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Panel 6: Vaccines

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Abstract

Objective—To update progress on the effectiveness of vaccine for prevention of acute otitis media (AOM) and identification of promising candidate antigens against *Streptococcus pneumoniae*, nontypeable *Haemophilus influenzae*, and *Moraxella catarrhalis*.

Review Methods—Literature searches were performed in OvidSP and PubMed restricted to articles published between June 2007 and September 2011. Search terms included *otitis media*, *vaccines*, *vaccine antigens*, and each of the otitis pathogens and candidate antigens identified in the ninth conference report.

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Conclusions—The current report provides further evidence for the effectiveness of pneumococcal conjugate vaccines (PCVs) in the prevention of otitis media. Observational studies demonstrate a greater decline in AOM episodes than reported in clinical efficacy trials. Unmet challenges include extending protection to additional serotypes and additional pathogens, the need to prevent early episodes, the development of correlates of protection for protein antigens, and the need to define where an otitis media vaccine strategy fits with priorities for child health.

Implications for Practice—Acute otitis media continues to be a burden on children and families, especially those who suffer from frequent recurrences. The 7-valent PCV (PCV7) has reduced the burden of disease as well as shifted the pneumococcal serotypes and the distribution of otopathogens currently reported in children with AOM. Antibiotic resistance remains an ongoing challenge. Multiple candidate antigens have demonstrated the necessary requirements of conservation, surface exposure, immunogenicity, and protection in animal models. Further research on the role of each antigen in pathogenesis, in the development of correlates of protection in animal models, and in new adjuvants to elicit responses in the youngest infants is likely to be productive and permit more antigens to move into human clinical trials.

Keywords

otitis media; vaccines; vaccine antigens; otitis pathogens; candidate antigens

The impact of acute otitis media (AOM) on child health far exceeds the discomfort and suffering associated with individual episodes of disease. Acute otitis media is among the largest drivers of antibiotic use in children, providing support for prevention of disease as an important strategy for reducing antibiotic prescribing and subsequently the emergence of resistance. Recurrent AOM is common, with as many as 20% to 30% of children suffering 3 or more episodes before their second birthday, with the potential for persistent middle ear effusion and conductive hearing loss and subsequent delay or impairment in speech and language development. Chronic suppurative otitis media (CSOM) also appears to have its origins in early onset, recurrent otitis media. Although now uncommon in developed countries, CSOM remains an important cause of acquired hearing loss globally and including countries such as India, Australia, and Greenland.^{1–5} Finally, AOM, its treatment, and its complications have a significant economic cost for society.

Methods

Literature searches were restricted to articles published between June 2007 and September 2011. OvidSP and PubMed were used to search for articles related to otitis media, vaccines, vaccine antigens, and specific pathogens. For example, keywords for the section on pneumococcal vaccines included *Streptococcus pneumoniae*, *pneumococ* vaccine*, *conjugate vaccine*, *protein antigens*, and *otitis media*; keywords for the section on *Haemophilus influenzae* vaccine included *Haemophilus influenzae*, *vaccines*, *protein D*, and *otitis media*. Searches were also conducted on each vaccine antigen discussed in the 2007 report to identify progress made since the prior panel report. Searches were limited to articles published in English. Explicit inclusion and exclusion criteria for individual publications were not defined for inclusion in the review. Only subjective methods were used to determine which articles to reference in this report. Each section of the report was

presented to the committee, as a whole, where members of the panel contributed comments about scientific quality as well as identified progress in specific areas that were not included in the summary presentations.

Results

Effectiveness of Pneumococcal Conjugate Vaccine on Incidence of AOM

Several randomized controlled studies have previously demonstrated the efficacy of pneumococcal conjugate vaccines in preventing pneumococcal AOM in young children and were enumerated in our previous report.⁶ Subsequently, a number of studies have estimated the population impact of the 7-valent pneumococcal conjugate vaccine (PCV7) on the incidence of AOM in the United States. Poehling et al⁷ evaluated the effect of PCV7 on rates of frequent otitis media and insertion of pressure-equalizing tubes (PET) in consecutive birth cohorts. Data from Tennessee Medicaid and from private insurance companies in New York were used to construct 4 birth cohorts (1998–1999, 1999–2000, 2000–2001, and 2001–2002). This study reported, for the 2000–2001 cohort, a 17% and 28% decline in frequent otitis media in Tennessee and New York children, respectively, since PCV7 introduction. Similarly, PET insertion by 2 years of age declined 16% and 23% since PCV7 introduction, in Tennessee and New York, respectively. Another study used data from a large private insurance company and compared rates of otitis media ambulatory visits before (1997–1999) and after PCV7 introduction (2004). Among children aged <2 years, a 43% reduction in the incidence of otitis media was observed. A similar 42% reduction in the incidence of related antibiotic prescribing was documented, for an overall 32% reduction in otitis-related costs.⁸ de Wals et al⁶ demonstrated that the decline in AOM in Quebec began prior to introduction of PCV7 and attempted to parse the portion attributable to vaccine compared with secular trends. These authors concluded that of the overall 25.2% decline in AOM episodes observed between 2007 and 2000, 13.2% was attributable to vaccine, with the largest impact in children younger than 2 years of age.

These studies have consistently reported that the overall number of visits for AOM has decreased since PCV7 introduction in the United States and Canada. Notably, most estimates exceeded the point estimates observed in individually randomized clinical trials. Understanding the methodologies of these studies and potential indirect effects of vaccination is necessary to interpret these findings. Specifically, changes in diagnostic criteria, intensity of seasonal respiratory infection with respiratory syncytial virus or influenza virus, and/or other secular trends likely influence the outcome of studies that rely on comparisons of time periods before and after vaccine introduction.

Finally, most studies have documented that after introduction of PCV7, nasopharyngeal carriage of pneumococcal vaccine serotypes has decreased substantially,⁹ resulting in a shift in serotype distributions both in carriage and in the etiology of AOM.¹⁰ For example, serotype 6C, a serotype first described in 2009, is now carried by ~3% of Massachusetts children.^{11,12} The population-based effects of the 13-valent pneumococcal conjugate vaccine on otitis media have yet to be elucidated.

Progress in the Identification of Vaccine Candidates

Streptococcus pneumoniae—Although conjugated polysaccharide vaccines have greatly helped to reduce the burden of pneumococcal diseases, differences in the global distribution of pneumococcal serotypes and increasing disease due to replacement serotypes suggest that a vaccine with coverage for a limited number of pneumococcal serotypes will not provide long-term, sustainable protection against pneumococcal otitis media. Vaccines composed of broadly conserved protein antigens would provide serotype-independent coverage and theoretically would not be associated with serotype replacement. Importantly, protein-based vaccines would be cheaper to produce than conjugate vaccines and therefore more affordable in resource-poor settings where need is greatest.¹³ Conventional wisdom suggests that as a result of the high rates of recombination and antigenic diversity in pneumococci, it is likely that a protein-based vaccine would require a combination of several protein antigens.

Pneumococcal Surface Protein A—Pneumococcal surface protein A (PspA) has been a promising vaccine candidate. Recent studies demonstrate sequence heterogeneity among pneumococcal (SP) isolates, and considerable work has gone into identifying PspA epitopes critical for eliciting broadly protective antibodies.^{14–16} Previous research focused primarily on the N-terminal alpha helical region of PspA; however, the proline-rich region is antigenically conserved and present in all PspA and the majority of PspC molecules. Recombinant fragments of the proline-rich region elicited cross-clade protective immunity to invasive infection.¹⁷ Research has also focused on adjuvants for PspA such as whole-cell pertussis,¹⁸ delivery systems such as outer membrane vesicles from *Salmonella*,^{19,20} DNA vectors,²¹ and mucosal delivery systems that use *Lactobacillus*^{22,23} or *Lactococcus*.²⁴ PspA conjugates have been prepared with polysaccharide capsule 23F, and immunization with such polysaccharide-protein conjugates enhances survival from invasive pneumococcal disease in animals beyond that achieved with either component alone.²⁵

