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MOLECULAR PATHOGENESIS OF PNEUMOCOCCAL PNEUMONIA

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1. ABSTRACT

The past two decades have witnessed an explosion of data on the molecular pathogenesis of pneumonia caused by *Streptococcus pneumoniae*, one of the most important pathogens currently plaguing man. Identification and functional analysis of genes and their proteins, elucidation of mechanisms involved in adherence, colonization, inflammation, and invasion, and an understanding of interactions with the host and with external factors have provided knowledge that can be used to attack this organism with small molecule or vaccine based strategies. Study of the pneumococcus has also led to insights into other pathogens that share a unique spectrum of respiratory disease. In this review we will discuss recent advances in our understanding of the pathogenesis of pneumonia due to *S. pneumoniae*, highlighting emerging themes common to other organisms such as *Haemophilus influenzae* and *Neisseria meningitidis*.

2. INTRODUCTION

Respiratory infections are the 6th leading cause of death in the world (1) and the leading cause of infectious deaths (2). *Streptococcus pneumoniae*, the pneumococcus, is the most common etiologic agent in community acquired pneumonia, as well as bacterial meningitis, otitis media, and sepsis (3,4,5,6). This spectrum of disease, unique among gram-positive bacteria, is shared by two gram-negative organisms, *Haemophilus influenzae* and *Neisseria meningitidis*. Although quite different genetically and physiologically, these prominent pathogens have intriguing similarities. All three are capable of asymptomatic carriage in the nasopharynx, but can cause invasive disease, particularly in the very young or very old, targeting the ear, lung, brain, and blood. They share certain structural features such as a polysaccharide capsule and phosphorylcholine moieties on their surfaces, and certain unusual physiological features such as natural

transformation of DNA and autolysis triggered by quorum sensing. Thus, consideration of the pathophysiology of one of these bacteria may yield insights into the pathogenesis of all three. While a strategy of vaccination has successfully controlled invasive disease from *H. influenzae* type b, problems due to the unique biology of the pneumococcus remain, and limiting the impact of this organism will continue to be a major challenge in the years to come.

3. PATHOGENESIS OF PNEUMOCOCCAL PNEUMONIA

In order to frame the discussion of pathogenesis in its proper context, a brief introduction to the gross pathology of pneumococcal pneumonia is necessary. Many of the gross pathological findings are now explainable by events occurring on the cellular and molecular level. The lung lesions characteristic of pneumococcus are classically described as lobar pneumonia beginning as peribronchial inflammation and progressing to complete consolidation of the alveolar spaces. In early studies it was divided into four overlapping stages: engorgement, red hepatization, gray hepatization, and resolution (7,8). The earliest stage, engorgement, which corresponds with the clinical onset of pneumonia, begins with congestion of the alveolar capillaries, exudation of a serous fluid into the alveolar space, and the presence of pneumococci within this fluid. This edema fluid acts as a culture medium for the pneumococcus allowing exponential growth under conditions where few leukocytes have been recruited and easing spread throughout the lung (9). As capillary engorgement and further leakage continue, macrophages and erythrocytes begin to pass into the alveoli leading to red hepatization, where the gross appearance of affected portions of the lung resembles that of the liver. Fibrin deposition and continued consolidation limit perfusion (10) leading to necrosis if the lesion does not resolve (11). The next stage, gray hepatization, is predicated on the influx of leukocytes into the lesion and phagocytosis through complement mediated opsonization. Grossly the lung is grayer in color as fibrin is deposited, red cells dissolve, the capillaries are compressed and leukocytes fill the alveoli. Few pneumococci are seen at this stage. Rabbits which are deprived of leukocytes do not progress to gray hepatization; rather, red hepatization progresses to frank hemorrhage and necrosis (11). As capsule specific antibodies are formed and allow more efficient opsonization for the now abundant leukocytes, the lesion begins the resolution phase with dissolution of the fibrin and clearance of cellular debris by monocytes leading to a return to normal lung architecture. Pneumococcal pneumonia is not a necrotic process in the normal host, and, paradoxically, the extent of clinical illness is not typically related to the degree of consolidation of the lung, as the patient is often well into recovery before resolution of the lesion begins. It is unknown why such intense inflammation characteristically resolves so perfectly.

