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Current concepts in host-microbe interaction leading to pneumococcal pneumonia

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Abstract

Purpose of review—Infection with *Streptococcus pneumoniae* (pneumococcus) results in colonization, which can lead to local or invasive disease, of which pneumonia is the most common manifestation. Despite the availability of pneumococcal vaccines, pneumococcal pneumonia is the leading cause of community and in-hospital pneumonia in the United States and globally. This article discusses new insights into the pathogenesis of pneumococcal disease.

Recent findings—The host-microbe interactions that determine whether pneumococcal colonization will result in clearance or invasive disease are highly complex. This paper focuses on new information in three areas that bear on the pathogenesis of pneumococcal disease: 1) factors that govern colonization, the prelude to invasive disease, including effects on colonization and invasion of capsular serotype, pneumolysin, surface proteins that regulate complement deposition, biofilm formation and agglutination; 2) the effect of co-infection with other bacteria and viruses on pneumococcal growth and virulence, including the synergistic effect of influenza; and 3) the contribution of the host inflammatory response to the pathogenesis of pneumococcal pneumonia, including the effects of pattern recognition molecules, cells that enhance and modulate inflammation, and therapies that modulate inflammation, such as statins.

Summary—Recent research on pneumococcal pathogenesis reveals new mechanisms by which microbial factors govern the ability of pneumococcus to progress from the state of colonization to disease and host inflammatory responses contribute to the development of pneumonia. These mechanisms suggest that therapies which modulate the inflammatory response could hold promise for ameliorating damage due to the inflammatory response in pneumococcal disease.

Keywords

Streptococcus pneumoniae; pneumococcal pneumonia; inflammation; colonization; surface proteins

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Conflicts of Interest

Neither author has a conflict of interest with data or findings discussed in this article.

Introduction

Streptococcus pneumoniae (pneumococcus) is an encapsulated Gram-positive microbe that colonizes the human nasopharynx throughout life. The principal virulence factor of pneumococcus is a polysaccharide capsule (CPS) that provides the bacterium with a mechanism of antigen variation and confers serotype specificity. The outcomes of pneumococcal infection range from asymptomatic nasopharyngeal colonization to local (otitis media) and distant invasive pneumococcal disease (IPD), including meningitis (CNS invasion), pneumonia (pulmonary invasion) and bacteremia/sepsis (bloodstream invasion). Since 2000, when immunization of infants and children with the seven-valent pneumococcal capsular polysaccharide conjugate vaccine (PCV7) began, there has been a dramatic decline in IPD in children as well as adults in countries where vaccine is used, due to herd immunity. Nonetheless, pneumococcus remains the most common and lethal cause of outpatient and inpatient pneumonias, which are associated with substantial morbidity, mortality and rising health care costs, particularly in the elderly [1–4]. This review will focus on recent studies on pneumococcal pathogenesis in three areas: 1) factors that govern colonization and progression to pneumonia; 2) the effect of co-infection with other microbes on pneumococcal virulence; and 3) the contribution of the host inflammatory response to the pathogenesis of pneumococcal pneumonia. We regret that due to space limitations we cannot review every recent article published on these topics and apologize in advance for omitting studies not covered here.

Factors that govern colonization: a prelude to pneumonia

The relationship between pneumococcal colonization and the development of IPD was recently discussed in an article that provides compelling evidence in support of the proposal that prevention of colonization is a functional marker of pneumococcal vaccine efficacy [5]. The centrality of colonization as a prelude to IPD places great importance on understanding factors that govern colonization.

The capsular polysaccharide has long been recognized as the main determinant of pneumococcal virulence. The propensity of given capsular types (serotypes) to colonize the nasopharynx has been previously associated with capsular size and structure [6] and recently, with growth in nutrient-restricted conditions [7]. Interestingly, a new study found that longer pneumococcal chain length facilitates adhesion [8], adding a new twist to the influence of capsular type and structure on colonization. Yet another twist has also emerged with the discovery that pneumococcal serotypes undergo microevolution due to mutations in the *wcjE* (O-acetyltransferase) gene, whereby colonizing strains exhibit phenotypic switching and become invasive strains [9]. Since the introduction of PCV7, the marked decrease in invasive disease with vaccine serotypes has been accompanied by an increase in disease with non-PCV serotypes [10–12]. Among non-PCV7 serotypes, serotypes 1, 3, and 19A have each emerged as major causes of severe pneumonia and empyema [13–17]. The longstanding association of serotype 3 virulence with its large capsule was reaffirmed in a recent capsule switching study [18], as was the association between more heavily encapsulated serotypes and mortality in IPD [19]. The virulence of serotype 1 pneumonia is

difficult to link to capsular structure, although other virulence determinants could be at play (see below). Serotypes 1, 3 and 19A are included in PCV13, which was introduced in 2010.

