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Panel 6: Vaccines

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Abstract

Objective. To review the literature on progress regarding (1) effectiveness of vaccines for prevention of otitis media (OM) and (2) development of vaccine antigens for OM bacterial and viral pathogens.

Data Sources. PubMed database of the National Library of Science.

Review Methods. We performed literature searches in PubMed for OM pathogens and candidate vaccine antigens, and we restricted the searches to articles in English that were published between July 2011 and June 2015. Panel members reviewed literature in their area of expertise.

Conclusions. Pneumococcal conjugate vaccines (PCVs) are somewhat effective for the prevention of pneumococcal OM, recurrent OM, OM visits, and tympanostomy tube insertions. Widespread use of PCVs has been associated with shifts in pneumococcal serotypes and bacterial pathogens associated with OM, diminishing PCV effectiveness against AOM. The 10-valent pneumococcal vaccine containing *Haemophilus influenzae* protein D (PHiD-CV) is effective for pneumococcal OM, but results from studies describing the potential impact on OM due to *H influenzae* have been inconsistent. Progress in vaccine development for *H influenzae, Moraxella catarrhalis*, and OM-associated respiratory viruses has been limited. Additional research is needed to extend vaccine protection to additional pneumococcal serotypes and other otopathogens. There are likely to be licensure challenges for protein-based vaccines, and data on correlates of protection for OM vaccine antigens are urgently needed.

Implications for Practice. OM continues to be a significant health care burden globally. Prevention is preferable to treatment, and vaccine development remains an important goal. As a polymicrobial disease, OM poses significant but not insurmountable challenges for vaccine development.

Keywords

otitis media, vaccines, children, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*

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Acute otitis media (AOM) is one of the most commonly diagnosed childhood infections and a leading diagnosis for the prescription of antibiotics.[1] The 3 primary bacterial pathogens that cause AOM are *Streptococcus pneumoniae*, nontypeable *Haemophilus influenzae* (NTHi), and *Moraxella catarrhalis*. Virtually all cases of AOM occur following or concurrent with a symptomatic viral respiratory tract infection.[2] The etiology of otitis media (OM) is continually undergoing changes associated with widespread use of pneumococcal conjugate vaccines (PCVs), which alter nasopharyngeal colonization patterns, particularly the distribution of pneumococcal serotypes. In clinical practice, AOM is managed empirically. Controversies exist regarding the use of antibiotics for treatment of OM, and antibiotic resistance is a major public health concern. Thus, new methods to prevent OM and

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Table 1. Etiology of Otitis Media Based on Culture and PCR of Middle Ear Fluids from Studies Published from July 2011 to June 2015.

			Middle Ear Fluid Culture (MEF Positive, %)			Middle Ear Fluid PCR (MEF Positive, %)		
Study Site	Diagnosis[a]	MEF, n	S pneumo	NTHi	Mcat	S pneumo	NTHi	Mcat
US[104]	OME	169	ND	ND	ND	12	47	34
US[104]	AOM	38	ND	ND	ND	6	76	30
Spain[12]	Complicated OM	105	20	42	2	39	61	ND
Russia[105]	AOM	541	20	3	2	ND	ND	ND
US[106]	AOM	530	29	37	20	ND	ND	ND
New Zealand[7]	Complicated OM	325	8	19	8	23	43	56
Israel[16]	Complicated OM	295	20	12	1	ND	ND	ND
Costa Rica[17]	AOM and recurrent AOM	456	19	25	5	ND	ND	ND

Abbreviations: AOM, acute otitis media; Mcat, *Moraxella catarrhalis*; MEF, middle ear fluid; ND, no data; NTHi, nontypeable *Haemophilus influenzae*; OM, otitis media; OME, otitis media with effusion; PCR, polymerase chain reaction; S pneumo, *Streptococcus pneumoniae*.
[a]Complicated OM includes recurrent OM, treatment failure, chronic OM, and OME.

its associated sequelae are urgently needed. Vaccines represent a promising approach to reduce the burden of OM globally. However, OM is a polymicrobial disease and poses significant challenges for vaccine development. The goal of this report is to provide an update on the role of conjugate and influenza virus vaccines in preventing OM and progress toward identification of new vaccine targets over the past 4 years.

