***Streptococcus pneumoniae*: transmission, colonization, and invasion**

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**Abstract |** *Streptococcus pneumoniae* has a complex relationship with its obligate human host. On the one hand, the pneumococci are highly adapted commensals and their main reservoir on the mucosal surface of the upper airways of carriers enables transmission. On the other hand, they can cause severe disease when bacterial and host factors allow them to invade essentially sterile sites such as the middle ear spaces, lungs, bloodstream and meninges. Transmission, colonization, and invasion depend on the remarkable ability of *S. pneumoniae* to evade or take advantage of the host inflammatory and immune responses. The different stages of pneumococcal carriage and disease have been investigated in detail in animal models and, more recently, in experimental human infection. Furthermore, widespread vaccination and the resulting immune pressure have shed light on pneumococcal population dynamics and pathogenesis. Here, we review the mechanistic insights provided by these studies on the multiple and varied interactions of the pneumococcus and its host.

**[H1] Introduction**

*Streptococcus pneumoniae* (also known as pneumococcus) is a Gram-positive, extracellular, opportunistic pathogen, which colonizes the mucosal surfaces of the human upper respiratory tract (URT) **[G].** Up to 27-65% of children and <10% of adults are carriers of *S. pneumoniae*, which involves a commensal relationship between the bacterium and the host1,2. Local spread, aspiration or seeding to the bloodstream results in invasive, inflammatory diseases3 (**FIG. 1**). *S. pneumoniae* is a leading bacterial cause of a wide range of infections, including otitis media, community-acquired pneumonia **[G]**, sepsis and meningitis. As all of these diseases are “dead ends” in the lifecycle of the organism, the bacterial factors that cause invasive diseases must also be adaptive for colonization and/or transmission.

In 2017, the World Health Organization included *S. pneumoniae* as one of twelve priority pathogens. The continued high burden of disease and rising rates of resistance to penicillin and other antibiotics have renewed interest in prevention. The widespread use of pneumococcal conjugate vaccines (PCV) has reduced invasive disease of serotypes with the capsular polysaccharide (CPS) types that are included in the vaccine4 **(BOX 1)**. The remarkable capacity of *S. pneumoniae* to remodel its genome through the uptake and incorporation of exogenous DNA (natural competence **[G]**) from other pneumococci or closely related oral streptococci has facilitated the spread of antibiotic resistance and evasion of vaccine-induced immunity. The prominence of *S. pneumoniae* as a cause of disease is due to the combination of high carriage rates, its genetic adaptability, and its ability to shift from a commensal to pathogenic interaction with its host. In this Review, we discuss the bacterial, environmental and host factors that contribute to the different stages of pneumococcal disease.

**[H1] Transmission of *S. pneumoniae***

Until recently, all that was known about pneumococcal ‘contagion’ was that spread requires close contact with a carrier(s) - especially young children, is more frequent during drier, colder months when airway secretions are more copious, and is more likely to occur in conjunction with viral infections of the upper respiratory tract5-7. This general ignorance about transmission was a consequence of a lack of tractable animal models and an inability to study human-to-human transmission in sufficient detail. In 2010, airborne transmission among closely housed ferrets co-infected with influenza A virus (IAV) was described8. Another group modeled murine transmission from ‘index’ pups colonized at 4 days of age to littermate ‘contact’ pups in the setting of IAV co-infection9. Similar to human transmission viral infection, close contact and younger age increased transmission. This infant mouse model has now enabled the study of the major steps during host-to-host spread, including exit from a colonized host (shedding), survival in the environment, and acquisition by a new host.

*[H3] Exit from the colonized host.* IAV-induced inflammation stimulates both the expression of mucin glycoproteins and the flow of mucus10,11.There are more pneumococci in nasal secretions of pups with IAV co-infection (**FIG. 2**) and only young mice shed *S. pneumoniae* at levels permissive for transmission12. Moreover, levels of shedding, correlate with the extent of URT inflammation in response to IAV infection – TLR2-deficiency, which is associated with an increased viral load and, subsequently, greater inflammation, results in higher rates of transmission and this effect is specific to the index mice12. Furthermore, the effect of IAV is recapitulated by intranasal treatment of the index mice with the TLR3 ligand polyIC **[G]**13.

The size of population bottlenecks in the infant mouse model during transmission was estimated by using marked isogenic bacterial strains13 In this study, all constructs colonized, shed and could be acquired in similar numbers by all pups. By contrast, after the index pup is simultaneously colonized withthe marked mutants, in the majority of transmission events only one of the mutants was successful. This tight population bottleneck during transmission, would explain the need for large numbers of shed pneumococci for at least one to be successful in reaching a new host. Accordingly, increasing the proportion of colonized index pups per cage to 50% made transmission to ~30% of contacts possible without the need for IAV co-infection14.

During early childhood, rhinorrhea is pronounced and clinical surveys demonstrate a relationship between secretion volume and *S. pneumoniae* density15. In the infant mouse model, dampening inflammation by intranasal dexamethasone **[G]** treatment or the use of *tlr2*-/- index mice reduces shedding and transmission16. The single pneumococcal toxin, pneumolysin (Ply), has strong pro-inflammatory effects and Ply-induced inflammation hastens clearance of bacteria from the URT17. A *ply* knock-out mutant and point mutant, in which the toxin is unable to oligomerize to form pores after membrane insertion, both demonstrated reduced URT inflammation, shedding and the ability to transmit to littermates16. Additionally, intranasal administration of the purified toxin, but not the inactive toxoid (PdB), can complement the inflammation, shedding and transmission defect of the *ply-* mutant. This is the first example of a pneumococcal factor that is specifically required for transmission. These findings with Ply also provide a link between pneumococcal virulence and transmission18, suggesting that factors such as Ply that contribute to the disease state by enhancing inflammation, in fact, also promote the transmission of *S. pneumoniae*.