4. STRUCTURAL CONSIDERATIONS

The role of the various structural components of *S. pneumoniae* in the pathogenesis of pneumonia is only now being elucidated. A brief review of these components will provide a basis for discussion of pathogenic mechanisms in later sections.

4.1. The polysaccharide capsule

In 1925 Avery and colleagues demonstrated that the soluble substance surrounding the pneumococcus was composed of polysaccharide (12,13). Over 90 distinct serotypes exist, each structurally and chemically different (14). Although polysaccharide does not play a role in adherence, invasion, or inflammation in the host (15), the capsule increases virulence by preventing phagocytosis. The degree of this effect on virulence appears to be dependent on the composition of the capsule and the underlying cell wall components rather than its thickness. Studies in mice indicate that differences in virulence with disparate capsule sizes (e.g. phase variation) are a result of changes in the thickness of the cell wall rather than the thickness of the capsule (16). Unencapsulated strains are referred to as “rough” due to the gross appearance of their colonies and are avirulent compared to their encapsulated parents (17). Although type specific antibody is effective at clearing pneumococcal pneumonia (18,19), the two currently licensed vaccines directed against the polysaccharide capsule cover only 7 or 23 of the more than 90 serotypes. Concern over shifts in the prevalence of targeted serotypes and the ability of pneumococcus to switch capsular types through transformation (20) has prompted a search for universally expressed protein antigens to include in future vaccine preparations (21).

4.2. The cell wall

Under the polysaccharide capsule is the pneumococcal cell wall, a dynamic structure consisting of a glycan backbone bearing more than a dozen structurally different peptides (22). Teichoic acid and lipoteichoic acid consist of extended repeat carbohydates differing only in their attachment to the cell surface; teichoic acid links directly to the peptidoglycan while lipoteichoic acid is hydrophobically anchored through its fatty acids to the plasma membrane. An unusual and important component of cell wall is the phosphorylcholine on the teichoic acid (23). This moiety acts as a docking site for a class of surface proteins known as choline binding proteins (24). Phosphorylcholine appears to be a key to the structural diversity of the surface of pneumococcus. Two chemically distinct variations of phosphorylcholine exist, one which typically has proteins bound to it (the choline binding proteins or CBPs), the other free. Recently is has been discovered that phosphorylcholine is not unique to pneumococcus but is also found on the surface of other respiratory pathogens such as *Neisseria spp.*, mycoplasmas, *H. influenzae*, and *Pseudomonas* (25,26). The presence of phosphorylcholine, the target of C-reactive protein (CRP) and a major constituent of surfactant, on many respiratory pathogens may point towards a common pathogenic mechanism and help explain some of the similarities in the clinical spectrum of disease.

4.3. Proteins involved in virulence

Much work in recent years has concentrated on the role of a variety of proteins in adherence, invasion, and

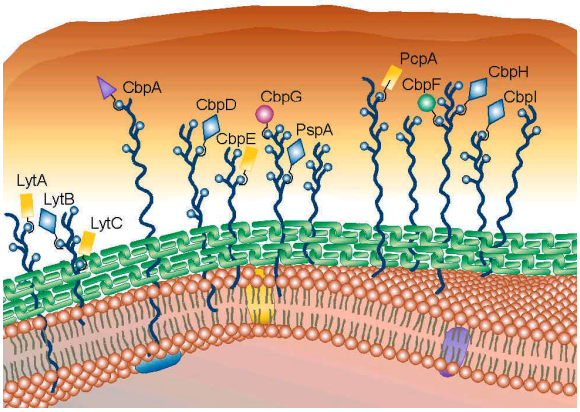

Figure 1. Choline binding proteins. This stylized representation of the pneumococcal cell wall shows the 12 identified choline binding proteins (Cbps) bound via a conserved binding domain to the choline component of teichoic acid or lipoteichoic acid (blue circles on dark blue structures) which are in turn anchored to either peptidoglycan (green) or the plasma membrane. CbpE is also known as phosphorylcholine esterase (Pce). In the *in vivo* situation certain Cbps such as CbpA and pneumococcal surface protein A (PspA) are more abundant than others, and differential expression due to phase variation would preclude all 12 from being present at any one time.

induction of inflammation. The function of all but a handful of these is unknown.

