular matrix, particularly collagen, the existence of this activity has remained contentious [9]. Recently, however, Becke *et al.* [9], using a combination of sophisticated analytical technologies, specifically atomic force microscopy-based single molecule force spectroscopy with lateral force microscopy, demonstrated that the pro-adhesive interaction of the RrgB protein with collagen-1 fibrils is strictly dependent on mechanical activation of RrgB. Under shear flow, but not equilibrium conditions, the D3 domain of RrgB serves as a 'molecular hook', anchoring the pilus protein via its D2 and D3 domains to collagen [9]. The authors

Table 2. Novel pneumococcal virulence factors

Virulence factor	Activity	Reference
PynA (Spr1057)	A pyrimidine nucleotidase that enzymatically inactivates potentially mutagenic pyrimidine derivatives	[4▪]
UbK (ubiquitous bacterial kinase)	A tyrosine kinase of unknown function, seemingly linked to cell-wall assembly	[5]
*Sp*FakB3	A fatty acid-binding protein, which promotes uptake of host polyunsaturated fatty acids at sites of infection	[6▪]

propose that this mechanism 'may contribute to pneumococcal virulence in shear force environments' [9].

Pro-inflammatory activity

Several DNA-associated proteins released during autolysis of pneumococci are reported to possess pro-inflammatory activity *in vitro* [10]. These proteins have been identified as: DNA chaperone factor, DnaK; the elongation factor Tu (EF-Tu, involved in the elongation phase of translation); and the glycolytic enzyme, glyceraldehyde-3-phosphate dehydrogenase [10]. These three proteins were found to activate the production of the pro-inflammatory cytokines, interleukin (IL)-6 and tumor necrosis factor-α on exposure to either isolated murine peritoneal macrophages or a human monocytic cell line (THP-1) via interaction with the Toll-like receptor (TLR), TLR4 [10]. The authors propose that these pro-inflammatory mechanisms may contribute to the pathogenesis of severe pneumococcal disease via exacerbation of inflammation-related tissue damage [10].

Immune-evasive activity

Recognized pneumococcal virulence factors to which ancillary immune-evasive activities have been attributed include plasmin-binding and fibronectin-binding protein (PfbA), pneumococcal surface protein A (PspA), and in the case of nonencapsulated strains of the pneumococcus (NESp), the oligopeptide-binding proteins AliC and AliD, as well as CbpAC, a variant of pneumococcal surface protein C (PspC).

PfbA

A novel immune-evasive activity has recently been described for the highly conserved pneumococcal adhesin, PfbA. This protein adhesin enables the pathogen to invade and take sanctuary in human erythrocytes [11]. More recently, Yamaguchi *et al.* [12▪] described that PfbA also interferes with phagocytosis and intracellular killing of the pneumococcus (TIGR4 strain) by isolated human blood neutrophils, as well as by the differentiated, neutrophil-like cell line HL-60. In addition, these investigators, using a hamster embryonic cell line (HEK293), transfected with the genes encoding the TLRs, TLR2 and TLR4 and those linked to activation of the transcription factor, nuclear factor kappa B (NFκB), observed that treatment of these cells with PfbA resulted in activation of NFκB [12▪]. The authors contend that blockade of TLR2, which promotes internalization of the pneumococcus, by PfbA, is the probable mechanism of inhibition of phagocytosis. This activity, in turn, may promote persistence of the pathogen, a contention supported by data derived from a murine sepsis model. In this model, infection with a PfbA-expressing strain of the pneumococcus resulted in significantly increased mortality relative to that observed with the wild-type, control strain [12▪].

PspA

Earlier studies reported that the pneumococcal endonuclease, endA, as well as the polysaccharide capsule, protected the pathogen against the trapping and/or bactericidal activity of neutrophil extracellular traps (NETs) [13,14]. More recently, a similar activity has been attributed to the major pneumococcal virulence factor, PspA, already known to protect the pathogen against complement activation by both the alternative and classical pathways [15▪▪]. In this recent study, decreased expression of PspA by the pneumococcus, as well as addition of an anti-PspA antibody to NETs, both increased NET-mediated trapping and killing of the pneumococcus [15▪▪]. With respect to possible mechanisms by which PspA protects the pneumococcus against NETs, the authors speculate that the anionic nature of the N-terminal region of PspA may repel the negative charge of the structural DNA lattice of NETs, preventing trapping of the pathogen [15▪▪].

