Pneumococcal Microbiology and Immunity

Barry M. Gray, MD

Pneumococcus is the quintessential encapsulated human pathogen. The pneumococcal capsule was identified early as the major virulence factor in many animal experiments. Vaccine development was attempted from the beginnings of pneumococcal research but was hampered by a lack of understanding of the diversity of capsular types. Neufeld's finding that strains could be typed with specific antisera by the capsular "swelling" test opened the door to serum therapy, to new fields of immunology and bacterial biochemistry, and eventually to the development of polysaccharide vaccines.

Many early experiments showed that killed or disrupted pneumococci or culture filtrates caused inflammation similar to that of infection with live cells. Cell wall constituents proved to be important virulence factors. The activity of powerful toxins was also observed and identified variously as hemolysins, leukotoxins, and the purpura-producing principle. The toxic effects were probably all attributable to what is now known as pneumolysin.

Meanwhile, Avery's studies of capsular transformation led directly to the discovery of DNA as the genetic material and ushered in a revolution in modern biology. The complete DNA sequences of several pneumococcal strains have been determined, revealing a genome of more than 2 million base pairs and 2,000 genes. Many proteins are devoted to sugar metabolism, some of which damage host cell surfaces and promote colonization. Some genes facilitate the uptake of foreign DNA, such as antibiotic resistance genes. More than 60 molecules are expressed on the cell surface, in addition to several proteins already under investigation as potential new vaccines.

POLYSACCHARIDE CAPSULE

The capsule is the major antigenic and structural feature of the pneumococcus. Unlike a candy-coated peanut, as suggested by some textbook illustrations, the capsule is a tangle of type-specific polymers intermingled with teichoic acids (C-polysaccharide), strands of peptidoglycan, other components of the cell wall, surface proteins, and various enzymes. Figure 1 shows the capsule covered with fine cationized ferritin and the location of pneumococcal surface protein A (PspA) tagged with larger immunogold beads. Figure 2 is an artist's conception of the capsule.

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distinct capsular types, some of which are closely related and antigenically cross-reactive, including the common types 6A/6B, 19A/19F, and 23A/23F. The types included in the 7-valent conjugate vaccine are 4, 6B, 9V, 14, 18C, 19F, and 23F, which account for approximately 85% of invasive pneumococcal infections. Pneumococci are able to switch capsular type by genetic exchange with a pneumococcus of a different type, but most switches occur among these commonly carried types.

Pneumococci also vary by how much capsular material they produce and the expression of other virulence factors in a coordinated process called phase variation. Small capsule variants are better able to adhere to epithelial cells and colonize mucosal surfaces, whereas large capsules are needed to impede phagocytosis and survive in the circulation and deep invasive sites.

Figure 2. Artist's conception of the pneumococcal capsule, showing the location of some of the major cell surface components, toxins, and enzymes, and the activity of pneumolysin and neuraminidase on the host cell. (Courtesy of Barry Gray, MD.)
<table>
<thead>
<tr>
<th>Vaccine Potential</th>
<th>Role in Pathogenesis</th>
<th>Virulence Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhaled vaccine recommended for children</td>
<td>High virulence determinant for children</td>
<td>23S rRNA in Chp vaccine</td>
</tr>
<tr>
<td>With irrigation &gt; 75% of children</td>
<td>Specific antibodies in lung, nasal, and pharyngeal tissues</td>
<td>23S rRNA in Chp vaccine</td>
</tr>
<tr>
<td>Less than 12 years old</td>
<td>Products of gain in function determinants</td>
<td>Phagocytic potential</td>
</tr>
<tr>
<td>Yes, licensed 23-S rDNA vaccine</td>
<td>Vaccine response</td>
<td>Host Response</td>
</tr>
<tr>
<td>No</td>
<td>Poor vaccine response</td>
<td>Specific antibodies, protein components</td>
</tr>
<tr>
<td>No antibodies</td>
<td>Poor vaccine response</td>
<td>Protein and carbohydrate components</td>
</tr>
</tbody>
</table>

