REVIEW ARTICLE

Aspects of pneumococcal infection including bacterial virulence, host response and vaccination

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Introduction and epidemiology

Pneumococcal infection—a continuing problem

The incidence of serious pneumococcal infection remains high: for pneumonia 68–260/100 000/year; for meningitis 1.2–2.8/100 000/year; and for bacteraemia 7–25/100 000/year (Morbidity and Mortality Weekly Review, 1981). The pneumococcus is the most common aetiological agent of community-acquired pneumonia, e.g., 76% of cases in a recent study from the United Kingdom (MacFarlane *et al.*, 1982). If only those cases serious enough for admission to an intensive therapy unit are considered, *Streptococcus pneumoniae* is still the most frequent pathogen and has a mortality rate of 75% (Woodhead *et al.*, 1985). Of 57 000 children under the age of 5 years admitted to hospital in 1983 with acute respiratory infection, some 700 died accounting for 10% of all deaths in this age group (Lancet, 1985). Fifteen million children died in underdeveloped countries before the age of five, and about one-third of these deaths resulted from acute lower respiratory-tract infections in which *S. pneumoniae* was probably the principal pathogen. (Lancet, 1985).

Although antibiotics to which this organism is exquisitely sensitive are readily available, the survival rate of invasive pneumococcal infection has not been improved as one might have expected in the antibiotic era. This was demonstrated in the study by Austrian and Gold (1964), who showed that antimicrobial agents did not alter the outcome for a group of patients who had passed a physiological “point-of-no-return”, and is further illustrated by a review of mortality in patients with pneumococcal bacteraemia over the last 50 years (summarised in table I). Although these studies are not fully comparable because of the different hospital popu-

lations studied, the consistent trend of high mortality, especially in those > 70 years old, is apparent.

*Carriage of *S. pneumoniae**

The pneumococcus is carried in the upper respiratory tract of many healthy individuals. Colonisation begins shortly after birth and by the age of 12 weeks has increased to rates similar to those found in mothers (Riley and Douglas, 1981). Duration of carriage varies slightly; in one study mean duration for serotype 3 was 8.2 weeks whereas that for serotype 19 was 6.0 weeks. Rates of carriage are highest in preschool children and decrease with increasing age. Among adults, the likelihood of pneumococcal carriage depends on the frequency of contact with children. Colonisation increases in frequency during the autumn in temperate countries (Hendley *et al.*, 1975). The carriage of one serotype does not necessarily confer resistance to reacquisition of another strain of the same serotype. In general, < 50% of carriage results in resistance to re-challenge, e.g., 42% for serotype 3 and 32% for serotype 19. The proportion of subjects resistant to colonisation with any one serotype increases with age because of previous colonisation episodes (Dowling *et al.*, 1971).

The spread of pneumococci is proportional to the frequency and intimacy of contact between people and transmission often occurs in conjunction with respiratory viruses. The organism may, therefore, spread rapidly in closed communities and in cramped accommodation such as military barracks. In the special environment of gold mines in South Africa, the rates of carriage of invasive serotypes among miners are high because the community constantly receives new nonimmune subjects who are at an increased risk of pneumonia for 6 months after their arrival in a compound; thereafter, the risk falls dramatically.

Table I. Changes in mortality from pneumococcal bacteraemia by age and with time

Place and time	Number of patients	Mortality (%) in age group				
		12-29	30-49	50-69	≥ 70	All
Boston, MA, USA 1929-35*	582	48	71	89	100	77
Brooklyn, NY, USA 1952-62†	529	8	12	24	54	25
Chicago, IL, USA 1967-70‡	325	6	19	41	61	28
London, UK 1970-84§	325	29	23	28	40	29

Data taken from studies of *Tilgham and Finland (1937), †Austrian and Gold (1964), Mufson *et al.* (1974) and §Gransden *et al.* (1985).

Predisposition to serious pneumococcal infection

Several host factors are consistently associated with an increased risk of invasive disease: the extremes of age; immunological deficiency, both inherited and acquired; metabolic abnormality; and chronic respiratory disease. Young children have poor responses to carbohydrate antigens and a peak of incidence is found in this age group. The incidence remains low in adult life increasing again among the elderly. Although isolated defects in the chemotactic responses of polymorphonuclear leucocytes (PMNLs) and monocytes, and immunoglobulin or complement deficiencies have been associated with an increased susceptibility to infection, the most significant compromise of immunological function numerically is splenectomy (Wara, 1981). This problem is compounded by a defect in the alternative complement pathway in patients with sickle-cell anaemia. As a result, pneumococcal bacteraemia is a serious threat for children with sickle-cell disease. Alcoholic cirrhosis and diabetes mellitus are the most significant metabolic abnormalities associated with bacteraemia and, in adults, chronic obstructive-airways disease is the most common predisposing respiratory disease. The results of three surveys detailing susceptibility to pneumococcal bacteraemia are summarised in table II (Gruer *et al.*, 1984; Burman *et al.*, 1985; Gransden *et al.*, 1985).