Methods

The PubMed database of the National Library of Medicine was used to search for articles related to vaccines and vaccine antigens for OM. The PubMed literature search was restricted to articles published between July 2011 and June 2015. Keywords included *Streptococcus pneumoniae, Haemophilus influenzae*, or *Moraxella catarrhalis* and vaccine, vaccine antigens, and OM. Searches were also conducted on each otopathogen and vaccine antigen discussed in the 2011 report of the 10th Research Conference on Recent Advances in Otitis Media.[3] Original research, reviews, and editorials were included, and searches were limited to articles published in English. Panel members reviewed literature in their area of expertise, and articles were included if they were judged to be scientifically sound; explicit inclusion, exclusion, and quality criteria were not used to select articles. Panel members met and discussed recent advances in the field and an early draft of the report at the Postsymposium Research Conference to the 18th International Symposium on Recent Advances in Otitis Media. The final draft was reviewed and approved by all panel members.

Discussion

Etiology of OM

Culture of middle ear fluid has been the gold standard for determining AOM etiology. **Table 1** shows results of cultures of middle ear fluid obtained by tympanocentesis or spontaneous perforation of the tympanic membrane. Biofilms are communities of bacteria encased in a matrix present in the middle ear.[4] Effusions recovered from the middle ear are often sterile by culture but contain abundant viable pathogens within biofilms, which can be detected by polymerase chain reaction.[5,6] Studies that use polymerase chain reaction to detect pathogens in middle ear fluids detect pathogens at higher proportions when compared with studies that use culture alone, particularly in the case of NTHi and *M catarrhalis* (**Table 1**).[7] Recognition of the role of biofilms in OM has important implications for understanding the etiology of OM and in designing more effective therapies as well as rational vaccine development strategies. Relying solely on culture as the end point in clinical trials of OM will assess only a subset of cases, and studies to assess vaccine efficacy and effectiveness should take into account the role of biofilms and culture-negative OM.

Effectiveness of Conjugate Vaccines for OM

Since 2000, many countries have implemented vaccine programs using the 7-valent PCV (PCV7; Prevnar). Earlier studies of PCV7 demonstrated a reduction in OM and nasopharyngeal colonization by the 7 pneumococcal vaccine serotypes, replacement by nonvaccine serotypes, and increases in the proportion of OM cases due to NTHi and *M catarrhalis*.[3,8-12] Since 2011, data are available that depict trends from regions where the vaccine has recently been introduced. Increasing use of the 13-valent PCV (PCV13) and the 10-valent pneumococcal vaccine with *H influenzae* protein D (Synflorix; PHiD-CV) as the carrier molecule has resulted in additional changes in nasopharyngeal colonization patterns and the distribution of pathogens causing OM.[13]

PCV7 and PCV13. An evaluation of 5 PCV vaccine trials with OM end points showed a combined vaccine efficacy against vaccine serotypes of 60%, an overall decrease of pneumococcal AOM of 25% to 52%, and an overall reduction in all cause AOM cases of −0.4% to 7%.[14] Observational studies demonstrate effectiveness of PCV

vaccines. A study in Israel examined AOM incidence from July 2004 to June 2013 and documented changes following the stepwise introduction of PCV7 (July 2009) and PCV13 (November 2010).[15] Incidence of AOM episodes caused by *S pneumoniae* decreased 77%, and there was a nonsignificant increase in AOM caused by nonvaccine serotypes when the PCV13 period was compared with the pre-PCV7 period.[15] A retrospective study of AOM in Israel showed that *S pneumoniae* was present in a significantly higher proportion of middle ear fluids when unvaccinated children were compared with PCV7- or PCV13-vaccinated children (69% vs 59% and 50%, respectively).[16] A Costa Rican study showed a lower frequency of *S pneumoniae* AOM (17.1% vs 25.5%) and a higher frequency of AOM due to NTHi (27.4% vs 20.8%) in PCV-vaccinated versus unvaccinated children.[17]

