The pneumococcus: host-organism interactions and their implications for immunotherapy and immunoprophylaxis

Conventional approaches to pneumococcal infection have focused separately on killing the organism and supporting the host. A capsular polysaccharide vaccine is also available, though this has been little used in practice.1 Whilst antibiotics and supportive care have greatly reduced overall mortality from *Streptococcus pneumoniae*, and will remain the keystone of the treatment of established infection, there remain two principal challenges from pneumococcal pneumonia – namely, the continuing high incidence of the disease and the high mortality in the first few days of hospitalisation.2-4

In recent years there has been an increased understanding of the interactions between the organism and the host, and specifically between the pneumococcus and the host, both in terms of how virulence factors of the organism contribute to disease pathogenesis and how the host’s response to infection, through the release of cytokines, may be deleterious. What are the prospects for using this knowledge to address these outstanding problems?

**Immunotherapy**
Cytokines are low molecular weight hormone-like polypeptides produced during immune reactions which are capable of mediating pathological as well as beneficial effects. Recombinant forms of several cytokines, and monoclonal antibodies raised against them, are now available and, as their roles in disease processes are beginning to be understood, their therapeutic potential is being considered. One area in which substantial understanding has been gained of the harmful consequences of cytokines induced by host-organism interactions is septic shock and the adult respiratory distress syndrome (ARDS). There is strong experimental evidence that tumour necrosis factor (TNF), interleukin (IL)-1 and IL-6 play an important role in the pathogenesis of these conditions,5,6 although trials of anti-TNF monoclonal antibody and IL-1 receptor antagonists in patients suffering from these conditions have so far been disappointing.8 Nevertheless, there is still considerable optimism that more targeted studies may prove beneficial in selected patients. Although the patients in these studies were acutely ill, and some had pneumonia, the results cannot be extrapolated to pneumococcal infection, for most of the patients were infected with Gram negative bacteria. Furthermore, our understanding of the relevance of these studies is limited by our lack of understanding of pneumococcal septicemia, in particular the extent to which ARDS or classical septic shock complicate this condition.

Only a limited number of studies have specifically examined the role of cytokines in pneumonia. A recent study by Moussa and colleagues9 measured plasma IL-6 and TNFz levels in patients admitted to hospital with community acquired pneumonia. They found raised levels of these cytokines in a percentage of the patients at some stage in their illness but observed little correlation with the outcome of infection. Marik et al10 also found raised levels of serum TNFz in inpatients with severe community acquired pneumonia but, in this case, in all patients studied. The levels remained stable over the first 12 hours of admission, were unaffected by antibiotics and, again, gave no prediction of outcome, unlike the APACHE II (disease severity) score. In contrast, two studies by Chollet-Martin and coworkers did find a positive correlation between cytokine levels and patient outcome.12,13 These studies principally concerned cytokine levels in patients with ARDS but included patients with pneumonia as a control group. Plasma levels of TNFz, IL-6, and IL-8 were raised in patients with pneumonia compared with controls. Levels were not as high as in patients with ARDS (without pneumonia) but were greatest in patients with both ARDS and pneumonia. In the overall patient group significantly higher levels of plasma IL-6, bronchoalveolar lavage fluid IL-8, and a trend towards higher plasma TNFz were found in the patients who died. In addition, lung injury scores were positively correlated with plasma TNFz and IL-6 amongst the patients with pneumonia. Thus there is some evidence of raised plasma levels of TNFz, IL-6 and IL-8 in patients with pneumonia, but the pathogenic and prognostic implications of their presence is not clear.

Several factors may contribute to the differences between these results. Firstly, the time point at which samples are obtained is very likely to be critical to the outcome of a study as cytokines are subject to regulatory mechanisms; thus non-elevated TNF levels, for example, may result from missing the peak of production and do not necessarily indicate that the cytokine is unimportant in disease pathogenesis. Secondly, the difficulties of obtaining accurate and meaningful plasma cytokine measurements are increasingly being recognised:14,15 endogenous cytokine binding proteins, ex vivo cytokine release, and inherent variations in what different types of assays measure may all contribute to conflicting results. It is essential that each group making cytokine measurements validates the ability of their assay system to detect cytokines in biological fluids and also establishes the normal range for the cytokine in their hands; such verification was not provided in some of the above studies.10,11

It should also be noted that, so far, only a few cytokines have been considered in pneumonia. It will be important to extend this range, especially as the combined effect of several cytokines is likely to be important in disease pathogenesis and outcome.16 Furthermore, these studies concern pneumonia in general and are not specific for the pneumococcus, or any other causative organism. Although logistically difficult, it would be interesting and potentially valuable to establish whether different cytokine profiles are associated with different pathogens.