4.3.1. Choline binding proteins

Choline binding proteins (CBPs) are a family of surface proteins bound to the choline component of cell wall teichoic acid or lipoteichoic acid (27) via a conserved ligand binding domain (28) (Figure 1). LytA, the major autolysin of pneumococcus, was the first described (29) and functions to lyse pneumococci during stationary phase or in response to penicillin. The two other known cell wall hydrolases, LytB (30) and LytC (31), affect colonization and virulence in an infant rat model, consistent with a hypothesized role in release of other proteins from the cell wall (32). Each autolysin possesses its own specificity; LytA is an amidase (29), LytB is a glucosaminidase (30), while LytC is a lysozyme (31). PspA is a surface protein involved in inhibition of complement activation (33,34) by a pathway independent of complement regulatory protein factor H (35). The likely function of PspA is to prevent deposition of C3b on the surface of the pneumococcus by interfering with complement factor B thus inhibiting complement mediated clearance of pneumococci (34). CbpA, the most abundant CBP, is an important adherence factor for pneumococcus. Mutants lacking this protein fail to adhere to epithelial cells *in vitro* and fail to colonize the nasopharynx in an infant rat model (27). The role of other CBPs is less well defined. PcpA has been characterized, but its function is unknown (36). Six new members of the CBP family were recently identified (32) and named CpbD, CpbE, CpbF, CbpG, CbpH, and CbpI. CbpD and CbpE appear to be involved in adherence, while CbpG, a putative serine protease, appears to have a role both in adherence and as a virulence determinant during sepsis (32). Inactivation of CbpE (also known as Pce), a teichoic acid phosphorylesterase, leads to an increase in virulence in a mouse model of sepsis (37).

4.3.2. Pneumolysin

Pneumolysin is a 53-kDa, multifunctional thiol-activated cytotoxin produced by nearly all clinical isolates of pneumococcus (38). Unlike most other gram-positive hemolysins, pneumolysin does not possess an N-terminal signal sequence for transport out of the cytoplasm. It has long been thought that pneumolysin release was dependent on autolysis of the bacteria by LytA and thus occurred only in late log phase or in stationary phase. However, recent evidence suggests that, at least in some strains, it can be released during early log phase by a LytA independent mechanism (39). Pneumolysin is a key component of inflammation through both its cytotoxic activity (40,41) and its ability to directly activate the classical complement pathway. Opsonic activity directed against pneumococcus is reduced through activation of the classic complement pathway by binding of pneumolysin to the Fc region of immunoglobulin G (42) in the absence of specific antibody (43). Pneumolysin appears to have a third, as yet unidentified function that contributes to virulence (44,45). Sepsis studies in mice using mutants with either loss of cytotoxic activity or loss of binding to Fc remain more virulent than mutants with a deletion of the pneumolysin gene entirely.

4.3.3. Other proteins

It is estimated that pneumococci possess over 500 surface proteins, many of which play a role in pathogenicity and virulence (46). The publication of the pneumococcal genome by the Institute for Genetic Research in 1997 (http://www.tigr.org/tdb/mdb/mdb.html) and the use of powerful new tools for molecular analysis such as signature tagged mutagenesis has allowed the tentative identification of 130 to 200 genes with largely unknown functions that may be involved in virulence (46,47). The role of some of these proteins is currently being explored. Some examples of recently characterized surface proteins are given here to illustrate the point. The predominant pneumococcal neuraminidase, NanA, has been demonstrated to play a role in persistence in the middle ear and development of otitis media in a chinchilla model (48), but does not contribute to virulence in a mouse intraperitoneal challenge model (49). Pneumococci produce a bacterial pyruvate oxidase, SpxB (50), and a superoxide dismutase, SodA (51), which are involved in the biology of reactive oxygen species and thereby in virulence in mouse models of infection. A recently identified IgA1 protease (52) likely has a role in virulence by protecting the pneumococcus from type-specific antibody during colonization, although the biological significance of this enzymatic activity in pneumococcus as well as other bacteria is yet to be proven (53).

5. COLONIZATION AND ADHERENCE

Streptococcus pneumoniae colonizes the nasopharynx of up to 40% of healthy children and adults

(54), often with multiple serotypes present in a single person (55,56). Carriage of a particular strain lasts 3-6 months depending on the serotype (56), and invasive disease typically occurs with acquisition of a new serotype (57). Acquisition of new serotypes occurs at an age dependent rate as frequently as every 4 months in infants (56). As the most common cause of community acquired pneumonia (7), the ability of the pneumococcus to adhere to and colonize the respiratory tract is a key early event in the pathogenesis of pneumonia. Perhaps of equal importance to the innate virulence determinants of pneumococcus is the influence of environmental factors such as cigarette smoke (58) and viral infections (59,60,61) on acquisition, carriage, and invasion in the host.