Marom et al analyzed an insurance claims database of a large managed health care plan in the United States from 2001 to 2011.[18] There was a trend toward reduction in AOM rates per child-year from 2001 to 2011 in children <2 years of age. These trends accelerated in 2010 in association with the introduction of PCV13. A 19% decline in tympanostomy tube insertion was also observed in 2010 to 2011. However, tympanic membrane perforations increased from 2001 to 2011.[18] Health registry data on children enrolled in FinOM study demonstrated a 34% (95% confidence interval, 1%-52%) reduction in tympanostomy tube placement in the PCV-vaccinated group for children aged 2 to 5 years but no reduction in children aged 6 to 13 years.[19] These data suggest that prevention of AOM, specifically early episodes, leads to reductions in complex and persistent OM as measured by tympanostomy tube insertion. Acute mastoiditis—a complication of OM that initially decreased after introduction of PCV7—increased with the emergence of pneumococcal serotype 19A.[20-22] Kaplan et al recently reported data suggesting that mastoiditis due to serotype 19A has begun to decline in the PCV13 era.[23] PCV13 was introduced relatively recently, and our understanding of the global impact of PCV13 on OM and complications of disease is still incomplete.

Pneumococcal isolates were collected from children undergoing myringotomy or tympanostomy tube insertion at 8 US children's hospitals; these data indicated a decline in PCV13 serotypes (mostly 19A) and that non-PCV13 serotypes represented ~60% of isolates collected between 2011 and 2013.[23] In Australia, serotype 19A was the most common serotype isolated from middle ear fluids after introduction of PCV7.[24] PCVs have also been associated with changes in the prevalence of antimicrobial resistance. In most studies, an overall increase in antibiotic resistance has been seen in association with PCV7 immunization that, to a large extent, is due to the increased prevalence of strains of serotype 19A.[24-27]

PCV7 and PCV13 mount effective and functional responses in infants and children.[28] Although some studies suggest that otitis-prone infants/children may mount a less effective response to the vaccine than non-otitis-prone children,[16] other studies have shown equally robust antibody responses to PCVs in both groups.[29-32]

PHiD-CV. A phase 3 double-blind study of 20,000 South American infants showed per-protocol efficacy of PHiD-CV against pneumococcal AOM and vaccine serotype pneumococcal AOM of approximately 56% and 67%, respectively, with an overall decrease of 16% in AOM incidence from any cause.[33,34] Leach et al studied indigenous Australian children vaccinated with PCV7 (2008 and 2009 birth cohorts) or PHiD-CV (2010 and 2010 birth cohorts) and followed them for subsequent OM.[35] More than 90% of the children had some form of OM. The authors observed a significant reduction in suppurative OM in PHiD-CV- versus PCV7-vaccinated children (51% vs 39%, $P = .0004$). Subtle differences between the PCV7 and PHiD-CV cohorts and the use of historical controls limit the evaluation of vaccine efficacy; however, the study demonstrates that indigenous Australian children suffer a disproportionate amount of ear disease despite current strategies for prevention of OM. The POET study examined an 11-valent PCV with a protein D carrier and demonstrated efficacy against OM due to *S pneumoniae* and NTHi.[36] While recent work indicates that the protein D component of PHiD-CV induces a strong antibody response, other studies have raised questions about the ultimate ability of a protein D–based vaccine to protect against NTHi disease, as described below.

The protein D component of the vaccine was demonstrated to be very immunogenic in Chilean,[37] Japanese,[38] and Mexican infants[39] when administered as part of a normal childhood vaccination series. Studies in Korean and Taiwanese infants examined the immunogenicity of PHiD-CV when coadministered with *H influenzae* type b vaccine and reported excellent immunogenicity of protein D.[40,41] Moreover, protein D has excellent immunogenicity in both premature and term infants when administered on a normal infant schedule.[42] A more recent report described the relative immunogenicity of PHiD-CV in Dutch children when coadministered with either DTPa-IPV-Hib (Pediacel) or DTPa-HBV-IPV/Hib (Infanrix) and found excellent immunogenicity of the protein D component of the vaccine in both groups.[43] Studies examined the ability of immunization with PHiD-CV to induce an anamnestic antibody response following boosting,[44] the immunogenicity of booster and catch-up doses of the vaccine,[45] and the effect of variation in age on the immunogenicity of individual booster doses.[46] In each instance, the protein D component of the vaccine demonstrated excellent immunogenicity.

A study of Dutch children compared the ability of immunization with PHiD-CV and PCV7 to prevent colonization with NTHi. PHiD-CV immunization had no demonstrable effect on the colonization, acquisition, or bacterial density of NTHi compared with PCV7.[47] Of concern, 3 of the 16 NTHi strains recovered from children in Australia strains completely lacked the gene that encodes protein D. No statistically significant reduction in AOM due to NTHi was observed in the Clinical Otitis Media and Pneumonia Study.[34] Collectively, these studies indicate that a protein

D–based vaccine might be less broadly protective for OM due to NTHi than originally envisioned.