The early use of bactericidal antibiotics by primary health-care physicians has decreased the mortality of pneumococcal infections but has not altered the attack rate. The introduction of an effective vaccine has proved a major advance in the prevention of pneumococcal disease in some patients. However, in many instances the patients most at risk of serious disease respond poorly to the vaccine. Over the last few years there has been a resurgence of interest in the antigenic structure and immunology of the pneumococcus. This review will focus on these studies and the way in which this

knowledge might be applied to improve the efficacy of vaccination.

Bacterial virulence determinants

Capsular polysaccharide (CPS)

Capsular polysaccharide is an essential virulence determinant of the pneumococcus in the absence of which the lethal inoculum in experimental infection is increased by a factor of 10^6 (Avery and Dubos, 1931). Pneumococcal capsular polysaccharide was the first non-protein antigen shown to be antigenic in man (Dochez and Avery, 1917). The capsule is produced in excess by bacteria in the logarithmic phase of growth and capsular antigens may be detected in the serum and urine of patients with invasive infections. The 83 different polysaccharides described have been classified in two systems; the American System numbers serotypes in the order of first isolation whereas the Danish system groups serotypes which are structurally and immunologically closely related, e.g., serotypes 6A and

Table II. Predisposing factors among patients with pneumococcal bacteraemia

Predisposing factor	Number of patients with stated predisposition among total number (n) in group studied		
	A n = 103	B n = 411	C n = 325
Chronic chest disorder	26	157	63
Alcohol abuse, cirrhosis	3	133	72
Haematological malignancy	9	39	28
Steroid therapy	0	0	28
Diabetes mellitus	2	22	11
Splenectomy or sickle cell disease	6	4	19

Data taken from the studies of: A, Gruer *et al.* (1984); B, Burman *et al.* (1985); C, Gransden *et al.* (1985).

6B differ only in the rhamnosyl-ribitol bond in their linkage (1-3 in 6A and 1-4 in 6B). These structural similarities cause difficulty in the production of monospecific antiserum for accurate serotyping, but work to the advantage of vaccine design in reducing the number of individual serotypes which must be included (Robbins *et al.*, 1983). The Danish system will be used throughout this review.

The capsular polysaccharides may be classified in two main structural groups: (i) linear heteropolymers composed of ≥ 2 monosaccharides as found in types 3 or 19F; (ii) multichained polysaccharides composed of 2-5 monosaccharides with additional residues. Invasiveness varies among strains and depends on the composition and quantity of capsule produced. Strains of both serotypes 3 and 37 produce large quantities of capsule and have a mucoid appearance on blood agar, but they differ in their ability to cause disease. The former, with a capsule composed of glucose and glucuronic acid, is among the most invasive types and is associated with a poor prognosis, whereas the latter, with a homopolymeric capsule of glucose, is rarely associated with infection. In experimental models with capsular mutant strains of the same serotype but with different amounts of capsule, virulence is found to be proportional to the quantity of capsule produced (Robbins *et al.*, 1983).

Cross-reactions occur with taxonomically unrelated organisms including *Escherichia coli*, *Pseudomonas aeruginosa* and *S. agalactiae* (Heidelberger *et al.*, 1985, 1986). All serotypes are antigenic and most are immunogenic, an important exception being serotype 6 in children. Generation of type-specific antibody heralds recovery from invasive infection (White, 1938). Although the capsule is a principal virulence determinant, the mechanism of its activity is less clear. CPS itself is not toxic to man or animals and its principal action is probably inhibition of phagocytosis. The presence of capsule presents a mechanical barrier preventing complement, deposited on the cell wall, from being opsonically active. In addition, CPS antigen has been shown to inhibit phagocytosis of opsonised bacteria by PMNLs as well as abolishing intracellular killing (Dhingra *et al.*, 1977; Schweinle, 1986).

C-polysaccharide (PnC)

C-polysaccharide is the major cell-wall component of the pneumococcus. Native C-polysaccharide is a ribitol teichoic acid containing phosphorylcholine and galactosamine; its detailed structure has been elucidated by Jennings *et al.* (1980). This antigen is covalently bound to the

peptidoglycan probably *via* the muramic acid residue.

