BACTERIAL OTITIS MEDIA: CURRENT VACCINE DEVELOPMENT STRATEGIES

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Abstract

Otitis media is the most common reason for children less than five years of age to visit a medical practitioner. Whilst the disease rarely results in death, there is significant associated morbidity. The most common complication is loss of hearing at a critical stage of the development of speech, language and cognitive abilities in children.

The cause and pathogenesis of otitis media is multifactorial. Among the contributing factors, the single most important are viral and bacterial infections. Infection with respiratory syncytial virus, influenza viruses, parainfluenza viruses, enteroviruses and adenovirus are most commonly associated with acute and chronic otitis media. *Streptococcus pneumoniae*, nontypeable *Haemophilus influenzae* and *Moraxella catarrhalis* are the most commonly isolated bacteria from the middle ears of children with otitis media.

Treatment of otitis media has largely relied on the administration of antimicrobials and surgical intervention. However, attention has recently focused on the development of a vaccine. For a vaccine to be effective against bacterial otitis media, it must, at the very least, contain antigens that induce a protective immune response in the middle ear against the three most common infecting bacteria. Whilst over the past decade there has been significant progress in the development of vaccines against invasive *S.pneumoniae* disease, these vaccines are less efficacious for otitis media. The search for candidate vaccine antigens for nontypeable *H. influenzae* are well advanced whilst less progress has been made for *M. catarrhalis*. No human studies have been conducted for nontypeable *H. influenzae* or *M. catarrhalis* and the concept of a tribacterial vaccine remains to be tested in animal models.

Only when vaccine antigens are determined and an understanding of the immune responses induced in the middle ear by infection and immunisation is gained will the formulation of a tribacterial vaccine against otitis media be possible.

Otitis Media

Otitis media is an inflammation of the middle ear without reference to etiology or pathogenesis. Clinically, otitis media presents in several forms that are characterised by both the duration of the disease and the type of exudate. The usual symptomatic presentation is known as acute otitis media and is characterised often by fever, irritability, otorrhea, lethargy, vomiting, diarrhoea and hearing loss in some children. The appearance of the tympanic membrane may reflect the presence of a middle ear effusion: the bulging membrane may be opaque and, in some instances, it may be erythematous. If the membrane has perforated, a bloodstained discharge from the ear may be present. However, it is important to realise that in up to half of the children who have tympanic membrane signs of otitis media, they may not have symptoms which demonstrate the insidious nature of the disease ¹⁻³. Otitis media with effusion, is usually associated with hearing loss. The tympanic membrane is more often opaque with little or no mobility. Otitis media with effusion may follow an episode of acute otitis media or it may occur independently ⁴. If it persists for more than three months, it is considered to be chronic.

The Burden of Disease

Otitis media is the most prevalent illness of childhood. In the United States, by three years of age more than 80% of children have had at least one episode of acute otitis media, and by seven years of age, almost 40% of children have had six or more episodes of acute otitis media ⁵. The peak incidence of acute otitis media occurs in the second half of the first year of life, with a second lower peak between the ages of four and five years ⁶. Whilst otitis media has low associated mortality, its high prevalence worldwide in infants and children make otitis media a major public health problem. The disease has a significant impact on the development of the child, its family and

the Health Care System. The annual cost to the Health Care System in the United States is estimated to exceed \$5 billion ⁷ and \$0.6 billion in Canada ⁸.

The sequelae of Otitis media

The most important sequelae for children living in developed countries, with ready access to medical care, is transient or long term hearing impediment. Children with prolonged periods of hearing loss due to recurrent acute otitis media or chronic otitis media may have impaired development of speech, language and cognitive skills and associated social problems. In addition, the disease places significant emotional and financial stress on families ^{9,10}. Rarely, suppurative complications do occur. However, for children living in communities with limited access to primary medical care and in developing countries, the burden of otitis media disease is much greater ^{11, 12}. A comprehensive review by Berman in 1995 ¹², demonstrated that depending on the study group, up to one-third of children in these communities had evidence of tympanic membrane perforation. Intracranial complications are probably less than would be predicted in these communities because perforation of the tympanic membrane for long periods of time provides effective drainage of the infection. However, such perforations have other complications, such as the progressive destruction of the ossicular chain resulting in deafness ¹¹.