AliC and AliD

Nonencapsulated *S. pneumoniae* (NESp) is recognized as an emerging human pathogen, which is not amenable to elimination by current capsular-targeted polysaccharide-vaccines. NESp also colonizes the nasopharynx and is mostly associated with noninvasive disease. However, little is known about the virulence mechanisms involved in colonization, as well as in the pathogenesis of mucosal and invasive disease. In this context, Bradshaw *et al.* [16▪▪] recently

identified the NESp oligopeptide-binding proteins, AliC and AliD, as well as a variant of pneumococcal surface protein C (CbpAC), as probable virulence factors. Using murine and chinchilla models of experimental colonization and invasive disease, expression of AliC and AliD augmented nasopharyngeal colonization, as well as development of pulmonary infection and otitis media [16▪▪]. Furthermore, using a whole blood model of pneumococcal killing, expression of AliC and AliD was associated with resistance of NESp to leukocyte bactericidal activity [16▪▪]. With respect to mechanisms of virulence, neither AliC nor AliD promoted adhesion to epithelial cells by NESp or biofilm formation. However, both of these oligopeptide-binding proteins, particularly AliD, significantly increased the expression of the potent pneumococcal immunosuppressive cytolysin, pneumolysin [16▪▪].

In the case of CbpAC, this virulence factor was essential in promoting adhesion of NESp to epithelial cells, while attenuating deposition of C3b on the surface of NESp. In addition, CbpAC bound nonspecifically to immunoglobulin A, possibly mimicking the polysaccharide capsule by forming a protective shield [16▪▪].

A summary of the activities of these ancillary pneumococcal virulence factors is shown in Table 3.

Although underscoring the resilience of the pneumococcus, the clinical significance of the aforementioned novel virulence factors, as well as the ancillary activities of previously recognized virulence factors, remains to be established. Accordingly, the remaining sections of this review focus on novel activities of two of the most prominent virulence factors of the pneumococcus in the immunopathogenesis of severe pneumococcal disease, namely, the polysaccharide capsule and the potent protein cytolysin, PLY.

PNEUMOCOCCAL CAPSULE

The pneumococcal capsule forms the outermost layer of the pneumococcus. It consists of polysaccharides, and varies in thickness, sometimes accounting for more than 40% of the volume of the pneumococcus, and is often considered the most important virulence factor of this organism [17▪▪,18]. Its main mechanism of action is interference with host opsonophagocytic clearance activity, although it also has a number of other activities that enable the pneumococcus to evade host defenses. In addition, the polysaccharide capsule determines the serotype of the organism and is the target for current pneumococcal vaccines [17▪▪]. Detailed reviews of

Table 3. Recognized pneumococcal virulence factors with novel ancillary virulence properties

Virulence factor	Primary virulence mechanism	Ancillary virulence mechanism	Reference
SPD_1609	Iron-binding protein	Indirect pro-adhesive activity involving increased expression of choline-binding protein A and neuraminidase A	[8]
RrgB (pilus backbone protein)	Major pilin subunit involved in adhesin and colonization	Complementary pro-adhesive activity mediated via binding of the D3 domain of RrgB to type 1 collagen under shear force conditions	[9]
DNA-associated proteins (DnaK, Tu and glyceraldehyde-3-phosphate dehydrogenase)	Roles in DNA chaperoning, elongation phase of translation, glycolysis	Pro-inflammatory activity via interaction with TLR4	[10]
PfbA (plasmin-binding and fibronectin-binding protein A)	Pro-adhesive, mediates invasion of erythrocytes	Interference with phagocytosis and intracellular killing of the pneumococcus via blockade of TLR2	[12▪]
PspA (pneumococcal surface protein A)	Protects against complement activation	Protects against entrapment and killing of the pneumococcus by neutrophil extracellular traps (NETs)	[15▪▪]
AliC and AliD	Oligopeptide-binding proteins, which are prominent surface proteins in nonencapsulated strains of the pneumococcus. In addition to substrate binding, they may compensate for loss of the polysaccharide capsule	Augment production of pneumolysin	[16▪▪]
CbpAC (variant of pneumococcal surface protein C, expressed by nonencapsulated strains of the pneumococcus)	PspC is a major adhesin of the pneumococcus	CbpAC is also pro-adhesive and counteracts deposition of C3b, as well as binding nonspecifically to immunoglobulin A	[16▪▪]

what is currently known about the capsule, its regulation and role in pathogenesis, as well as the mechanisms of capsule synthesis and the genetic basis for serotype differences, have been published elsewhere [17▪▪]. Accordingly, the current review focuses only on very recent advances in research involving the capsule.

Emerging novel capsular serotypes and mechanisms of capsule modification

The capsule is encoded by the *cps* locus and currently approximately 98 structurally and distinct capsular polysaccharide types have been described, although new serotypes are being discovered, especially from previously understudied regions of the world. In this context, Van Tonder *et al*. [19▪] recently analyzed the *cps* loci of more than 18 000 genomes from the Global Pneumococcal Sequencing (GPS) project, which identified nine putative novel *cps* loci (9X, 11X, 16X, 18X1, 18X2, 18X3, 29X, 33X, 36X) that could code for novel serotypes, which were found in ~2.6% of the genomes. These will undergo further biochemical and serological investigation, to determine if they do indeed encode novel polysaccharides.

