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Prospects for a vaccine against otitis media

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Otitis media is a major cause of morbidity in 80% of all children less than 3 years of age and often goes undiagnosed in the general population. There is evidence to suggest that the incidence of otitis media is increasing. The major cause of otitis media is infection of the middle ear with microbes from the nasopharynx. The anatomical orientation of the eustachian tube, in association with a number of risk factors, predisposes infants and young children to the infection. Bacteria are responsible for approximately 70% of cases of acute otitis media, with *Streptococcus pneumoniae*, nontypeable *Haemophilus influenzae* and *Moraxella catarrhalis* predominating as the causative agents. The respiratory viruses, respiratory syncytial virus, rhinovirus, parainfluenza and influenza, account for 30% of acute otitis media cases. Over the past decade, there has been a profound increase in the reported resistance to antibiotics, which, with increased disease burden, has focussed attention on vaccine development for otitis media. A polymicrobial formulation containing antigens from all major pathogens would have the greatest potential to deliver a sustained reduction in the disease burden globally. The disappointing outcomes for otitis media seen with the polysaccharide pneumococcal conjugate vaccine have raised major challenges for the vaccination strategy. Clearly, more knowledge is required concerning immune mechanisms in the middle ear, as well as vaccine formulations containing antigens that are more representative of the polymicrobial nature of the disease. Antigens that have been extensively tested in animal models are now available for testing in human subjects.

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Otitis media (OM) is an infectious disease of the young, commonly affecting more than three-quarters of all children before they reach 3 years of age [1]. OM, which can be caused by a variety of etiological agents, including nontypeable *Haemophilus influenzae* (NTHi), *Streptococcus pneumoniae*, *Moraxella catarrhalis*, respiratory syncytial virus (RSV), rhinovirus, parainfluenza (PIV) and influenza is responsible for significant morbidity. Some 5% of children are predisposed to recurrent episodes of OM that is associated with repeated courses of antibiotics, the need for general surgery for tympanostomy tube placement, hearing impairment and long-term effects on speech and language development that lead to learning and behavioural difficulties as these children reach school age [2-4]. Although OM has a low associated mortality in the developed world, its

high prevalence globally in infants and children makes OM a significant burden to patients, caregivers and the healthcare system. It is the primary reason for preschool children to visit a general practitioner, accounting for 16 million visits in 2000 [5] and responsible for over half of the antibiotics dispensed to children under 3 years of age in the USA [6]. In 2005, it was estimated that the national cost of acute otitis media (AOM) in the USA was between US\$3.8 billion and 5.7 billion; 40% of national expenditure for OM is generated by children 1-3 years of age whilst two-thirds of the total cost is attributable to chronic ear infections progressing from AOM [7]. Over a period of 10-20 years, there has been a reported 68% increase in OM incidence in a population-based study in Finland [8] and a 15% increase in the reported incidence of OM

in children in the USA [9]. A rising trend in OM has also been observed in Australia over the last 30 years, with OM progressing from being the fourth most frequent problem managed by general practitioners in 1971 to the third most frequent problem in 2001 [10].

In the last decade, resistance to one or more antimicrobials has escalated dramatically, largely due to the overuse of antibiotics [11]. The indiscriminate use of antibiotics in poorly diagnosed cases of AOM and the use of multiple courses of antibiotics in difficult to treat cases of OM have promoted the selection and spread of resistant bacteria. Treatment of OM caused by antibiotic-resistant pathogens often requires the use of more expensive and potentially more toxic drugs, perpetuating a vicious cycle of inadequate management, culminating in an increased number of infections with a decreased response to commonly used antibiotics, and sometimes treatment failure. The increased number of treatment failures [12] associated with inadequate coverage of the implicated pathogens may be the result of the shift in OM microbiology in the pediatric population following the introduction of pneumococcal vaccination [13]. There has been an observed increase in β -lactamase-producing *H. influenzae* and a decrease in resistant *S. pneumoniae* in the USA [13], initiating a guideline recommendation for high-dose amoxicillin/clavulanic acid for the treatment of AOM in patients who have failed previous treatment with amoxicillin [14].

In order to break this cycle, it is necessary to favor the prevention of OM rather than its treatment. Attention has been focused on developing a more effective vaccine against some of the pathogens responsible for middle-ear disease as a long-term strategy to reduce both antibiotic dependence and disease burden.

Otitis media

Despite the existence of precise clinical definitions, the diagnosis and management of AOM have generated considerable debate and differences in opinion amongst health professionals [15–17]. AOM is defined as the presence of middle-ear effusion accompanied by signs and symptoms of acute infection [18]. Otitis media with effusion (OME) is defined as a condition of inflammation of the middle ear with fluid collection in the middle-ear space. The signs and symptoms of acute infection are not present and the tympanic membrane is not perforated [19]. There is often a clinical continuum between AOM and OME. It is important to discriminate between the two conditions because OME is usually not treated with antimicrobials. Persistent middle-ear fluid (MEF) results in decreased mobility of the tympanic membrane and serves as a barrier to sound conduction and consequently hearing loss.

Acute otitis media

Almost two-thirds (62%) of children have had at least one episode of AOM by 1 year of age and approximately 80% by 3 years of age, with a peak incidence between 6 and 18 months [1]. At least 80% of AOM cases resolve within 2–14 days and with minimal benefits from antimicrobials [20]. The 'Clinical Practice Guideline: Diagnosis and Management of Acute Otitis

Media' recommends analgesia for the management of AOM for children older than 2 years of age, with the introduction of antibiotics if the child deteriorates or is not improving within 48–72 h [18]. In severe cases of certain diagnosis, antibacterial therapy is recommended. There is no consensus for the observation period prior to antibiotic treatment for children younger than 2 years of age with a recommendation for immediate treatment on clinical considerations along with certainty of diagnosis. Judicious use of antibiotics is recommended with the global prevalence of antibiotic-resistant bacteria and the increased risk of carriage of potential respiratory pathogens [21].

Recurrent AOM

Recurrent AOM is defined as three or more episodes within a 6-month period, or four or more episodes within a 12-month period. It commonly affects 10–20% of children by 12 months of age with approximately 40% of 7-year olds having experienced six or more episodes of AOM [22]. The natural history of AOM is for the number of infections to decrease with time; however, some very young children may have a propensity for AOM, in which case it may be necessary to have tympanostomy tubes inserted.

Otitis media with effusion

More than half of children have experienced OME by 1 year of age. Recurrent OME occurs in 30–40% of children and 5–10% of episodes last 1 year or longer [23]. The extensive literature on the role of antibiotics in the management of OME suggests a benefit that is short-lived [24]. For those children with effusions persisting for more than 3 months who have an increased risk for developmental difficulties, surgical intervention should be considered [19].