Recent studies reveal new aspects of the long recognized relationship between pneumococcal invasiveness and evasion of complement via reduced capsular deposition of complement component 3 (C3) and/or C3 degradation products. These studies tie expression of pneumococcal surface proteins that mediate adhesion and invasion to capsular type [20]; link factor H binding, which leads to reduced C3 deposition and phagocyte binding, to capsular type [21]; and show that serotype 1 is highly resistant to C3 deposition and phagocytosis [22]. Pneumococcal surface proteins including PspA, PspC, and CbpA have been shown, respectively, to impair C3 deposition and CR3-mediated phagocytosis and to inhibit C reactive protein binding to phosphorylcholine [23;24], and C3 deposition by binding C4b-binding protein [25], and factor H [26;27]. The role of pneumococcal cell-wall hydrolases LytC and LytB in invasion was recently linked to evasion of C3 deposition [28], while enolase, a surface plasminogen-binding protein was shown to inhibit C3 deposition by binding C4b-binding protein in human, but not mouse serum [29]. The latter is a reminder that human and mouse C3 differ [30], and that caution should be exercised in extending mouse studies to humans. Other proteins recently shown to impact pneumococcal invasion include sialidase (NanA), which stimulated proinflammatory cytokines and extracellular trap production by human neutrophils [31], and a pilus component that facilitated pneumococcal uptake by macrophages and accelerated dissemination in mice via complement receptor 3 [32].

There is a robust literature on pneumolysin (PLY), a cholesterol dependent cytolysin that binds TLR4 and has been proposed as a 'universal' pneumococcal vaccine candidate [33–36]. Although PLY is thought to be released by pneumococcal cells upon autolysis, a recent study provides evidence for a PLY export mechanism that builds on the discovery a few years ago of PLY in the cell wall without cell lysis [37;38]. The role of PLY in pneumococcal growth and dissemination *in vivo* is highlighted in several recent studies. Intranasal infection of mice with a serotype 3 PLY deletion mutant resulted in bacterial dissemination and death as did the wild type strain, but a strain engineered to produce less PLY conferred resistance to lethal challenge and skewed the lung cellular profile towards B and T cells, rather than neutrophils [39]. Although another study showed that PLY increased lung permeability and impaired bacterial clearance in the setting of Flt3L-induced dendritic cell activation [40], in others, the immune-stimulating properties of PLY controlled pneumococcal growth via TLR4 and MyD88 [41] as did activation of the NLRP3 inflammasome and IL-1 β expression [42]. The aforementioned engineered serotype 3 strain led to lower levels of IL-6 and IL-17 in the lungs and reduced dendritic cell cytokine release *ex vivo* [39]. It is important to note that some pneumococcal strains express non-hemolytic PLY. Such strains had an early growth advantage in the blood [43] and an impaired ability to activate the NLRP3 inflammasome [44]. Interestingly, some serotype 1 strains express a non-hemolytic PLY [45;46], perhaps in part explaining its propensity for invasiveness [47]. The lack of PLY hemolytic activity appears to enable pneumococcus to escape innate immune surveillance, highlighting previous data showing the importance of PLY in promoting early bacterial clearance [41].

Several recent studies show that pneumococcal biofilm formation affects nasopharyngeal colonization. In one study, pneumococcal serine-rich repeat protein (PsrP), a surface protein and lung-specific virulence factor, promoted bacterial aggregation and biofilm structures in the nasopharynx and lungs of mice [48]. Another study showed that biofilm-derived bacteria exhibited increased PsrP and CbpA expression, the transparent phenotype and increased nasopharyngeal adhesion with reduced invasiveness in mice [49]. However, in other studies, bacteria with the opaque phenotype formed extracellular matrixes, grew in biofilms and were invasive [50], and convalescent sera from pneumonia patients bound planktonic-, but not biofilm-grown pneumococcal (TIGR4) proteins [51], but the biofilm-associated protein, PsrP, was expressed by both bacterial types. Given that genetic material is exchanged between co-colonizing strains of pneumococcus during biofilm growth [52] and this depends on fratricide [53], the phenotype of one strain could be affected by another. A recent article comprehensively reviewed pneumococcal biology in the nasopharynx, including biofilms [54].