Histidine Triad Family (Pht)—The Pht family (PhtA, PhtB, PhtD, and PhtE) proteins are well conserved across pneumococcal serotypes and have been described as virulence factors. Recent data suggest Phts are regulators of metal homeostasis.²⁶ As such, they may play a role in ion storage, particularly zinc, specifically regulating availability when the bacterium faces ion-restricted environments, as is the case during early stages of infection. The potential of the Pht protein family as vaccine candidates was recently evaluated with several animal challenge models.²⁷ PhtD immunization was shown to prevent nasopharyngeal colonization by several different serotypes, to reduce the bacterial load in lungs of nonlethally challenged animals, and to prevent death from several serotypes in otherwise lethally challenged mice. The functionality of purified anti-PhtD antibodies from naturally exposed humans was also demonstrated by protection against lethal challenge after passive transfer in mice. Finally, a significant additive effect on protection was observed using vaccination-induced anti-PhtD in combination with passively transferred anti-polysaccharide antibodies.²⁸

Pneumococcal Surface Adhesion A—Pneumococcal surface adhesion A (PsaA) is a member of the family of metal-binding lipoproteins that is present in all pneumococci and

viridans streptococci. Oral and intranasal vaccination with the full-length protein stimulated high titers of serum IgG and mucosal IgA and was protective for colonization with a subset of *S pneumoniae* challenge strains (although titers did not always correlate with protection) but failed to protect against intraperitoneal challenge.²⁹ Its failure to protect against intraperitoneal challenge and the variability in strain protection suggest its potential is limited. It has been incorporated with StkP and PcsB into a multiantigen candidate vaccine (IC47), which recently completed phase I human trials under the auspices of Intercell (Vienna, Austria).

Pneumococcal Pilus Subunits—*Streptococcus pneumoniae* strains carrying type 1 and type 2 pili have increased in recent years.^{30,31} Of the type 1 pilus subunits, protein sequence similarity indicates that RrgC is the most conserved, followed by RrgA and RrgB. High variability in RrgB makes this a less than optimal vaccine candidate.³² There are 2 clades of RrgA; these 2 variants share 84% sequence identity and demonstrate cross-protection upon passive immunization in mice.³³ However, there are concerns with a vaccine strategy focused on pili. Within a population of pneumococci containing the type 1 pilus operon, only a subset of strains (approximately 30%) express pili at a given point in time.³⁴ Biphasic phenotypic expression of pili is likely to allow nonpiliated members of the population a selective advantage under adverse conditions such as immune selection pressure. Moreover, pili encoding loci are not present in all strains and might be lost under vaccine-induced selection pressure.^{32,35}

Pneumolysin—Pneumolysin (Ply) is pore-forming toxin that is critical for virulence, immunogenic, and conserved across *S pneumoniae* serotypes. Native pneumolysin is not surface expressed and is associated with toxicity. Oloo and colleagues³⁶ used a structure-based approach to design nontoxic forms of pneumolysin that retained immunogenic and protective epitopes.

Additional Protein Targets—Pneumococcal serine-rich repeat protein (PsrP) is a serine-rich repeat protein that functions as an adhesion and promotes the formation of *S pneumoniae* aggregates.^{37,38} Passive immunization with antiserum against recombinant PsrP resulted in lower *S pneumoniae* titers in the lungs and blood but did not affect colonization.³⁷ Heat shock protein caseinolytic protease (Clp) delivered through the mucosal route increased survival in a murine sepsis model and decreased bacterial counts in a pneumonia model.³⁹ Pneumococcal choline binding protein A (PcpA) is regulated in a manganese-dependent fashion.⁴⁰ Immunization with recombinant PcpA provides protection in pneumonia and sepsis models but does not affect nasopharyngeal colonization. Sortase A (SrtA) plays a key role in pathogenesis and is highly conserved at the DNA level.⁴¹ SrtA intraperitoneal immunization protects against intraperitoneal challenge but not lung infection or colonization. Bacterial counts in the lung were reduced following intranasal immunization. Polyamine transport operon (potD) is membrane associated and antigenically conserved among pneumococci.⁴² Recombinant PotD was used for mucosal immunization of mice. Significantly lower counts of bacteria were observed in nasal washes and brain tissues but not in the lungs and olfactory bulbs. Pneumococcal protective protein A (PppA)

expressed on *Lactococcus lactis* generates mucosal and systemic immunity and may provide protection against multiple *S pneumoniae* serotypes.⁴³

Genomic Approaches—Small peptide surface display libraries were used to identify highly conserved and crossprotective proteins in SP. A protein (PcsB) analogous to the cell wall separation protein of group B streptococcus and a serine/threonine protein kinase (StkP) were studied as potential vaccine targets.⁴⁴ These proteins have been combined with PsaA and completed phase I human studies that demonstrated immune response to each protein in adults.

Phage display libraries were used to identify peptide mimics of the polysaccharide capsule of serotypes 6B and 9V.⁴⁵ These mimics protected mice from lethal challenge with *S pneumoniae*. A monoclonal antibody, Dob1, has also been identified that binds polysaccharide from serotypes 6A, 6B, 6C, and 19A and is capable of opsonizing *S pneumoniae* from these serotypes.⁴⁶ The Dob1 epitope has been proposed as a simple chemical structure that could be used in vaccines. Nevertheless, the surface expression of Dob1 is variable and dependent on the capsule expression.⁴⁷

Multiple Subunit Vaccines—Pneumolysin has been used as a mucosal adjuvant to induce high levels of mucosal and serum antibodies specific for PsaA.⁴⁸ A fusion protein of PsaA and a nontoxic derivative of pneumolysin were conjugated to cell wall polysaccharide and used to immunize mice through intranasal and subcutaneous routes.⁴⁹ Mice were protected from colonization through both routes and pneumonia through the subcutaneous route. The trivalent conjugate was superior to bivalent conjugates and mixtures of each antigen. Intraperitoneal and intranasal immunization with the combination of adenosine triphosphate (ATP)–dependent caseinolytic protease (ClpP), a Ply mutant, and putative lipote-protein ligase (Lpl) was demonstrated to be as effective as PCV7 and the 23-valent pneumococcal polysaccharide vaccine (PPV23) in pneumonia and sepsis murine models.⁵⁰ Immunization with recombinant ZmpB, nontoxic Ply, and DnaJ, in combination, provided better protection against colonization and invasive pneumococcal infection than single antigens.⁵¹

Killed Whole-Cell Vaccines—Whole-cell vaccines have been modified for testing in humans by mutating the pneumolysin (PdT) and examining alternative means to ethanol for preparation.⁵² Killed whole-cell vaccines do not prevent colonization but rather reduce the duration of carriage. As epidemiologic studies indicate that risk of pneumococcal otitis media is associated with the acquisition of a new strain, it is not clear how effective these vaccines will be for the prevention of AOM in children.⁵³

Haemophilus influenzae

Several nontypeable *H influenzae* (NTHi) protein antigens continue to be investigated as possible vaccine antigens. Recent progress has been made in characterizing several of these antigens in terms of their roles in bacterial pathogenesis, their potential usefulness as vaccine antigens, and their roles as targets of the host immune response to NTHi.

P6—Although regarded as a highly conserved antigen, a recent study of 151 NTHi respiratory isolates reported variant P6 proteins in 9% of strains.⁵⁴ Another study from the same group used molecular modeling, site-directed mutagenesis, and nuclear magnetic resonance (NMR) spectroscopy to characterize the P6 protein and concluded that P6 was not a transmembrane protein at all and was unlikely to be exposed on the bacterial surface or accessible to host antibody.⁵⁵ Noda and coworkers⁵⁶ attempted to identify P6 peptides that might be incorporated into a universal vaccine and identified promiscuous T-cell epitopes that could, in theory, provide broad-based protection against NTHi disease. Using P6 as a model vaccine antigen, Kodama and coworkers reported studies investigating a variety of different immunization protocols intended to induce a protective mucosal antibody response in a murine model of NTHi nasopharyngeal colonization. They found that administration of CpG oligodeoxynucleotides,⁵⁷ fms-like tyrosine kinase receptor-3 ligands,⁵⁸ α -galactosylceramide,⁵⁶ or plasmid DNA encoding P6 given with immunostimulatory complexes⁵⁹ was each capable of inducing an enhanced mucosal antibody response in the nasopharynx and more rapid clearance of NTHi from the nasopharynx. Sabirov and coworkers⁶⁰ reported a series of studies characterizing the antibody response of children with NTHi otitis media directed against P6. They reported that breastfed infants experienced a lower incidence of AOM and had higher levels of P6-specific serum antibodies, and these antibody levels correlated with the level of serum bactericidal activity. In a subsequent study of otitis-prone children and non-otitis-prone controls, the authors found that otitis-prone children had lower levels of anti-P6 antibodies in their acute phase sera and demonstrated a much decreased antibody response to P6 (and OMP 26 and protein D) in their convalescent sera, providing a possible explanation for the increased risk of infection in otitis-prone children.⁶¹