Pathogenesis of OM

Etiology

The causes and pathogenesis of OM are multifactorial [25]; however, paramount to the disease process is a dysfunctional eustachian tube that potentially favors OM in infants and young children. High rates of nasopharyngeal colonization with potentially pathogenic bacteria and viruses [26], socio-economic status and repeated exposure to large numbers of children whether at home or in childcare [27] are reported to be the most significant.

Microbiology

Bacterial and viral infections are the single most important cause of OM. A viral upper respiratory tract infection may modify or overwhelm the protective mechanisms of the mucosal membranes and initiate the cascade of events that lead to bacterial pathogens gaining access to the usually sterile environment in and around the middle-ear [28]. Bacteria are isolated in approximately 70% of MEF samples from children with OM [28]. Three microbes are reported to predominate as the cause of AOM: *S. pneumoniae* (25–50%), NTHi (15–30%) and *M. catarrhalis* (3–20%) [29]. Colonization of the nasopharynx with these

microbes at an early age and the early onset of OM are closely correlated [26,30]. The true indication of bacterial involvement in OM may be underestimated as PCR-based assay systems detect the presence of bacterial DNA in up to 80% of culturally sterile MEFs [31–33].

In a review of the importance of respiratory viruses in AOM, it has been reported that respiratory viruses alone account for 30% of AOM cases or with concurrent bacterial pathogens in approximately 15% of cases [28]. RSV is the most commonly detected virus in MEF. Other viral agents include rhinovirus, influenza virus and PIV. Enterovirus and adenovirus are also reported occasionally. Using conventional viral culture and viral antigen detection, the same viruses have been identified in nasopharyngeal aspirates from children with AOM. When sensitive techniques such as PCR are used, rhinovirus has also been found to be as common as RSV [34], and interestingly, has been associated with an increase in antibiotic failure in the treatment of AOM when co-infecting with bacteria [35]. The PCR assay has substantially increased the detection of viruses with a threefold increase in sensitivity compared with viral culture [36]. This has allowed a broader range of viruses to be detected in MEF samples from children with AOM [37], and indeed has enabled the detection of the newly discovered human respiratory pathogens metapneumovirus (hMPV), which has been implicated in OM [38] and coronavirus (HCoV-NL63) [39]. PCR used in a retrospective study of nasal washings collected over a 20-year period detected hMPV in 5% of specimens from children with upper respiratory infections who had previously tested culture-negative for viruses. AOM was found in 50% of these children. hMPV was detected at a similar frequency to other viral middle-ear pathogens [40].

Immunology

Host defense mechanisms in the middle ear associated with OM are poorly understood. Animal studies have shown that the middle-ear cavity and eustachian tube have immune characteristics similar to other mucosal sites [41,42]. This suggests that the induction of a mucosal immune response may be most relevant in providing protection against infectious agents that cause OM. In adult human subjects it is known that mucosal immune competence may be down-regulated by persistent nasopharyngeal carriage [43] or neonatal colonization [CRIPPS *ET AL*; KALGOORLIE OM PROJECT, UNPUBLISHED DATA] and this down regulation may be antigen specific, shifting the host–parasite relationship in favor of higher levels of bacterial colonization in the nasopharynx, a major risk factor for OM [44,45]. Other studies have suggested a number of subtle systemic immunodeficiencies that are associated with proneness to OM, but none of these are conclusive to causal linkage. More recently, genetically determined functional polymorphisms observed in both leukocyte Fcγ and mannose-binding lectins suggest that these warrant further study with respect to disease susceptibility [46]. Clearly further studies are needed in order to determine the relationship between mucosal immune competence, nasopharyngeal colonization, MEF inflammatory and immunological parameters, and OM.

Preventative strategies

The surgical approach

The ‘Clinical Practice Guidelines’ recommend that surgical intervention should only be performed on children with OME lasting 4 months or more with persistent hearing loss and other signs and symptoms, for recurrent or persistent OME in children at risk regardless of hearing status, or when there has been structural damage to the tympanic membrane or middle ear [19]. Tympanosotomy tube insertion is the treatment of choice followed by adenoidectomy. Both procedures have been demonstrated to reduce OME prevalence. However, outcomes from surgery are highly variable and given that the benefits are short-term (20–50% of OME cases relapse after tube extrusion) [19], the implications of surgery and the sequelae of indwelling tubes (7% of cases experience recurrent otorrhea or obstruction) and long-term sequelae after tube extrusion, including tympanosclerosis (32%), must be considered [47].

Vaccination approach

OM is a polymicrobial disease and as such, any vaccine should target the primary infectious pathogens *S. pneumoniae*, NTHi, *M. catarrhalis*, RSV, rhinovirus, PIV and influenza, and also protect infants from 2 months of age. Ideally, it should target the two levels of OM development: the viral destruction of the respiratory tract epithelium and the multiplication of bacteria in the middle ear. The feasibility of producing a vaccine that is capable of protecting against all OM pathogens is doubtful; nevertheless, a combined bacterial–viral vaccine formulation for OM should be the long-term objective. For now, the general consensus is that an effective OM vaccine will need to contain antigens that induce a protective response in the middle ear against all three of the most common infecting bacteria. Although this may not provide unequivocal protection against OM, because of the high frequency of bacterial OM, the actual numbers of episodes prevented by this vaccine could be substantial. Strong rationale would suggest that preventing OM in the first year of life with an effective treatment for AOM and the prevention of subsequent infection could have a significant human and economic impact. Universal vaccination would be the optimum outcome due to the universal nature of the disease and the difficulty in assessing those children who are predisposed to recurrent OM. This could be achieved by incorporating the OM vaccine into the national immunization schedules where appropriate.

The challenge is to identify the appropriate antigens, namely those antigens able to generate an appropriate immune response to prevent disease, which are also conserved among strains, and present them in such a way as to generate a protective immune response in infants and young children without causing immune-mediated damage to the middle ear. TABLE 1 lists many of these antigens, and summarizes the current status of vaccine research and development.

The nasopharynx is colonized by a broad variety of microorganisms, including commensal bacteria, as well as potential pathogens such as *S. pneumoniae*, NTHi and *M. catarrhalis*. The concern arises that in such a finely tuned ecological environment an

Table 1. Summary of vaccines and vaccine candidate antigens for bacterial otitis media.