**Table**

Pneumococcal Virulence Factors and Host Response

**Legend**

- **CFP**: Choline binding protein
- **CFP (pCC)**: Choline binding protein (pCC)
CELL WALL CARBOHYDRATES

Pneumococcal cell walls are composed of multiple layers of peptidoglycan, in which the repeating units of muramic acid and glucosamine are cross-linked via the short "stem" peptides on the muramic acid. Peptidoglycan chains are assembled by six enzymes known as penicillin-binding proteins. These penicillin-binding proteins are the site of action of penicillins and cephalosporins. When peptidoglycan chains are broken during autolysis or by the action of β-lactam antibiotics, muramic acid peptides are released. These are highly inflammatory, and act in the host to stimulate tumor necrosis factor (TNF) and interleukins (ILs), especially IL-1 and IL-6. Antibodies to peptidoglycan do not protect against infection but probably promote recovery from disease.

The pneumococcus has a species-specific cell wall teichoic acid also known as C-polysaccharide. This consists of repeating units of polyglycerolphosphate, contains phosphoryl choline, and is essential for cellular integrity and normal cell division. C-reactive protein gets its name from reacting with C-polysaccharide. It binds to the phosphoryl choline on teichoic acid and also to host phosphoryl choline determinants that are exposed by damage to cell surfaces. C-reactive protein fixes complement and incites chemotaxis and inflammation. Humans also make antibodies to phosphoryl choline in response to pneumococcal colonization and infection. Although both anti-phosphoryl choline antibodies and C-reactive protein are protective in mouse models of infection, they play little or no role in protecting humans.

CELL SURFACE PROTEINS AND ENZYMES

PspA is the first of a family of choline-binding proteins to be investigated as a potential vaccine. At one end, PspA binds factor B and inhibits alternative complement activation. The other end of PspA binds to choline of cell wall lipoteichoic acid, anchoring the protein within the capsule and also blocking phosphoryl choline determinants that would otherwise act as activator surfaces. Pneumococcal surface protein C (PspC; also called choline-binding protein A) is a multifunctional adhesin that binds to secretory IgA, complement factor H, and oligosaccharides expressed on cytokine-activated host cells. PspC is being evaluated in mice as a potential vaccine. Pneumococcal surface adhesin A (PsaaA) is a metal-binding lipoprotein that probably functions mainly as a magnesium and zinc transporter but is also necessary for adherence and colonization.

Several autolytic enzymes have been implicated in virulence, including cell wall amidase and autolysin. Pneumococci that are defective in autolysis are generally avirulent, but antibodies to autolysin do not contribute to immunity. It appears that the main effect of these enzymes is to promote cell lysis, resulting in release of intracellular pneumolysin, along with cell wall materials.

Pneumococci produce two distinct neuraminidases, enzymes that cleave terminal N-acetyl-neuraminic moieties from host cell surfaces. Although neuraminidases have minimal direct toxicity, their action exposes sugar sequences, especially galactosamine-galactose units that pneumococci use for adherence to host cells. This is necessary for colonization and may contribute to invasion. When large amounts of neuraminidase are secreted into the circulation, the enzyme exposes the T antigen on red blood cells, platelets, and glomerular cells. Naturally occurring antibodies bind to the T antigen, fix complement, and damage the cells, causing pneumococcal hemolytic-uremic syndrome. Antibodies to neuraminidase do not by themselves offer much protection against experimental infections, but it is possible that they attenuate colonization, invasion, or hemolysis.

Pneumococci produce an immunoglobulin A protease that cleaves human serum and secretory IgA1 at the hinge region. Although a pathogenic role has not been established, this enzyme may facilitate colonization. Humans make antibodies to the protease, which in turn may impair colonization.