Etiology of Otitis media

The cause and pathogenesis of otitis media are multifactorial. Factors which contribute to prevalence and chronicity of otitis media include upper respiratory tract viral and bacterial infection, age of the child, male gender, allergy, eustachian tube dysfunction, exposure to pollution, day care environments and impaired immunity. The single most important factor is upper respiratory tract viral and bacterial

infection. A viral upper respiratory tract infection and subsequent tracking of virus and/or bacteria along the eustachian tube to the middle ear often initiates acute otitis media. Virus or virus antigen alone has been reported to be isolated from the middle ear of children with acute otitis media in between 5% and 25% of cases ^{6, 13, 14}. Bacteria are isolated between 40 and 70% of cases ¹⁵⁻¹⁷. In cases of acute otitis media, concurrent viral and bacterial presence occurs in up to 17% of cases ¹⁸. With better detection methods, this may indeed be much higher ¹⁹. However, in up to about one third of cases, no causative infectious agent is determined and whilst there is still some debate with respect to this observation, it is most likely due to the sensitivity and other technical difficulties of culture and detection.

It is now well established that there is a highly significant correlation between upper respiratory tract viral infection and bacterial otitis media ²⁰. The predominant viral upper respiratory tract infections associated with increased risk of acute otitis media are respiratory syncytial virus, influenza virus (type A or B), parainfluenza viruses, enteroviruses and adenovirus. In addition, there is a high correlation between the bacteria isolated from the middle ear of children with acute otitis media and the predominant organism carried in the nasopharynx ²¹. Three upper respiratory tract commensal bacteria are responsible for almost all bacterial otitis media: *Streptococcus pneumoniae*, nontypeable *Haemophilus influenzae* and *Moraxella catarrhalis* ^{6, 19, 22-26}. The remainder of this review will focus on otitis media caused by these bacteria and the pursuit of an effective vaccine strategy.

Vaccination as a treatment of choice

The sheer burden of otitis media in the community necessitates the widespread administration of antimicrobial agents to affected children, often for periods of many

months. With concerns growing about increased microbial antibiotic resistance, attention has been focused on the possibility of vaccination against some of the pathogens responsible.

The availability of new protein-polysaccharide conjugate technology has enabled the production of vaccines against *S. pneumoniae* which are immunogenic in young children and highly efficacious against invasive disease caused by *S. pneumoniae* ²⁷. ²⁸. In addition, these conjugate vaccines are able to induce mucosal SIgA specific antibodies in saliva and nasopharyngeal secretions and reduce nasopharyngeal colonisation ²⁹. Despite these positive indicators, efficacy estimates against acute otitis media from the few studies that are currently available, are low and clearly indicate that an effective otitis media vaccine will need to contain antigens which induce a protective response in the middle ear against all three of the most common infecting bacteria. To this end, our group has been undertaking a protein antigen search program for nontypeable *H. influenzae*, *M. catarrhalis*, and *S. pneumoniae* and testing the vaccine potential of candidate antigens with a rodent model of acute otitis media.

The rodent model of acute otitis media

The study of bacterial otitis media using animal models has not been easy. Researchers are mindful that these bacteria specifically colonise and cause disease in humans. Rodent models have been established in the gerbil, chinchilla, guinea pig, rat and mouse ³⁰⁻³³ and each has its own inherent strengths and weaknesses. Some are suited to studying the pathology associated with otitis media, others for colonisation studies and some have been developed to study the effect of immunisation on infection. In addition, some of these rodent species are only suited to studies with a

single bacteria pathogen. For example, the chinchilla is an excellent model for investigation of nontypeable *H. influenzae* infections but is less acceptable for similar studies with *S. pneumoniae*. To date, the rat seems to be a useful species for studying immunisation-induced immune responses for all three bacteria ³⁴⁻³⁷, although all studies reported to date have involved single-pathogen induced otitis media and no studies have yet reported outcomes for a mixed bacterial infection.

Immunisation against acute otitis media in the rodent model

We have established bacterial clearance models for middle ear infections in rats ³⁷ (authors unpublished data). The models assess the ability of an induced immune response to clear an infection, compare the efficacy of mucosal and systemic immune responses and investigate the impact on the inflammatory responses. Studies with whole killed cells and potential vaccine antigens for each of these pathogens have provided some interesting results. The immunisation regimens had a significant impact on the capacity to clear infection ³⁸⁻⁴⁰ with clearance of nontypeable *H. influenzae* appearing to be primarily controlled by a mucosal response, whereas, the systemic response appeared more efficacious against *S. pneumoniae* ⁴⁰.

In addition to investigating the impact of immunisation with whole killed cell formulations on bacterial clearance, the rat model has been highly successful in providing evidence on the potential protective efficacy of protein antigens. A number of leading vaccine candidates has been evaluated. In the rat model, the nontypeable *H. influenzae* antigens OMP26, LB1 and LPD have all demonstrated potential protective efficacy following mucosal immunisation ⁴¹. These antigens have separately been evaluated in the chinchilla model using a parenteral immunisation

protocol and resulted in similar beneficial effects on both nasopharyngeal carriage and middle ear infection^{41, 42}. Similarly, the rat model has been used to demonstrate the differences in protective efficacy for protein antigens such as CD, OMP E and others from *M. catarrhalis* (authors unpublished results).