Phosphorylcholine is the immunologically dominant epitope, an antigen not usually present among bacteria. Cross-reactions have been found with only a few strains of *S. mitior*, *S. salivarius* and *E. coli* (Mold *et al.*, 1982a, b). However, it is widely distributed among eukaryotic parasitic species including *Fasciola*, *Ascaris*, *Toxocara* (Sugane and Oshima, 1983) and *Leishmania* (Pritchard *et al.*, 1985), as well as some fungi (Pepys and Longbottom, 1971). C-polysaccharide is also important because it can activate the alternate complement pathway (see *Host defences*).

Phosphorylcholine is an essential growth factor for pneumococci and, although bacteria continue to grow when choline is substituted by another amino-alcohol, physiological defects soon appear: inability to undergo genetic transformation; resistance to phages; and, most significantly, the inability to divide so that long chains are formed (Briles and Tomasz, 1973). This latter change arises from the resistance of the ethanolamine-containing teichoic acid to pneumococcal N-acetylmuramic acid-L-alanine amidase (autolysin). Interaction with choline residues is essential for the enzymatic hydrolysis of the amide bond between N-acetyl muramic acid and L-alanine (Holtje and Tomasz, 1975).

F antigen

Phosphorylcholine is also incorporated into the F antigen, so called because it is a Forssman antigen capable of evoking sheep red-cell haemolysins when injected into rabbits. Forssman antigens are widely distributed in nature being found in the tissues of mammals, fish and birds and in bacteria. F antigen is an amphipathic molecule structurally similar to C-polysaccharide, with additional covalently attached lipid material which is a mixture of eight fatty acids one of which may be a hydroxyacid. Lipid makes up 5.7% of the mol. wt of the antigen and 15% of choline incorporated into the organism may be found in the F antigen (Briles and Tomasz, 1973).

This pneumococcal antigen is located in the small-vesicle fraction of the plasma membrane (Horne and Tomasz, 1985) as are lipoteichoic acids (LTAs) of other organisms. It has been suggested, therefore, that the F antigen is the functional equivalent of the more conventional membrane LTAs which contain a polyglycerophosphate backbone (Knox and Wicken, 1973). F antigen is a powerful inhibitor of pneumococcal autolysin. Regulation of murein hydrolase activity is a

function confirmed in other membrane teichoic acids with a polyglycerophosphate backbone. F antigen produced in cells grown in a medium in which choline has been replaced by ethanolamine is a significantly poorer inhibitor of autolysin. All pneumococci are prone to lysis during the stationary phase of growth, a state that is preceded by release of F antigen. It is thought that the loss of this powerful regulatory antigen results in the unres-trained activity of the N-acetylmuramic acid-L-alanine amidase (Horne and Tomasz, 1985).

Purpura-producing principle

Purpura-producing principle was first described by Julianelle and Reiman in 1926 as a component derived from pneumococcal cell walls. This material, which was capable of producing purpura in mice, rabbits and guinea pigs, has a mol. wt of 2.6×10^7 , is a complex of peptidoglycan and teichoic acid, and has been shown to be produced by the action of autolysin on the bacterial cell wall (Chetty and Kreger, 1985). Treatment with lysozyme results in inactivation implying that the β -1, 4-glycosidic linkage is required. It is not known whether the teichoic-acid (PnC) component is needed for biological activity.

Protein antigens and toxins

Pneumolysin. This sulphhydryl-activated toxin has strong sequence homology with the streptolysin-O toxin of *S. pyogenes* and with tetanolysin of *Clostridium tetani*. It is of 53–56 Kda, has a pI of 5.2, and has been found in the cytoplasm of almost all clinical isolates tested (Walker *et al.*, 1987). It has haemolytic activity, which may be inhibited by cholesterol and antibody raised to streptolysin-O, and may have an important role in the pathogenicity of pneumococcal infection. Thus, purified toxin has been shown to inhibit the respiratory burst of PMNLs and monocytes, and the activation of the alternate complement pathway (Nandoskar *et al.*, 1986). Injection of toxoided pneumolysin into mice significantly increased their survival time on lethal challenge with virulent organisms (Paton and Ferrente, 1983; Paton *et al.*, 1983, 1984; Ferrente *et al.*, 1984).

Pneumococcal surface protein A. This protein has been found on the surface of many clinical and laboratory strains of pneumococcus. Experiments with monoclonal antibodies directed against this protein have shown that passive immunisation may be protective. No function has been attributed to this antigen, although its loss is associated with loss of virulence (McDaniel *et al.*, 1987).