PNEUMOlysIN

The major toxin of pneumococci is pneumolysin, a multifunctional protein that binds to cholesterol and leads to pore formation in host cells. It belongs to a family of structurally similar
toxins, including streptolysin O from *Streptococcus*, perfringolysin from *Clostridium*, and protective antigen from *Bacillus anthracis*. Pneumolysin subunits are produced within the bacterial cell but it is not clear whether they become toxic only when the pneumococcal cell dies and releases its contents. Pneumolysin monomers bind to cholesterol on cell membranes and bind sequentially to other monomers, forming rings and arcs of 20 to 80 subunits that destabilize the membranes and cause lysis. Pneumolysin also binds IgG and activates complement. At sublethal doses, the toxin inhibits the respiratory burst of neutrophils, causes leakage of lysozyme, and induces apoptosis (programmed cell death). Pneumolysin is toxic to a wide variety of cells, including red blood cells, leukocytes, and vascular and brain microvascular endothelial cells. In the brain, it appears to be the main inducer of cytotoxicity, whereas cell wall components are also active in disruption of pulmonary and vascular endothelia.10,22 Pneumolysin also induces production of TNF-α and IL-1β and nitric oxide in macrophages, and the combination of pneumolysin and hydrogen peroxide from pneumococci inhibits the cilia on ependymal cells of the ventricular system of the brain.23,24 Antibodies to pneumolysin are protective against experimental infections in animals, and circumstantial evidence suggests that the same is true for humans.25

**INNATE IMMUNITY**

Nearly every child carries at least one pneumococcal strain by the second year of life, yet the development of systemic disease or mucosal infections, such as otitis media, is relatively unusual.26 Host defenses begin at the mucosal boundary. Bacteria of all types are continually being entrapped in the mucus blanket, killed by lysozyme and other defensive proteins, and carried away by the action of epithelial cilia. Organisms adhering to epithelial cells may not get a foothold before the cells are shed, washed away, and replaced by new cells with fresh slippery surfaces. Alpha-lactalbumin in human milk is bactericidal for pneumococci and may afford some protection for nursing infants.27 Human milk also contains oligosaccharides that bind to pneumococcal adhesins and prevent epithelial attachment of the bacteria.28 Similarly, the plant sugar xylitol has been used in chewing gum or syrup to prevent otitis media by impeding epithelial attachment and inhibiting pneumococcal growth.29

Pneumococci that breach the mucosal barrier activate complement via the alternative pathway or by antibodies that recognize common bacterial surface components.24,25 Macrophages in the submucosa engulf small numbers of penetrating bacteria, and neutrophils are recruited to areas of inflammation where larger numbers of bacteria have begun to cause tissue damage. Pulmonary macrophages are particularly efficient at clearing pneumococci that reach the alveoli.

When pneumococci overwhelm the local defenses, they multiply, produce toxins, and autolysen. Inflammation-inducing products are recognized by macrophage IL-1 receptors called Toll-like receptors (TLR), after their resemblance to the Toll molecules originally found in *Drosophila* fruit flies.30 At least 10 TLRs are known in humans and TLR2 is activated by many kinds of bacteria, including pneumococci.31 The TLR activation triggers the secretion of TNF, nitric oxide, IL-1, IL-6, and IL-8. In small amounts, these mediators stimulate dendritic cells, T helper cells, and B cells to effect an antibody response. They also amplify the inflammatory pathways and stimulate recruitment of neutrophils to the site of infection. When all goes well, the invaders are localized, ingested, and killed. The host subsequently develops an antibody response that prevents further attacks by that particular pneumococcal type or strain.

Alternatively, with an inadequate host response, the bacteria continue to multiply at initial sites of infection and in the spleen and bloodstream, with possible dissemination to other tissues. Pneumococci multiplying in alveoli spread locally in the lungs via the pores of Kohn and may breach the alveolar–capillary barrier and spill over into the circulation. The direct effects of pneumolysin and to some extent the host inflammatory response contribute to endothelial disruptions and breaches of the blood–brain barrier.32 Another mode of invasion is transcytosis (active transport of pneumococci through the cell), which appears to be mediated by PspC and
the host cell receptor for platelet-activating factor.\textsuperscript{33} Pneumococci occasionally bypass the bloodstream and invade the meninges directly from nasal infection or otitis media.\textsuperscript{34} Treatment with β-lactam antibiotics causes rapid breakdown of the bacteria, releasing more toxins and cell wall remnants, resulting in more inflammation and tissue damage.