All these experiments have investigated the impact of immunisation in an acute infection model and under conditions where only one pathogen induces the infection. They have demonstrated that in the different models, the outcomes are similar for the nontypeable *H. influenzae* studies. In these studies, antigens have been delivered both mucosally and parenterally and the induced immune responses and their impact on clearance of infection differed for the parenteral immunisations in the rat and chinchilla models. Such differences in results from animal model studies help highlight some of the significant challenges facing the development of an effective combination vaccine against these three pathogens.

Programming of the inflammatory response in the middle ear by immunisation

TNF α is important in the acute-phase response through induction of physiological changes associated with eliminating the infecting organism, limiting tissue damage and activating repair processes. While other cytokines and chemokines, such as IFN γ , IL-1, IL-6, IL-10, the macrophage inflammatory proteins, monocyte chemoattractant protein and others^{43, 44} all play important roles in the infection response and cross-talk between various chemoattractant receptors, knockout and depletion studies have demonstrated that host defence is less compromised by the absences of any one of these compared to TNF α . Whilst TNF α is a critical factor in host defence against

infection, it has the potential to be both beneficial and detrimental to host survival. For example, anti-TNF α treatment of mice with *S. pneumoniae* results in accelerated bacterial proliferation and death from bacteraemia⁴⁵. Furthermore, *S. pneumoniae* cell wall proteins induce brain microvascular endothelial cells to produce TNF α causing damage to the blood brain barrier which results in meningitis from bacterial invasion⁴⁶. Under these circumstances, anti-TNF α treatment prevents the bacteria from crossing the blood brain barrier and causing meningitis⁴⁶. However, its role in septic shock has serious detrimental outcomes⁴⁷. Thus, while TNF α is essential in the response to infection, its levels must be regulated to prevent harm.

What is known in the middle ear?

In a rodent model of acute otitis media, we have monitored levels of TNF α in the middle ear fluid of naïve and immunised animals in response to infection with *S. pneumoniae*, nontypeable *H. influenzae* and *M. catarrhalis*. Both the magnitude and kinetics of the TNF α response to infection were different for each infecting bacteria and immunisation modulated the TNF α response differently for each bacteria (authors unpublished observations). Animals immunised against nontypeable *H. influenzae* had significantly higher levels of TNF α earlier in the infection and these levels declined more rapidly when compared with non-immune animals. In contrast, *S. pneumoniae* infection induced a higher but later and more sustained level of TNF α in the middle ear fluid of naïve animals. This response was significantly suppressed in immune animals. *M. catarrhalis* infection resulted in mid-range levels of TNF α in middle ear fluid that did not appear to be regulated by immunisation (unpublished observations). This different response is consistent with the observation of Melhus and Ryan (2000)⁴⁸ who reported that mRNA expression in the middle ear mucosa of rats appeared

much later for *S. pneumoniae* infection compared with nontypeable *H. influenzae* infection.

Is a tribacterial vaccine feasible?

Given that the kinetics and immune modulation of the pro inflammatory cytokine TNF α as a result of immunisation appears to be pathogen specific, the question must be raised as to whether or not it is indeed feasible to be able to produce a combined formulation for all three bacteria that is efficacious and does not cause more serious sequelae, particularly in cases of mixed infections. For example, the rapid increase in TNF α , influx of polymorphonuclear neutrophils and an elevated activation state of the phagocytes in immune animals in response to nontypeable *H. influenzae* (authors unpublished observations) may compromise the mucosal barrier in such a way as to promote bacteraemia by a concurrent *S. pneumoniae* infection. Similarly, it is not known what effect changes in TNF α levels have on the virulence of *M. catarrhalis* infections. These questions are vital for otitis media whereby an adverse inflammatory response in the confines of the middle ear cavity will, at the very least, cause permanent damage and hearing loss or, at worse, bacteraemia and/or meningitis that could result in death. To further examine this, studies were conducted in an acute mouse lung infection model using a whole killed cell vaccine formulation of all three bacteria. These preliminary studies were conducted in the lung for ease of experimentation and previous experience had informed us that the lung and middle ear behaved similarly.

A formulation containing whole killed *S. pneumoniae*, nontypeable *H. influenzae* and *M. catarrhalis* in the ratio 1:1:3 was prepared and mice were immunised mucosally with this vaccine⁴⁹. Twenty-one days after immunisation, the mice were challenged

intratracheally with live *S. pneumoniae*, nontypeable *H. influenzae* or *M. catarrhalis*. The results show that immunisation with the tri-bacterial formulation induced protective immunity against for each of the challenge bacteria independently (Figure 1). The ratio of the bacteria in the vaccine formulation was critical for the efficacy of the vaccine. For example, when the ratio of bacteria was altered to 1:1:1 protection against *M. catarrhalis*, the challenge was lost (Figure 2).