Pilin and OMP P5—Novotny and coworkers⁶² continued their studies of OMP P5 and the type IV pilin protein of NTHi as potential vaccine candidates. This group constructed a chimeric peptide vaccine consisting of a P5-derived B-cell epitope fused to the N-terminus of recombinant soluble pilin and reported protection against infection in a passive immunization model. In subsequent work, this group reported that transcutaneous immunization with an OMP P5 peptide, a pilin-derived peptide, and the P5-pilin fusion peptide just described were each capable of providing protection in the chinchilla otitis media model and also enhanced resolution of preformed biofilms.⁶³

Protein D—The prior Vaccine Report included the results of the POET study, which employed an 11-valent pneumococcal polysaccharide vaccine with a protein D carrier and demonstrated protection against both vaccine-type pneumococcal otitis and disease due to NTHi.^{64,65} These clinical studies failed to demonstrate a clear correlation between serum antibody levels in protein D-immunized children and the observed protection seen against NTHi disease. To better understand the potential mechanisms of protection, investigators constructed a protein D knockout mutant. They demonstrated that the mutant exhibited reduced adherence, diminished amounts of ChoP in NTHi biofilms, and diminished survival in vivo, and antibody against protein D had similar effects on the phenotype of the bacteria.⁶⁶ A functional assay measuring inhibition of protein D phosphodiesterase activity in serum samples of children immunized with the protein D conjugate vaccine was

described, and a modest, but imperfect, correlation was noted between serum protein D antibody levels and enzymatic inhibition.⁶⁷ A follow-up clinical study of the protective ability of the protein D conjugate vaccine against NTHi nasopharyngeal carriage in a population of young children demonstrated marginal protection.⁶⁵

HMW1/HMW2 and Hia—Winter and coworkers⁶⁸ continued studies of the NTHi high molecular weight proteins and their potential as vaccine candidates. One study reported that the Hia autotransporter proteins of NTHi are targets of opsonophagocytic antibodies and that shared epitopes recognized by such antibodies are present on the Hia proteins of unrelated NTHi strains. Another study from that same group reported the construction of recombinant adenovirus vaccines that expressed the HMW1/HMW2 or Hia proteins of prototype NTHi strains and demonstrated the immunogenicity of the constructs given by either the parenteral or intranasal route in the chinchilla model.⁶⁹

Lipooligosaccharide—Hong and coworkers⁷⁰ continued their investigations of NTHi lipooligosaccharide as a potential vaccine candidate. They described a mucosal vaccination approach with a detoxified lipooligosaccharide-tetanus conjugate vaccine. They reported that intranasal immunization of chinchillas with the conjugate vaccine was associated with decreased nasopharyngeal colonization and decreased otitis media development in an NP challenge model and with earlier clearance of bacteria and lesser disease severity in an intrabullar challenge model.

Moraxella catarrhalis

Moraxella catarrhalis is the third most frequent bacterial cause of otitis media.^{71,72} Furthermore, as the distribution of bacterial pathogens continues to change with the anticipated development and use of vaccines for *S pneumoniae* and NTHi, continued monitoring of the etiology of otitis media will be critical as the relative role of *M catarrhalis* in otitis media is likely to increase.

A challenge in identifying and testing new vaccine antigens of *M catarrhalis* is the absence of a model system that simulates human infection and reliably predicts protective immune responses. Despite this limitation, the past 4 years have brought substantial progress in identifying vaccine antigens by using various complementary model systems, including in vitro cell culture models, adherence assays, immunoassays with human samples, the mouse pulmonary clearance model, and others.^{73–76}

A genome mining approach, which has been successful in several bacterial species, has been applied to *M catarrhalis* and has resulted in the identification of several new promising vaccine antigens.^{77–79} Ruckdeschel et al⁷⁷ examined genome sequences from *M catarrhalis* to identify open reading frames that encode 348 putative surface-expressed proteins. Three *Moraxella* surface proteins, *msh22*, *msh75*, and *msh78*, were shown to be conserved among a collection of clinical isolates; are transcribed during in vitro growth; and are expressed during carriage in the respiratory tract of patients with chronic obstructive pulmonary disease (COPD). These genes exhibited homology to cytochrome c, class II, succinic semialdehyde dehydrogenase, and an outer membrane nitrite reductase from *Neisseria*, respectively. A subset of patients with COPD demonstrated systemic and mucosal antibody

responses to each protein after acquisition and clearance of *M catarrhalis*. Thus, these proteins appear to be expressed in the respiratory tracts and immunogenic.⁷⁷ A follow-up study demonstrated that antisera to recombinant Msp22 and Msp75 recognizes the native protein in heterologous strains. Both subcutaneous and intranasal immunization with recombinant proteins resulted in enhanced clearance of *M catarrhalis* in the murine pulmonary clearance model.⁷⁸ Oligopeptide permease A (OppA) was also identified in the report by Ruckdeschel et al.⁷⁷ Despite the predicted periplasmic location of OppA based on homology analyses, OppA was shown to express epitopes on the surface of *M catarrhalis* by flow cytometry and whole-cell enzyme-linked immunosorbent assay (ELISA). OppA is highly conserved among *M catarrhalis* isolates from subjects with otitis media or COPD. Intranasal immunization with recombinant OppA results in enhanced clearance in the mouse pulmonary clearance model.⁷⁹

Another approach has also identified MCR 1416 (Msp22) as a promising vaccine candidate. Using an ANTIGENome technology, which expresses epitope-sized peptides and uses selected sera from children with otitis media and healthy individuals, 214 antigen candidates were identified. Twenty-three were selected by in vitro and in vivo studies for additional characterization. Eight of the 23 candidates have been tested in the *Moraxella* pulmonary clearance model, and 3 of these antigens have induced faster bacterial clearance compared with adjuvant or with the previously characterized antigen OmpCD, which served as a positive control. The most significant protection data were obtained with the antigen MCR_1416 (Msp22), which was further investigated for its biological function by in vitro studies suggesting that MCR_1416 is a heme binding protein (Sanja Selek, personal communication, 2011).

As the lipooligosaccharide (LOS) molecule of *M catarrhalis* is relatively conserved, the detoxified LOS is a potentially viable vaccine candidate.^{80,81} Table 1 briefly summarizes current potential vaccine antigens under development and includes several adhesins and integral membrane proteins that have excellent potential.

Viral Vaccines

As viral upper respiratory infection (URI) is the most common predisposing factor for the bacterial invasion of the middle ear resulting in AOM, protection against AOM through the use of vaccines against viral causes of URI is thought to be a potential strategy. However, since the last report, there has not been substantial progress in the development and testing of viral vaccines that provide protection against AOM. There is additional new information on the continued benefit of influenza vaccines in the protection against AOM. No other viral vaccines have been reported in a similar fashion.

Marchisio et al⁸² reported the use of a newer formulation of an inactivated, injectable influenza vaccine that had the neuraminidase and hemagglutinin antigens of the donor viruses integrated into the lipid membrane of the virosome (Inflexal V; Berna Biotech, Milan, Italy). The vaccine was tested in Italian children aged 1 to 5 years with a history of recurrent AOM and previously unvaccinated against influenza. Half of the children (n = 90) received the vaccine, whereas the other half (n = 90) received no vaccine. The number of children experiencing at least 1 AOM episode was significantly smaller in the vaccinated

group, as was the mean number of AOM episodes, the mean number of AOM episodes without perforation, and the mean number of antibiotic courses. The mean duration of bilateral otitis media with effusion was also significantly shorter. The vaccine had significantly greater efficacy in preventing AOM in the absence of a history of recurrent perforation. Based on this study, it is not clear if this new virosome formulation of the vaccine is better than the standard injectable vaccine for protection against AOM.