Neuraminidase. This virulence factor, possessed by many pathogens, has the capacity to damage the host by cleaving terminal sialic-acid residues in glycoproteins, glycolipids and oligosaccharides expressed on host-cell membranes and in body fluids. The concentration of N-acetylneuraminic acid in cerebrospinal fluid can be correlated with coma and adverse outcome in pneumococcal meningitis. Purified pneumococcal neuraminidase is a protein of 107 Kda that is predominantly cell-wall associated. Although it was previously thought that there were several isoenzymes, recent work demonstrated that it is highly susceptible to proteolytic cleavage during purification, resulting in apparent mol. wt heterogeneity (Lock *et al.*, 1988). The pathogenic significance of this enzyme in pneumococcal infection has not been established.

Neutrophil elastase inhibitor. Despite the massive influx of PMNLs during pneumococcal infection and the release of toxic products, there is rarely any derangement of alveolar architecture after recovery. This may be associated with neutrophil elastase inhibitors, two of which are known to be produced by *S. pneumoniae*. One is a negatively charged substance of low mol. wt that depends on an ionic interaction with the cationic elastase. It is substantially inhibited by NaCl in physiological concentrations and unlikely, therefore, to be important *in vivo*. A second inhibitor, located on the outside of the bacterial cell membrane has a mol. wt of 140 000. It inhibits elastase non-competitively and is not inhibited by NaCl. Its action is specific; it does not inhibit other elastases or other neutrophil enzymes (Vered *et al.*, 1984). It is sensitive to trypsin and the cleavage products are also biologically active, protecting mice from alveolar damage by neutrophil-granule extracts (Vered *et al.*, 1985). Interaction with elastase is by non-covalent bonds.

Host defences

Early events

The integrity of the respiratory epithelium is an important defence against invasion. Mice infected with influenza virus and subsequently challenged with aerosolised virulent pneumococci are resistant to infection until the influenza infection has progressed sufficiently to produce histological damage to the epithelium. *S. pneumoniae* does not adhere to ciliated epithelial cells, but 4–6 days after influenza virus infection the ciliated cells have desquamated. The bacteria then attach readily to the basal cells (Plotkowski *et al.*, 1986).

In the alveolar space, macrophages provide

defence against inhaled particulate matter. However, they are not able to phagocytose capsulate pneumococci efficiently even when the organisms have been opsonised with 80% non-immune serum (Jonsson *et al.*, 1985). Once infection is established, there is a massive influx of PMNLs, the stimulus for this being the bacterial cell wall. This response is dose dependent and may occur as a result of chemotactic factors generated *via* the interaction of C-polysaccharide with the alternate complement pathway (Vial *et al.*, 1984).

The role of the spleen

Patients who have undergone splenectomy are at greatly increased risk of overwhelming bacterial infections and *S. pneumoniae* is responsible for >50% of these episodes. The outcome varies according to the underlying medical condition for which splenectomy was performed. The prognosis is best for those patients who have had splenectomy after trauma, with a poorer prognosis for patients with portal hypertension, sickle-cell anaemia, Hodgkin's disease or other diseases of the reticuloendothelial system (Wara, 1981). The spleen contributes to resistance to pneumococcal infections in several ways. First, it provides a large phagocytic mass for the clearance of poorly opsonised particles; this is important in the early stage of infection before the appearance of specific antibodies. When IgM and IgG appear, pneumococci are preferentially cleared by the liver (Schulkind *et al.*, 1967). The spleen also contributes by enhancing the production of serotype-specific antibodies. Splenectomised patients respond less well to vaccination and infection, and antibody levels decline more quickly. In the mouse model, type-specific antibody concentrations reach a peak 5 days after vaccination, and elevated levels are sustained for >28 days. In splenectomised mice, the peak is not reached until 14 days. All antibody responses to serotype-3 organisms are abolished by splenectomy (Cohn and Schiffman, 1987).

Antibody responses to S. pneumoniae

Capsular polysaccharides. Antibody to CPS is considered to be essential for the survival of an animal challenged with virulent pneumococci. It is the centrality of this antibody response which has made these polysaccharide antigens the main target for vaccine development. In natural infection, type-specific antibody is produced by the sixth day of infection and can usually be detected the first day after crisis (White, 1938). In healthy persons, anti-

CPS antibodies vary with age of the person and in concentration and class specificity also. High IgG₂ antibody activity can be found in cord blood and in serum from adults. IgG activity is strongest in children and almost absent in adults. The concentration of IgA antibodies increase steadily with age (Rynnel-Dagöö *et al.*, 1986).