These results clearly demonstrate potential for the successful development of an efficacious tri-bacterial vaccine. Research is now being conducted to determine efficacy in the otitis media model and against mixed bacterial challenges. Inflammatory and immune responses in the middle ear will be determined and monitored in these studies.

Lead Protein Antigens

The decision on which antigens or how many to include in a vaccine formulation remains the subject of much discussion. Antigen searches for *S. pneumoniae* and nontypeable *H. influenzae* have been more extensively reported than for *M. catarrhalis* and as yet, there is very little work that has investigated the potential of combining the key vaccine antigens from all three bacteria.

The current vaccines for *S. pneumoniae* are based on the polysaccharide components. Unfortunately, this results in serotype-specific protection covering only those serotypes included in the particular formulation. Since there are over 90 known capsular serotypes for *S. pneumoniae*, this vaccine will need to be improved to provide much broader specificity. This will most likely be achieved through inclusion of protein antigens. For *S. pneumoniae* the most studied candidates have been PspA,

PsaA, CbpA and pneumolysin⁵⁰. Issues associated with these antigens include problems with between strain heterogeneity and some are virulence factors. The identification of antigenically conserved regions, in addition to elimination of the toxic-associated elements with virulence factors such as pneumolysin, are examples of major impediments in the development of a vaccine incorporating these antigens.

Similar issue with strain heterogeneity have been a problem for many protein antigens from nontypeable *H. influenzae* and *M. catarrhalis*. Many proteins from nontypeable *H. influenzae* have been investigated as potential vaccine candidates, including P1, P2 P4, transferrin binding protein b, D15, HMW1, HMW2, Hia and several other minor proteins^{51,52}, but none of these have provided the same level of protection as OMP26, lipoprotein D, LB1-conjugates or P6. LB1 peptides are highly conserved regions of the P5-fimbrin protein and are an example of where the problem with strain heterogeneity has been overcome. As indicated above, studies of *M. catarrhalis* antigens have not been as extensively researched. While there appears to be strain-specific immunity to *M. catarrhalis* post-infection, the protein profiles on sodium dodecyl sulfate polyacrylamide gel electrophoresis are relatively similar. Proteins such as CD, OMP E, UspA1, UspA2, the transferrin-binding proteins and lactoferrin-binding proteins have been the most studied⁵³. Conserved protective epitopes or single antigens that may constitute components of a vaccine are still under investigation, with CD and OMP E attracting the most attention in recent years^{54,55}.

Conclusions

Prevention of bacterial otitis media caused by *S. pneumoniae*, nontypeable *H. influenzae* and *M. catarrhalis* will require development of a multivalent vaccine. While there remains a concerted effort to identify the most suitable antigens for each

of these bacteria, studies must be undertaken to investigate the issues associated with (1) combining antigens from each of the pathogens, (2) the importance of immune regulation of the inflammatory responses in the presence of a mixed bacterial infection in the middle ear, and (3) whether a vaccine should not only prevent infection and disease but also rid the nasopharynx of carriage. The latter is important to the significance of carriage in protecting the nasopharynx from other opportunistic pathogens.

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Figure 1. Enhancement of clearance of *S. pneumoniae*, nontypeable *H. influenzae* and *M. catarrhalis* from (A) the bronchoalveolar lavage (BAL) and (B) lung homogenate following mucosal immunisation with a tribacterial formulation of killed bacteria. Balb/c mice (n=4-5 per group) were immunised as described ⁴⁹ with a 1:1:3 ratio of *S.pneumoniae*:nontypeable *H. influenzae*:*M. catarrhalis* by intra-Peyer's patch inoculation on day 0 and an intra tracheal boost on day 14 such that each mouse received either 5×10^7 or 1.5×10^8 equivalent colony forming units. On day 21 the mice received a pulmonary challenge with live bacteria⁴⁹. Bacteria were recovered from the BAL and lung homogenate at 4 hours post challenge for nontypeable *H. influenzae* and *M. catarrhalis* and 5 hours post challenge for *S. pneumoniae* groups. Bacterial clearance was compared to that in the non immunised control groups (* $P < 0.05$).

Figure 2. Enhancement of clearance of *S. pneumoniae*, nontypeable *H. influenzae* and *M. catarrhalis* from (A) the bronchoalveolar lavage (BAL) and (B) lung homogenate following mucosal immunisation with a tribacterial formulation of killed bacteria with a 1:1:1 ratio of *S.pneumoniae*:nontypeable *H. influenzae*:*M. catarrhalis* as described for figure 1. Bacterial clearance was compared to that in the non immunised control groups (* $P < 0.05$).

Figure 1

Figure 2

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