At present intervention studies have been confined to animal models. Ziegler-Heitbrock et al17 found that injection of *Srv pneumoniae* into pigs caused a sharp and transient rise in serum IL-6 levels which peaked at four hours. Pretreatment of the pigs with the monococyte activator muramyl tripeptide phosphatidylethanolamine (MTP-PE) prevented this rise and also protected the animals from death. Other animal studies have also shown a beneficial effect of granulocyte colony stimulating factor (GCSF), a neutrophil activator, in protecting rodents against pneumococcal infection, although in each case the GCSF therapy commenced before the pneumococcal challenge, limiting the usefulness of these studies.18,19 The use of “immune activators” may seem paradoxical in light of other studies suggesting that high levels of cytokines may be deleterious to the host. These apparently diametrically
opposed lines of investigation emphasise the point that cytokines can mediate both beneficial and harmful effects. Indeed, local cytokine release is known to be important in pulmonary defence. It is also worth noting that anti-TNF therapy was associated with increased mortality in a murine model of peritonitis. In this regard it is interesting that GCSF was beneficial in a canine model of bacterial sepsis whereas monoclonal antibodies directed against leucocyte CD11/18 adhesion molecule, which inhibits neutrophil function, were harmful. Although the pneumococcus was not the subject of these last two studies, they illustrate that risks are involved in attempting to neutralise individual components of the immune system.

Clearly this is a complex field, but it is important not to be daunted as the potential benefit of immunotherapeutic intervention is a goal worth pursuing. Mortality from bacteraemic pneumococcal pneumonia is consistently over 20% and mortality in the first few days of hospitalisation has not changed since the pre-antibiotic era. However, before we can contemplate immunotherapeutic intervention in human pneumococcal infection we need to have a much greater understanding of the role of cytokines in pneumococcal infection and to clarify the point at which the immune response ceases to be beneficial to the patient and cytokine release becomes pathological. This will entail using reliable assays to measure a wide range of cytokines at various time points during the course of pneumonia and correlating levels with the causative organism, and with clinical signs such as hypotension and neutropenia as well as outcome of infection.

Immunoprophylaxis

Given that the prospects for such immunotherapy are not imminent, what means do we have to prevent pneumococcal infection, and how can an increased knowledge of the interactions between the pneumococcus and its host be used to improve the pneumococcal vaccine?

The currently available vaccine is composed of polysaccharides from the capsules of 23 serotypes of Str. pneumoniae. Capsular polysaccharide (CPS) was chosen as the antigen because of the key role played by the capsule in the pathogenicity of Str. pneumoniae. Purified CPS is a T-independent antigen — that is, it can elicit an antibody response by stimulating B cells directly without the help of T lymphocytes. In contrast, most vaccines are composed of protein antigens — for example, diphtheria, tetanus — which require T cell help to elicit immunity — that is, they are T-dependent. T-independent antigens have certain properties which make them less than ideal immunogens: (1) they do not elicit a memory booster response; thus on second and subsequent encounter of the antigen there is no enhanced/faster response; and (2) they are very poor immunogens in children under two years of age. However, T-independent antigens can be converted into T-dependent ones by their conjugation to a protein carrier. This approach has successfully been used to improve the Haemophilus influenzae type b (Hib) vaccine leading to the licensing and now routine use of the conjugate vaccine.

Pneumococcal conjugate vaccines are under development but work is less advanced than with Hib. Most work to date has been performed in animals. Type-specific CPS conjugated to diphtheria, tetanus or pertussis toxoids, as well as other proteins such as bovine serum albumin, have been shown to be immunogenic and to have enhanced specific immunogenicity compared with the 23-valent vaccine or the CPS alone. The conjugates elicited a T-dependent antibody response, producing IgG, memory B cells, and an enhanced secondary response. The influence of polysaccharide chain length, polysaccharide to protein ratio, and the effect of different carriers and adjuvants on the immunogenicity of the conjugates are all under investigation. In addition, types 16B and 19F conjugate vaccines (but not type 14) were shown to protect chinchillas against otitis media.