Bacteria	Vaccine	Manufacturer	Stage of development	Opsonizing or bactericidal antibody detected	Protection demonstrated in an animal model of OM	Protection demonstrated in human subjects against OM
<i>Streptococcus pneumoniae</i>						
	Pneumovax 23™	Aventis Pasteur	Licensed			No – children as a booster
	Prevenar™	Wyeth-Lederle	Licensed			Yes – infants, children
	Glyconjugate vaccine (4, 6B, 9V, 14, 18C, 19F, 23F) conjugated to meningococcal B OMP	Merck	Phase III			Yes – infants
	Glyconjugate vaccine (1, 4, 5, 6B, 9V, 14, 18C, 19F, 23F) conjugated to CRM197	Wyeth-Lederle	Phase III			Yes – infants, toddlers
	Glyconjugate vaccine (1, 4, 5, 7F, 9V, 19F, 23F) conjugated to tetanus protein and (3, 6B, 14 and 18C) conjugated to diphtheria toxoid	Aventis Pasteur	Phase II			Yes – infants
	Glyconjugate vaccine (1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, 23F) conjugated to <i>H. influenzae</i> -derived protein D	GlaxoSmithKline	Phase III			Yes – infants
	Glyconjugate vaccine (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F) conjugated to CRM197	Wyeth-Lederle	Phase I/II	-	-	-
	PspA	Aventis Pasteur	Preclinical	-	Yes	-
	PsaA	Aventis Pasteur	Preclinical	-	-	-
	PspA + PsaA + PdB		Preclinical	-	-	-
	Neuraminidase		Preclinical	-	Yes	-
	BVH-3 & BVH-11	GlaxoSmithKline	Phase I	Yes	-	-

Table 1. Summary of vaccines and vaccine candidate antigens for bacterial otitis media.

Bacteria	Vaccine	Manufacturer	Stage of development	Opsonizing or bactericidal antibody detected	Protection demonstrated in an animal model of OM	Protection demonstrated in human subjects against OM
<i>Haemophilus influenzae</i> (nontypeable)						
	Glyconjugate vaccine (1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, 23F) conjugated to <i>H. influenzae</i> -derived protein D	GlaxoSmithKline	Phase III			Yes – infants
	Subunit detoxified LOS conjugated to TT		Phase I	Yes	Yes	-
	Subunit detoxified LOS conjugated to HMW protein from <i>H. influenzae</i> (nontypeable)		Preclinical	Yes	Yes	-
	OMP P6		Preclinical	Yes	Yes	-
	Recombinant P6 protein conjugated to adamantylamide dipeptide		Preclinical	-	Yes	-
	Recombinant P6 protein conjugated to cholera toxin		Preclinical	-	-	-
	OMP 26		Preclinical	-	Yes	-
	OMP P5-derived peptides		Preclinical	-	Yes	-
	OMP P2 subunit		Preclinical	Yes	-	-
	OMP P1		Preclinical	Yes	Yes	-
	OMP Hin47	Aventis Pasteur	Phase I	-	Yes	-
	HMW1/HMW2		Preclinical	Yes	Yes	-
	Hap conjugated to mutant cholera toxin		Preclinical	-	Yes	-
	Recombinant Hap		Preclinical	-	Yes	-
	Nonenzymatically active rP4		Preclinical	Yes	-	-
<i>Moraxella catarrhalis</i>						
	Adherence proteins UspA1, CD, MID, Mcap		Preclinical	Yes – UspA and CD	-	-
	Protein involved in hemagglutination Hag		R & D	-	-	-

Table 1. Summary of vaccines and vaccine candidate antigens for bacterial otitis media.

Bacteria	Vaccine	Manufacturer	Stage of development	Opsonizing or bactericidal antibody detected	Protection demonstrated in an animal model of OM	Protection demonstrated in human subjects against OM
	Proteins involved in iron acquisition LbpA/LbpB, TbpA/TbpB, CopB, B1		Preclinical	Yes - TbpB and CopB	-	-
	Protein involved in serum resistance UspA2		Preclinical	Yes	-	-
	Protein involved in phase variation UspA1/2		R & D	-	-	-
	Highly conserved proteins E, CD, G1		R & D	-	-	-
	Detoxified LOS conjugated to either TT or CRM		Preclinical	Yes	-	-

CD: Outer membrane protein CD; CopB: Catarrhalis outer membrane protein B; CRM: Cross reactive material; Hag: Heat modifiable haemagglutinin; Hap: *Haemophilus* autotransporter protein; HMW: High molecular weight; Lbp: Lactoferrin-binding protein; LOS: Lipooligosaccharide; McaP: Outer membrane protein McaP; MID: *M. catarrhalis* IgD-binding protein; OM: Otitis media; OMP: Outer membrane protein; PdB: Pneumolysin; Psp: Pneumococcal surface protein; Tbp: Transferrin-binding protein; TT: Tetanus toxoid; Usp: Ubiquitous surface protein.

external force, such as vaccination, may unfavorably influence colonization and/or elimination of microorganisms creating a niche for new pathogens to colonize the nasopharynx. A notable example is the accumulating evidence suggesting that the use of pneumococcal vaccines may be exerting a selective pressure to perpetuate the emergence of penicillin resistance and multiple antibiotic resistance among nonvaccine serotypes in the nasopharynxes of the pediatric population [48–50]. Several studies have demonstrated an increase in nasopharyngeal carriage by nonvaccine pneumococci among vaccinated children [51–56]. The increase in carriage of nonvaccine serotypes may represent a replacement of serotypes or the unmasking of resident nonvaccine serotypes. Currently, antimicrobial resistance is identified predominantly among serotypes included in the conjugate vaccine; of concern is the possibility that capsular transformation may serve as a mechanism for spreading antibiotic resistance to nonvaccine serotypes [57]. The appearance and increased prevalence of nonvaccine variants of major antibiotic-nonsusceptible clones [57] may have significant implications in middle-ear disease with the observation that the nonvaccine strains may be as virulent as the vaccine strains and capable of causing AOM [52,56,58]. In a trial of a conjugate vaccine in Finland [56], there were 33% more episodes of AOM in the vaccine group caused by nonvaccine serotypes than in the control group after the booster dose of vaccine. The potential implications for the risk of pneumococcal disease would be that, without intervention, another group of serotypes would become established over time, diminishing the efficacy of the vaccine. Pneumococcal vaccination may also induce replacement by other

potential pathogens, such as *Staphylococcus aureus* [59,60], *Streptococcus pyogenes* [61,62], NTHi or *M. catarrhalis* [13]. A study from the Netherlands [59] and one from Israel [60] reported an inverse relationship in colonization patterns between serotypes of *S. pneumoniae*, including vaccine serotypes, and *S. aureus* in children. This may negate the desired effect of pneumococcal vaccination by increasing *Staphylococcus* diseases and possibly promoting an increase of multidrug-resistant *S. aureus* infections. The potential also exists for a more invasive and virulent organism such as *S. pyogenes* (group A β -hemolytic *Streptococcus*), which currently ranks as the fourthmost predominant pathogen, causing approximately 2–5% of pediatric AOM, to cause the more serious sequelae of OM, mastoiditis [61,62]. The full ramifications of any unintended consequences of immunization with conjugated vaccines are yet to be elucidated and ongoing surveillance of isolates from the nasopharynx is critical.