Effectiveness of Viral Vaccines for OM

Respiratory viral infection is one of the most important precipitating factors leading to AOM.[1,2] Therefore, vaccines that prevent respiratory viral infections would likely prevent AOM. However, influenza is the only OM-associated respiratory virus for which there is a licensed vaccine.

Influenza vaccines may fail to prevent influenza infection due to mismatches with circulating influenza strains; it has been suggested that while nonneutralizing influenza vaccines may fail to prevent influenza infection, they could protect from secondary bacterial infections such as OM.[48] Heikkinen et al used pooled data from randomized controlled trials of influenza virus vaccines and reported that during influenza seasons, the efficacy of the live attenuated influenza vaccine (LAIV) against all-cause AOM was 7.5% when compared with placebo.[49] They concluded that the combination of LAIVs and PCVs could further reduce the incidence of AOM in children. However, in a small study in 92 children, pandemic influenza A/H1N1 vaccine did not reduce the rate of AOM as compared with that of unvaccinated children.[50] In 2015, the Cochrane Collaboration concluded that overall influenza vaccination results in a small reduction in AOM and that the benefits may not justify the use of influenza vaccine as a strategy to prevent AOM due to vaccine safety concerns.[51]

Mina et al reported that mice inoculated with LAIV, which causes mild infection of the nasopharynx, had decreased pneumococcal and staphylococcal clearance from the nasopharynx and increased bacterial colonization rates.[52] LAIV increased transmigration to and persistence of pneumococci within the middle ear.[53] The authors suggest that live attenuated vaccine may increase risk of AOM. More data are needed to determine whether these data can be extrapolated to humans.

Seppälä et al reported reduction in AOM rates in children after oral live attenuated poliovirus vaccination, which could be due to the prevention of viral infections caused by nonpolio viruses.[54] However, large-scale impact of the oral polio vaccine on burden of AOM in children is unknown.

Progress in Identification of New Vaccine Candidates

S pneumoniae

Due to limitations of PCVs,[55] there has been considerable interest in the development of pneumococcal vaccines that target common and conserved surface proteins that could supplement or eventually replace PCVs. In theory, protein-based vaccines would be simpler and cheaper to produce than conjugate vaccines. Candidate pneumococcal antigens and vaccines are summarized in **Table 2**.

Several pneumococcal protein antigens have been studied in animal models. Immunization with pneumococcal surface adhesion A (PsaA) protected against OM in a murine model.[56] A fusion of PsaA and nontoxic pneumolysin (Ply) produced a more robust Th1/Th17 mucosal response than each protein alone or immunization with a mix of the 2 proteins.[57] A fusion protein consisting of the choline binding protein A peptide and L460D, a nontoxic pneumolysoid, was shown to be more broadly protective against pneumococcal disease than pneumolysoid alone in mice, with a reduction in OM from 55% to 25%.[58] Two conserved proteins of unknown function, SP1298 and SP2205, were not directly tested for prevention of AOM but were used to immunize mice, and they showed a reduced pneumococcal load in the nasopharynx, lungs, and blood.[59] Immunization of chinchillas with the chimeric protein RrgB321, a component of pneumococcal pilus 1, did not protect against experimental OM, although a delay in development of disease was seen.[60]

Some laboratories have attempted to develop whole-cell pneumococcal vaccines. A live whole-cell vaccine lacking FtzY (to stop multiplication of the bacteria) produced serotype-independent protection against OM that was CD4+ T cell dependent.[61] A whole-cell vaccine composed of an ethanol-killed capsule-deficient *S pneumoniae* mutant provided protection against colonization with serotype 4 and 19F strains.[62]