Fewer studies have been conducted in humans using CPS conjugated to these common protein carriers. Such vaccines have been shown to be immunogenic in adults and children with sickle cell disease and to elicit higher levels of anti-CPS antibodies and a response in a greater proportion of subjects than the CPS alone, or CPS as a component of the pneumococcal vaccine. The CPS conjugates elicited booster responses in children but not in adults, in keeping with Hib conjugates, and may indicate that maximal antibody responses were achieved in adults following their first immunisation, or that they were hyperimmune to the tetanus toxoid carrier, or that too short an interval was allowed between immunisations.

Thus studies are limited to measuring antibody responses, although the elicited antibody has been shown to protect passively immunised mice against lethal challenge with Str. pneumoniae, protection studies remain to be reported and will require much larger study populations.

Whilst these results are generally encouraging, pneumococcal conjugate vaccines utilising these common protein carriers potentially present several problems. Firstly, pneumococcal conjugate vaccines will require the physical linking of the protein to many different CPS moieties — a more complicated task than for Hib, with just one CPS. In addition, high levels of free, unconjugated CPS have been shown to inhibit the anti-CPS antibody response to CPS protein vaccine, although low levels (up to 10%) did not have this effect. Secondly, most pneumococcal conjugate vaccines developed so far use the same protein carriers as those used in the Hib and meningococcal vaccines and which are also used routinely in their own right. Repeated use of the same toxoid has the potential of inducing Arthus-type hypersensitivity reactions at the site of injection, caused by the deposition of immune complexes. Such reactions have been reported in adult volunteers receiving toxoid conjugated vaccines, although the reactions were generally not debilitating or prolonged and it appears that adverse reactions may be less common in children as predicted from experience of Hib conjugate vaccines. Thirdly, it is recognised that the introduction of a new antigen on a carrier with which the recipient has previously been immunised can result in suppression of the immune response to both the novel antigen and the carrier. Thus, repeated use of the same toxoids conjugated to different T-independent polysaccharides could have the opposite effect to that desired, although this has not been reported to date in the context of pneumococcal conjugate vaccines.

These latter two points indicate the need to develop novel carriers for the pneumococcal CPS antigens. Obvious candidates for such carriers are proteins derived from the pneumococcus itself, which might potentially have the additional advantage of conferring species-specific immunity. In recent years it has become increasingly apparent that, although a critical factor, CPS is not the only virulence determinant of Str. pneumoniae; a number of protein and other polysaccharide components are also considered to play a part in pathogenesis and may thus have protective immunogenic potential. The most extensively studied of these is pneumolysin. There is considerable evidence to suggest that pneumolysin is a virulence determinant of the pneumococcus: firstly, pneumolysin has been shown to have a variety of detrimental effects on host cells and func-

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tions in vitro and causes a severe lobar pneumonia when injected into the apical lobe bronchus of rats.\textsuperscript{30-35} More direct evidence comes from the demonstration that laboratory mutated strains of pneumococcal deficient in pneumolysin production have reduced virulence compared with wild type organisms\textsuperscript{57,58} and that immunisation with pneumolysin or its toxoid protected mice against subsequent challenge with virulent pneumococci.\textsuperscript{59,60} Such procedures have similarly been used to implicate autolysin (which causes the self-destruction of pneumococci in the stationary phase of growth and is therefore responsible for the release of intracellular pneumococcal toxins), and pneumococcal surface protein A as virulence, and potentially protective, immunogenic factors.\textsuperscript{61-65} Other pneumococcal products which may contribute to the pathogenicity of the organism include neuraminidase,\textsuperscript{66-68} hyaluronidase,\textsuperscript{69} a neutrophil elastase inhibitor,\textsuperscript{70,71} various proteases,\textsuperscript{72,73} and an inhibitor of the respiratory burst of neutrophils.\textsuperscript{74,75}

Conjugate vaccines using pneumococcal proteins as the carrier for CPS are currently under investigation. Studies in mice have shown that a toxoid of pneumococcal conjugated type 19F CPS enhanced the immunogenicity of the CPS as well as eliciting anti-pneumolysin antibodies.\textsuperscript{60} The highest antibody responses to both the CPS and pneumolysin were observed in animals that received three doses of the conjugate, indicating that immunological memory had been induced. Even if not used as the carrier in a conjugate vaccine, inclusion of one or more of these pneumococcal proteins in a vaccine formulation may be a potentially fruitful way of improving the pneumococcal vaccine.