As epidemiologic studies show that the prevalence of different serotypes is highly variable, the role of capsule type and amount on shedding and transmission was tested using isogenic serotype-switch and *cps*-promoter switch mutants19. Some serotype-switch mutants colonized at wildtype levels but were shed and transmitted poorly in infant mice. Mutants with lower expression of CPS and thinner capsules were also shed and transmitted poorly. The capsule layer shields underlying surface adhesins and mutants with reduced shedding and transmission showed increased binding to URT mucins in an *in vitro* assay. Encapsulation, therefore, may facilitate shedding by allowing escape from the mucus that lines the airway surface, with a thicker capsule or capsule of certain serotypes being more effective.

*[H3] Survival in the environment****.*** The extent of airborne transmission (as demonstrated by the ferret studies) versus contact-dependent transmission (as shown by the infant mouse model) is unclear. A number of recent reports have examined factors that affect survival of *S. pneumoniae* outside the host. Transmission through secretions of carriers could involve direct person-to-person contact or spread involving bacteria on contaminated surfaces. As evidence of the latter, in the mouse model the co-housed dam is not colonized but has large numbers of *S. pneumoniae* on her teats and in cage-switch experiments can serve as a source of contagion9,16. *S. pneumoniae* can also be easily cultured from common objects such as soft toys, recently handled by colonized children20. Under ambient, nutrient-sufficient conditions, such as in *ex vivo* human saliva, pneumococci can survive for days21. Under nutrient poor-conditions, such as in airway surface fluid, bacterial expression of Ply increases *ex vivo* survival16. This effect can be explained by toxin-dependent inflammation and consequently increased nutrients levels in secretions. Capsule expression from the *cps* locus increases survival in nutrient-poor environmental conditions, perhaps by providing a reserve of glycans22. Furthermore, pneumococci survive desiccation for many days and biofilm bacteria better retain viability *in vitro* than planktonic bacteria20,23.

*[H3] Acquisition by the new host*. Given the importance of PCV in reducing transmission from immunized children, the infant mouse model has been used to explore the role of immunity for spread24. Pre-existing *S. pneumoniae* colonization of contact pups inhibits the acquisition of a new strain13. This bacterial interference could affect the frequency of co-colonizing strains. Passive immunization of contact pups with anti-capsular polysaccharide IgG is also sufficient to block acquisition, although this effect requires high levels of antibody and can be overcome by a large inoculum25. The protective activity of specific antibodies during acquisition is independent of Fc fragment **[G]** -mediated effects but requires their agglutinating function **[G]**, which could facilitate mechanical clearance by the mucocilliary flow **[G]**. However, *S. pneumoniae* evades clearance that is mediated by IgA1, the most abundant immunoglobulin on mucosal surfaces of the human URT26. The pneumococcal protease ZmpA (also known as Iga) with cleaves the hinge region of human IgA1 and this eliminates the agglutinating activity of this immunoglobulin25. Thus, PCV likely is effective because it induces IgG, which is not sensitive to the protease, at levels high enough to reach the mucosal surface and block pneumococcal acquisition. In a model of experimental human colonization with *S. pneumoniae*, levels of CPS-specific memory B cells correlate with protection from acquisition27. Such memory B cells can quickly differentiate into antibody-secreting plasma cells following antigen exposure. Furthermore, the efficacy of *S. pneumoniae* agglutinatination of airway secretions after PCV vaccination correlates with protection during experimental human colonization28. An additional effect of immunity demonstrated in the infant mouse model is a decrease in shedding by index pups24. Moreover, immunity in either the index or contact pups alone is sufficient to reduce rates of transmission, indicating that decreased shedding and protection from acquisition both contribute. These experiments were carried out with serotype-specific antibody. It is unclear whether immunity to other *S. pneumoniae* surface targets can block transmission. In this regard, immunization with Ply shows no effect on shedding and transmission, even though the toxin is required for spread between pups16. This result is not unexpected as Ply is not secreted, is not present on the cell surface, and might be released only when pneumococci are lysed within the phagosome and therefore are not exposed to antibody29. When pneumococci are killed by lysozyme within the phagosome, the released Ply forms pores, which enable bacterial products to access the host cell cytosol and trigger the production of proinflammatory chemokines and cytokines30-32. In this manner, *S. pneumoniae* responds to an influx of professional phagocytes, when it finds itself in a host that is no longer hospitable. Triggering inflammation and mucus secretions drive its transit to a new, more hospitable, host.

**[H1] Colonization by *S. pneumoniae***

Nasopharyngeal carriage is the source *S. pneumoniae* spread between hosts and the first step towards invasive disease. Several bacterial factors are required for *S. pneumoniae* to colonize and persist on the mucosal surface at a density and duration that is sufficient for transmission to occur (**FIG. 3**). For example, *S. pneumoniae* expresses two enzymes, PgdA and Adr, which modify its peptidoglycan rendering it resistant to the lytic effects of lysozyme, which is abundant on the mucosal surface of the URT33. The main featuresthat facilitate colonization are adherence to host cells and tissues, subversion of mucosal innate and adaptive immunity, and evasion of clearance by mucociliary flow.