Patients with pneumococcal bacteraemia differ markedly in the concentration of antibody found in serum. One study of 50 adults with bacteraemia showed that some had developed invasive disease despite the presence of preformed specific antibody to CPS. In others antibody concentrations usually rose, but occasionally fell or remained unchanged, in the convalescent period. In those patients with low antibody concentrations, free CPS antigen could be detected in serum (Riley and Douglas, 1981).

After vaccination there is a significant rise in total IgG, predominantly of IgG₁ and IgG₂ subclasses. In one study, after vaccination with type-3 polysaccharide, the geometric mean titre of IgG₂ was significantly higher than those of IgG₁, IgG₃ or IgG₄. Only the IgG₂ post-vaccination titre was statistically significantly greater than the pre-vaccination concentration (Chudwin *et al.*, 1987).

Children differ in their responses to CPS: for example, serotype-6 strains are often carried for prolonged periods because immune response to this polysaccharide is poor; invasive disease with strains of this type is, therefore, common. Conversely, in adults, type 6 is a good immunogen and invasive disease consequently rare (Robbins *et al.*, 1983). The rank order for the most frequently isolated serotypes from blood in adults is as follows: 8, 4, 3, 14, 7, 12, 9, 1, 18, 19, 6 and 23 which together account for 79.8% of isolates. In children, the rank order is: 14, 6, 23, 19, 4, 9, 7, 1, 3, 8 and 15, the first four types being responsible for 60% of all invasive infections (Robbins *et al.*, 1983).

Studies of mice immunised with an optimal dose of type-3 pneumococci show that the number of plaque-forming cells (PFC) per spleen rises rapidly to a maximum after 4 days; the numbers fall quickly thereafter (Baker *et al.*, 1981). The levels of antibody in the serum do not, however, decline as rapidly owing to the development of extra-splenic antibody synthesis. The initial response to CPS is mostly in the IgM fraction and, of the two types found, one fixes complement and the other requires the addition of class-specific facilitating antiserum. The proportions of IgG₁, IgG₂ and IgA vary depending on the strain of mouse studied (Baker and Stashak, 1969; Barthold *et al.*, 1974; Pasanen *et al.*, 1979).

CPS is a T cell-independent antigen but treatment of mice with antilymphocyte serum (ALS) results in a delay in the splenic PFC followed by a later enhancement. This is reflected in higher total-antibody concentration after treatment with ALS, an effect eliminated when ALS was absorbed with thymocytes. Since the overall effect cannot be demonstrated in athymic nude mice, it is suggested that T cells are important in the enhancement of antibody responses, although not required for its initiation. It has been proposed that antibody responses to type-3 pneumococci are regulated by two types of T cell, suppressor (T_S) and contrasuppressor cells (T_{CS}) (Baker *et al.*, 1981; Braley-Mullen 1986a). The T_{CS} belong to a unique T cell subset; they are Igh restricted, have antigen-binding receptors and carry the phenotype $Lyt1^+ 2^- L3T4^- I-J^+ I-A^+$. Contrasuppressors, which must be compatible with both T_S and B cells, have no activity in the absence of T suppressor cells (Braley-Mullen, 1986a, b).

After vaccination of man, anti-CPS antibody-secreting cells can be detected only when peripheral blood-derived mononuclear-cell (PBMC) cultures are depleted of T cells. In EBV-stimulated cultures depleted of T cells, anti-CPS antibody-secreting cells can be detected in small numbers in PBMC taken before vaccination. The numbers increase significantly after vaccination, in contrast with the situation occurring when T cells are not removed, when there is a decrease in the number of secreting cells (Heilmann, 1987).

C-polysaccharide. Antibodies to PnC have the advantage of being common to all 83 serotypes. Naturally occurring and monoclonal antibodies and myeloma proteins with antiphosphorylcholine (PC) specificity have all been shown to protect mice challenged with virulent pneumococci. Significant levels of antibody to PnC are uncommon in the first 5 months of life (Briles *et al.*, 1987), but increase steadily thereafter so that nearly all children develop some antibody (IgM) by the age of 4 years (Gray *et al.*, 1983). Antibody levels increase with age and develop in response to carriage and infection (Gray *et al.*, 1983). Of the 83 types of pneumococcus, 56 were shown to produce a measurable change in the anti-PnC antibody level in response to carriage. Stimulation of these antibodies need not necessarily be by pneumococci because similar epitopes are found on other bacteria, fungi and parasites (Pepys and Longbottom, 1971).

Children less than 2 years old respond briskly to PnC, in contrast to their poor antibody response to CPS. A study of 124 patients hospitalised with pneumonia showed that three of six who had *S.*

pneumoniae bacteraemia had a significant rise in anti-PnC antibody concentration. Of 44 patients in whom a positive culture was also obtained, 17 had a significant rise in PnC antibody levels, in comparison to only one of 35 patients whose pneumonia was thought to be caused by another organism (Holmberg *et al.*, 1985).