In the case of capsule modification, Middleton *et al*. [20] investigated the effect of a recombinant, *Paenibacillus*-derived 3-specific glycosyl hydrolase, Pn3Pase, which targets the capsular polysaccharide of serotype 3 pneumococcus, one of the most virulent serotypes of the pneumococcus. Degradation of the capsule by Pn3Pase was associated with increased susceptibility of the bacterium to phagocytosis by macrophages and complement-mediated killing by neutrophils *in vitro*, and reduced nasopharyngeal colonization and development of sepsis in a mouse model *in vivo*. Although the authors considered that enzymatic hydrolysis of the capsular polysaccharides may represent a novel therapeutic approach to pneumococcal infections, acquisition of Pn3Pase by serotype 3 via horizontal transfer from commensal organisms may also enable the pathogen to evade capsule-targeted host defenses. Conversely, Nahm *et al*. [21▪] demonstrated that a nonvirulent oral streptococcal strain SK95, and a pneumococcal strain, D39, produced chemically identical capsular polysaccharides and that the transfer of *cps* loci from oral streptococcal strains to nonencapsulated, nonvirulent, pneumococci was associated with the development of virulence in those organisms, as assessed in a mouse model. Ye *et al*. [22▪], using pneumococcal *lytr* gene mutants, documented that pneumococcal LytR is involved in the attachment of both capsular polysaccharide and teichoic acid, both important for the virulence of the pneumococcus, to the cell wall of the microorganism.

Dennis *et al*. [23] isolated a high frequency of mucoid pneumococci, which expressed high levels of capsular polysaccharides, from the sputum of children with cystic fibrosis. These authors studied biofilm formation and maturation in mucoid and nonmucoid pneumococcal isolates from children with and without cystic fibrosis and found that while nonmucoid strains 19A and 19F had greater levels of biofilm initiation and adherence to epithelial cells, mucoid strains had significantly greater biofilm maturation. Furthermore, highly encapsulated mucoid phase variants of a serotype 3 pneumococcal strain predominated during late adherence and were more efficient in colonizing $CFTR^{-/-}$ mice than wild-type mice, indicating that over-expression of capsule may enhance the development of mature biofilms and pneumococcal colonization in patients with cystic fibrosis. Li and Zhang [24] have extensively reviewed phase variation or spontaneous reversible phenotypic variation in colony opacity, encapsulation, and pilus expression among pneumococci.

Novel insights into capsule biosynthesis

As part of its pathophysiology, *S. pneumoniae* is highly dependent on host nutrients, including purines, pyrimidines, carbon sources, amino acids, and other compounds. Much is known about the metabolism of the pneumococcus [25], which allows an understanding of how the microorganism adapts to the varying host environment when causing infection, and this understanding may lead to the development of potentially novel treatment strategies. Nakamaya *et al*. [26], had previously documented that deletion of *cadA*, a gene encoding lysine decarboxylase, an enzyme that catalyzes cadaverine synthesis, resulted in an attenuated pneumococcal phenotype, while in the current study, they showed that *cadA* deletion (*ΔcadA*) was associated with downregulation of genes encoding proteins involved in polyamine biosynthesis and transport, as well as reduced capsule production. These same authors later characterized the transcriptome and metabolome of *ΔcadA*, and identified specific mechanisms by which polyamines may regulate capsular polysaccharide synthesis [27]. The authors concluded in both these studies that as polyamine metabolism was required for capsule synthesis, it may represent a target for novel therapy.

Other investigators [28], studied the effects of various exogenous carbohydrate sources on capsule production and gene expression in several

pneumococcal serotypes. These authors also concluded that knowing the effects of different carbon sources on different pneumococcal serotypes may not only lead to a better understanding of pneumococcal disease pathogenesis but also to potential novel treatment strategies. In addition, Carvalho *et al*. [29] studied the interplay between capsule expression and uracil metabolism in *S. pneumoniae* strain D39 and proposed a model of how uracil may act as a signal for the production of different capsular amounts.