*[H3] Adherence to the nasopharynx.* The first defense that *S. pneumoniae* encounters in the nasopharynx is mucus entrapment. The glycocalyx overlying the URT epithelium is composed of gel-like mucin glycoproteins and contains antimicrobial peptides and immunoglobulins34. *S. pneumoniae*, as other residents of the URT, is found predominantly along this mucus layer35. While the mucus layer keeps the bacteria away from the underlying cell surface, adherence to mucin glycans helps the bacteria to remain in the nasopharynx and provides a favorable niche and nutrients. On the other hand, CPSs, which are almost all negatively charged, repel the sialic-acid rich mucopolysaccharides in mucus36. By avoiding entrapment in the nasal mucus, *S.* *pneumoniae* might access and attach to the surface of epithelial cells. Much of our understanding of *S. pneumoniae*-host cell interactions comes primarily from models that use cultured human epithelial cells. *S. pneumoniae* uses several surface components for binding, but their relative importance in natural carriage has not been established. Examples of these adhesins are surface located pneumococcal adhesion and virulence A (PavA), PavB, and enolase (Eno), all of which bind to the extracellular matrix protein fibronectin and plasminogen 37-39. Phosphorylcholine (ChoP) moieties on cell wall teichoic acid bind to the platelet-activating factor receptor (PAFR) and Choline-binding protein A (CbpA; also known as PspC) binds the secretory component on the polymeric immunoglobulin receptor40,41. CbpA also binds the host proteins Factor H and vitronectin. Other major classes of host cell receptors include carcinoembryonic antigen-related cell adhesion molecule (CEACAM) and intercellular cell adhesion molecule (ICAM-1)42. *S. pneumoniae* increases expression of many of its epithelial surface receptors and thereby adherence in response to inflammatory stimuli43. The surface-exposed lipoproteins putative proteinase maturation protein A (PpmA)44 and streptococcal lipoprotein rotamase A (SlrA)45 have also contribute to adherence to epithelial cells. The choline-binding protein L (CbpL) facilitates migration of *S. pneumoniae* from the nasopharynx to the lungs and blood46. *S. pneumoniae* encodes at least 10 extracellular glycosidases some of which have been shown to enhance adherence by modifying host glycoconjugates to reveal glycan receptors47. In addition, two of these surface glycosidases, neuraminidase A (NanA) and the β-galactosidase BgaA, themselves have lectin domains **[G]** and seem to function as adhesins independently of their enzymatic activities48,49. *N*-acetylglucosamine-β-(1,3)-galactose inhibits pneumococcal adherence to epithelial cells and *S. pneumoniae* is one of many pathogens that bind to *N*-acetylglucosamine-β-(1,4)-galactose50,51. These adhesive interactions with the epithelial surface may be needed for colonization but also comprise the initial step in the invasion process (see below).

*[H3] Interactions with the nasopharyngeal flora*. The success of *S. pneumoniae* as a colonizer requires interactions with the nasopharyngeal microbiota, which likely are extensive and complex. These interactions can either be cooperative or competitive52. For example, detection of Gram-negative peptidoglycan through the sensor Nod1 by neutrophils triggers killing of *S. pneumoniae*53. During experimental human colonization, increased microbiota diversity is associated with increased acquisition of *S. pneumoniae* following intranasal challenge54. *S. pneumoniae* colonization was also found to promote microbial heterogeneity in these studies. Similarly, during the first two years of life, *S. pneumoniae* colonization was associated with less stable microbiome profiles55. Co-colonizing pneumococci compete with one another through a diverse array of bacteriocins **[G]** (pneumocins) and related peptides with antimicrobial activity56-58. Lysis of susceptible strains not only allows for predation but also provides a source of DNA for the adaptation of the predator.

In general, inflammatory conditions in the URT favor the presence of *S. pneumoniae*. A common and important example is infection with URT viruses. Nasal inflammation in response to infection with respiratory viruses such as IAV, modulates the expression of proinflammatory chemokines, up-regulates epithelial receptors used for *S. pneumoniae* adherence, compromises the integrity of the epithelium, and provides a more nutrient-rich, inflammatory milieu. Together these effects of viral co-infection increase the susceptibility to acquisition and the density of colonizing *S. pneumoniae*59-61. Recent data from murine models and clinical studies have shown that the live attenuated influenza vaccine also increases numbers of colonizing *S. pneumoniae*62-64. A higher pneumococcal density in the nasopharynx is likely to facilitate transmission and also micro-aspiration to the lungs and, thereby, increase the likelihood of progression to disease65.

*[H3] Bacterial and host factors involved in clearance.* Individual carriage episodes typically last for weeks to months. Using a model for calculating the duration of carriage episodes from a longitudinal carriage study, and combining these results with whole genome sequence data, it was recently estimated that *S. pneumoniae* genomic variation accounts for 63% of the variation in carriage duration, whereas measured host traits (such as age and previous carriage) accounted for less than 5%. Serotype was found to have a major influence on carriage duration66. This pan-genome-wide association study also identified prophage sequences as having the greatest negative impact on carriage duration, independent of serotype.

One important characteristic that enables *S. pneumoniae* to successfully thrive in this competitive niche is its ability to evade and sometimes hijack host responses during colonization. In mouse models, acquisition of the organism leads to a mild acute inflammatory response in the URT that is ineffective at completely clearing the organism67. By contrast, pre-existing inflammation is the most closely associated susceptibility factor in the human challenge model68. Many of the factors contributing to the eventual clearance of *S. pneumoniae* have been delineated. Studies in mice suggest that clearance requires TLR2-dependent responses that result in the recruitment of additional macrophages from the monocyte pool into the nasal lumen. A positive feedback and additional recruitment of macrophages is required for the gradual elimination of the infection 69. The cellular immune responses to *S. pneumoniae* are greatly accelerated by cytosolic sensing of the pathogen, which requires the pore-forming function of Ply29,30. Accordingly, *ply*-deficient mutants, or mutants unable to form pores, show prolonged colonization and diminished production of key inflammatory mediators needed for clearance, including IL-1, CXC and CC-motif chemokines and type 1 interferons **[G]**29,30,32. These macrophage-dependent responses are dysfunctional in both infant and aged mice, which might explain the higher incidence of infection among the very young and old70,71. The importance of cellular clearance mechanisms is likely a consequence of the inability of specific antibodies that are induced during carriage to clear the organism once it is established on the mucosal surface72,73.