Models of vaccine efficacy

OM is a disease of humans, and as such, there are limitations in the currently available animal models of human infection and their ability to address such factors as the pathogenesis of infection and the immune response to infection. A review of animal models used to study OM has reported that rabbits, gerbils, monkeys, ferrets, guinea-pigs, mice, chinchillas and rats have all been used successfully, with the rat and chinchilla model systems being used most extensively [63]. The animal model used by investigators largely depends on the nature of the scientific investigation. The rat model has been primarily used to study immunological

responses in the middle ear to infection, screening of potential protein antigens for immunization and the assessment of mucosal immunization, focusing on the immunological response in the clearance of pathogens from the middle ear. Infection is established by direct inoculation into the middle ear. Although the chinchilla model is thought to more closely replicate human disease, the lack of the widespread availability of the animals and molecular reagents has limited studies. However, despite these limitations, the model has provided useful knowledge with respect to the pathogenesis of the disease, immune mechanisms in the middle ear and the evaluation of new vaccine antigens. The chinchilla is an excellent model of NTHi OM due to its sustainability in the nasopharynx and middle ear but is less acceptable for similar studies with *S. pneumoniae* and *M. catarrhalis*. The use of the mouse model is increasing in popularity with the increasing availability of knockout and transgenic mice and readily available reagents. More importantly, the mouse is widely used as an animal model for human diseases. Although animal models have provided useful data in assessing vaccine antigens, ultimately, it will be necessary to test a potential vaccine in humans to draw meaningful conclusions regarding the likely efficacy of a vaccine under development.

In addition to animal models, it has been recommended that human nasopharyngeal colonization should be assessed in efficacy studies [64]. Previous studies with conjugate vaccines for *H. influenzae* type b and pneumococcus have demonstrated a reduction in nasopharyngeal colonization [49,65]. It would not be unreasonable to suggest that vaccines for OM may confer protection by this mechanism in part. Regardless of its predictive value, the human nasopharyngeal colonization model may offer some insight into human mucosal immune responses and the effect of candidate vaccine antigens on nasopharyngeal colonization. In this context, in late 2005, a clinical trial was opened to examine the effect of pneumococcal vaccination on nasopharyngeal colonization in infants with AOM [201].

Viral vaccines

Influenza vaccines

The influenza vaccines licensed for use in young children (>6 months of age) are a preparation of inactivated split or subunit virus vaccines. These vaccines are administered intramuscularly. Different study designs and risk cohorts and a lack of standardized criteria for diagnosis have offered conflicting evidence regarding the effectiveness of the influenza vaccine in preventing OM. Three comparable studies have shown a 30–36% reduction in OM after influenza vaccination [66–68], whilst a fourth study that was limited by a lack of influenza epidemic during the second season showed no difference compared with placebo groups [69]. A systematic review assessing the effects of live attenuated and inactivated influenza vaccines against AOM in healthy children up to 16 years of age suggested no difference with placebo or standard care, but lacked statistical power [70]. In June 2003, a cold-adapted, trivalent, intranasal influenza vaccine (FluMist™, MedImmune Vaccines Inc.) was approved in the USA. The vaccine relies on the use of an attenuated strain

that multiplies exclusively in the nose. Results of clinical trials in children and adults have demonstrated that the vaccine reduces the incidence of influenza and it is associated with a 30% decrease in febrile OM in children [71]. As a live intranasal vaccine, it is anticipated that it would offer superior mucosal immunity compared with the currently available vaccines that are less immunogenic in very young children. Its principal advantage, in comparison with other influenza vaccines, is that it is administered nasally, which has greater acceptance and ease of administration than the intramuscular route. Studies in healthy children 12 months to 17 years of age showed FluMist™ to be safe in children over 3 years of age with an observation that younger children have an increased risk of reactive airway disease [72]. Therefore, for safety reasons, it is licensed for use in people 5–49 years of age, and as such, is not currently available for use against AOM during the peak age of disease burden. An intranasal inactivated virosomal influenza vaccine has demonstrated a 44% reduction in new AOM episodes in children under 5 years of age with recurrent AOM and may prove to be a viable alternative [73]. It is unlikely that influenza will be included in a polymicrobial otitis vaccine considering the unpredictability of determining the influenza strain that will be dominant in any given year. It is generally accepted that an additional benefit of the influenza vaccine and any new formulations will be the capacity of the vaccine to reduce the incidence of OM as a secondary benefit.

RSV vaccines

The National Institute of Allergy and Infectious Diseases (NIAID), in its initiative to accelerate the development of vaccines to vaccine-preventable disease, identified a vaccine to RSV infection as a high priority [202]. Despite substantial efforts over the last 10 years towards producing a vaccine for RSV infection in children, a vaccine is not currently available. Several problems inherent in RSV vaccine development have dampened the enthusiasm of industry in progressing a strategy for RSV:

- The potential of infants failing to achieve protective immunity following vaccination owing to immunologic immaturity and/or suppression of the immune response by maternally derived antibody
- A successful vaccine would need to protect against subgroup A and B strains of RSV and finally
- The legacy of failed RSV vaccines in the 1960s, one of which actually produced severe lower respiratory tract symptoms (bronchoconstriction and pneumonia) in infants [74]. Two immunized infants died as toddlers as a consequence of subsequent RSV infection. The cause of the deaths was attributed to bronchopneumoniae.

A comprehensive review on treatments for RSV disease has reported that RSV protein vaccine candidates, recombinant RSV subunit vaccine candidates, protein vaccine candidates for maternal immunization and live attenuated vaccine candidates have all been evaluated in humans [75]. Three types of RSV subunit vaccines have been evaluated in clinical trials: purified

F glycoprotein vaccines (PFP-1, -2 and -3); BBG2Na, a peptide from the G glycoprotein conjugated to the albumin-binding domain of streptococcal protein G; and copurified F, G and matrix (M) proteins [75]. Of this group, the purified fusion proteins PFP-1, -2 and -3 have been shown to be safe and immunogenic in children over 12 months of age [76–78]. PFP-2 was well tolerated and immunogenic in women in their third trimester of pregnancy, with all 35 infants being born healthy and having higher vaccine-specific anti-F IgG binding antibody to RSV at birth, 2 and 6 months of life compared with infants of nonvaccinees [79]. Anti-F IgA and IgG concentrations in breast milk were elevated in mothers who received the vaccines.

MEDI-524^{TM} (Numax) is a novel recombinant humanized IgG1 monoclonal antibody (mAb), currently being developed to potentially prevent serious lower respiratory disease caused by RSV in pediatric patients at high-risk of RSV disease. MEDI-524 (a derivative of palivizumab, the current standard of care for RSV disease) has exhibited enhanced neutralizing activity compared with palivizumab in an animal model [80]. An ongoing worldwide, Phase III trial assessing the efficacy of MEDI-524 in reducing RSV disease in high-risk infants (≤ 6 months of age) commenced in late 2004 [203]. The comparative trial is designed to assess MEDI-524 against lower respiratory tract illness and OM. A follow-up trial is anticipated to start in mid-2006 [204]. MEDI-524 will be available in an inhaled formulation.