**SPECIFIC IMMUNITY**

Opsonization and killing of pneumococci are enormously facilitated by type-specific antibodies directed against capsular polysaccharides.\textsuperscript{2,4,5,38} Although some phagocytosis can occur with IgG alone, mediated by Fc receptors on the phagocytes, it is greatly enhanced by activation of complement via the classic pathway. IgG antibodies are more efficient than IgM in this regard. Individual responses vary considerably, depending on age and circumstances of exposure. Most adults make antibodies after acute infections, and some make antibody in response to asymptomatic colonization.\textsuperscript{36} Infants generally respond poorly to natural exposure and to the unconjugated 23-valent polysaccharide vaccine, but respond well to the new conjugate vaccines.\textsuperscript{37}

Different conjugate vaccines appear to stimulate varying responses with respect to fine specificity, avidity, and opsonic capacity.\textsuperscript{38} This may be particularly important when considering protection of infants against poorly immunogenic serotypes and against cross-reacting serotypes. For example, the type 19F antigen in the conjugate vaccine elicits an antibody response comparable to that of other types, but the opsonic capacity of the antibodies is poor. This is reflected in the overall low efficiency of the conjugate vaccine against otitis media due to types 19F and 19A. In contrast, conjugate vaccine efficacy was excellent (84%) for type 6B but only modest (57%) against disease due to the related type 6A.\textsuperscript{39}

Specific immune responses to pneumococci are not limited to the capsular polysaccharides. Humans make antibodies to various surface proteins, including PspA and the surface adhesin PsA.\textsuperscript{40,41} Antibodies to these antigens are highly protective in mice,\textsuperscript{16,42} and antibody to PsA may be associated with lower risk of nasopharyngeal carriage and otitis media.\textsuperscript{43} Patients with uncomplicated pneumococcal pneumonia have higher levels of antibody to pneumolysin than do patients with bacteremic pneumonia, suggesting that these antibodies may prevent more serious forms of pneumococcal disease.\textsuperscript{29} These virulence molecules are attractive vaccine candidates because they may afford protection against serotypes not included in capsular polysaccharide vaccines. They are also likely to be immunogenic in children, and they may act locally at mucosal surfaces to reduce the risk of colonization and development of disease.

**MUCOSAL IMMUNITY**

Antibodies to pneumococcal capsular polysaccharides are detected in nasopharyngeal secretions and middle ear fluids of infants beginning at approximately 6 months of age.\textsuperscript{44} Immunization with conjugate vaccines results in salivary IgG derived from serum and IgA produced locally at the mucosal surface.\textsuperscript{45} Both are thought to contribute to the reduction in pneumococcal carriage observed by 9 to 12 months of age in vaccinees.\textsuperscript{46} A role for secretory antibodies to pneumolysin and surface proteins has also been suggested.\textsuperscript{47,48} Intranasal vaccines against PspA and PsA are highly effective in preventing nasopharyngeal carriage in a mouse model,\textsuperscript{16} and various strategies for mucosal immunization may be considered for protection of humans in the future.

**REFERENCES**


**CORRECTIONS**

There is an error in Table 5 on page 104 of the article “Pediatric Tuberculosis” in the February 2002 issue. The maximum daily dose of rifampin should be 600 mgm instead of 600 mg.

On page 142 of “Resident’s Column: What Every Resident Should Know About Tuberculosis,” the sentence, “In areas with multiple-drug resistance, ethambutol is added for the first 2 months.” should read “In areas with high rates (> 4%) of resistance to isoniazid, ethambutol is added for the first 2 months.”