[H3] *Immunizing effect of colonization.* Colonization increases anti-capsular (serotype specific) and anti-protein (non-serotype specific) antibody levels74-77. Experimental data from murine models show that colonization is an immunizing event and protects against subsequent colonization and disease78,79. Experimental human carriage studies have confirmed that colonization increases nasal, lung and serum antibody levels 74,80,81.Moreover, these studies corroborated observations in murine models demonstrating the protective effect of colonization against reacquisition of the same strain up to one year following the first colonization episode75. Serotype- or strain-specific immunity seems to be required for this protection, as challenge of volunteers following a known natural carriage episode with a strain of a different serotype did not result in increased protection27. These infection studies also showed that colonization increases levels of *S. pneumoniae*-specific CD4+ T memory cells in the blood and lungs in humans80. In mice, anti-pneumococcal CD4+ T cells are sufficient and the TH17 response necessary for efficient clearance82,83. The importance of TH17 immunity in natural colonization has yet to be confirmed, although a low ratio of TH17 to T regulatory cells (Tregs) correlates with colonization in children and increases with age as colonization frequency decreases84.

**[H1] Invasive pneumococcal disease**

From an evolutionary perspective, stable nasopharyngeal colonization ought to be the principal *modus operandi* of *S. pneumoniae*, as this enables ready transmission to new hosts. As noted above, induction of proinflammatory chemokines and cytokines, upregulation of target receptors and damage to the respiratory epithelium caused by viral infection of the upper respiratory tract increases bacterial loads in the nasopharynx. This facilitates bacterial transmission but also increases the likelihood of penetration of host tissues and progression to localized or invasive disease. Progression to invasive disease is more likely in young children, the elderly, and in patients with specific lifestyle traits and comorbidities. There are also marked differences in the capacity of specific *S. pneumoniae* strains to cause invasive disease, which is unsurprising given the vast genetic and phenotypic heterogeneity of this bacterium. *S. pneumoniae* factors and pathways that contribute to tissue adherence and invasion are outlined in **FIG. 4**.

*[H3] Niche adaptation.*Translocation from the nasopharynx to deeper tissues exposes *S. pneumoniae*  to distinct micro-environmental niches, requiring extensive adjustments to gene expression patterns. The importance of such adaptations for pathogenesis was initially suggested by genome-wide screens, for example by signature-tagged mutagenesis **[G]**, which showed that, in addition to known virulence genes, a large number of metabolic and transporter genes were required either for colonization or for local or invasive infections, but not necessarily for growth of *S. pneumoniae* *in vitro*85-87. Subsequent studies using genomic microarray analysis identified substantial differences in expression patterns of these non-traditional ‘virulence’ genes between pneumococci growing in distinct host niches (nasopharynx, lungs and blood) and compared to cells grown *in vitro*88,89.

Acquisition of metal ions, particularly transition metals such as iron (Fe), manganese (Mn) and zinc (Zn), is crucial for growth and survival of *S. pneumoniae* in multiple host niches, where availability of free ions may be restricted. These metals are essential co-factors for many metabolic and other enzymes, and in the case of Mn, also mediate resistance to oxidative stress90. Unsurprisingly, genes encoding the metal-binding components of ATP-binding cassette (ABC) transporters responsible for uptake of Fe (*piuA*, *piaA* and *pitA*), Mn (*psaA*) and Zn (*adcA* and *adcAII*), are preferentially expressed in the host environment, and the respective *S. pneumoniae* knock-out mutants are heavily attenuated *in vivo* in models of both carriage and invasive disease91-93. Indeed, the absolute requirement for *psaA* *in vivo* makes it a valid target for novel antimicrobials94. Certain metals may also be deleterious in excess and hence intracellular concentrations must be strictly regulated by coordination of uptake and efflux systems90. In addition, excess Zn released into the extracellular compartment by leukocytes poses a particular problem for invading pneumococci. Zn can compete with Mn for the metal binding site in PsaA95, but unlike Mn, which is passed from PsaA to the PsaBC transporter for uptake, Zn binds irreversibly to PsaA thereby blocking the transport pathway and starving the bacterium of Mn96. Thus, host Zn release contributes to nutritional immunity and may explain why dietary zinc deficiency increases rates of pneumococcal disease97,98. PspA also interacts with host lactoferrin, an iron-sequestering glycoprotein, and this protects the bacterium from killing by apolactoferrin (the iron-free form of lactoferrin)99. Recent work has shown that the variable capacity of different *S. pneumoniae* strains to bind lactoferrin depends on PspA and differences in CPS100.

Optimal utilization of carbon sources available in distinct host niches is also critical for pathogenesis. *S. pneumoniae* is totally dependent on carbohydrates as a carbon source and its genome encodes roughly 30 carbohydrate-specific phosphotransferase systems (PTS) and ABC transporters capable of importing a wide range of sugars101. Many of these have previously been shown to contribute to growth and survival *in vivo*102. Although glucose is available in the blood, free sugars may be in low abundance at sites such as the mucosa of the upper and lower respiratory tract. In these niches, pneumococci scavenge sugars by sequential cleavage of host cell surface *N*-linked glycoconjugates, which is mediated by surface-associated exoglycosidases such as NanA, BgaA, and a β-*N*-acetylglucosaminidase, StrH. The released sugars (sialic acid, galactose and *N*-acetylglucosamine) may then be taken up by the relevant ABC and PTS transporters and metabolized. At the same time, mannose residues are unmasked on the core glycan structure, which may function as surface receptors for pneumococcal adherence103. *S. pneumoniae* also has a surface-associated endoglycosidase EndoD104, which releases the residual Man3Glc*N*Ac2 structure from host glycoconjugates. Terminal mannose can also be released from high mannose *N*-glycans by SpGH92 and taken up by the mannose PTS. Meanwhile, residual Man5Glc*N*Ac2 is also released from these host structures by EndoD and taken up along with Man3Glc*NA*c2 by an ABC transporter, with further deconstruction occurring in the pneumococcal cytoplasm104. The various released sugars can have substantial intracellular effects by regulating carbohydrate metabolism through the catabolite repressor CcpA102. Sialic acid released by NanA has been shown to act as a signal, increasing bacterial loads in the nasopharynx of mice colonized with *S. pneumoniae*, facilitating invasion of nasal tissue and progression to pneumonia and meningitis105,106. Such signaling involves the two-component response regulator **[G]** CiaR and requires sialic acid uptake by the transporter SatABC and this results in increased pneumococcal resistance to antimicrobial reactive oxygen species107. NanA can also trigger TGF-β signaling pathways, leading to endothelial cell invasion108.