Live-attenuated vaccine candidates have been tested in infants [81,82]. Recently, a recombinant RSV vaccine with multiple mutations (rA2cp248/404/1030 δ SH) has been shown, in a Phase I study, to be appropriately attenuated for young infants and has the potential to induce protective immunity in this population group [83]. Furthermore, it was observed that antibody might not be a significant mediator of protection induced by this live vaccine. Further work is planned to determine whether the recombinant vaccine can induce protective immunity against wild-type RSV. The same group of investigators have also been involved in a test-of-concept trial evaluating a combination RSV and PIV3 live, attenuated, intranasal vaccine for safety, viral replication and immunogenicity in children 6–18 months of age [84]. The results showed that the bivalent RSV/PIV3 vaccine was safe and immunogenic, and warrants further development. There have been no studies reporting the impact of these formulations on OM in infants and young children. Further insight into vaccine efficacy, RSV pathogenesis and the host's immune response to infection may become possible with the establishment of the chinchilla and mouse animal models of RSV infection of the nasopharynx and eustachian tube [85].

PIV vaccines

The human PIVs consist of four serotypes: PIV3 ranks second only to RSV as the leading cause of bronchiolitis and pneumoniae in infants under 6 months old; PIV1 and 2 cause illness after 6 months of age whereas PIV4 has been associated with mild upper respiratory tract illness in children and adults [202].

Although development of a vaccine to prevent PIV infections has been a high priority at the NIAID, a licensed vaccine is not yet available, with prospective manufacturers daunted with the prospect of developing a multivalent vaccine. To date, live-attenuated PIV3 vaccines that have been investigated in humans have been shown to be satisfactorily attenuated, immunogenic, and phenotypically stable in infants and young children [86,87]. The impact of PIV3-cp45 on OM is doubtful, with 3 out of 32 seronegative vaccine recipients developing OM in Phase I studies [86], and no demonstrated difference in the occurrence of AOM or serous OM in 6–18-month-old healthy children in Phase II studies [87].

Bacterial vaccines

Pneumococcal vaccines

Composition

In 1983, the 23-valent pneumococcal polysaccharide vaccine, 23PPV (Pneumovax 23^{TM} [Aventis Pasteur, Lyon, France]), (serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F) was licensed in the USA. The pneumococcal serotypes included in the vaccine were chosen on the basis of the relative distribution of the individual serotypes that cause approximately 90% of invasive pneumococcal infections [88].

With the advent of the *H. influenzae* type b vaccine against meningitis in the 1980s came the technology to conjugate the pneumococcal polysaccharides to protein carriers. In 2000, a four-dose regimen of a 7-valent pneumococcal conjugate vaccine (PCV7) (Prevenar^{TM}/Prevnar^{TM}, Wyeth-Lederle Vaccines) (serotypes 4, 6B, 9V, 14, 18C, 19F and 23F), conjugated to a non-toxic diphtheria-toxin variant (CRM197), was licensed for use in infants and toddlers in the USA. Since then, marketing approval has been granted in the European Union, Australia and several other countries. Those serotypes included in the vaccine are estimated to cause 80% of invasive pneumococcal disease in children and approximately 60% of pneumococcal AOM [89,90]. PCV7 has the potential coverage of over 85% of the pneumococcal isolates for the USA, 60–70% for Europe, approximately 55% for Asia [91] and around 48% in Scotland [92]. However, serotype prevalence may vary not only with geographical location but also with time.

Other PCVs are currently under development that differ with regard to the nature of the carrier protein and the number of included serotypes. These new formulations attempt to reduce the influence of any increase in the load of a carrier might have on the immunogenicity of other concomitantly injected conjugate vaccines that use the same protein carrier and the PCV itself, and to increase the serotype coverage of pneumococcal vaccines. These new vaccines include nine serotypes (contains those serotypes of the 7-valent vaccine with two additional serotypes, 1 and 5) or 11 serotypes (contains those serotypes of the 9-valent vaccine with two additional serotypes, 3 and 7F), which are conjugated to one of several carrier proteins, including CRM197, tetanus protein, diphtheria toxoid, *Neisseria meningitidis* group B outer-membrane protein complex (OMPC) and *H. influenzae* protein D.

Immune response to pneumococcal vaccination

Functional immunity to pneumococcus is thought to be a result of opsonizing antibody. Young children less than 2 years of age are unable to respond effectively to polysaccharide antigens due to immaturity of the immune system that is unable to make effective T-cell independent responses. This renders the polysaccharide vaccine virtually useless against pneumococcal OM and invasive disease in children of this age when they are most prone to OM. 23PPV has been used as a booster after primary immunization with a PCV in order to reduce immunization costs (23PPV being less expensive than pneumococcal conjugated vaccine) and to induce higher serum [52,93] and salivary antipneumococcal antibodies, in higher concentrations [94] than a conjugate booster.

Unlike the polysaccharide vaccines, the PCVs are able to elicit a T-cell-dependent antibody response to the protein carrier and polysaccharides, making them highly efficacious against invasive disease in children younger than 2 years of age. They are able to induce a primary immune response in young children after one or more doses. PCVs have been demonstrated to induce both humoral and mucosal responses. The IgG serum antibodies are functionally active, as demonstrated by their high avidity and opsonophagocytic activity, and consist of subclass IgG1 [95,96], but after booster immunization usually consist of both IgG1 and IgG2 [95]. Several studies have reported both IgA and IgG mucosal responses as measured by antibody in saliva [93,97–99]. The antibody response is predominantly of subclass IgA1 [94], and the antibodies persist for only a short period [97,100]. There is a positive correlation between serum and salivary IgG levels suggesting that salivary IgG has diffused from serum [98]. Furthermore, serum serotype-specific pneumococcal anticapsular IgG responses have been reported to be correlated inversely with nasopharyngeal acquisition of pneumococcus [101]. Consistent with this observation, several studies have shown a significant reduction in nasopharyngeal carriage in vaccine-serotype pneumococci in infants as a result of pneumococcal conjugate vaccination [51,53,55,102,103]. As the burden of nasopharyngeal colonization is a major risk factor of AOM [44,45], it might be predicted that an immune response that reduces nasopharyngeal colonization would be efficacious against AOM. However, in some studies where there were apparent drops in vaccine carriage, there were compensatory rises in nonvaccine serotypes or other bacterial pathogens [51–53,55,56]. Modification of the microbial ecology of the nasopharynx by vaccination could at least partly explain the different efficacy observed for AOM compared with invasive disease [104] following vaccination.