NONENCAPSULATED PNEUMOCOCCI

Thus, while the capsule has been considered to be essential for the virulence of clinical isolates of pneumococci, recent studies, as mentioned above, have indicated the emergence of NESp strains. The virulence of some of these strains appears to be related to genes located in the *cps* locus that encode novel proteins that compensate for the lack of capsule [30▪▪]. These novel proteins enhance both colonization of the host and virulence of the NESp during otitis media and pneumonia in animal models. Clearly, current pneumococcal vaccination strategies do not protect against NESp strains, and may in fact, be enhancing their emergence (together with nonvaccine serotypes) and would require novel vaccines for prevention. These strains may even have enhanced adherence for colonization as they do not have a capsule masking their surface adhesins. Mohale *et al*. [31▪] characterized (by capsular locus sequence analysis) nontypeable pneumococci (NTPn) associated with carriage and compared them with previously described invasive NTPn obtained from previous cross-sectional studies and laboratory-based surveillance studies, respectively. In that study, the NTPn represented 3.7% of carriage isolates and 0.1% of invasive isolates ($P < 0.001$), whereas 24% of individuals were co-colonized. Ninety-one percent of carriage NTPn had complete deletion of the capsular locus, whereas 9% had capsule genes, compared with 44 and 56% in invasive NTPn. The authors further identified 293 and 275 genes that were specifically associated with carriage and invasive NTPn. These, and other, differences in the carriage and invasive NTPn may explain their success in either colonization or in invasive disease, respectively.

PNEUMOLYSIN

Notwithstanding the well recognized mechanisms by which pneumolysin (PLY) contributes to the immunopathogenesis of invasive pneumococcal disease (IPD), recent insights have identified novel prothrombotic, as well as immunosuppressive activities, of the toxin.

Prothrombotic activities of pneumolysin

The prothrombotic potential of PLY is associated with the following:

(1) activation of human platelets *in vitro*, resulting in upregulation of the adhesion molecule, CD62P (P-selectin) [32];
(2) formation of neutrophil : platelet heterotypic aggregates via interaction of CD62P on platelets with its counter-receptor P-selectin glycoprotein ligand-1 (PSGL-1) on neutrophils [33];
(3) activation of NETosis [34].

All of these activities have the potential to promote intravascular occlusion, which may contribute to the pathogenesis of cardiovascular events, which occur commonly in patients with pneumococcal community-acquired pneumonia [35,36▪].

Immunosuppressive activity of pneumolysin

Subramanian *et al*. [37▪▪] have recently described a novel immunosuppressive activity of PLY mediated via binding of the toxin to human dendritic cells and murine macrophages via a cholesterol-independent mechanism. In this setting, PLY interacts with the mannose-receptor C type 1 (MRC-1), a receptor expressed predominantly on anti-inflammatory, M2-like alveolar macrophages, which promotes uptake of viral and microbial pathogens [37▪▪]. In this study, exposure of dendritic cells and macrophages to either recombinant PLY or PLY-expressing strains of the pneumococcus resulted in polarization of naïve T cells towards an immunosuppressive regulatory T-cell (Treg) phenotype. These effects were dependent on the D4 domain of the toxin and were not observed following exposure of the dendritic cells or macrophages to either a pneumolysoid, or to non-PLY-expressing strains of the pneumococcus. Disabling the expression of MRC-1 on dendritic cells also abolished the immunosuppressive effects of PLY. Intriguingly, PLY–MRC-1 interactions promoted internalization of the pneumococcus into the secure environment of nonlysozomal compartments, enabling intracellular persistence of the pathogen. The *in-vivo* significance of these findings was established in a murine model of experimental airways infection, using either animals in which the *MRC-1* gene had been deleted, or wild-type mice treated with MRC-1 antibodies. Both strategies resulted in decreased

bacterial loads in the airways [37▪▪]. The authors concluded that these novel insights into PLY–MRC-1 interactions have 'important implications for future vaccine design' [37▪▪].

CONCLUSION

This review adds to the array of virulence factors, and their mechanisms of action, that enable the pneumococcus to establish itself in the human host, evade host defenses, and ultimately cause mucosal and invasive disease. Ongoing research into mechanisms of pneumococcal competence is likely to reveal even more pathogenic mechanisms utilized by this formidable human pathogen.

Acknowledgements

None.

Financial support and sponsorship

C.F. is supported by the National Research Foundation of South Africa.

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ▪ of special interest
- ▪▪ of outstanding interest

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One of two good articles reviewing major virulence factors of the pneumococcus that allow successful interaction of the microorganism with the human host.

3. ▪ Brooks LRK, Mias GI. *Streptococcus pneumoniae's* virulence and host immunity: Aging, diagnostics, and prevention. Front Immunol 2018; 9:1366.

One of two good articles reviewing major virulence factors of the pneumococcus that allow successful interaction of the microorganism with the human host.

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This study has identified PynA as a novel, potential virulence factor of the pneumococcus.

5. Pelletier A, Freton C, Gallay C, *et al.* The tyrosine-autokinase UbK is required for proper cell growth and cell morphology of *Streptococcus pneumoniae*. Front Microbiol 2019; 10:1942.
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