The role of biofilms in the ability of *S. pneumoniae* to persist at various sites of infection is not well understood and their contribution to invasive disease remains controversial. Most studies of pneumococcal biofilms have been carried out *in vitro*, and *in vivo* data are limited. Pneumococcal biofilm structures have been detected in biopsies from patients with otitis media109 and in the middle ear cleft of chinchillas co-infected with *S. pneumoniae* and *Haemophilus influenzae*110.In biopsies from volunteers colonized in experimental human studies, *S. pneumoniae* is found in microcolonies68, although it has not been determined whether these have characteristics of biofilms such as an extracellular matrix. The production of an extracellular matrix has a major impact on the ability of *S. pneumoniae* that has been grown in a biofilm *in vitro* to subsequently translocate from the nasopharynx to the lungs in a murine infection model111. A recent report has also suggested that NanA-mediated cleavage of sialic acid promotes biofilm formation *in vivo* and increases carbon availability during colonization112. Murine experiments suggested that the pneumococcal serine-rich repeat protein (PsrP) is particularly important for bacterial attachment to lung cells and biofilm formation by intra-species interaction113. PsrP seems to be required for bacterial persistence in the lower airway but not for nasal colonization or survival in the bloodstream during sepsis114.

Quorum sensing (QS) **[G]** and phase-variation also have an important role in modulating pneumococcal niche adaptations. It has been known for many years that *S. pneumoniae* colonies can switch between ‘transparent’ and ‘opaque’ phenotypes, in a process known as phase variation. These variants differ in levels of expression of key virulence proteins such as PspA and CbpA, as well as CPS and cell wall teichoic acid. The transparent phenotype is favored in the nasopharyngeal niche and the opaque in the blood115. A more recent study has shown that the underlying mechanism involves a type I restriction-modification system **[G],** SpnIII, within a genetic locus containing inverted repeats that enable spontaneous rearrangement of alternative specificity domain genes. This generates six different SpnIII target specificities, each with distinct genome-wide DNA methylation patterns, gene expression profiles, and virulence phenotypes116. Moreover, pneumococci were shown to readily switch between SpnIII alleles during progression of disease in a murine model116. Differentially expressed genes included the CPS biosynthesis locus *cps*, various sugar transporters, the Mn transporter *psaBCA*, and *luxS*. The *luxS* gene is of particular interest, as it is involved in the synthesis of the ubiquitous quorum sensing molecule autoinducer 2 (AI-2), which is an important regulator of biofilm formation and virulence in pneumococci117. Recent studies show that AI-2 accumulating in the extracellular compartment is sensed by the pneumococcal fructose-specific PTS component FruA, leading to upregulation of the galactose ABC transporter and the Leloir pathway **[G]**105. Galactose is an important carbon source for *S. pneumoniae* in the respiratory tract and AI-2-mediated quorum sensing seems to be essential for its uptake and metabolism. Up-regulation of the Leloir pathway increases the availability of activated sugar precursors, leading to increased production of CPS and a hyper-virulent phenotype105.

*[H3] Penetration of tissues.*Invasive pneumococcal disease requires breaching of epithelial and/or endothelial barriers and penetration of tissues, ultimately providing access to the bloodstream, and in the case of meningitis, breaching the blood brain barrier (BBB). Invasion involves interaction between ChoP moieties and PAFR on the surface of cytokine-activated respiratory epithelial and vascular endothelial cells, followed by hijacking of the PAF receptor recycling pathway to gain entry40. An alternative route involves interaction between the pneumococcal surface protein CbpA and pIgR on human respiratory epithelial cells. Subversion of the pIgR recycling pathway enables internalization and transmigration of *S. pneumoniae* across polarized epithelial cell monolayers41. Interestingly, another region of CbpA has been shown to bind to the laminin receptor on brain microvascular endothelium and this facilitates ChoP-PAFR-dependent penetration of the BBB during development of pneumococcal meningitis118. CbpA, as well as laminin receptor and PAFR, are also necessary for invasion of cardiomyocytes and formation of cardiac microlesions, which can occur as a complication of invasive pneumococcal disease119. Recently, RrgA, the tip adhesin of the pneumococcal pilus-1, has also been shown to interact with pIgR and platelet endothelial cell adhesion molecule 1 (PECAM-1) on brain microvascular endothelium, and antibody blockade or deletion of these two receptors reduced brain invasion in a mouse meningitis model120. Currently, the relative importance of these uptake mechanisms and the extent of cooperation between them are uncertain. Furthermore, many *S. pneumoniae* strains are not piliated and thus cannot use RrgA-dependent pathways. It should also be emphasized that bacteremia is not an essential prerequisite for meningitis, as localized infections such as sinusitis or mastoiditis can also lead to meningitis. When modeled in mice, meningitis may also develop as a consequence of interaction of pneumococci colonizing the nasopharynx with gangliosides on the surface of olfactory neurons, triggering cell invasion and direct entry of pneumococci into the central nervous system by retrograde axonal transport121. Such non-hematogenous spread is stimulated by exogenous sialic acid106.

Regardless of the mechanism or site of invasion, the pneumococcal capsule impedes adherence to and invasion of host cells, because it may sterically hinder interactions between cell wall ChoP or surface proteins and their cognate host receptors122. However, pneumococci markedly reduce capsule thickness when in close contact with epithelial cells and during the invasion process123. This process of capsule shedding has recently been shown to depend on the major pneumococcal autolysin LytA and is triggered by exposure to cationic antimicrobial peptides that are released by the host cells124.