Efficacy of pneumococcal vaccination

In clinical trials to date, PCVs have been shown only to be modestly beneficial against OM. Both Finnish and Californian trials have reported a 6% OM efficacy point estimate [105,106]. In the Californian study, PCV7 demonstrated a decrease in AOM visits by 8.9%, AOM episodes by 7% and recurrent episodes by 9.3% [106]. In the two Finnish trials,

PCV7 [56] and a 7-valent PCV conjugated to an outer membrane protein complex (OMPC) of *N. meningitidis* (PncOMPC) [52] showed a similar reduction of 56 and 57%, respectively, in AOM due to vaccine-type pneumococci. PCV7 reduced the frequency of episodes due to serotypes that cross-react with those in the vaccine by 51% [56]. The overall reduction of AOM irrespective of aetiology was 6% for PCV7; however, there was an increase of 33% in the cases of pneumococcal AOM caused by nonvaccine serotypes [56]. Of significance was a 24% reduction in tympanostomy tube procedures [105], suggesting a reduction of severity of disease might have occurred.

It is well documented that onset of OM during the first 6 months of life is one of the strongest risk determinants for recurrent OM and chronic OME [45]. An ongoing Phase II study is investigating neonatal PCV7 immunization of high-risk infants in Papua New Guinea [205]. It is hypothesized that a three-dose regimen over 3 months in newborns may stimulate antibody to protect against invasive disease and reduce the burden of early carriage.

Two Dutch studies with PCV7 followed by a booster of 23PPV in children 1–7 years of age failed to show any protection of vaccination against recurrent AOM [53,107]. Overall nasopharyngeal carriage of pneumococci was not affected by vaccination because of concurrent increase in nonvaccine serotypes [53]. Therefore, the data does not lend support to the use of PCV7 in the management of recurrent AOM in previously unvaccinated toddlers and children. Furthermore, a recent study showed that combined PCV7 and 23PPV does not prevent recurrence of OME among children 2–8 years of age with a history of persistent OME [108]. It may be that the PCV protects against AOM and the progression to an otitis-prone condition, when given in early infancy however, once the recurrence is established, is no longer beneficial.

A 9-valent pneumococcal vaccine conjugated to CRM197 (PNCRM9) has been shown to decrease OM by 17% in children 1–3 years of age [109]. An 11-valent mixed carrier vaccine, PncDT11 (polysaccharide antigens of serotypes 1, 4, 5, 7F, 9V, 19F and 23F conjugated to tetanus protein, and serotypes 3, 6B, 14 and 18C conjugated to diphtheria toxoid) has been reported to be safe and immunogenic in infants from 2 months of age [110,111]. Its impact on OM was not addressed. Immunogenicity studies are also currently being conducted against a 13-valent PCV in healthy infants [206].

A 11-valent pneumococcal vaccine conjugated to *H. influenzae*-derived protein D (Pn-PD) has been shown to be safe in infants and induces antibody responses to all components of the vaccine, including protein D [112]. More recently, Phase III studies have demonstrated Pn-PD to confer protection against AOM caused by vaccine pneumococcal serotypes (52.6%), as well as AOM caused by NTHi (35.3%) and any pneumococci (51.5%) [113].

The reasons for the disappointing impact of pneumococcal vaccines on AOM despite good antibody responses may be multifactorial:

- Serotype replacement following vaccination may result in middle-ear disease caused by pneumococci of nonvaccine serotypes.
- Pneumococcal vaccination may also induce replacement by other pathogens involved in middle-ear disease such as NTHi or *M. catarrhalis*.
- Suboptimal IgG responses to some serotypes. Animal [114] and human [52,56] studies have shown that protection from disease is dependent upon serotype-specific humoral pneumococcal anticapsular IgG concentrations. Analysis of combined results from the two Finnish studies [115] showed a clear correlation between IgG concentrations 4 weeks after vaccination and protection against AOM caused by serotypes 6B, 19F and 23F. The serotype efficacy ranged from 25% for 19F to 84% for 6B.

The results of the studies of pneumococcal conjugate vaccination in children raise important questions regarding the nature of the immune response in the middle ear. Why does a vaccine with excellent outcomes against an invasive disease perform so poorly against infection with the same microbes in the ear? There is now a body of evidence to suggest that the middle ear is, at least in part, dependent on mucosal immunity and that a mucosal immunization approach may be more efficacious than immunization by a systemic route. In this context, further studies are needed to better characterize immune mechanisms in the middle ear. Additional studies will be required to determine the most efficacious route of immunization and choice of adjuvant. A mucosal strategy should not be discounted.

Maternal immunization against pneumococcal disease

A Cochrane review has assessed the effect of pneumococcal vaccination during pregnancy for preventing pneumococcal disease during the infant's first months of life [116]. The review found there was not enough information to conclude whether vaccination with the 23PPV during pregnancy led to fewer infant infections. However, it has been shown in the chinchilla OM model that maternal heptavalent pneumococcal polysaccharide-protein vaccine immunization reduces the burden of early infant OM and invasive pneumococcal disease [117].

Immunization against NTHi AOM

The only study conducted to date that has shown vaccine-induced protection against NTHi AOM is the 11-valent pneumococcal vaccine conjugate to the *H. influenzae*-derived protein D [113]. Although 35.3% efficacy was observed with this vaccine against AOM caused by NTHi, there was no correlation between efficacy and anti-protein D antibodies. Further studies are required to determine the nature of NTHi immunity stimulated by protein D. Other conserved NTHi proteins that stimulate cellular immunity or antibodies that block attachment may need to be added to the vaccine composition to improve efficacy against AOM caused by NTHi. Furthermore, it will also be important in future trials to control for the possible interaction between the reduction in pneumococcal

AOM and NTHi infections. It may be necessary to include a trial arm where only the carrier protein-D and additional NTHi antigens are tested to determine the true effect of these antigens on NTHi AOM.

Pneumococcal protein antigens

In parallel to the development of conjugate vaccines, other pneumococcal antigens common to all serotypes are also being tested. The virulence proteins, pneumococcal surface-adhesin A (PsaA), pneumococcal surface-protein A (PspA) and neuraminidase, yield encouraging results in animal models. PspA elicits protection against OM in a rat model [118]. PspA is serologically variable, however, a recombinant form of the protein has been shown to be immunogenic in humans [119]. The immunity to PspA is sufficiently crossreactive for it to be considered as a vaccine candidate. PspA and recombinant lipidated PsaA reduced pneumococcal carriage in mice [120]. Issues regarding antigen heterogeneity and toxicity associated with some virulence factors such as pneumolysin have hampered further development of a vaccine incorporating these antigens. However, a combination vaccine of PspA, PsaA and PdB (a mutant form of pneumolysin) has been shown to produce a synergistic protective response in mice [121]. As these proteins appear to function at different stages of the pathogenic process, it is anticipated that a combined pneumococcal vaccine may provide protection against a wider variety of strains over immunization with any one of the protein antigens. Neuraminidase affords protection against *S. pneumoniae* nasopharyngeal colonization and OM in chinchillas [122].