Bracco Neto et al⁸³ investigated the efficacy and safety of 1 vs 2 doses of live attenuated influenza vaccine (LAIV) in 3200 influenza vaccine-naïve Brazilian children aged 6 to <36 months over a 2-year period. The 2-dose regimen in year 1 was significantly effective against all febrile episodes of AOM (34% efficacy compared with placebo) and all episodes of influenza-associated AOM caused by strains antigenically similar to those in the vaccine (74% efficacy). The 1-dose regimen was also similarly effective. A similar trend was observed in the second year of study. However, this study does not allow us to compare the efficacy of live, intranasal vaccine with that of killed vaccine for protection against AOM. Nonetheless, this study from a country with developing economy produced results similar to the previous studies in the developed nations of North America and Europe.

Although influenza and pneumococcal vaccines independently show protection against AOM, it may be postulated that the combined effect of both vaccines may be more robust. However, this assumption was proven not to be correct. Jansen et al⁸⁴ evaluated the effects of influenza vaccination with or without heptavalent pneumococcal conjugate vaccination on respiratory tract infections (RTIs) in 579 Dutch children aged 18 to 72 with a previous history of physician-diagnosed respiratory infection. The children were assigned to 2 doses of parenteral inactivated trivalent subunit influenza plus heptavalent pneumococcal conjugate vaccination (TIV+PCV7), influenza plus placebo vaccination (TIV+plac), or control hepatitis B virus vaccination plus placebo (HBV+plac). During influenza seasons, febrile RTIs were reduced by 24% in the combined TIV+PCV7 group and by 13% in the TIV-alone group compared with the control group. However, episodes of AOM were reduced by 57% in the TIV+PCV7 group and by 71% in the TIV-alone group. The reasons for the reduced efficacy for the combined vaccines as compared with TIV alone are not clear.

Measles is well known to cause suppurative otitis media as a complication. Although measles has been well controlled through the use of a live, injectable vaccine for the past several decades, its impact on AOM and other long-term otologic complications has not been published. Recently, Arnold et al⁸⁵ studied the influence of measles vaccination on the incidence of otosclerosis in Germany. The pathologic process of otosclerosis is characterized by an inflammatory lytic phase followed by an abnormal bone remodeling at very specific sites of predilection. There is a clear genetic predisposition, with about half of all cases occurring in families with more than one affected member. N, H, and F measles proteins as well as measles virus RNA have been demonstrated in osteoblasts, chondroblasts, and macrophages of the inflammatory phase of the disease. In the absence of official data, the investigators reconstructed the rate of vaccination coverage between 1974 and 2004 using information from the Robert Koch Institute and from the literature. Between 1993 and 2004, the incidence of hospital treatments for otosclerosis decreased to a

significantly greater extent in the vaccinated patients than in the unvaccinated patients. The decline was much greater in men than in women. A comparable effect could not be demonstrated in patients with otitis media, suggesting that the pathogenesis of these 2 otologic conditions is different.

Discussion

The current report provides evidence for the effectiveness of pneumococcal conjugate vaccines in the prevention of otitis media. The data come from observational studies that compare the incidence before and after vaccine introduction and demonstrate a greater decline than reported in the clinical efficacy trials of PCV7. Investigators have suggested this enhanced effect may be the result of indirect effects that accrue with vaccine uptake in the community and the redistribution of carriage serotypes, as well as the effect of prevention of early disease on subsequent risk of developing additional episodes, secular changes in diagnostic criteria, use of influenza vaccine in the community, and disease incidence unrelated to pneumococcal conjugate vaccine. Despite the potential for confounding and the lack of certainty as to the size of the effect, the reductions in episodes of AOM confirm the effectiveness of vaccination for the prevention of AOM as well as identify unmet challenges in moving forward to extend protection to additional serotypes and additional pathogens.

Prevention of AOM would have its highest value in children at risk for recurrent otitis media; children at risk for development of CSOM, including indigenous peoples; and children at risk for comorbidities associated with recurrent AOM such as persistent otitis media with effusion, conductive hearing loss, and language delay. The challenge is that studies to date suggest that pneumococcal conjugate vaccine is not effective when initially administered to children who are already suffering from recurrent otitis media,⁸⁶ suggesting that prevention of early episodes is critical or that otitis-prone children may have suboptimal immune response to vaccines. Dagan and colleagues (personal communication, 2011) recently reported that early disease is more often due to vaccine serotypes of *S pneumoniae*. They hypothesize that nonvaccine serotypes, NTHi, and *M catarrhalis* often are “opportunistic” invaders that follow damage to the middle ear after early, recurrent otitis occurs. Clinical trials of PCV in California and Finland reported efficacy for children beginning after the primary series of immunizations at 7 months of age.^{87,88} Poehling et al⁸⁹ reported a decline in invasive pneumococcal disease in the first 90 days of life, prior to expected protection from the direct effect of immunization, presumably as a result of indirect protection. Prevention of early episodes is potentially one strategy for reducing the burden of middle ear disease in childhood. Both the induction of immunity early in life as well as the impact of early disease on subsequent risk should be a focus of research activity for both industry and government (National Institutes of Health [NIH]). Indeed, maternal immunization strategy needs to be investigated with respect to the benefit to the infant.

Despite progress in identifying and characterizing promising candidate antigens from *S pneumoniae*, NTHi, and *M catarrhalis*, moving them from animal studies to human studies is a critical step that has limited progress in the prevention of AOM for a number of reasons. Among them, feasibility and enthusiasm for a vaccine that targets primarily AOM pathogens

is a significant hurdle for the pharmaceutical industry, which may consider prevention of AOM to be of limited marketplace potential. The pneumococcal conjugate vaccines have been developed primarily to address invasive pneumococcal disease, usually defined as invasive disease and pneumonia. PHiD-CV, a pneumococcal conjugate vaccine that incorporates protein D as a carrier as well as to elicit protection against disease due to NTHi, was developed to extend prevention to include middle ear pathogens beyond the pneumococcus. Further work defining the value of AOM prevention and where such a vaccine strategy fits into the priorities for child health would provide guidance to industry as to whether such vaccines are likely to be viewed as costeffective and can be fiscally responsible investments. If the model is to build on a pneumococcal polysaccharide conjugate vaccine, then encouraging industry-academic collaborations will be a critical piece in developing a future vaccine combination that will include AOM pathogens among the targets for prevention.

The lack of correlates of protection for protein antigens has also limited the enthusiasm for moving such candidates into human trials. Progress is evident in that both PhtD (GlaxoSmithKline, Research Triangle Park, North Carolina) and IC47 (a combination of 3 pneumococcal protein antigens: StkP, PcsB, and PsaA) have completed phase I immunogenicity trials that demonstrated immune responses in adults (see clinical trials.gov). However, the lack of established correlates of protection for pneumococcal AOM and the absence of functional correlates in animal studies for protection present challenges for moving protein antigens forward into clinical efficacy trials as immunogenicity, in and of itself, is not currently predictive of success. The need for clinical efficacy trials will itself be a challenge as enrollment in tympanocentesis studies has been difficult both from a regulatory (institutional review board) and from a population perspective. Potentially, nasopharyngeal carriage may provide an end point that provides insight into both direct and indirect effects.

One strategy to enhance the potential for development of effective vaccines for the prevention of AOM would be increased collaboration among investigators as well between industry and academia. Candidate antigens should be evaluated in different models of AOM, both singly and in combination. For those where significant protection is observed in more than one animal model system, the role of the candidate antigen in disease pathogenesis should be defined in detail and the mechanism of action for the protective antibody understood with the goal of developing correlates of immunity. This would permit immunogenicity studies to evaluate both antibody quantity and function as end points and give greater security in the likelihood of success when moving from phase I studies to phase II/III studies. Of course, barriers such as intellectual property rights, NIH funding for what may seem like duplication when evaluating identical antigens in 2 or more models, and limited appreciation of the burden of middle ear disease on child health and specifically its global impact must be solved if we are to hasten the pace of progress.