Several pneumococcal virulence factors that directly damage host tissues or induce host inflammatory responses also facilitate tissue invasion. One of the most notable examples is the pore-forming toxin Ply, which, in addition to wide-ranging pro-inflammatory effects, directly lyses or induces apoptosis of diverse cell types, including lung epithelium and endothelial cells at the BBB125. Ply also inhibits mucociliary clearance in human lungs, separates tight junctions between cells, which enables tissue penetration, and exposes new sites for pneumococcal attachment126. The pneumococcal pyruvate oxidase SpxB and α-glycerophosphate oxidase GlpO produce hydrogen peroxide, which also contributes to tissue damage in the lung and at the BBB127. Surface-exposed hydrolytic enzymes, including neuraminidases, hyaluronate lyase128 and metalloproteases129 can also directly damage host tissues. Two glycolytic enzymes, enolase and glyceraldehyde-3-phosphate dehydrogenase, are also surface-exposed and function as plasminogen-binding proteins, along with the choline-binding protein CbpE. They sequester and activate host plasminogen at the pneumococcal surface and facilitate adherence to and penetration of the extracellular matrix130,131. An overview of pneumococcal surface proteins and other factors contributing to adherence and invasion is provided in **Table 1**.

*[H3] Evasion and subversion of host defenses***.** *S. pneumoniae* expresses a plethora of factors that mediate immune evasion and subversion (Table 1). As an extracellular pathogen, *S. pneumoniae* must evade neutrophil-mediated killing to survive the acute inflammation that accompanies tissue invasion. Neutrophils can readily kill phagocytized pneumococci by releasing serine proteases from neutrophil granules132. One mechanism to evade neutrophil recruitment involves CbpE (also known as Pce), which functions as a ChoP esterase. CbpE cleaves ChoP moieties on host-derived platelet-activating factor (PAF), which is a potent activator of neutrophils133. Many of the virulence determinants of *S. pneumoniae* target components of the complement system to minimize opsonophagocytosis **[G]** and clearance of invading pneumococci (reviewed in 134). The CPS is undoubtedly the most important defense against the host immune system. For example, although non-encapsulated pneumococci can colonize the URT and cause superficial eye infections, they rarely cause invasive infection. CPS covers deeper bacterial surface structures and thereby inhibits binding of immunoglobulins, complement components and C-reactive protein. It reduces opsonization with C3b and inactivated C3b (iC3b) and physically impairs interactions between C3b, iC3b and Fc regions of immunoglobulins with their receptors on phagocytic cells135. Capsular serotypes differ in the efficacy with which they inhibit opsonophagocytosis and the level of inhibition correlates with their ability to cause invasive disease. Studies of capsule-switch mutants have shown an inverse relationship between the amount of C3b and iC3b deposition and binding to factor H, which inhibits the alternative complement pathway136. Increased levels of factor H in nasal lavages of asymptomatic individuals infected with an URT virus predispose to acquisition of *S. pneumoniae*137. Factor H mainly binds CbpA on the pneumococcal surface. CbpA can also bind directly to C3 and in some strains the classical complement pathway inhibitor C4b-binding protein (C4BP) in an interaction that is inhibited by CbpA binding to vitronectin134,138,139. Thus, CPS and CbpA on the pneumococcal surface both are important for resistance to opsonophagocytosis.

PspA also interferes with complement deposition by binding factor B and blocking formation of or accelerating the dissociation of the alternative pathway C3 convertase140. Furthermore, Ply released from the bacterium activates the classical complement pathway through a domain with structural similarity to the Fc component of IgG, thereby depleting serum opsonic activity125,141. The combined functions of PspA and Ply are essential for *S. pneumoniae* to successfully cause septicemia142. Other pneumococcal proteins that interfere with opsonophagocytosis include the exoglycosidases NanA, BgaA and StrH, presumably by deglycosylating human glycoproteins that are important for complement deposition143. Plasminogen binding and activation by Eno and GAPDH bind and activate plasminogen and this can also contribute to immune evasion through the degradation of complement pathway components134. *S. pneumoniae* grows in both short- and long-chain forms. Short-chain forms have a minimal surface area and are less likely to trigger complement activation, and therefore are more likely to evade opsonophagocytic clearance during invasive disease144.

**[H1] Conclusions and outlook**

*S. pneumoniae* has proven to be a truly resilient foe. It has overcome selective pressure from multiple classes of antibiotics and now seems to be adapting to the immune pressure of widespread immunization. These developments demonstrate that we cannot be complacent and further insights are needed to combat pneumococcal disease. This review has highlighted the current state of our understanding of the three key stages in the pathogenesis of *S. pneumoniae* — transmission, colonization and invasion. In particular, our understanding has profited from progress in defining the molecular events involved in invasion and new models of transmission in infant mice and of carriage in humans. Further progress will likely come from a broader perspective that takes into account pneumococcal ecology. In this regard, there are now more than 8000 publicly available whole-genome sequences of *S. pneumoniae*, which are providing a more comprehensive view of the species and the remarkable extent of its diversity. Additional insight will come from studies of the interactions of *S. pneumoniae* with other members of the microbiota and a better understanding of its niche in the human URT.

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**Competing interests**

The authors declare no competing interests.

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**Box 1. *Streptococcus pneumoniae* vaccination**

*Streptococcus pneumoniae*has a high genetic diversity and certain lineages are particularly successful. An important source of strain-to-strain variation is the structure of the capsular polysaccharide (CPS), which is the major virulence determinant and immunodominant surface structure of *S. pneumoniae*. Currently, 97 immunologically and structurally distinct CPS types are recognized but only a relatively small subset of these types is commonly found to cause carriage and disease. Therefore, CPS-based vaccines target only a limited number of serotypes. When covalently conjugated to an immunogenic protein carrier, CPS is recognized as a T cell-dependent antigen, which stimulates a more effective humoral immune response (including immunoglobulin class switching, affinity maturation and memory) than polysaccharide-alone antigens, particularly in young children. Since its introduction in 2000, the pneumococcal conjugate vaccine (PCV) has been highly effective in preventing invasive pneumococcal diseases. An unexpected benefit of the high levels of serotype-specific immunoglobulin G generated by PCV have been reduced rates of carriage in and transmission from immunized children, which also protects unimmunized populations (herd immunity)145. However, the protection elicited by PCV is incomplete, as current formulations contain only 10 to 13 of the 97 known CPS types146. A further issue is the rising prevalence of non-vaccine serotypes in carriage and disease (‘serotype replacement’) as a consequence of the immune pressure from widespread use of PCV147,148. Current efforts to improve prevention through vaccination are directed at increasing the number of serotypes covered by PCV or adding conserved pneumococcal proteins that induce serotype-independent immunity.