5.1. Receptors in the upper respiratory tract

Adherence of pneumococcus to the respiratory tract is a complex process with expression of up to five different constellations of surface proteins depending on the target cell type (62). The pneumococcus prefers sialylated sugars for adherence (63), but whether recognition of sialic acid is an early step to bring the bacterium within close apposition of a preferred receptor or not is poorly understood. Studies of primary adherence have identified GlcNAc(β1-3)Gal as a binding lectin on buccal epithelial cells (64) and the nasopharynx (65,66). It has been suggested that the neuraminidase of *S. pneumoniae* acts to cleave terminal sialic acid exposing cryptic receptors, thus enhancing adherence (67). The observation that neuraminidase activity in the chinchilla model of otitis media allows pneumococcus to track up the eustachian tube supports this concept (68). Viruses with neuraminidase activity, such as influenza viruses and parainfluenza viruses, may act synergistically with pneumococcus in this fashion. This alteration of receptors may prime the nasopharyngeal epithelium for adherence by different serotypes not previously encountered by the host or may allow proliferation of existing colonizing strains.

5.2. Regulation of surface composition

The pneumococcus is able to sense its environment and respond by altering the face it presents to the world. Several mechanisms involved in this chameleon act are now beginning to be understood.

5.2.1. Phase variation

There are differences in the ability of morphological variants of a pneumococcal strain to bind to cells in the nasopharynx (69). It was recognized that these morphological phenotypes, termed opaque, semi-transparent, and transparent because of their appearance when viewed on transparent solid agar, were the result of a process termed phase variation. Pneumococci undergo a spontaneous change in expression of a variety of proteins at a strain-specific rate resulting in these different phenotypes. Transparent variants are able to colonize the nasopharynx more efficiently and more stably (69), while opaque variants demonstrate increased virulence and improved survival in the blood due to differences in cell wall proteins (16). Opaque variants have a thicker capsule than transparent variants, but this is thought to be a response to the decreased amount of teichoic acid seen in these variants rather than a mechanism to improve virulence by adding more capsule (16). One could hypothesize that the transparent phenotype is utilized for colonization and invasion results from spontaneous variation to the more virulent opaque phenotype which is more suited for survival in the bloodstream.

5.2.2. Two-component signal transduction systems

Many bacteria sense their environment through two-component signal transduction systems and respond to changes in the environment by regulating transcription. Environmental stimuli such as oxygen levels, nutrients required for growth, or concentration of peptides utilized in quorum sensing may act to trigger these systems. Binding of a ligand to a surface-exposed histidine kinase leads to phosphorylation of a response regulator which in turn can alter gene transcription by binding DNA. Several two-component systems have been identified in pneumococcus and appear to contribute in a complex manner to regulation of transformation, autolysis, and adherence (70,71,72,73,74). For example, a two-component system regulating phosphate uptake, PnpR-PnpS, has recently been identified (70). Deletion of an involved gene, *pstB*, not only decreased phosphate uptake but had effects on transformation and penicillin tolerance as well.

5.3. Role of phosphorylcholine in adherence

Pneumococcus appears to express several different ligands in its cell wall for adherence, including some of the recently described choline binding proteins. CbpA is a multifunctional protein possessing a role in adherence both in the upper and lower respiratory tract (27). CbpD and CbpE contribute to colonization in the infant rat model, although their exact role is unknown (32). The putative serine protease CbpG also contributes to colonization, perhaps by altering or freeing other surface associated molecules to improve ligand-cell interactions (32). Although the presence of phosphorylcholine and the role of CBPs in adherence was thought to be unique to the pneumococcus, mycoplasmas (75), *P. aeruginosa*, *H. influenzae*, and *Neisseria spp.* also utilize phosphorylcholine on their cell surface (26). In *H. influenzae* it is found on lipopolysaccharide (LPS), while *Neisseria* display it on pili. Phosphorylcholine moieties on both pneumococcus (27) and *H. influenzae* (76) undergo phase variation and modulate adherence and colonization (25,27,32). The location of phosphorylcholine on *Neisseria*, the pili, would imply a role in adherence as well. Thus a diverse group of mucosal pathogens, some of which share a clinical spectrum of disease with the pneumococcus, have independently learned to exploit this structure.