An inactivated whole-cell vaccine that has undergone a phase 1 clinical study in healthy US adults demonstrated an acceptable safety and reactogenicity profile along with encouraging immune responses.[63] This vaccine is appealing in that it incorporates numerous pneumococcal antigens and, in preclinical models, demonstrates 2 mechanisms of protection: protection against nasopharyngeal carriage via a T cell–mediated mechanism and protection against pneumococcal disease via humoral antibody responses.[64] GSK Biologicals is developing a novel pneumococcal vaccine that involves the addition of 2 pneumococcal proteins to its PCV10 Synflorix: pneumococcal histidine triad protein D (PhtD) and pneumolysin.[65] This vaccine candidate has undergone a number of clinical trials and is currently being evaluated in infants in the Gambia for an impact on carriage of nonvaccine serotypes.[66] GSK is also evaluating these 2 proteins for their ability to vaccinate children against AOM.[67] Sanofi Pasteur is developing a novel pneumococcal vaccine that includes 3 proteins: PhtD, pneumolysoid, and pneumococcal choline binding protein A. This candidate has also been assessed in a number of trials, including in a small study in Bangladeshi infants where the vaccine was found to be immunogenic but did not appear to affect nasopharyngeal carriage.[68] Finally, Genocea has used its antigen discovery platform to identify T-cell antigens associated with protection against pneumococcal nasopharyngeal carriage and has initiated clinical studies to assess a trivalent protein vaccine candidate.[69]

Ren et al and Xu et al investigated natural antibodies to pneumococcal proteins in children.[70,71] They observed that the titers against PhtD, pneumococcal choline binding protein A, and Ply were significantly lower in serum and middle ear fluid in OM prone as compared with non-OM-prone children.[56,70] Verhaegh et al screened antibodies

Table 2. Protein Vaccine Antigens of *Streptococcus pneumoniae* at Various Stages of Development.

Antigen	Molecular Mass, kDa	Putative Function and Other Features	Reference[a]
Ply/pneumolysoid	53	Cholesterol-dependent toxin	57, 58
PhtD; PhtE	95; 115	Histidine-triad proteins	70, 71
PcpA	79	Choline binding protein, adhesin	70, 107
NanA	110	Neuraminidase A, sialidase activity	No new information
PsaA	35	Manganese ABC transport substrate binding protein	57, 70, 71
CbpA/PspC	79; smaller fragments for fusion also being used	Choline binding protein, binds complement factors and polymeric IG receptor	58
SP1298[b]	35	DHH subfamily 1 protein, cyclic-AMP phosphodiesterase	59
SP2205[b]	73	DHH subfamily 1 protein, cyclic-AMP phosphodiesterase	59
Pilus-1		Adherence	60
Whole-cell vaccine		Not applicable	No publications, under development

[a]References from July 2011 to June 2015.
[b]Newly identified vaccine antigen from previous report.

against 18 pneumococcal proteins, including PhtD and Ply, and showed that there were no differences in IgG, IgA, and IgM levels when comparing children with recurrent AOM and chronic OM with effusion.[72] Another study demonstrated that otitis-prone children have a lower percentage of memory B cells and immunoglobulin production to pneumococcal protein antigens, such as pneumolysin and PhtD/PhtE, when compared with non-otitis-prone children.[73]

Nontypeable H influenzae

PCVs have been associated with an increased proportion of disease associated with NTHi strains.[74] An effective NTHi vaccine candidate will be surface exposed, conserved in heterologous strains, immunogenic, and expressed during the disease state.[75] A number of vaccine candidates have been investigated since 2011 and are summarized in **Table 3**.

Kodama and colleagues tested the efficacy of nasal vaccination with outer membrane protein 6 (P6) in combination with the chemokine CCL20 in a murine model.[76] The addition of CCL20 resulted in significant increases in IgA and IgG titers and a subsequent increase in nasopharyngeal clearance of NTHi. The same group tested the efficacy of fms-like tyrosine kinase receptor 3 ligand as a mucosal adjuvant with P6 protein. This pairing also increased dendritic cell recruitment and nasopharyngeal clearance of NTHi.[77,78] Han and colleagues compared the P6 response in mice when delivered with macrophage-derived chemokine or with Freund's adjuvant. Combination of P6 with macrophage-derived chemokine increased serum IgG titers; however, there was no statistical difference in survival rate when the 2 adjuvants were compared.[79] Hybridoma cell lines producing monoclonal antibodies against P6 have been developed, although efficacy has not been tested to date.[80]

The outer membrane protein P5 is highly conserved in all NTHi strains sequenced[81-83] and has been shown to be an effective vaccine candidate in experimental models of OM.[84] Host responses to immunization with P5 have been described in transcutaneous immunization of NTHi-infected chinchillas with a chimeric immunogen that includes P5 and the type IV pilus PilA.[84] The authors observed a significant reduction in the signs of OM and resolution of mucosal biofilms when animals were immunized with the P5 chimeric immunogen.