**Fig. 1.** **The lifecycle of *Streptococcus pneumoniae* and the pathogenesis of pneumococcal disease.** *Streptococcus pneumoniae* colonizes the mucosa of the upper respiratory tract (URT). This carriage is the prerequisite for both transmission to other individuals and for invasive disease in the carrier. Carriers can shed *S. pneumoniae* in nasal secretions and thereby transmit the bacterium. Dissemination beyond the URT, either by aspiration, bacteremia or local spread, can lead to invasive diseases, such as pneumonia, meningitis and otitis media.

**Fig. 2.** **Bacterial and host factors affecting pneumococcal shedding from carriers.** *Streptococcus pneumoniae* is found predominantly in the mucus layer overlying the epithelial surface of the upper respiratory tract. Inflammation (indicated by the presence of neutrophils), which is induced by the pore-forming toxin pneumolysin or by co-infection with influenza virus and other respiratory viruses, stimulates secretions and increase shedding. By contrast, agglutinating antibodies, such as anti-capsule IgG and IgA1, decrease shedding, unless they are cleaved by the human IgA1-specific pneumococcal protease. Capsule type and amount also influence mucus-association and numbers of shed bacteria.

**Fig. 3. Molecular mechanisms of pneumococcal colonization of host surfaces.** Key functions that enable *Streptococcus pneumoniae* colonization are establishing the first contact with the epithelium and epithelial receptors, interaction with the complement system, mucus degradation, metal binding, impairment of neutrophil activity and the pro-inflammatory effects of the toxin, Ply.The pneumococcal enzymes NanA, BgaA and StrH degrade mucus and thereby inhibit mucociliary clearance. Furthermore, the LytA autolysin-facilitated release of Ply damages the epithelium and reduces ciliary beating. Negatively-charged capsular polysaccharide (CPS) inhibits bacterial mucus entrapment. CPS and several pneumococcal proteins, including PspA, CbpA, Eno and Pht, directly and indirectly block complement deposition. PspA also binds to lactoferrin to acquire iron and blocks the antimicrobial effect of apolactoferrin. PepO, which is released from the pneumococcal surface, binds to C1q leading and thereby depletes complement components. Pneumococcal CbpE (also known as Pce) impairs neutrophil recruitment by degrading PAF, a host-derived inflammatory phospholipid. CbpA interacts with factor H interactions to facilitate adherence and subsequent internalization of *S. pneumoniae* via cell glycosaminoglycans. CbpA also binds to pIgR to promote adherence. The IgA protease subverts mucosal humoral immunity by cleaving IgA1. Phosphorylcholine on teichoic acid mimics host PAF and allows binding to its receptor. Piliated strains express a tip adhesin, RrgA. Other *S. pneumoniae* adhesins include Eno and PavA.

**Fig. 4. Stages in pneumococcal adherence and invasion.**A. Several steps are required for invasion of the respiratory tract. *Streptococcus pneumoniae* evades entrapment in mucus and mucociliary clearance by negatively charged CPS and proteolytic degradation of secretory IgA1 by Iga. NanA, BgaA and StrH degycosylate mucus, and also unmask glycan targets for adhesins on the epithelium. Finally, Ply inhibits ciliary beating. Adherence to the apical surface of epithelial cells is mediated by diverse surface structures, including ChoP, CbpA, RrgA at the tip of pili **[Au:OK?]** , PavA and PsrP. *S. pneumoniae* binds through ChoP to PAFR and through CbpA to pIgR and by subverting the respective host receptor recycling pathways induces its endocytosis, which is followed by release of pneumococci at basolateral surface. Alternatively, Ply and H2O2, directly damage the epithelium and Hyl and plasmin, which is bound to the pneumococcal surface through Eno, GAPDH or CbpE, degrade the extracelluar matrix. This breaks down the epithelial barrier and provides a pathway for paracellular invasionChoP-PAFR and CbpA-pIgR interactions also enable pneumococci to traverse the endothelium and to enter the bloodstream. Upregulation of PAFR by inflammatory cytokines amplifies ChoP-PAFR-mediated invasion. CPS and other virulence factors, including PspA, CbpA and Ply, facilitate evasion of opsonophagocytosis **[Au:OK?]**. B. To penetrate the blood brain barrier,*S. pneumoniae* uses ChoP-PAFR, CbpA-pIgR and CbpA-laminin receptor binding. Strains that express pili also use RrgA to bind to pIgR and PECAM-1. Similarly to invasion of the respiratory tract, Ply, H2O2 generated by GlpO and activated plasmin bound to the pneumococcal surface proteins Eno, GAPDH and CbpE can compromise the blood brain barrier.