In mice, as in man, the response to PnC is dominated by the phosphorylcholine (PC) portion of the molecule. Antibodies are restricted to one of three major idiotype families, T15, M603 and M511 (named by the corresponding prototypic idiotype bearing mouse-myeloma protein). All of the antibodies have a similar V_H region (T15), and each of the three families of antibodies has a distinct V region in its light chain. All of the V_H regions expressed in mice are derived from the germ-line sequence. Those variants which do occur have arisen by somatic mutation, and this occurs only in IgA and IgG. IgM molecules express the germline T15 V exclusively (Crews *et al.*, 1981). It is not known if human responses are clonally restricted in the same way.

Immunisation of rabbits with non-capsulate pneumococci conferred some degree of active and passive immunity (Tillet, 1928) to different capsulate types in mice. Rabbit polyclonal and mouse monoclonal anti-PC antibodies have been shown to protect mice from fatal challenge (Yother *et al.*, 1982). Amongst the different idiotypes, T15 antibodies are much more protective than the other families, and, of these, IgG antibodies are superior to IgM (Briles *et al.*, 1984). The mechanism of this protection was thought to involve enhancement of opsonisation (McDaniel *et al.*, 1984). Similar protection against challenge with capsulate organisms of different serotypes has also been shown for C-reactive protein (CRP) (Yother *et al.*, 1982).

Antibodies to C-polysaccharide are almost universal in man and, therefore, are not protective in the same way as anti-CPS. PnC is situated deep to the capsule and is not available for opsonisation. Anti-PnC antibody from vaccinated subjects fails to opsonise capsulate strains (Musher *et al.*, 1986) and CRP is not able to enhance opsonisation of capsulate strains above normal serum (Chudwin *et al.*, 1985), but can do so for serotype 27, which is unique in having a PnC-like epitope in its capsule. The ability of any one serum to opsonise cannot be correlated to CRP or anti-PnC antibody concentrations present (Chudwin *et al.*, 1985).

These contradictory results may have arisen from problems with handling capsulate organisms, exposing cell-wall antigen to opsonisation with CRP or anti-PnC antibody. However, increased survival

in mice protected with proteins with anti-PC activity has been reproduced on many occasions. It may be that there is a fundamental difference in the immune response between man and mouse. The human response to PC-containing pathogens may be with idiotypes which are not protective, as in the case of the mouse idiotypic families M603 and M511. There has been insufficient study of human anti-PC responses to answer this question. Finally, the role of CRP and anti-PC antibodies may not be *via* complement-dependent opsonisation, but rather by limiting host damage caused by PnC released from infecting organisms.

Complement

The role of complement in host resistance to pneumococcal infection is critical. During the course of acute infection patients with severe disease have decreased levels of circulating C3 and factor B, indicating the consumption of complement together with alternate complement-pathway activation or rapid turnover of factor B (Reed *et al.*, 1976). Kindreds with inherited deficiencies of C3 are, with one exception, at an increased risk of pneumococcal bacteraemia and septicaemia (Alper *et al.*, 1970). In the same way, increased susceptibility is found in patients with C3b inactivator deficiency and combined C2-factor B deficiency (Alper *et al.*, 1970; Abramson *et al.*, 1971; Newman *et al.*, 1978). For mice treated with cobra (*Naja naja*) venom factor, the lethal dose of *S. pneumoniae* by intraperitoneal injection is reduced by up to 50 times (Winkelstein *et al.*, 1975; Winkelstein, 1981). As in infection with other gram-positive bacteria, opsonisation by the deposition of C3b on the bacterial surface is the main way in which complement proteins act; complement is unable to produce bacterial lysis in pneumococci. Although the membrane attack-complex forms normally, it is inserted 10–15 nm into the peptidoglycan, well short of the 100-nm murein layer of pneumococci (Joiner *et al.*, 1983).

The alternate pathway. Pneumococci are potent activators of the alternate pathway. PnC is the active cell-wall component because removal of teichoic acid from peptidoglycan diminished complement activation (Winkelstein and Tomasz, 1978). The plasma membrane is also a weak activator of the alternate pathway, an activity residing in the F antigen (Hummell *et al.*, 1985). Pneumococcal capsule can also activate the alternate complement pathway. Serotypes 7, 12, 14 and 25 do so readily but not type 1. Serotypes 3, 4 and 8 also activate this pathway but lose the capacity when cross-reacting antibody is removed. Comple-

ment activation by the capsule is 50–100 times less efficient than with PnC (Fine, 1975).