Selected Future Research Objectives

1. Induction of early immune protective immune responses
2. Evaluation of maternal immunization for prevention of AOM

3. Development of correlates of protection for acute bacterial otitis media
4. Definition of the role of potential vaccine antigens in the pathogenesis of AOM
5. Evaluation of the candidate antigen in human trials

Implications for Practice

The recurrent nature of acute otitis media continues to be burdensome to children and families, especially those who suffer from frequent recurrences and in disadvantaged populations where disease progresses to chronic suppurative otitis media with associated impacts on hearing loss and educational potential. PCV7 has reduced the burden of vaccine-serotype disease as well as shifted the pneumococcal serotypes carried in the nasopharynx toward those with lower disease-causing potential. Antibiotic resistance remains a challenge to successful therapy with ceftriaxone-resistant pneumococci present in the community and increasing emergence of β -lactamase-negative, amoxicillin-resistant NTHi identified globally. The next-generation PCV13 has been introduced, and early data suggest efficacy against invasive pneumococcal disease and carriage of SP19A, the multidrug resistance isolate that has been associated with both treatment failure in AOM⁹⁰ and the increasing number of cases of pneumococcal mastoiditis.⁹¹ Promising data on an 11-valent pneumococcal polysaccharide conjugate vaccine with protein D as a carrier was published in 2006,⁶⁵ but additional confirmation of efficacy against NTHi otitis media with the licensed formulation, PHiD-CV (a 10-valent conjugate), is pending data from the COMPAS trial in South America.

A number of candidate protein antigens have had progress to human trials since 2007, including PhtD and IC47 (StkP, PcsB, and PsaA), with demonstration of immunogenicity in adults to date, but their protective efficacy in phase II and III trials remains to be demonstrated. Multiple candidates have demonstrated the necessary requirements for candidate vaccine antigens: conservation among isolates, surface exposure, immunogenicity in animals, and protection in animal models of disease or specifically experimental otitis media. Further research of the role of each antigen in the pathogenesis of disease, in the development of correlates of protection in animal models, and in new adjuvants developed to elicit response in the youngest infants is likely to be productive and permit more antigens to move into clinical trials in humans. Systematic cross-comparisons of methods and conditions would also be valuable to resolve some of the inconsistency in the protection data obtained by different research teams.

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References

1. Jensen RG, Homoe P, Andersson M, Koch A. Long-term follow-up of chronic suppurative otitis media in a high-risk children cohort. *Int J Pediatr Otorhinolaryngol*. 2011; 75:948–954. [PubMed: 21592588]
2. Koch A, Homoe P, Pipper C, Hjuler T, Melbye M. Chronic suppurative otitis media in a birth cohort of children in Greenland: population-based study of incidence and risk factors. *Pediatr Infect Dis J*. 2011; 30:25–29. [PubMed: 20700079]
3. Leach AJ, Morris PS. The burden and outcome of respiratory tract infection in Australian and Aboriginal children. *Pediatr Infect Dis J*. 2007; 26:S4–S7. [PubMed: 18049380]
4. Menon S, Bharadwaj R, Chowdhary A, Kaundinya DV, Palande DA. Current epidemiology of intracranial abscesses: a prospective-5 year study. *J Med Microbiol*. 2008; 57:1259–1268. [PubMed: 18809555]
5. Morris PS, Leach AJ. Acute and chronic otitis media. *Pediatr Clin North Am*. 2009; 56:1383–1399. [PubMed: 19962027]
6. de Wals P, Carbon M, Sevin E, Deceuninck G, Ouakki M. Reduced physician claims for otitis media after implementation of pneumococcal conjugate vaccine program in the province of Quebec, Canada. *Pediatr Infect Dis J*. 2009; 28:e271–e275. [PubMed: 19710582]
7. Poehling KA, Szilagyi PG, Grijalva CG, et al. Reduction of frequent otitis media and pressure-equalizing tube insertions in children after introduction of pneumococcal conjugate vaccine. *Pediatrics*. 2007; 119:707–715. [PubMed: 17403841]
8. Zhou F, Shefer A, Kong Y, Nuorti JP. Trends in acute otitis media–related health care utilization by privately insured young children in the United States, 1997–2004. *Pediatrics*. 2008; 121:253–260. [PubMed: 18245415]
9. O'Brien, KL.; Dagan, R.; Makela, PH. Nasopharyngeal carriage. In: Siber, GR.; Klugman, KP.; Makela, PH., editors. *Pneumococcal Vaccines*. Washington, DC: ASM Press; 2008. p. 279–300.
10. Pelton SI, Leibovitz E. Recent advances in otitis media. *Pediatr Infect Dis J*. 2009; 28:S133–S137. [PubMed: 19918136]
11. Huang SS, Hinrichsen VL, Stevenson AE, et al. Continued impact of pneumococcal conjugate vaccine on carriage in young children. *Pediatrics*. 2009; 124:e1–e11. [PubMed: 19564254]
12. Nahm MH, Lin J, Finkelstein JA, Pelton SI. Increase in the prevalence of the newly discovered pneumococcal serotype 6C in the nasopharynx after introduction of pneumococcal conjugate vaccine. *J Infect Dis*. 2009; 199:320–325. [PubMed: 19099489]
13. Levine OS, Cherian T, Hajjeh R, Knoll MD. Progress and future challenges in coordinated surveillance and detection of pneumococcal and Hib disease in developing countries. *Clin Infect Dis*. 2009; 48:S33–S36. [PubMed: 19191617]
14. Darrieux M, Miyaji EN, Ferreira DM, et al. Fusion proteins containing family 1 and family 2 PspA fragments elicit protection against *Streptococcus pneumoniae* that correlates with antibody-mediated enhancement of complement deposition. *Infect Immun*. 2007; 75:5930–5938. [PubMed: 17923518]
15. Darrieux M, Moreno AT, Ferreira DM, et al. Recognition of pneumococcal isolates by antisera raised against PspA fragments from different clades. *J Med Microbiol*. 2008; 57:273–278. [PubMed: 18287288]
16. Goulart C, Darrieux M, Rodriguez D, et al. Selection of family-1 PspA molecules capable of inducing broad-ranging cross-reactivity by complement deposition and opsonophagocytosis by murine peritoneal cells. *Vaccine*. 2011; 29:1634–1642. [PubMed: 21211592]
17. Daniels CC, Coan P, King J, et al. The proline-rich region of pneumococcal surface proteins A and C contains surface-accessible epitopes common to all pneumococci and elicits antibody-mediated protection against sepsis. *Infect Immun*. 2010; 78:2163–2172. [PubMed: 20194601]

18. Oliveira ML, Miyaji EN, Ferreira DM, et al. Combination of pneumococcal surface protein A (PspA) with whole cell pertussis vaccine increases protection against pneumococcal challenge in mice. *PLoS ONE*. 2010; 5:e10863. [PubMed: 20523738]
19. Muralinath M, Kuehn MJ, Roland KL, Curtiss R III. Immunization with *Salmonella enterica* serovar Typhimurium-derived outer membrane vesicles delivering the pneumococcal protein PspA confers protection against challenge with *Streptococcus pneumoniae*. *Infect Immun*. 2011; 79:887–894. [PubMed: 21115718]
20. Xin W, Li Y, Mo H, Roland KL, Curtiss R III. PspA family fusion proteins delivered by attenuated *Salmonella enterica* serovar Typhimurium extend and enhance protection against *Streptococcus pneumoniae*. *Infect Immun*. 2009; 77:4518–4528. [PubMed: 19687204]
21. Ferreira DM, Oliveira ML, Moreno AT, Ho PL, Briles DE, Miyaji EN. Protection against nasal colonization with *Streptococcus pneumoniae* by parenteral immunization with a DNA vaccine encoding PspA (Pneumococcal surface protein A). *Microb Pathog*. 2010; 48:205–213. [PubMed: 20206678]
22. Ferreira DM, Darrieux M, Silva DA, et al. Characterization of protective mucosal and systemic immune responses elicited by pneumococcal surface protein PspA and PspC nasal vaccines against a respiratory pneumococcal challenge in mice. *Clin Vaccine Immunol*. 2009; 16:636–645. [PubMed: 19279169]
23. Campos IB, Darrieux M, Ferreira DM, et al. Nasal immunization of mice with *Lactobacillus casei* expressing the Pneumococcal Surface Protein A: induction of antibodies, complement deposition and partial protection against *Streptococcus pneumoniae* challenge. *Microbes Infect*. 2008; 10:481–488. [PubMed: 18403234]
24. Hanniffy SB, Carter AT, Hitchin E, Wells JM. Mucosal delivery of a pneumococcal vaccine using *Lactococcus lactis* affords protection against respiratory infection. *J Infect Dis*. 2007; 195:185–193. [PubMed: 17191163]
25. Csordas FC, Perciani CT, Darrieux M, et al. Protection induced by pneumococcal surface protein A (PspA) is enhanced by conjugation to a *Streptococcus pneumoniae* capsular polysaccharide. *Vaccine*. 2008; 26:2925–2929. [PubMed: 18440673]
26. Rioux S, Neyt C, Di Paolo E, et al. Transcriptional regulation, occurrence and putative role of the Pht family of *Streptococcus pneumoniae*. *Microbiology*. 2011; 157:336–348. [PubMed: 20966093]
27. Godfroid F, Hermand P, Verlant V, Denoel P, Poolman JT. Preclinical evaluation of the Pht proteins as potential cross-protective pneumococcal vaccine antigens. *Infect Immun*. 2011; 79:238–245. [PubMed: 20956575]
28. Denoel P, Godfroid F, Hermand P, Verlant V, Poolman J. Combined protective effects of anti-PhtD and anti-pneumococcal polysaccharides. *Vaccine*. 2011; 29:6451–6453. [PubMed: 21315695]
29. Wang S, Li Y, Shi H, et al. Immune responses to recombinant pneumococcal PsaA antigen delivered by a live attenuated *Salmonella* vaccine. *Infect Immun*. 2010; 78:3258–3271. [PubMed: 20479086]
30. Regev-Yochay G, Hanage WP, Trzcinski K, et al. Re-emergence of the type 1 pilus among *Streptococcus pneumoniae* isolates in Massachusetts, USA. *Vaccine*. 2010; 28:4842–4846. [PubMed: 20434550]
31. Zahner D, Gudlavalleti A, Stephens DS. Increase in pilus islet 2-encoded pili among *Streptococcus pneumoniae* isolates, Atlanta, Georgia, USA. *Emerg Infect Dis*. 2010; 16:955–962. [PubMed: 20507746]
32. Moschioni M, Donati C, Muzzi A, et al. *Streptococcus pneumoniae* contains 3 rlrA pilus variants that are clonally related. *J Infect Dis*. 2008; 197:888–896. [PubMed: 18269316]
33. Moschioni M, Emolo C, Biagini M, et al. The two variants of the *Streptococcus pneumoniae* pilus 1 RrgA adhesin retain the same function and elicit cross-protection in vivo. *Infect Immun*. 2010; 78:5033–5042. [PubMed: 20823200]
34. Basset A, Turner KH, Boush E, Sayeed S, Dove SL, Malley R. Expression of the type 1 pneumococcal pilus is bistable and negatively regulated by the structural component RrgA. *Infect Immun*. 2011; 79:2974–2983. [PubMed: 21576325]