An innovative, subunit vaccine, PGCvax™, is based on two novel pneumococcal surface proteins (BVH-3 and -11). Studies in a mouse model demonstrated that the proteins are highly conserved throughout the pneumococcal species and are able to elicit protective antibodies [123]. Phase I clinical studies have shown the recombinant vaccines to be well tolerated and immunogenic in adults [202]. It is planned to extend the Phase I study to include infants.

The potential advantages of a pneumococcal protein vaccine over a polysaccharide-protein conjugate vaccine include:

- The use of formulations that have a combination of proteins likely to target more than one mechanism of pneumococcal carriage and colonization
- Less expense, abrogating the cost of conjugation and product purification

NTHi vaccine candidates

The development of a vaccine to NTHi has been given the highest priority by the NIAID. However, progress has been hindered by the lack of clinical studies identifying an *in vitro* correlate of immunity to NTHi infection. Recommendations for the characterization of the protective immune responses to NTHi include bactericidal antibody and/or inhibition of adherence to relevant host cells, and protective responses in at least two animal models [64]. Preclinical studies have identified various antigens as vaccine candidates.

Lipooligosaccharide (LOS) is a major surface antigen and virulence factor of NTHi, capable of eliciting bactericidal and opsonic antibodies in animals [124,125]. A potential vaccine candidate, 9274LOS, is structurally heterogeneous [126] and shares common epitopes among NTHi LOSs [124]. When prepared as a detoxified protein conjugate (i.e., dLOS linked to tetanus toxoid [TT] or high molecular weight [HMW] protein from NTHi) it was shown to have high immunogenicity in mice and rabbits and induced complement-mediated bactericidal activity against NTHi in rabbits [127]. Furthermore, it was able to elicit immunity against NTHi OM in a chinchilla model [128] and significantly enhance NTHi clearance in the mouse nasopharynx [129]. A Phase I clinical trial has demonstrated that it is well tolerated in adults [130].

P6 is highly conserved among all *H. influenzae* strains. Immunization with P6 induces protection in the pulmonary clearance model in the rat [131], mouse [132] and chinchilla models of OM [133]. It is a target of human bactericidal antibody, which is associated with protection from OM [133]. Intranasal immunization with native P6 [134] and recombinant P6 protein coupled to adamantylamide dipeptide (AdDp) as mucosal adjuvant confers antigen-specific protection against OM by NTHi in a mouse model [135], whilst P6 coupled to cholera toxin induced P6-specific sinus mucosal and systemic immunological responses mainly of the IgA and IgG isotypes in rats [136]. A recent study has shown that maternal intranasal immunization with P6 induced specific immune responses in sera and colostrums of mother mice and protective antibodies were transferred from mother to neonate mice through transplacental transfer during pregnancy and through breast milk after birth [137]. Furthermore, intranasal immunization with P6 has been shown to modulate cellular responses with an increase in memory T cells in middle-ear mucosa [138] and decreased concentrations of tumour-necrosis factor- α in middle-ear effusions [134]. Recent data has shown that P6 of NTHi contains a single immunodominant T-cell epitope and that both B- and T-cell responses are important in the ability of P6 to induce protection in the murine model [132]. It would follow that a clinical study to determine if T cells from humans would also recognize the T-cell epitope should be conducted.

OMP26 is highly conserved and is associated with protection against NTHi strains following parenteral and mucosal immunization in the chinchilla and rat models, respectively [139,140]. Furthermore, these studies demonstrated that OMP26 induced a high-titer and specific response in both animal models. OMP26 is a strong vaccine candidate, as demonstrated by its efficacy as an immunogen against OM caused by NTHi in two diverse rodent models.

OMP P5 fimbriae adhesin-derived peptides (LB1 and LPD-LB1(f)_{2,1,3}) protect against both homologous and heterologous strains in the chinchilla nasopharyngeal clearance model and the OM model in the rat by inducing a specific antibody response [139,141]. As described above for OMP26, the demonstrated efficacy of P5-derived vaccine candidates in two animal models provides strong support for further evaluation for their inclusion in a vaccine directed against NTHi-induced OM.

Lipoprotein D is highly conserved in *H. influenzae* strains. Lipoprotein D is used predominantly as a carrier protein for NTHi [139,141], whilst protein D (a recombinant deacylated form of LPD) has been used more recently as a carrier protein for pneumococcal antigens [112,113]. In this formulation, significant protection (35.3%) was observed against AOM caused by NTHi [113].

The OMP P2 constitutes nearly 50% of the total OMP of NTHi; however, it has not previously been considered as a strong vaccine candidate because antibodies directed against it have been shown to be highly strain-specific [202]. Further studies into the conserved regions of the P2 porin protein showed that antibodies to loop 6 have bactericidal activity against multiple strains of NTHi supporting the concept of using conserved regions of the P2 protein as vaccine antigens [142].

HtrA/H91A (Hin47) is the nonproteolytic recombinant form of the *H. influenzae* HtrA stress-shock protein. The vaccine relies on an adhesin-receptor technology and is combined with an adjuvant. It has been shown to be partially protective in the chinchilla model of NTHi-induced OM [143]. Although Phase I testing in adults have shown Hin47 to be safe and immunogenic, it is yet to be assessed in the pediatric population [202].

HMW1 and HMW2 are a family of HMW-adhesins that are encoded by genes that are 80% identical and found in approximately 75% of NTHi strains [202]. The HMW proteins are targets of human opsonizing antibodies that demonstrate protection against NTHi in the chinchilla OM model [144]. The HMW-adhesin, Hap, when conjugated to mutant cholera toxin, reduced nasopharyngeal colonization when mice were challenged intranasally with a heterologous strain of NTHi [145]. A recombinant form of the cell-binding domain of the Hap protein was capable of eliciting cross-reacting antibodies and also reduced nasopharyngeal colonization suggesting that it may be worthy of further investigation as a vaccine candidate [146].

The NTHi P4 lipoprotein is highly conserved and elicits antibodies that are broadly cross-reactive and have bactericidal activity [147,148]. A recombinant form of P4 has been shown to reduce nasopharyngeal colonization in a mouse model after intranasal immunization [149]. However, as a highly specific acid phosphomonoesterase, its inclusion in a vaccine for infants is challenging, therefore, a nonenzymatically active recombinant P4 protein has been developed and shown to generate bactericidal antibodies [150].

***Moraxella catarrhalis* vaccine candidates**

To date, the major focus of investigation in identifying *M. catarrhalis* vaccine antigens has been on OMPs and LOSs. Significantly, UspA, CD, and CopB elicit bactericidal antibodies [151–153]. Immunization with UspA [151], recombinant CD [154], or CopB [153] enhanced pulmonary clearance of *M. catarrhalis* in a mouse model. *M. catarrhalis* serotype A dLOS and serotype B dLOS conjugated to protein carriers elicited strong bactericidal antibodies in animal models [155,156].

and conferred protection in a pulmonary infection model [157]. There have been no human studies investigating the prospect of immunizing against OM caused by *M. catarrhalis*.