Studies of the proteolytic degradation of C3b covalently bound by pneumococcal capsule indicate variation among serotypes. Serotypes 3 and 4 were shown to have C3b, iC3b and C3d whereas serotypes 6A and 14 have only iC3b (Hostetter, 1986). Many studies have sought, therefore, to explain the differences in virulence among serotypes by the ability to fix complement by the alternate pathway. Although such differences are readily demonstrated they do not form a consistent pattern.

Alternate-pathway activation will take place in the absence of specific antibody but the process is enhanced when bacteria are incubated with immunoglobulin isolated from non-immune serum. The specificity of this antibody is not known, but the effect can be removed if serum is absorbed with *E. coli* indicating that the phenomenon may arise from cross-reacting antibodies. (Bjornson *et al.*, 1985). This activity is related to the F(ab') portion of the molecule as the enhancement still occurs when the Fc fragment is removed (Bjornson and Lobel, 1986). It is the absence of this cross-reacting antibody which is responsible for the defect in the alternate pathway found in patients with sickle-cell disease. Complement activation by cell-wall teichoic acid and F antigen results in the deposition of C3b on the cell wall. However, at this site it is not available to the phagocyte receptors, so limiting its effectiveness in the phagocytic process (Brown *et al.*, 1983).

The classical pathway. Antibodies to CPS of the correct subclass fix complement *via* the classical pathway. In-vitro experiments with rabbit antibodies against the capsular polysaccharide have been shown to activate C1, C4, C2 and C3 sequentially, depositing C3b opsonically on the surface of the organism (Paramen *et al.*, 1979). Paradoxically, experiments with convalescent human serum have failed to show significant activation of the classical pathway, due in part to the class-specific responses to infection. The ability to opsonise can be correlated with total anti-pneumococcal IgG₁ and IgG₂ (Chudwin *et al.*, 1987). The classical pathway may also be activated by antibodies to other pneumococcal components such as PnC. Interaction of PnC with anti-PnC antibodies or CRP and complement results in activation of the classical pathway, a mechanism possibly important in the early stage of infection when CRP levels are high.

Vaccination

The first attempt to produce a vaccine against *S. pneumoniae* was made in 1911 by Almroth Wright

who had previously developed a successful vaccine against typhoid. He initiated six mass-inoculation experiments involving more than 50 000 subjects in the South African mines. The vaccine itself was made without regard to serotype and too few organisms were used in its formulation, thus explaining its failure. His work was carried on by Spencer Lister who showed that recovery from pneumococcal infection was accompanied by the development of antibodies to the infecting organism; he concluded that pneumococci existed as a number of serotypes and showed that a minimum of 10^{12} organisms was required to stimulate an optimum antibody level. Lister attempted vaccination trials among South African miners, and although he showed a reduction in the attack rate in vaccinated persons from one compound in comparison to unvaccinated ones in another, criticism of the study design left the issue of efficacy a subject of debate (reviewed by Austrian, 1981*a, b*). In 1945, MacLeod showed that a vaccine containing CPS from strains of serotypes 1, 2, 5 and 7 was highly effective in preventing illness caused by these serotypes among military recruits. This promising development was followed by the production of a hexavalent vaccine, but interest in a pneumococcal vaccine waned after the introduction of penicillin.

The current vaccine is composed of 23 serotype-specific capsular polysaccharides (50 μ g each of types 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F and 23F), a dosage based on the work of MacLeod who showed that 30–60 μ g of types 1, 2, 5 and 7 was required to produce type-specific protection in military recruits (MacLeod *et al.*, 1945). Most healthy adults show at least a two-fold increase in type-specific antibodies within 3 weeks of vaccination. In 23 normal subjects tested 10 years after vaccination, antibody responses to serotypes 4 and 7F remained elevated; but for 1, 3, 12F, 14 and 19F serotypes, only eight subjects maintained elevated anti-capsular antibodies (Mufson *et al.*, 1987). There is no clear antibody level which might be described as protective; studies of pre- and post-vaccination serum showed that, although there were significant rises in total specific immunoglobulin, concentrations could not be correlated with changes in the opsonic ability of serum (Chudwin *et al.*, 1985).

The choice of serotypes for inclusion in the vaccine has been based on surveys of isolations in Europe and North America, taking into account the cross-reactions noted above. Serotypes representing isolates in Brazil, Senegal and Israel were

also included in the evaluation (Robbins *et al.*, 1983). The unavailability of reliable serotype data from Third World countries may mean that the vaccine will be less efficient than predicted, if the distribution of serotypes there differs markedly from those in Europe and North America. There is no doubt that pneumococcal vaccination is highly efficient in a young, immunologically competent population at high risk of pneumococcal disease (Schwartz, 1982). The efficacy is less clear when there is compromise of the immune system.