Another important but seemingly underaddressed issue of pneumococcal immunisation concerns the appropriate route of administration of the vaccine. Although immunisation is standardly parenteral,\textsuperscript{76} Streptococcus pneumoniae infects the host via the mucosa, suggesting that oral immunisation might be a reasonable or more rational approach. The existence of a common mucosa-associated lymphoid tissue permits antibody production at mucosal sites other than the site of vaccination – hence oral immunisation can elicit an immune response in the respiratory mucosa.\textsuperscript{77,78} Interest in oral vaccines for combating a number of infectious diseases has increased over the last few years as new molecular techniques have been developed, although the possibility of inducing tolerance rather than protective immunity is a potential problem. One current approach involves packaging the antigens of interest into non-degradable particles to potentiate antigen delivery.\textsuperscript{79,80} Another utilise attenuated bacterial strains capable of only limited replication, such as \textit{Salmonella typhimurium} \textit{aro A}, as vectors for carrying the genes encoding protective antigens from the pathogen of interest.\textsuperscript{79,81} Such oral vaccines have several potential advantages: firstly, as well as inducing systemic immunity they can elicit local T cell reactivity and secretory IgA and thus might reduce colonisation with the pathogen. Secondly, oral immunisation is a much more convenient route of vaccination than parenteral administration, which is likely to result in greatly enhanced usage of the vaccine. Thirdly, animal studies have suggested that the mucosal immune system may be less susceptible to age-related dysfunction than the systemic system. Thus, murine splenic immune responses to different antigens, including pneumococcal antigens, have been shown to decline with age, whilst mucosal responses have remained constant.\textsuperscript{82} If this is also the case for human subjects, oral immunisation would appear to be a highly appropriate route for immunisation since the elderly are one of the key target populations for the pneumococcal vaccine.

Studies of oral vaccination against pneumococcal in-

fection have to date only been reported in animals.\textsuperscript{83,84} For example, oral immunisation of rats by inoculation of live \textit{S. pneumoniae} into the gastrointestinal tract resulted in a specific serum IgG response which was associated with protection against acute otitis media.\textsuperscript{85} Furthermore, a reduction in the incidence of otitis media was only observed in the rats that had been immunised by the gastrointestinal, as opposed to any other, route (and that had manifested a serum IgG response). Similarly, oral immunisation of mice with a bacterial vaccine comprising lysates of several respiratory pathogens including \textit{S. pneumoniae} significantly decreased mortality on subsequent challenge with virulent pneumococci.\textsuperscript{86,87} Stable expression of a modified (toxoided) pneumolysin gene has been achieved in \textit{Salmonella typhimurium} \textit{C5 Aro A} and oral vaccination of mice with this construct was shown to elicit anti-pneumolysin serum IgA and IgG;\textsuperscript{88} protection studies were not included in this report. These results are encouraging and suggest that this innovative field could prove fruitful.

Conclusions

It is too early to know whether cytokines will have a place in the management of pneumococcal infection. There are many potential pitfalls in disturbing the intricate counterbalances of the immune system, but this is a rapidly developing field in which advances can be expected, and it is possible that a small number of selected patients may eventually be shown to benefit from this treatment. However, reliable cytokine measurements which can be interpreted with expertise will need to be available to identify the patients who may benefit. In contrast, vaccination is a well established form of immunological intervention, and there are real prospects of the pneumococcal vaccine being improved. Immunisation offers the only realistic hope of reducing the incidence and overall burden of pneumococcal disease, although concerted efforts will be needed to implement and assess immunisation programmes. New developments in vaccination should not be neglected in the rush to find a use for cytokines.

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