Additional work has focused on NTHi protein E and protein F. Singh and colleagues demonstrated that peptides corresponding to surface-exposed regions of protein E were immunogenic in mice and that antibodies to these peptides bound protein E on the bacterial cell surface.[85] Mice immunized with a truncated form of the highly conserved protein F adhesin cleared NTHi infections significantly earlier than mice treated with a control peptide.[86]

Serum antibody responses were measured to outer membrane proteins D, P6, and 26 of NTHi in otitis-prone and non-otitis-prone children. Otitis-prone children mount less IgG serum antibody response toward all 3 protein antigens, possibly linked to recurrent infections.[87] Recent work defined outer membrane protein 26 T- and B-cell epitopes, which will improve existing NTHi vaccines.[88]

Winter and coworkers examined the ability of antisera raised against purified high molecular weight (HMW) proteins HMW1/HMW2 or recombinant Hia proteins to mediate opsonophagocytic killing of a large panel of NTHi strains.[89] The 3 HMW1/HMW2 antisera mediated killing of 48 of 65 HMW1/HMW2-expressing strains, and the 2 Hia antisera mediated killing of 15 of 24 Hia-expressing strains. Thus, a vaccine formulated with a limited number of HMW1/HMW2 and Hia proteins might provide protection against disease caused by most NTHi strains.

Davis and coworkers reported on the prevalence, distribution, and sequence diversity of *hmwA* among a large

Table 3. Protein Vaccine Antigens of *Haemophilus influenzae* at Various Stages of Development.

Antigen	Molecular Mass, kDa	Putative Function and Other Features	Reference[a]
Protein D	42	Adherence to epithelial cells	42-47
Protein E	16	Multifunctional adhesin	72, 108
Protein F	30	Binds vitronectin and laminin	109, 110
OMP P5	27	Binds factor H, adherence to epithelial cells	84
PilA	15	Twitching motility, adherence, competence, biofilm formation	84
OMP P6	16	Structural role	77-80
OMP26	26	Skp family of translocation proteins	88
HMW1; HMW2	125; 120	Adherence	89
Hia	114	Adherence	89

[a]References from July 2011 to June 2015.

collection of commensal and OM derived NTHi.[90] *hmwA* was detected in 61% of NTHi and was significantly more prevalent among OM isolates than commensal isolates. Almost all of the *hmwA*-positive isolates possessed 2 *hmw* loci. The authors also determined the DNA sequence of the *hmwA* binding region of 33 isolates and found that the average amino acid identity across all *hmwA* sequences was 62%. Phylogenetic analyses of the *hmwA* binding revealed 4 distinct sequence clusters, and the majority of *hmwA* sequences (83%) belonged to 1 of 2 dominant sequence clusters. These data would be important to consider in any vaccine development efforts involving the HMW1/HMW2 proteins.

M catarrhalis

Prevention of bacterial OM will also require vaccines directed at *M catarrhalis*. Several surface proteins of *M catarrhalis* are in various stages of development as vaccine antigens[91] and are summarized in **Table 4**. Since the previous report, a promising *M catarrhalis* antigen, substrate-binding protein, has been identified.[92] Immunization of mice with substrate-binding protein induces enhanced clearance in the mouse pulmonary clearance model.[92] Smidt et al[93] conducted a comprehensive antigen screen to identify potential *M catarrhalis* vaccine candidates. Three antigens induced significantly faster clearance than OmpCD or adjuvant alone in a mouse pulmonary clearance model. The most efficacious antigen, Msp22, was determined to be a heme binding protein.[93] Immunization with a polypeptide shared by filamentous hemagglutinin-like proteins MhaB1 and MhaB2 induced antibodies that interfered with *M catarrhalis* colonization of the chinchilla nasopharynx.[94] Advances in characterization of other putative vaccine antigens include oligopeptide permease A[93,95] and detoxified lipooligosaccharide.[96,97]