**Table 1. Major pneumococcal virulence factors**

|  |  |  |
| --- | --- | --- |
| **Virulence factor** | **Description** | **Function in pathogenesis** |
| Capsular polysaccharide (CPS) | * Major surface antigen * 97 structurally distinct serotypes | * Prevents entrapment by mucus during colonization * inhibits opsonophagocytosis by preventing interaction of iC3b and 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 * Peptidoglycan fragments | Pathogen-associated molecular patterns | Promote inflammation |
| Pneumolysin (Ply) | * Pore-forming toxin * TLR-4 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 TLR-4, 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 and SpsA) | 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 platelet-activating factor (PAF) * Binds plasminogen |
| CbpG | * CBP * Serine protease | * Cell-attached form promotes adherence * Extracellular form degrades fibronectin * Important for mucosal and invasive disease |
| CbpL | CBP | * Binds collagen, elastin and C-reactive protein * Promotes dissemination from nasopharynx to lungs and blood by inhibiting phagocytosis |
| NanA | * Neuraminidase * LPXTG | * Cleaves terminal sialic acid from host mucin and cell surface glycoconjugates * Unmasks receptors for adhesins * Important role in otitis media * Triggers TGF-β signaling to facilitate endothelial invasion |
| BgaA | * β-Galactosidase * LPXTG | Sequential cleavage of sugars from host glycoconjugates |
| StrH | * β-*N*-acetylglucosaminidase * LPXTG | Sequential cleavage of sugars from host glycoconjugates |
| EndoD | * Endo-*N*-acetylglucosamindase * LPXTG | Sequential cleavage of sugars from host glycoconjugates |
| Hyl | * Hyaluronate lyase * LPXTG | * Degradation of extracellular matrix * Facilitates tissue penetration |
| PrtA | * Serine protease * LPXTG | * Cleaves lactoferrin * Possible adhesin |
| ZmpA (also known as Iga) | * Metalloprotease * LPXTG | Cleaves human IgA1 |
| ZmpB | * Metalloprotease * LPXTG | Possible adhesin |
| ZmpC | * Metalloprotease * LPXTG | Cleaves human matrix metalloprotease 9 |
| PepO | Endopeptidase | * Binds fibronectin and plasminogen * Facilitates adherence and invasion * Binds C1q to inhibit classical complement pathway |
| 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 * RrgC | * LPXTG proteins * Structural components of pilus-1 * Encoded by *rlrA* pathogenicity islet * RrgA is tip adhesin | * Adhesins * Bind to a range of glycans * Facilitate colonization and biofilm formation * RrgA also binds pIgR and PECAM-1 on endothelium of the blood brain barrier, which promotes brain invasion |
| PsaA | * Lipoprotein * Solute-binding component of Mn-specific ABC transporter | * Manganese uptake in host environment * Essential for pneumococcal resistance to oxidative stress *in vivo* |
| * AdcA * AdcAII | * Lipoproteins * Solute-binding components of a single Zn-specific ABC transporter | Zinc acquisition *in vivo* |
| * PiuA * PiaA * PitA | * Lipoprotein * Solute-binding components of iron-specific ABC transporters | Iron acquisition *in vivo* |
| * SlrA * PpmA | * Lipoproteins * Peptidyl-prolyl isomerases | Contribute to nasopharyngeal colonization |
| * PhtA * PhtB * PhtD * 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 * PavB | * Fibronectin-binding proteins * NCSP | * Adherence 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 H2O2 |
| GlpO | α-Glycerophosphate oxidase | Generates H2O2 |
| SodA | Mn-dependent superoxide dismutase | Resistance to oxidative stress |
| * Etrx1 * Etrx2 | * Surface-exposed thioredoxin-family lipoproteins | Resistance to oxidative stress |
| * SpMsrAB2 | * Methionine sulfoxide reductase | Redox partner of Etrx1 and Etrx2 |

CBP, choline binding surface protein

LPXTG, sortase-anchored surface protein

NCSP, non-classical surface protein lacking secretion signals or anchorage motifs

**Glossary Terms**

**Upper respiratory tract**. Includes the nasal cavity, paranasal sinuses, mouth, pharynx, and larynx and forms the major passages above the trachea.

**Community-acquired pneumonia**. Infection of the lung acquired outside of hospitals or nursing facilities.

**Natural competence**. The endogenous ability of a [bacterium](https://en.wikipedia.org/wiki/Cell_(biology)) to alter its genes by taking up extracellular [DNA](https://en.wikipedia.org/wiki/DNA) from its environment through [transformation](https://en.wikipedia.org/wiki/Transformation_(genetics)).

**PolyIC**. Polyinosinic:polycytidylic acid is an agonist of toll-like receptor 3 and mimics dsRNA found in some viruses.

**Dexamethasone**. An anti-inflammatory corticosteroid.

**Fc fragment**. The tail region of an antibody; interacts with cell-surface receptors and some proteins of the complement system.

**Agglutinating function**. The clumping of antigens through multi-valent binding by antibodies.

**Mucocilliary flow**. A non-immunological defense mechanism that involves ciliary action and the flow of mucus; clears the respiratory tract from pathogens and particles

**Lectin domains**. Carbohydrate-binding domains on proteins

**Bacteriocins**. Proteinaceous or peptidic toxins produced by bacteria to inhibit the growth of similar or closely related bacteria.

**Type 1 interferons**. A group of signaling proteins expressed and released by host cells to regulate immune responses to pathogens.

**Signature-tagged mutagenesis**. A genetic technique using DNA signature tags (molecular barcodes) to identify mutants in mixed populations.

**Two-component response regulator**. The transcription factor component of a stimulus-response mechanism for bacteria to sense and respond to environmental changes.

**Quorum sensing (QS)**. A system of stimuli and responses, which is correlated to microbial population density.

**Restriction-modification system**. A bacterial defense system, **[Au:OK?]** in which restriction endonuclease cleave and inactivate specific target sequences in foreign DNA (for example, from phages); cleavage sites in host DNA are protected by methylation.

**Leloir pathway**. The predominant route of cellular galactose metabolism.

**Opsonophagocytosis**. Process by which a microorganism is labeled (opsonized) by host immune factors to facilitate uptake by phagocytic cells

**Subject terms**

[Biological sciences / Microbiology / Bacteria / Bacterial pathogenesis](http://subjects.npd.nature.com/products/nrmicro#631/326/41/2531)

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Health sciences / Diseases / Infectious diseases / Bacterial infection

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**ToC blurb**

Many people carry *Streptococcus pneumoniae* on the mucosa of the upper respiratory tract and carriage is the prerequisite for later tissue invasion and transmission to a new host. In this Review, Weiser *et al*. summarize the mechanisms that allow pneumococci to be transmitted and its progression from colonizer to pathogen.