6. INFLAMMATION AND INVASION

Following adherence and asymptomatic colonization, *S. pneumoniae* will infrequently cause more invasive disease such as pneumonia. How the pneumococcus changes from a passive colonizing agent in the nasopharynx to a destructive invader of the lower respiratory tract is incompletely understood (66). Regardless of whether it is by spontaneous variation to a more suitable form or the result of capitalization on

Table 1. Summary of events occurring during pneumococcal pneumonia

Stage	Engorgement	Red Hepatization	Gray Hepatization	Resolution
Time[1]	0-4 hours	4-24 hours	1-5 days	5-10 days
Pneumococcus	Rapid growth and spread through lung. Cell wall binds CD14 and toll-like receptor 2 initiating cytokine cascade and induces NF-kappa B	Activates alternative pathway of complement. Immobilizes and degrades soluble C3	Most pneumococci phagocytosed by neutrophils through complement mediated opsonization	Combats type specific antibody: CbpA binds secretory IgA, specific protease cleaves IgA
Host	Induction of TNF-alpha, IL-1, IL-6. Capillary congestion, leak. Serous exudate	Upregulation of PAF and CD18. NF-kappa B, TNF-alpha, IL-1 peak. Influx of erythrocytes, neutrophils and macrophages. Fibrin deposition	C-reactive protein acts as opsonin. IL-6 , nitric oxide, leukotriene B4 peak. Influx of leukocytes, erythrocytes dissolve. Fibrin deposition continues	Most pneumococci cleared. Capsule specific antibody provides efficient opsonization. Fibrin dissolves, monocytes clear debris. Neutrophils apoptose. Inflammatory mediators no longer present
Pathology	Involved lobe distended, moist. Pleura shiny. Prominent pulmonary edema	Capillaries engorged. Erythrocytes fill alveoli. Resembles liver	Capillaries compressed limiting perfusion. Leukocytes, fibrin fill alveoli giving gray appearance	Exudate removed. Lung architecture begins to return to normal

Abbreviations: CbpA = choline binding protein A, IgA = immunoglobulin A, TNF = tumor necrosis factor, IL = interleukin, PAF = plate activating factor. [1]The time course (arbitrarily defined here) is variable depending on inoculating dose and host factors, and the stages overlap as various areas of the lung undergo the process at different times.

opportunities presented by external forces such as antecedent viral infection, the pneumococcus can gain access to the alveolar space and set in motion a series of events leading to inflammation and clinical pneumonia (Table 1) (24). Some of the mechanisms operative at the molecular level are beginning to be appreciated in the pathogenesis of this infection. Invasion of the bloodstream directly from the nasopharynx can occur as well. Pneumococci co-opt the polymeric immunoglobulin receptor, which is typically involved in transport of immunoglobulins to the apical cell surface, and utilize it for translocation across epithelial cells (77). It is not known how this pathway impacts on disease.

6.1. Receptors in the lower respiratory tract

Although the pneumococcus can bind to many epithelial cell types in the nasopharynx, it cannot adhere to the ciliated epithelium lining the tracheo-bronchial tree and utilizes an entirely different set of cell surface receptors in the alveolus. The difficulty reaching the alveolus directly from the nasopharynx without binding anywhere in between and the need for a different set of surface proteins for adherence between nasopharynx and lung may partially explain why the incidence of pneumonia is so infrequent compared to the rate of carriage. Once in the alveolus several receptors may be utilized. The major described ligand in the lower respiratory tract is CbpA (27) (for the purposes of this review we are defining the bacterial moiety as the ligand and the cellular structure as the receptor). It utilizes the receptor for platelet activating factor (PAF) in adherence to both pneumocytes and endothelial cells and has recently been demonstrated to bind to cell surface associated C3 (78). Other as yet unidentified ligands can bind to the human milk protein lacto-*N*-neotetraose (65), as well as GalNAc(β1-3)Gal and GalNAc(β1-4)Gal bearing targets (63,79,80). These interactions are poorly understood at this time.