35. Donati C, Hiller NL, Tettelin H, et al. Structure and dynamics of the pangenome of *Streptococcus pneumoniae* and closely related species. *Genome Biol.* 2010; 11:R107. [PubMed: 21034474]
36. Oloo EO, Yethon JA, Ochs MM, Carpick B, Oomen R. Structure-guided antigen engineering yields pneumolysin mutants suitable for vaccination against pneumococcal disease. *J Biol Chem.* 2011; 286:12133–12140. [PubMed: 21296887]
37. Rose L, Shivshankar P, Hinojosa E, Rodriguez A, Sanchez CJ, Orihuela CJ. Antibodies against PsrP, a novel *Streptococcus pneumoniae* adhesin, block adhesion and protect mice against pneumococcal challenge. *J Infect Dis.* 2008; 198:375–383. [PubMed: 18507531]
38. Sanchez CJ, Shivshankar P, Stol K, et al. The pneumococcal serine-rich repeat protein is an intra-species bacterial adhesin that promotes bacterial aggregation in vivo and in biofilms. *PLoS Pathog.* 2010; 6:e1001044. [PubMed: 20714350]
39. Cao J, Chen T, Li D, et al. Mucosal immunization with purified ClpP could elicit protective efficacy against pneumococcal pneumonia and sepsis in mice. *Microbes Infect.* 2008; 10:1536–1542. [PubMed: 18930162]
40. Glover DT, Hollingshead SK, Briles DE. *Streptococcus pneumoniae* surface protein PcpA elicits protection against lung infection and fatal sepsis. *Infect Immun.* 2008; 76:2767–2776. [PubMed: 18391008]
41. Gianfaldoni C, Maccari S, Pancotto L, et al. Sortase A confers protection against *Streptococcus pneumoniae* in mice. *Infect Immun.* 2009; 77:2957–2961. [PubMed: 19433540]
42. Shah P, Briles DE, King J, Hale Y, Swiatlo E. Mucosal immunization with polyamine transport protein D (PotD) protects mice against nasopharyngeal colonization with *Streptococcus pneumoniae*. *Exp Biol Med.* 2009; 234:403–409.
43. Medina M, Villena J, Vintini E, Hebert EM, Raya R, Alvarez S. Nasal immunization with *Lactococcus lactis* expressing the pneumococcal protective protein A induces protective immunity in mice. *Infect Immun.* 2008; 76:2696–2705. [PubMed: 18390997]
44. Gieffing C, Meinke AL, Hanner M, et al. Discovery of a novel class of highly conserved vaccine antigens using genomic scale antigenic fingerprinting of pneumococcus with human antibodies. *J Exp Med.* 2008; 205:117–131. [PubMed: 18166586]
45. Smith CM, Lo Passo C, Scuderi A, et al. Peptide mimics of two pneumococcal capsular polysaccharide serotypes (6B and 9V) protect mice from a lethal challenge with *Streptococcus pneumoniae*. *Eur J Immunol.* 2009; 39:1527–1535. [PubMed: 19499518]
46. Park S, Parameswar AR, Demchenko AV, Nahm MH. Identification of a simple chemical structure associated with protective human antibodies against multiple pneumococcal serogroups. *Infect Immun.* 2009; 77:3374–3379. [PubMed: 19451241]
47. Mills MF, Marquart ME, McDaniel LS. Localization of PcsB of *Streptococcus pneumoniae* and its differential expression in response to stress. *J Bacteriol.* 2007; 189:4544–4546. [PubMed: 17449619]
48. Douce G, Ross K, Cowan G, Ma J, Mitchell TJ. Novel mucosal vaccines generated by genetic conjugation of heterologous proteins to pneumolysin (PLY) from *Streptococcus pneumoniae*. *Vaccine.* 2010; 28:3231–3237. [PubMed: 20188176]
49. Lu YJ, Forte S, Thompson CM, Anderson PW, Malley R. Protection against pneumococcal colonization and fatal pneumonia by a trivalent conjugate of a fusion protein with the cell wall polysaccharide. *Infect Immun.* 2009; 77:2076–2083. [PubMed: 19255193]
50. Wu K, Zhang X, Shi J, et al. Immunization with a combination of three pneumococcal proteins confers additive and broad protection against *Streptococcus pneumoniae* infections in mice. *Infect Immun.* 2010; 78:1276–1283. [PubMed: 20038538]
51. Gong Y, Xu W, Cui Y, et al. Immunization with a ZmpB-based protein vaccine could protect against pneumococcal diseases in mice. *Infect Immun.* 2011; 79:867–878. [PubMed: 21098102]
52. Lu YJ, Yadav P, Clements JD, et al. Options for inactivation, adjuvant, and route of topical administration of a killed, unencapsulated pneumococcal whole-cell vaccine. *Clin Vaccine Immunol.* 2010; 17:1005–1012. [PubMed: 20427625]
53. Syrjanen RK, Auranen KJ, Leino TM, Kilpi TM, Makela PH. Pneumococcal acute otitis media in relation to pneumococcal nasopharyngeal carriage. *Pediatr Infect Dis J.* 2005; 24:801–806. [PubMed: 16148847]