New insights into regulation of the pneumococcal biofilm phenotype have begun to emerge. A biofilm producing CPS-deficient strain expressed less *lytA*, *IgA1*, and *psaA* than a CPS-expressing strain [55] and bacteria from mouse nasopharynx, lungs and blood each had different gene expression profiles [56]. The latter suggests a role for niche-specific host factors in governing pneumococcal growth and phenotype. This is highlighted by a study in which serotype 3 strains from the ears of mice did not disseminate, while bloodstream-derived bacteria invaded the lungs and blood but failed to colonize the nasopharynx after intranasal infection [57]. Host factors influencing these phenotypes remain to be identified. However, a recent study showed that binding of an agglutinating CPS-specific monoclonal antibody enhanced pneumococcal quorum sensing, competence-induced transformation and fratricide and expression of competence and fratricide genes [58]. Notably, this and other agglutinating antibodies did not induce phagocyte-mediated bacterial killing *in vitro* although they were protective in mice [59–61], suggesting that antibody-mediated agglutination might regulate pneumococcal growth in certain tissues. In support of this idea, mucosal tissues are replete with antibody and antibody-mediated effects on fungal metabolism have been described [62]. On the other hand, serotype-specific agglutinating polyclonal rabbit antibody was recently shown to enhance pneumococcal killing by phagocytes via capsular C3 deposition [63]. Thus, antibody agglutination of pneumococcus could benefit the host via several different mechanisms, underscoring the nearly century old observation that serotype-specific agglutination induced bacterial clearance in rabbits with pneumococcal pneumonia [64].

Co-infection and pneumococcal virulence

The presence of other microbes in the nasopharynx can influence pneumococcal colonization and invasiveness. Recent studies show that pneumococcal adherence was inhibited *in vitro* by bacteriocins produced by *S. mitis* and *S. salivarius* from healthy children [65], while *in vivo*, the density of pneumococcus in the nasopharynx correlated positively with presence of *Haemophilus influenzae*, but negatively with *Staphylococcus aureus* [66]. The latter provides a potentially mechanistic explanation for the observation that colonization with *S. aureus* has risen in the pneumococcal conjugate vaccine era [67]. In

one study, virulent pneumococcal serotypes emerged during nasopharyngeal competition with *Haemophilus influenzae* [68]. However, in another, pneumococcal colonization correlated negatively with α -hemolytic streptococci during asymptomatic viral upper respiratory infection (URI), but in viral URI with otitis media, pneumococcal and non-typeable *Haemophilus influenzae* colonization each increased while α -hemolytic streptococci decreased [69]. In a study utilizing a mouse model of otitis media, co-infection with influenza A resulted in an increase in bacterial growth and middle ear inflammation compared to pneumococcal infection alone [70], implicating viral inflammation in bacterial growth. In other studies, an influenza A-associated increase in pneumococcal colonization led to increased type 1 IFN production, inhibition of macrophage recruitment, sepsis and death [71], and the mortality of mice co-infected with pneumococcus and the 2009 pandemic H1N1 influenza strain increased due to lung damage due to defective lung repair mechanisms [72]. These findings highlight the central role that an excessive, virally induced host inflammatory response plays in pneumococcal pathogenesis and are consistent with clinical data showing that pneumococcal co-infection was strongly correlated with severe disease in the 2009 H1N1 influenza pandemic [73].

The role of host inflammation in pneumococcal pneumonia

The pathogenesis and clearance of bacteria in pneumococcal pneumonia are governed by complex inflammatory responses that stem from host pattern receptor and signaling molecules, phagocytes and cells that govern innate and acquired immunity. Some such responses control pneumococcal growth, while others are dispensable or even detrimental. For example, one study showed that pneumococcal binding to TLR2 triggers inflammation in mice, but TLR2 deficiency did not affect mortality or bacterial growth in the lungs [74], while another found that TLR2 was redundant with Nod2, which recruited monocytes/macrophages *via* CCL2 [75]. Meanwhile, TLR2 was implicated in mortality due to excess inflammation in mice with serotype 3 pneumonia following influenza [76]. Along the same lines, while one study found that type 1 IFN signaling was essential for pneumococcal nasopharyngeal clearance [77], another showed that in the setting of influenza A co-infection, the type 1 IFN response impaired macrophage recruitment, resulting in a higher nasopharyngeal bacterial burden [71]. These studies underscore the central role that viral inflammation plays in dysregulated immune responses to pneumococcus.