6.2. Induction of inflammation

Adherence, invasion, and death of pneumococci in the lower respiratory tract can all contribute to inflammation by activating the cytokine, complement, and coagulation cascades. The primary inflammatory stimulant is the cell wall, while the polysaccharide capsule is relatively non-reactive (81,82). Binding of peptidoglycan to CD14, a cell surface receptor known to initiate an inflammatory response to endotoxin (83), and toll-like receptor 2 initiates the cytokine cascade. This causes swelling and leakage of capillaries leading to the early pathologic appearance of engorgement and red hepatization. However, cell wall components can also trigger cytokine release by a CD14 independent pathway specific to lung but not fully understood at this time (84). This pathway leads to induction of NF-kappa B and induction of TNF-alpha, Il-1, IL-6, and IL-8 (85). TNF-alpha and IL-1 levels peak in the lungs at 12 hours following infection then begin to decrease, while IL-6 plateaus at 12 hours and remains elevated for several days (86).

The presence of cell wall components (teichoic acid and peptidoglycan) directly activates the alternative pathway of complement which provides a chemotactic

signal for accumulation of leukocytes (87). Cell wall components also upregulate the chemokine PAF and the leukocyte adhesion molecule CD18 (88,89). The massive influx of leukocytes and the resulting slow clearance of bacteria due to the inefficient opsonization by complement heralds the change in pathologic appearance from red hepatization to gray hepatization. The role of complement in inflammation (90) and clearance of pneumococci is well supported by animal studies of complement depletion where hypocomplementemia predisposes to pneumococcal pneumonia but not pneumonia from *Klebsiella pneumoniae* or *Staphylococcus aureus* (91). The pneumococcus has developed strategies to decrease the effectiveness of complement, both by binding to immobilized C3 (78) and by degrading soluble C3 (92). C-reactive protein, a constituent of normal human serum and bronchoalveolar fluid which appears as an acute phase reactant in response to inflammatory stimuli (93), can also bind directly to phosphorylcholine on the cell wall and act as an opsonin during early inflammation (94).

Reduction of inflammation and resolution of pneumonia is dependent on the appearance of anti-capsular antibodies. These provide for efficient opsonization for phagocytosis resulting in clearance of bacteria manifested pathologically as the passage from the stage of gray hepatization to that of resolution (19,95). Pneumococci attempt to combat type-specific antibody by at least two mechanisms. CbpA binds specifically to secretory immunoglobulin A (IgA) (96), and pneumococcus produces a protease which can cleave IgA (52). Presence of type specific antibody from prior exposure or vaccination effectively prevents invasive disease and accompanying inflammation.

6.3. Invasion

Induction of inflammation appears to be a key prerequisite for invasion into respiratory epithelial and vascular endothelial cells. Inflammation triggers both an increase in expression of the PAF receptor and in permissiveness for binding (97) allowing pneumococcus to engage the PAF receptor using phosphorylcholine. Invasion takes place by transcytosis in vacuoles while bound to the PAF receptor (98). Approximately 70% of bacteria are internalized by this pathway. Although the PAF receptor on type II pneumocytes in the alveoli binds transparent and opaque variants with approximately equal efficiency (66), transparent variants are more likely to be transcytosed (98). This is somewhat of a paradox, as it appears that transparent variants have an advantage in invasion and transport through cells, yet opaque variants demonstrate improved survival in the blood.

6.4. The role of antecedent viral infection

It has long been appreciated that respiratory viral infections, particularly influenza, predispose to secondary bacterial complications such as pneumonia. There are likely many factors involved with no one mechanism predominating. Autopsy studies during the influenza pandemic of 1957-1958 in patients who died of secondary bacterial pneumonia demonstrated that bacteria adhered to areas of the tracheo-bronchial tree where viral-mediated denudation had occurred. Destruction of ciliated epithelial cells, a cell type which does not normally support adherence of bacterial pathogens, allowed infection through binding to exposed basement membrane (99). Studies in mice (100) and human volunteers (101) demonstrate increased binding of pneumococcus to epithelial surfaces following influenza virus infection, and *in vitro* study of human respiratory epithelium revealed that adherence of pneumococcus was enhanced following adenovirus infection (102). It has been hypothesized that the inflammatory response to influenza is creating a milieu favorable for pneumococcal adherence and invasion of the lower respiratory tract through cytokine mediated upregulation of receptors, one candidate being the PAF receptor (103,104). Influenza virus and similar viruses also act synergistically by suppressing the local immune response and decreasing phagocytosis (105). Further study of the exact mechanisms involved in viral-bacterial synergy is needed.