As described in the 2011 report, the lack of good animal models for *M catarrhalis* remains a challenge to vaccine development.[3] The mouse pulmonary clearance model is the most widely used model for assessing vaccine antigens of *M catarrhalis*. The model yields reproducible results, and immunization with selected antigens induces enhanced clearance, which has been interpreted as a protective response. However, the model does not simulate human disease, and induction of enhanced clearance in the mouse model has not yet been correlated with protection in humans. *M catarrhalis* is cleared readily from the middle ear of chinchillas after direct instillation. Since the last report, 3 studies have successfully used the chinchilla model to study *M catarrhalis* colonization and/or infection. As part of a study of global transcriptome expression by *M catarrhalis*, Hoopman et al inoculated chinchillas intranasally and demonstrated nasopharyngeal colonization that persisted for 72 hours.[98] Brockson et al demonstrated that intranasal coinfection with respiratory syncytial virus and NTHi predisposed to *M catarrhalis* induced ascending OM in the chinchilla.[99] *M catarrhalis* was cultured from the nasopharynx and middle ear in the majority of animals for up to 17 days. Finally, Shaffer et al demonstrated nasopharyngeal colonization of chinchillas by *M catarrhalis* for up to 7 days as part of evaluating MhaB1 and MhaB2 as potential vaccine antigens.[94] These approaches show promise in testing *M catarrhalis* vaccine antigens in the chinchilla, which has been a valuable model in evaluating vaccine antigens of NTHi and *S pneumoniae*.

Vaccine Delivery Methods

Multiple studies utilize a subcutaneous route to explore novel vaccine candidates for the prevention of OM.[58,92,94,97] The ability to deliver bacterial antigens for the prevention of OM via an intranasal route also continues to garner momentum.[56,61,83,93] As an example, Xu et al investigated intranasal immunization using PsaA protein of *S pneumoniae* delivered with chitosan in the form of nanoparticles and demonstrated greater protection against AOM following middle ear challenge with a serotype 14 pneumococcus compared with delivery of naked PsaA.[56] There has also been a great deal of recent activity in the assessment of alternative delivery methods, including transcutaneous and maternal immunization for OM vaccine candidates.

Transcutaneous

Since 2011, there have been 3 reports of transcutaneous immunization to prevent OM due to NTHi. Novotny et al

Table 4. Vaccine Antigens of *Moraxella catarrhalis* at Various Stages of Development.

Antigen	Molecular Mass, kDa	Putative Function and Other Features	Reference[a]
MID/Hag	200	Adhesin, binds IgD, hemagglutinin	111
MchA1, MchA2; MhaB1, MhaB2	184; 201	Filamentous hemagglutinin-like adhesin	94
McmA	110	Metallopeptidase-like adhesin	
OppA	~80	Oligopeptide permease	93, 95
Msp 75	~75	Homology to succinic dehydrogenase	
McaP	66	Adhesin and phospholipase B	
UspA2	62 (oligomer)	Binds complement, vitronectin, and laminin	112, 113
OMP E	50	Putative fatty acid transport	
OMP CD	45	OMP A–like protein, binds mucin, adhesin	93
M35	~35	Porin, conserved with one variable loop	114
SBP2[b]	~30	Substrate binding protein of an ABC transporter	92
OMP G1a	~29	Lipoprotein, putative copper transport protein	
OMP G1b	~29	Surface molecule	
OlpA	24	Homologous with Neisseria Opa adhesins	115
Msp 22	~22	Surface lipoprotein	93
Type IV pili	16	Adhesin, transformation, biofilm formation	116
Lipooligosaccharide	2.5-4	Detoxified form is potential vaccine antigen	96, 97, 117, 118

[a]References from July 2011 to June 2015.
[b]Newly identified vaccine antigen from previous report.

immunized chinchillas by rubbing a chimeric OMP P5 and type IV pili immunogen onto the inner surface of their outer ears.[84,100] The authors demonstrated protective efficacy against development of OM and rapid resolution of established disease.[84,100] Novotny et al combined the chimeric OMP P5 and type IV pili immunogen and a bacterial DNA-binding protein, integration host factor, and demonstrated that this combination of immunogens resulted in significantly earlier eradication of NTHi from planktonic and adherent populations in the middle ear, disruption of mucosal biofilms, and rapid resolution of signs of disease as compared with controls.[101]

Maternal Immunization

Immunization of pregnant women has been proposed as a potentially effective immunization strategy to protect infants from OM. van Santen et al reported that maternal influenza vaccination and infant receipt of PCV confer greater protection from OM than PCV alone.[102] Conversely, Daly and colleagues reported discouraging results of a randomized trial of pregnant women given either an investigational 9-valent PCV vaccine or a placebo in the last trimester of pregnancy; all infants received Prevnar at 2, 4, 6, and 12 months.[103] Immunizing pregnant women with PCV-9 increased infants' risk of AOM in the first 6 months of life. The authors attributed this outcome to decreased infant antibody responses to the vaccine serotypes delivered in Prevnar due to dampening by high levels of passively acquired pneumococcal antibodies and/or altered B lymphocyte immune responses when exposed to these polysaccharide antigens in utero.