54. Chang A, Kaur R, Michel LV, Casey JR, Pichichero M. *Haemophilus influenzae* vaccine candidate outer membrane protein P6 is not conserved in all strains. *Hum Vaccines*. 2011; 7:102–105.
55. Michel LV, Kalmeta B, McCreary M, Snyder J, Craig P, Pichichero ME. Vaccine candidate P6 of nontypable *Haemophilus influenzae* is not a transmembrane protein based on protein structural analysis. *Vaccine*. 2011; 29:1624–1627. [PubMed: 21215345]
56. Noda K, Kodama S, Umemoto S, Abe N, Hirano T, Suzuki M. Nasal vaccination with P6 outer membrane protein and alphagalactosylceramide induces nontypeable *Haemophilus influenzae* – specific protective immunity associated with NKT cell activation and dendritic cell expansion in nasopharynx. *Vaccine*. 2010; 28:5068–5074. [PubMed: 20478344]
57. Kodama S, Hirano T, Abe N, Suzuki M. Local application of CpG oligodeoxynucleotide enhances nontypeable *Haemophilus influenzae* specific mucosal IgA responses in the middle ear. *Acta Otolaryngol*. 2007; 127:809–815. [PubMed: 17762989]
58. Kodama S, Hirano T, Noda K, Abe N, Suzuki M. A single nasal dose of fms-like tyrosine kinase receptor-3 ligand, but not peritoneal application, enhances nontypeable *Haemophilus influenzae* specific long-term mucosal immune responses in the nasopharynx. *Vaccine*. 2010; 28:2510–2516. [PubMed: 20117272]
59. Kodama S, Hirano T, Noda K, Umemoto S, Suzuki M. Nasal immunization with plasmid DNA encoding P6 protein and immunostimulatory complexes elicits nontypeable *Haemophilus influenzae* specific long-term mucosal immune responses in the nasopharynx. *Vaccine*. 2011; 29:1881–1890. [PubMed: 21237276]
60. Sabirov A, Casey JR, Murphy TF, Pichichero ME. Breast-feeding is associated with a reduced frequency of acute otitis media and high serum antibody levels against NTHi and outer membrane protein vaccine antigen candidate P6. *Pediatr Res*. 2009; 66:565–570. [PubMed: 19581824]
61. Kaur R, Casey JR, Pichichero ME. Serum antibody response to three nontypeable *Haemophilus influenzae* outer membrane proteins during acute otitis media and nasopharyngeal colonization in otitis prone and non-otitis prone children. *Vaccine*. 2011; 29:1023–1028. [PubMed: 21129398]
62. Novotny LA, Adams LD, Kang DR, et al. Epitope mapping immunodominant regions of the Pila protein of nontypeable *Haemophilus influenzae* (NTHi) to facilitate the design of two novel chimeric vaccine candidates. *Vaccine*. 2009; 28:279–289. [PubMed: 19699813]
63. Novotny LA, Clements JD, Bakaletz LO. Transcutaneous immunization as preventative and therapeutic regimens to protect against experimental otitis media due to nontypeable *Haemophilus influenzae*. *Mucosal Immunol*. 2011; 4:456–467. [PubMed: 21326197]
64. Lim, DJ.; Bluestone, CD.; Casselbrant, ML., editors. Recent advances in otitis media: report of the ninth research conference. http://www.hei.org/otitis2007/9th_IOMS_FINAL_Res_Conf_Report.pdf. [Accessed August 14, 2012]
65. Prymula R, Kriz P, Kaliskova E, Pascal T, Poolman J, Schuerman L. Effect of vaccination with pneumococcal capsular polysaccharides conjugated to *Haemophilus influenzae* derived protein D on nasopharyngeal carriage of *Streptococcus pneumoniae* and *H. influenzae* in children under 2 years of age. *Vaccine*. 2009; 28:71–78. [PubMed: 19818722]
66. Johnson RW, McGillivray G, Denoel P, Poolman J, Bakaletz LO. Abrogation of nontypeable *Haemophilus influenzae* protein D function reduces phosphorylcholine decoration, adherence to airway epithelial cells, and fitness in a chinchilla model of otitis media. *Vaccine*. 2011; 29:1211–1221. [PubMed: 21167861]
67. Toropainen M, Raitolehto A, Henckaerts I, et al. Pneumococcal *Haemophilus influenzae* protein D conjugate vaccine induces antibodies that inhibit glycerophosphodiester phosphodiesterase activity of protein D. *Infect Immun*. 2008; 76:4546–4553. [PubMed: 18644877]
68. Winter LE, Barenkamp SJ. Antibodies specific for the Hia adhesion proteins of nontypeable *Haemophilus influenzae* mediate opsonophagocytic activity. *Clin Vaccine Immunol*. 2009; 16:1040–1046. [PubMed: 19474261]
69. Winter LE, Barenkamp SJ. Construction and immunogenicity of recombinant adenovirus vaccines expressing the HMW1, HMW2, or Hia adhesion protein of nontypeable *Haemophilus influenzae*. *Clin Vaccine Immunol*. 2010; 17:1567–1575. [PubMed: 20685934]

70. Hong W, Peng D, Rivera M, Gu XX. Protection against nontypeable *Haemophilus influenzae* challenges by mucosal vaccination with a detoxified lipooligosaccharide conjugate in two chinchilla models. *Microbes Infect.* 2010; 12:11–18. [PubMed: 19782149]
71. Broides A, Dagan R, Greenberg D, Givon-Lavi N, Leibovitz E. Acute otitis media caused by *Moraxella catarrhalis*: epidemiologic and clinical characteristics. *Clin Infect Dis.* 2009; 49:1641–1647. [PubMed: 19886799]
72. Casey JR, Adlowitz DG, Pichichero ME. New patterns in the otopathogens causing acute otitis media six to eight years after introduction of pneumococcal conjugate vaccine. *Pediatr Infect Dis J.* 2010; 29:304. [PubMed: 19935445]
73. Murphy TF. Vaccine development for *Moraxella catarrhalis*: rationale, approaches and challenges. *Expert Rev Vaccines.* 2009; 8:655–658. [PubMed: 19485745]
74. Murphy TF, Parameswaran GI. *Moraxella catarrhalis*, a human respiratory tract pathogen. *Clin Infect Dis.* 2009; 49:124–131. [PubMed: 19480579]
75. Tan TT, Riesbeck K. Current progress of adhesins as vaccine candidates for *Moraxella catarrhalis*. *Exp Rev Vaccines.* 2007; 6:949–956.
76. Mawas F, Ho MM, Corbel MJ. Current progress with *Moraxella catarrhalis* antigens as vaccine candidates. *Expert Rev Vaccines.* 2009; 8:77–90. [PubMed: 19093775]
77. Ruckdeschel EA, Kirkham C, Lesse AJ, Hu Z, Murphy TF. Mining the *Moraxella catarrhalis* genome: identification of potential vaccine antigens expressed during human infection. *Infect Immun.* 2008; 76:1599–1607. [PubMed: 18227159]
78. Ruckdeschel EA, Brauer AL, Johnson A, Murphy TF. Characterization of proteins Msp22 and Msp75 as vaccine antigens of *Moraxella catarrhalis*. *Vaccine.* 2009; 27:7065–7072. [PubMed: 19786139]
79. Yang M, Johnson A, Murphy TF. Characterization and evaluation of the *Moraxella catarrhalis* oligopeptide permease A as a mucosal vaccine antigen. *Infect Immun.* 2011; 79:846–857. [PubMed: 21134967]
80. Yu S, Gu XX. Biological and immunological characteristics of lipooligosaccharide-based conjugate vaccines for serotype C *Moraxella catarrhalis*. *Infect Immun.* 2007; 75:2974–2980. [PubMed: 17371852]
81. Yu S, Xie H, Datta A, Naidu N, Gu XX. Galactose residues on the lipooligosaccharide of *Moraxella catarrhalis* 26404 form the epitope recognized by the bactericidal antiserum from conjugate vaccination. *Infect Immun.* 2008; 76:4251–4258. [PubMed: 18559429]
82. Marchisio P, Esposito S, Bianchini S, et al. Efficacy of injectable trivalent virosomal-adjuvanted inactivated influenza vaccine in preventing acute otitis media in children with recurrent complicated or noncomplicated acute otitis media. *Pediatr Infect Dis J.* 2009; 28:855–859. [PubMed: 19564812]
83. Bracco Neto H, Farhat CK, Tregnaghi MW, et al. Efficacy and safety of 1 and 2 doses of live attenuated influenza vaccine in vaccine-naïve children. *Pediatr Infect Dis J.* 2009; 28:365–371. [PubMed: 19395948]
84. Jansen AG, Sanders EA, Hoes AW, van Loon AM, Hak E. Effects of influenza plus pneumococcal conjugate vaccination versus influenza vaccination alone in preventing respiratory tract infections in children: a randomized, double-blind, placebo-controlled trial. *J Pediatrics.* 2008; 153:764–770.
85. Arnold W, Busch R, Arnold A, Ritscher B, Neiss A, Niedermeyer HP. The influence of measles vaccination on the incidence of otosclerosis in Germany. *Eur Arch Otorhinolaryngol.* 2007; 264:741–748. [PubMed: 17297608]
86. Veenhoven R, Bogaert D, Uiterwaal C, et al. Effect of conjugate pneumococcal vaccine followed by polysaccharide pneumococcal vaccine on recurrent acute otitis media: a randomised study. *Lancet.* 2003; 361:2189–2195. [PubMed: 12842372]
87. Black S, Shinefield H, Fireman B, et al. Efficacy, safety and immunogenicity of heptavalent pneumococcal conjugate vaccine in children. *Pediatr Infect Dis J.* 2000; 19:187–195. [PubMed: 10749457]
88. Eskola J, Kilpi T, Palmu A, et al. Efficacy of a pneumococcal conjugate vaccine against acute otitis media. *N Engl J Med.* 2001; 344:403–409. [PubMed: 11172176]