As has been noted, development of antibody to CPS requires a functional splenic mass and patients with splenectomy respond less well to the vaccine than normal subjects. However, protective levels of antibody can be stimulated in 80% of patients, but these levels may decline rapidly (Giebink *et al.*, 1984). There is probably little difference in the antibody level developed following vaccination among groups splenectomised for traumatic, haematologic or other immunological indications. Timing of vaccination with respect to splenectomy is probably not critical, but because antibody responses afterwards may be impaired, vaccination before operation is preferred. Revaccination of splenectomised patients may be beneficial, because it has been shown that the percentage of subjects with a protective level of antibody to serotypes 6A, 14 and 19 can be increased from 74, 44 and 79% to 92, 66 and 92%, respectively (Kaplan *et al.*, 1986).

Children less than 2 years old respond poorly to polysaccharide antigens. Serotypes included in the vaccine may be classified as strongly immunogenic in children of all ages, as age-dependent immunogens (19F and 18C), or as uniformly poor immunogens (6A and 23F) (Leinonen *et al.*, 1986). In a large study of children less than 5 years old in Papua New Guinea, where acute respiratory infection is the commonest cause of death, children were vaccinated between 6 months and 5 years of age. The efficiency of vaccination in reducing death from acute lower respiratory tract infections was estimated at 59% and 50% in children less than 5 and 2 years, respectively. There was a significant difference in the mortality in the vaccinated group compared with that in a placebo-control group (Riley *et al.*, 1986).

Other trials of vaccine efficiency in high-risk groups (such as the elderly) have often been disappointing. In one study of 89 patients, there was no difference in bacteraemic rates in vaccinated subjects compared with control subjects (Forrester *et al.*, 1987). Moreover, 65% of blood-isolate types from non-vaccinated patients were included in the vaccine compared with 69% of those in the

vaccinated group. A randomised double-blind placebo-controlled trial in 2295 high-risk patients (>55 years old and with chronic cardiac, pulmonary, hepatic or renal disease, alcoholism or diabetes mellitus) has been performed. Of 63 proven or probable invasive infections, 27 were placebo and 36 vaccine recipients. In the vaccine group 14 infections were caused by serotypes contained in the vaccine. Vaccine failure probably occurred because subjects did not make, or sustain, serum antibodies to their infecting organism (Simberkoff *et al.*, 1986). A recent study of elderly subjects without immune compromise showed a 70% vaccine efficiency. It is clear, therefore, that vaccination provides useful protection to those patients immunologically competent to respond, but for patients at highest risk alternative vaccine strategies must be found (Sims *et al.*, 1988).

New developments

Alternative vaccine strategies may be considered in three main areas: development of pneumococcal protein vaccine candidates; enhancement of responses to CPS vaccine by conjugation with protein antigens; and enhancement of PnC-antibody responses. These approaches are not necessarily mutually exclusive and the provision of an improved vaccine might involve several of these strategies.

Several groups are investigating protein antigens including pneumococcal surface-protein A, the loss of which results in a loss of virulence. Mice may be partially protected by immunisation with this antigen and passive immunity with monoclonal

antibody against surface protein is also partially protective (McDaniel *et al.*, 1987). Pneumolysin, which has been cloned and sequenced, is a protein antigen known to protect mice.

Linking an oligosaccharide from serotype-6 material to the non-toxic diphtheria protein from mutant strain CRM197 rendered it highly immunogenic (Porro *et al.*, 1985). This method might be used to improve the antibody responses to other poorly immunogenic types.

Antibodies to PC have been shown to be protective in mouse models. Attempts to enhance immunity have been made with purified PnC, ovalbumin-PC, and KLH-PC. An idiotype vaccine has been developed to the PC epitope (Wallick *et al.*, 1983; Ward *et al.*, 1987). All of these strategies have enhanced mouse resistance to infection but, as has been noted, their applicability to man is still controversial.

Concluding remarks

S. pneumoniae is still one of the most important organisms infecting man. Much has been learnt about the organism and human immune responses to it in the last 100 years. However, our understanding of some aspects of pathogenesis, notably events occurring early in the course of infection, is poor. Growth areas in research are likely to include: factors transforming carriage into invasive infection; the role of alveolar macrophages and PMNL in the lung; the role of preformed anti-PC antibodies, CRP, and antibodies to protein antigens which might enhance species-specific resistance to infection.

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