Recent studies show that pneumococcal infection stimulates macrophages and epithelial cells to produce CXC chemokines, which induce neutrophil recruitment to the lungs [78;79], and that cathepsin G and neutrophil elastase are important mediators of neutrophil killing [80]. While these studies advance our understanding of neutrophil recruitment and killing, experimental depletion of neutrophils in murine pneumococcal pneumonia models has previously revealed serotype-specific effects ranging from deleterious to a beneficial effect on lethality [81–83]. In another recent study, pro-inflammatory responses to pneumococcus were modulated by MAPK-dependent induction of COX-2 in response to pneumococcal pneumonia, leading to prostaglandin release [84], revealing a novel mechanism by which the inflammatory consequences of neutrophil killing are modulated.

While bacterial killing can induce inflammatory damage, a recent study demonstrated that cathepsin D mediated alveolar macrophage apoptosis-associated pneumococcal killing in the lungs [85]. Similarly, a protective CPS-specific monoclonal antibody also mediated alveolar macrophage apoptosis, albeit without early bacterial killing as lung inflammation was markedly reduced even though bacterial burdens were similar in control mice that died [86]. Thus, monoclonal antibody-treated mice, which were not protected after macrophage depletion, were spared the inflammatory consequences of early bacterial killing, perhaps because macrophage uptake prevented bacterial binding to host receptors. In another serotype 3 model, the beneficial effect of alveolar apoptosis stemmed from a TNF-related apoptosis-inducing ligand (TRAIL) mediated reduction in lung inflammation [87]. In contrast, administration of GM-CSF in a serotype 19 pneumonia model increased nitric oxide-mediated bacterial killing and reduced apoptosis-mediated killing [88]. These observations suggest that immune modulation via apoptosis and immune stimulation via bacterial killing can each confer a host benefit in pneumococcal pneumonia, with the caveat that additional factors, including the inoculum [89] are likely to influence the mechanism of bacterial clearance.

T-cells are recruited to the lungs in pneumococcal pneumonia. However, data on their role in host defense against pneumococcus is conflicting. Previous studies showed that the Th17 response is important for clearance of nasopharyngeal colonization in naïve [90] and immunized mice [91]. However, CD4⁺ T-cells were dispensable in a serotype 3 pneumonia model in immunized [92] and naïve mice in which CD8⁺ T- cells were required for survival [93]. In the latter, CD8⁺ T cells controlled the inflammatory response in the lungs in an IFN- γ and perforin-dependent manner and regulated the IL-17 response via TGF- β , with CD4⁺T and Th17 cells being dispensable for survival. The latter was also shown in another study in which CD4⁺ T-cell deficient mice were more resistant to serotype 2 pneumonia despite having similar bacterial burdens to wild type mice 48 hrs after infection [94].

The foregoing observations underscore the relationship between an inability to regulate inflammation and the pathogenesis of pneumococcal pneumonia. In keeping with this concept, age associated susceptibility to pneumonia in mice was a function of an increase in pro-inflammatory cytokines as well as host ligands to which pneumococcus binds [95]. In patients, an increase in cardiovascular events following admission for pneumonia in a cohort of > 50,000 elderly patients [96], and cardiac complications, including early mortality in community acquired pneumonia [97], were hypothesized to stem from inflammation driven by pneumococcus, leading to the proposal that adjunctive anti-inflammatory agents be considered in treating pneumonia [97]. In support of this, in mice, hMG-CoA inhibitor (statin) therapy decreased neutrophil and macrophage influx, chemokine levels and bacterial burdens in the lungs [98]. Statins also prolonged survival, and decreased lung inflammation, PAFr expression, pneumococcal adherence and PLY-mediated cytotoxicity in a model of sickle cell anemia [99]. These studies provide new insight into and a possible mechanistic explanation for the retrospective clinical finding that patients with pneumococcal pneumonia who were on a statin at the time of admission had lower mortality even though they were older with more comorbidities [100]. Macrolides provided no benefit in the foregoing study [101], although azithromycin ameliorated inflammation in post-influenza pneumococcal pneumonia and improved survival over ampicillin in mice [76].

Conclusion

The pathogenesis of IPD involves a complex array of microbial factors and host responses. A theme of recent studies is that modulation of the host inflammatory response is critically important in controlling bacterial growth and tissue damage. As our understanding of the factors that drive the immune response to pneumococcus deepens, new therapeutic targets and immunotherapeutic agents that modulate the inflammatory response are likely to emerge as adjuncts to current antimicrobial agents or stand alone therapies.

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Key Points

- Pneumococcus expresses an array of factors that impact its interaction with the host.
- Biofilm formation and niche-specific gene expression play important roles in colonization with pneumococcus and pathogenesis of pneumococcal pneumonia.
- Colonization with pneumococcus and outcomes in pneumococcal pneumonia are impacted by the presence of certain other microbes.
- Regulation of host inflammation is key to control of bacterial growth as well as host damage in pneumococcal pneumonia.