6.5. Death of the pneumococcus – role in pathogenesis

The inflammatory response in pneumococcal pneumonia peaks at the time when anti-capsular antibody appears and bacteria are being actively cleared by the immune system. In the pre-antibiotic era this often prompted an abrupt shift from toxemia to “lysis by crisis” where the patient would go from near death to apparent recovery in a matter of several hours (7). Clearance by leukocytes after efficient opsonization with specific antibody or killing of bacteria with antibiotic therapy triggers LytA and autolysis, releasing peptidoglycan and other cell wall components into the already damaged lung in a final burst of inflammatory mediators. There appears to be a concentration dependent effect in animal models where exceeding a threshold value of cell wall components found in 10^6 bacteria per milliliter triggers a brisk inflammatory response that subsequently declines in conjunction with recovery (106). It is at this stage that pneumolysin may play a major role in pathogenesis, since it is retained in the cytoplasm until lysis occurs. New evidence that a LytA independent pathway for release of pneumolysin exists and allows release during early log phase of growth (39) may alter this thinking, however. Release of other enzymes during lysis, such as the neuraminidase, and induction of hydrogen peroxide and nitric oxide contribute to the inflammatory burst at death (107). Support for the role of autolysis in pathogenesis of inflammation comes from studies of autolysin deficient pneumococci which show reduced tissue damage in animal models (108).

6.6. Correlates in other bacteria

Although inflammation is triggered by different structures in gram-negative bacteria (the outer membrane protein lipopolysaccharide - LPS) and in pneumococcus (cell wall constituents), certain comparisons between the mechanisms of inflammation nevertheless hold true. Both systems can utilize CD14 to engender induction of cytokines, although pneumococcus has independent pathways such as that involving PAF receptor that may be more important (84). There is conflicting evidence as to whether LPS of *H. influenzae* binds PAF receptor (87,109).

However, it does possess phosphorylcholine in its structure (25) which is involved in both adherence and virulence (26). Thus, it may utilize this pathway in a manner similar to pneumococcus. C-reactive protein can bind directly to the choline moieties on *H. influenzae* as it does with *S. pneumoniae* (93). However, C-reactive protein does not appear to opsonize *H. influenzae* as it does the pneumococcus; rather it triggers activation of the alternate complement pathway and direct complement mediated lysis of the bacteria. *H. influenzae* and *N. meningitidis* both possess autolysins similar to pneumococcus which may contribute to inflammation during clearance or lysis through antibiotic mediated mechanisms.

7. OPPORTUNITIES FOR INTERVENTION

The unfolding story of how pneumococcus causes disease in humans has been driven by a need for more effective interventions. Dissection of the molecular pathways responsible for adherence, colonization, invasion, induction of the inflammatory response, and evasion of the immune system will provide targets for directed drug or vaccine therapies for this common pathogen.

7.1. Difficulties with antibiotic therapy

Since the introduction of sulfonamides and penicillin in the first half of the twentieth century, antibiotic therapy has been the primary intervention in control of pneumococcal disease (110). Clever adaptation of the bacteria to antibiotic pressure in the population has outstripped our ability to attack the pneumococcus with chemotherapeutic agents.

7.1.1. Antibiotic resistance

Antibiotic resistance could be detected in clinical isolates as early as 1941, a mere 12 years after the discovery of penicillin (110,111). The mechanism of resistance is decreased binding of drug to the bacterial cell wall synthesis enzymes (112,113), commonly known as penicillin binding proteins. Because of a decreased affinity of penicillin for the active site of the penicillin binding proteins, a higher concentration of penicillin must be delivered to the bacterium, leading to clinical resistance when antibiotic levels in tissues fall below this minimum inhibitory concentration. Penicillin resistant strains make up for the changes in affinity of these enzymes for cell wall building blocks by activation of genes (*MurM* and *MurN*) which produce branched chain cell wall peptides rather than the typical linear structures (114). Although the mechanism is not known, it is clear that the capacity to produce these branched chain cell wall precursors is a prerequisite for expression of penicillin binding proteins with lowered affinity for penicillin, as interruption of *MurMN* leads to complete inhibition of expression of penicillin resistance. Isolates highly resistant to penicillin and cephalosporins are already common and are increasing in prevalence (115). The knowledge that resistance mechanisms for vancomycin, currently the antibiotic of last resort for multidrug resistant pneumococcus, exist in nature coupled with the ability of pneumococcus to take up DNA by transformation presents a frightening scenario for the near future when we may be faced with invasive pneumococcal infections for which there is no chemotherapeutic cure.