89. Poehling KA, Talbot TR, Griffin MR, et al. Invasive pneumococcal disease among infants before and after introduction of pneumococcal conjugate vaccine. *JAMA*. 2006; 295:1668–1674. [PubMed: 16609088]
90. Pichichero ME, Casey JR. Emergence of a multiresistant serotype 19A pneumococcal strain not included in the 7-valent conjugate vaccine as an otopathogen in children. *JAMA*. 2007; 298:1772–1778. [PubMed: 17940232]
91. Ongkasuwan J, Valdez TA, Hulten KG, Mason EO Jr, Kaplan SL. Pneumococcal mastoiditis in children and the emergence of multidrug-resistant serotype 19A isolates. *Pediatrics*. 2008; 122:34–39. [PubMed: 18595984]
92. Balder R, Krunkosky TM, Nguyen CQ, Feezel L, Lafontaine ER. Hag mediates adherence of *Moraxella catarrhalis* to ciliated human airway cells. *Infect Immun*. 2009; 77:4597–4608. [PubMed: 19667048]
93. LaFontaine ER, Snipes LE, Bullard B, Brauer AL, Sethi S, Murphy TF. Identification of domains of the Hag/MID surface protein recognized by systemic and mucosal antibodies in adults with chronic obstructive pulmonary disease following clearance of *Moraxella catarrhalis*. *Clin Vaccine Immunol*. 2009; 16:653–659. [PubMed: 19321697]
94. Hallstrom T, Nordstrom T, Tan TT, et al. Immune evasion of *Moraxella catarrhalis* involves ubiquitous surface protein A-dependent C3d binding. *J Immunol*. 2011; 186:3120–3129. [PubMed: 21270401]
95. Bullard B, Lipski S, Lafontaine ER. Regions important for the adhesin activity of *Moraxella catarrhalis* Hag. *BMC Microbiol*. 2007; 7:65. [PubMed: 17608944]
96. Balder R, Hassel J, Lipski S, Lafontaine ER. *Moraxella catarrhalis* strain O35E expresses two filamentous hemagglutinin-like proteins that mediate adherence to human epithelial cells. *Infect Immun*. 2007; 75:2765–2775. [PubMed: 17371858]
97. Plamondon P, Luke NR, Campagnari AA. Identification of a novel two partner secretion locus in *Moraxella catarrhalis*. *Infect Immun*. 2007; 75:2929–2936. [PubMed: 17420235]
98. Lipski SL, Holm MM, Lafontaine ER. Identification of a *Moraxella catarrhalis* gene that confers adherence to various human epithelial cell lines in vitro. *FEMS Microbiol Lett*. 2007; 267:207–213. [PubMed: 17166229]
99. Mawas F, Ho MM, Huskisson R, Saydam M, Corbel MJ. Physico-chemical characterisation and immunogenicity of a multi-valent candidate vaccine against non-typeable *Haemophilus influenzae* and *Moraxella catarrhalis*. *Vaccine*. 2007; 25:4801–4808. [PubMed: 17507119]
100. Singh B, Blom AM, Unal C, Nilson B, Morgelin M, Riesbeck K. Vitronectin binds to the head region of *Moraxella catarrhalis* ubiquitous surface protein A2 and confers complement-inhibitory activity. *Mol Microbiol*. 2010; 75:1426–1444. [PubMed: 20199596]
101. Lipski SL, Akimana C, Timpe JM, Wooten RM, Lafontaine ER. The *Moraxella catarrhalis* autotransporter McaP is a conserved surface protein that mediates adherence to human epithelial cells through its N-terminal passenger domain. *Infect Immun*. 2007; 75:314–324. [PubMed: 17088358]
102. Becker PD, Bertot GM, Souss D, Ebsen T, Guzman CA, Grinstein S. Intranasal vaccination with recombinant outer membrane protein CD and adamantylamide dipeptide as the mucosal adjuvant enhances pulmonary clearance of *Moraxella catarrhalis* in an experimental murine model. *Infect Immun*. 2007; 75:1778–1784. [PubMed: 17101651]
103. Liu DF, McMichael JC, Baker SM. *Moraxella catarrhalis* outer membrane protein CD elicits antibodies that inhibit CD binding to human mucin and enhance pulmonary clearance of *M. catarrhalis* in a mouse model. *Infect Immun*. 2007; 75:2818–2825. [PubMed: 17403868]
104. Augustyniak D, Mleczko J, Gutowicz J. The immunogenicity of the liposome-associated outer membrane proteins (OMPs) of *Moraxella catarrhalis*. *Cell Mol Biol Lett*. 2010; 15:70–89. [PubMed: 19851720]
105. Brooks MJ, Laurence CA, Hansen EJ, Gray-Owen SD. Characterization of the *Moraxella catarrhalis* opa-like protein, OlpA, reveals a phylogenetically conserved family of outer membrane proteins. *J Bacteriol*. 2007; 189:76–82. [PubMed: 17041038]
106. Luke-Marshall NR, Sauberan SL, Campagnari AA. Comparative analyses of the *Moraxella catarrhalis* type-IV pilus structural subunit PilA. *Gene*. 2011; 477:19–23. [PubMed: 21256201]

107. Luke NR, Jurgisek JA, Bakaletz LO, Campagnari AA. Contribution of *Moraxella catarrhalis* type IV pili to nasopharyngeal colonization and biofilm formation. *Infect Immun*. 2007; 75:5559–5564. [PubMed: 17908808]
108. Ren D, Yu S, Gao S, et al. Mutant lipooligosaccharide-based conjugate vaccine demonstrates a broad-spectrum effectiveness against *Moraxella catarrhalis*. *Vaccine*. 2011; 29:4210–4217. [PubMed: 21501641]
109. Cox AD, St Michael F, Cairns CM, et al. Investigating the potential of conserved inner core oligosaccharide regions of *Moraxella catarrhalis* lipopolysaccharide as vaccine antigens: accessibility and functional activity of monoclonal antibodies and glycoconjugate derived sera. *Glycoconjugate J*. 2011; 28:165–182.

Table 1Potential Vaccine Antigens of *Moraxella catarrhalis*

Antigen	Molecular Mass, kDa	Putative Function and Other Features	Mouse Pulmonary Clearance ^a	Reference ^b
MID/Hag	200	Adhesin, binds IgD, hemagglutinin	Yes	82–95
MchA1, MchA2	184, 201	Filamentous hemagglutinin-like adhesin		96, 97
MhaB1, MhaB2 MchA	110	Metallopeptidase-like adhesin		98
OppA	~80	Oligopeptide permease	Yes	79
UspA2	62 (oligomer)	Binds complement, vitronectin, and laminin	Yes	94, 99, 100
Msp 75	~75	Homology to succinic dehydrogenase	Yes	77, 78
McaP	66	Adhesin and phospholipase B		101
OMP E	50	Possible fatty acid transport		
OMP CD	45	OMP A-like protein, binds mucin, adhesin	Yes	102–104
M35	36.1	Porin, conserved with one variable loop		
OMP G1a	~29	Lipoprotein putative copper transport protein		
OMP G1b	~29	Surface molecule		
OlpA	24	Homologous with <i>Neisseria</i> Opa adhesins		105
Msp 22	~22	Surface lipoprotein	Yes	77, 78
Type IV pili	16	Adhesin, transformation, biofilm formation		106, 107
Lipooligosaccharide	2.5–4	Detoxified form is potential vaccine antigen	Yes	81, 108, 109

^aIndicates that immunization with antigen induces enhance clearance in the mouse pulmonary clearance model.

^bReferences from 2007 to present.