Implications for Practice

Several studies demonstrate the positive impact of PCV7, PCV13, and PHiD-CV on pneumococcal OM, recurrent OM, OM visits, and tympanostomy tube insertions following introduction. While effective for pneumococcal OM, PHiD-CV may be less broadly protective for NTHi than originally envisioned. Neither PCV7 nor PCV13 appears to have substantial impact on development of complex OM in indigenous Australian children. There are limitations to the overall impact of PCVs because of serotype replacement.[55] Emerging *S pneumoniae* serotypes prevalent as causes of OM are 35B, 23A, 23B, 15A, 15B/C, 16F, and 21. A 15-valent PCV is in development, but there are technical limitations to the number of serotypes that can be included in vaccines, and the complexity of manufacturing has resulted in relatively high prices for multivalent PCVs that make them unaffordable for many developing countries. Of concern, there are significant challenges to licensure of new pneumococcal vaccines; PCV13 and PHiD-CV were approved on the basis of immunologic noninferiority and safety as compared with PCV7.[55] Licensing criteria and correlates of protection for protein-based and whole-cell vaccines have not been defined.

Future efforts are needed to develop more effective influenza vaccines with robust, long-lasting cross-strain protection. Since the last review in 2011, there are no additional studies on the use of passive or active immune prophylaxis against respiratory syncytial virus infection for protection against AOM. Research is needed to develop vaccines against other respiratory viruses, including rhinoviruses, respiratory syncytial virus, parainfluenza viruses, and adenoviruses.

There are ongoing needs for vaccines that specifically target NTHi and *M catarrhalis*–induced OM due to the shifting microbiology of OM after broad use of PCVs. Additional important research goals include better integration of our understanding of those aspects of the microbiology of the nasopharynx and OM that could confound clinical trial outcomes, such as the culture-negative status of middle ear fluids despite robust biofilms and the potential presence of *H haemolyticus* in the nasopharynx; and the need for more sophisticated testing after delivery of an NTHi vaccine antigen to detect loss of cells that express that antigen (instead of just +/− scoring) to better gauge the effectiveness of the immunization on nasopharyngeal colonization (and more akin to current methods to demonstrate the loss of vaccine serotypes of the pneumococcus after delivery of whatever valent PCV is being used).

Critical Future Research Objectives

1. Identification of correlates of protection for AOM.
2. Urgent need to move several already well-characterized candidate vaccine antigens from animal studies to human trials.
3. Further development of alternative vaccination delivery methods, including maternal immunization.
4. Testing of *M catarrhalis* vaccine candidates in novel animal models.
5. Continued development of noncapsular vaccine antigens for pneumococcal OM.
6. Identification and characterization of additional candidate vaccine antigens of bacterial and viral OM pathogens.

Author Contributions

Melinda M. Pettigrew, panel chair, coordination of postsymposium meeting, substantial contributions to writing and editing; **Mark R. Alderson**, participation in postsymposium meeting and substantial contributions to writing and editing; **Lauren O. Bakaletz**, contributions to conception and design of postmeeting conference, participation in postsymposium meeting, and substantial contributions to writing and editing; **Stephen J. Barenkamp**, participation in postsymposium meeting and substantial contributions to writing and editing; **Anders P. Hakansson**, participation in postsymposium meeting and substantial contributions to writing and editing; **Kevin M. Mason**, participation in postsymposium meeting and substantial contributions to writing and editing; **Johanna Nokso-Koivisto**, participation in postsymposium meeting and substantial contributions to writing and editing; **Janak Patel**, participation in postsymposium meeting and substantial contributions to writing and editing; **Stephen I. Pelton**, participation in postsymposium meeting and substantial contributions to writing and editing; **Timothy F. Murphy**, panel cochair, coordination of postsymposium meeting, participation in postsymposium meeting and substantial contributions to writing and editing.

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