7.1.2. Tolerance

Antibiotic tolerance, the ability of a bacterium to survive in the presence of antibiotic, neither growing nor undergoing lysis, has been described in pneumococcus for penicillin (108,116,117) and more recently for vancomycin (74,118). Although induction of inflammation may be dampened without autolysis and release of pneumolysin and other enzymes, eradication may become difficult, particularly in areas of the body with poor host defenses such as the meninges (108,119). In addition, because tolerance gives the bacteria the ability to survive longer in the presence of the antibiotic, there is more opportunity for development of resistance mechanisms (74). The clinical significance of this emerging trait is yet to be determined.

7.2. Role of vaccines

Although pneumococcal vaccines have been studied since experiments with whole killed pneumococci were done in South African gold miners in 1911 (120), enthusiasm for their use has been tempered by their insufficient efficacy in the elderly and the very young. The immune systems of these two important target groups do not handle polysaccharide antigens well, and all licensed vaccines have been exclusively polysaccharide based until the year 2000. Following the blueprint from the successful conjugate vaccine against *H. influenzae*, a new seven-valent polysaccharide-protein (the protein portion is a non-toxic analogue of diptheria toxin) conjugate vaccine has recently entered use. This vaccine has proven effective against invasive pneumococcal disease and otitis media (121,122) in the less than two year old age group not covered by the 23-valent polysaccharide vaccine (123). However, the impact on otitis media may be minimal as it reduced the overall incidence of otitis media by only approximately 6% despite decreasing culture proven pneumococcal otitis media by 34%. Although the current formulation of the heptavalent vaccine covers the majority of invasive serotypes in the United States, it does not cover those prevalent in other parts of the world. The ability of pneumococcus to switch capsular types through transformation raises concerns that the pattern of carriage and of resulting invasive disease may change in response to broad coverage with the vaccine (20). Clearly other approaches need to be explored.

7.3. Future directions for intervention

A great deal of research in the pneumococcal field is currently directed at defining protein antigens which are conserved across subtypes and can provide protection from either colonization or invasive disease (21,124). Study in animal vaccine challenge models has identified a number of potential candidates including pneumolysin (125,126), the CBPs (32,46), PspA (33), proteinase maturation protein A (127), and a set of four novel pneumococcal histidine triad proteins (128). It seems likely that combination vaccines containing a few selected proteins will be more effective than single antigen preparations (129), but just which proteins will be most effective is not yet known.

In addition to traditional vaccines, intervention via small molecule approaches may be beneficial. Specific targeting of receptors using oligosaccharides which could be sprayed into the nasopharynx has been proposed (65). However, the failure of a clinical trial of this concept (130) indicates that application in humans may require improved knowledge of the receptor and ligand structures. Potentially, drugs designed specifically to inhibit receptors such as PAF receptor may find a clinical niche (65). Finally, there is ample evidence to suggest that agents designed to attenuate the acute host response may be beneficial adjuncts to specific therapy. The use of steroids to down-modulate the inflammatory response has gained acceptance for some types of bacterial meningitis (131) and pneumonias. It is not unreasonable to assume that a better understanding of the pathways involved in pneumococcal induction of inflammation could lead to more specific agents to be used in pneumococcal pneumonia. For instance, strategies used to down-regulate the production of nitric oxide in animal models of pneumonia and sepsis have shown promise. Further research in such areas is necessary to impact on this widely prevalent disease.

8. PERSPECTIVE

Although much has been discovered over the last two decades, the study of pneumococcal pathogenesis remains in its infancy. A framework for the important players has been defined, but only a small portion of the genome is well characterized and undoubtedly many surprises lie ahead. A strategy of carefully defining the steps of pathogenesis and using this knowledge to design novel therapeutics and vaccines will eventually bring this highly adapted, lethal pathogen under our control.

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