Expert commentary

OM is a multifactorial disease that primarily affects three-quarters of the developed world's children under 3 years of age. Although associated mortality is low, the burden of the disease on humanity is enormous. The predominant cause of OM is infection of the middle-ear with microbes that are mainly commensals of the nasopharynx. The anatomical orientation of the eustachian tube in association with a number of risk factors predisposes infants and young children to middle-ear infection. Although there has been a significant body of research undertaken towards the development of a suitable vaccine against the disease, the polymicrobial nature of the infection presents major obstacles, particularly with respect to formulation, trialling and manufacture of a vaccine. At present, the price of complex and multivalent conjugates, such as the PCV, are high, with a four-dose regimen of the PCV7 costing in excess of US\$200 [158]. The limitation to development of this technology is likely to be commercial. It has proven to be very expensive to develop a pneumococcus vaccine. The involvement of the pharmaceutical industry in the development of new respiratory vaccines has been stalled due to the consolidation within the pharmaceutical industry, adverse experiences with live-attenuated viral vaccines (e.g., rotavirus and RSV), and the formidable costs in vaccine development, clinical trials and manufacturing facilities. Complicating the matter further is a US FDA requirement that before a new PCV may be licensed, it must be demonstrated to be 'noninferior' to existing vaccines [159,160]. With the next generation of PCVs to enter the market, it may not be feasible to conduct additional efficacy trials comparing a candidate PCV to PCV7 for licensure. An alternative and more cost-effective method for comparing vaccines would be to conduct clinical trials based on comparing immunological responses where parameters that best correlate with protection have been quantified. To this end, it will be necessary to develop functional assays to measure correlates of OM protection to the various pathogens presently under investigation, for instance, NTHi, *S. pneumoniae*, *M. catarrhalis*, influenza virus, RSV and PIV.

There is a general consensus that the successful vaccine for OM will most likely include more than one antigen. A strategy in the first instance would be the development of a formulation containing antigens of the two major bacterial agents, NTHi and *S. pneumoniae*. This approach has been exemplified by the recent use of an NTHi protein as a carrier for pneumococcal polysaccharides that was shown to be partially effective in reducing the incidence of OM caused by NTHi and *S. pneumoniae*. For NTHi, OMP26 and the OMP P5 fimbriae-derived peptides could be considered as lead vaccine candidates. Although the choice is somewhat more complex for *S. pneumoniae*, PspA could be considered the leading vaccine candidate for this bacterium, at this point in time. Efficacy may be improved by the addition of PsaA and PdB to the

formulation, as well as ensuring an appropriate representation of proteins from PspA family 1 and 2. These antigens are well characterized, immunogenic and efficacious in animal models and are now available for human studies. It is feasible that this approach could see the development of a highly efficacious vaccine available within the next 5 years given an appropriate investment by the vaccine industry. It might be predicted that additional NTHi antigens and replacement of pneumococcal polysaccharides conjugated to a protein carrier with pneumococcal protein antigens will increase efficacy. The next strategy would be to add to this core formulation additional antigens as they become available, particularly for *M. catarrhalis* and RSV. To date, there has been the development of a live-attenuated RSV vaccine that is well tolerated in infants and demonstrates protection by challenge studies [83]. Clinical testing of the vaccine candidates was conducted by a consortium of investigators as part of an agreement between industry (Wyeth Vaccines Research) and government laboratories (NIAID and National Institutes of Health). A combination vaccine of RSV and PIV3 may have some impact on the incidence of OM [84].

Although the rapid development of this approach will present a serious challenge for the current paradigm concerning clinical trials that exist within the regulatory authorities worldwide, it is feasible that an efficacious vaccine against the major causative microbes for OM could be achieved within the next decade. This will only be achieved with the fostering of co-operative agreements between industry, government laboratories and academics to progress the evaluation of the significant number of promising vaccine candidates in human trials. It is most likely that the formulations will not induce sterile immunity against OM pathogens. Indeed, this may not be desirable given that they are part of the commensal flora of the nasopharynx and sterile immunity may result in replacement flora with greater pathogenic potential. The aim should be to reduce nasopharyngeal load and induce appropriate immune responses when microbes ascend the eustachian tube.

There is substantial evidence that indicates that OM often goes unrecognized in the community and as a result the incidence and subsequent sequelae such as hearing loss may be higher than that reported. The failure to develop programs to screen routinely for OM and research improved treatment strategies is a global public health disgrace.

Five-year view

Over the next 5 years, it could be expected that a highly efficacious multivalent vaccine containing both NTHi and *S. pneumoniae* antigens will be produced commercially. Antigens for these microbes are currently available and needing clinical trial. By 2011, it is feasible that a vaccine containing additional antigens for *M. catarrhalis* and RSV could be available. However, this will require significant commitment from the vaccine industry and more flexible regulatory approaches to trialling these formulations. Regrettably, tens of millions of children will continue to develop major hearing disabilities as a result of OM over the next 5 years.

Key issues

- Otitis media (OM) is a major cause of morbidity in the world's children.
- OM is caused by microbial infection of the middle ear as a result of the anatomical orientation of the eustachian tube in association with a number of risk factors in infants and young children.
- The predominant microbes that cause infection are nontypeable *Haemophilus influenzae* (NTHi), *Streptococcus pneumoniae*, *Moraxella catarrhalis*, respiratory syncytial virus (RSV), rhinovirus, parainfluenza (PIV) and influenza.
- The potential exists for the rapid commercial development of a polyvalent vaccine for NTHi and *S. pneumoniae*.
- Candidate vaccine antigens have been identified in animal models for NTHi and *S. pneumoniae*.
- Promising research is being undertaken towards the identification of vaccine antigens for *M. catarrhalis*, RSV and PIV.
- Some clinical trials for RSV and PIV have been conducted, but efficacy for OM have not been determined.
- There needs to be a commitment from the vaccine industry, research institutions and regulatory authorities to work in partnership to rapidly trial new vaccine formulations specifically developed for OM.
- Further research is necessary to characterize the nature of the immune response in the ear and particularly what differentiates it from the respiratory tract. Mucosal immunity needs to be explored further.
- Significant investment in public health initiatives is required to provide for early diagnosis of OM and interventions.
- The vaccine has to be affordable and accessible to the children of the world and in sustained supply.
- Pathogen replacement following vaccination is a major concern and ongoing surveillance studies will be required following the introduction of a polymicrobial vaccine for OM.
- Vaccination offers the greatest potential to deliver a sustained reduction in the burden of OM in children globally.

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