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Panel 8: Vaccines and Immunology

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Abstract

Objective.—To review and highlight significant advances made towards vaccine development and understanding of the immunology of otitis media (OM) since the 19th International

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⁵.AUTHOR CONTRIBUTIONS

Each author compiled literature searches and drafted a section(s) of the manuscript. All authors contributed to drafting of the final manuscript.

⁶.DISCLOSURES

Competing interests: LSK has received investigator-initiated grants and travel support from Pfizer and GSK to attend conferences that are not related to this work. LLH has received institutional funding from GSK, Pfizer, and Merck. TFM has patents for *Moraxella catarrhalis* vaccines and LSK holds patents for pneumolysin as a vaccine antigen. SIP has received investigator-initiated research grants through Boston Medical Center from Merck Vaccines and Pfizer and personal honorarium for participation in advisory boards, expert consultation and symposia from GlaxoSmithKline, Pfizer, and Merck Vaccines. All other authors declare no competing interests.

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Symposium on Recent Advances in Otitis Media (ISOM) in 2015, as well as identify future research directions and knowledge gaps.

Data Sources.—PubMed database, National Library of Medicine.

Review Methods.—Key topics were assigned to each panel member for detailed review. Draft reviews were collated, circulated, and thoroughly discussed when the panel met at the 20th ISOM in June 2019. The final manuscript was prepared with input from all panel members.

Conclusions.—Since 2015 there have been a number of studies assessing the impact of licensed pneumococcal vaccines on OM. While these studies have confirmed that these vaccines are effective in preventing carriage and/or disease caused by vaccine serotypes, OM caused by non-vaccine serotype pneumococci and other otopathogens remains a significant health care burden globally. Development of multi-species vaccines is challenging but essential to reducing the global burden of OM. Influenza vaccination has been shown to prevent acute OM, and with novel vaccines against nontypeable *Haemophilus influenzae* (NTHi), *Moraxella catarrhalis* and Respiratory Syncytial Virus (RSV) in clinical trials, the potential to significantly prevent OM is within reach. Research into alternative vaccine delivery strategies has demonstrated the power of maternal and mucosal vaccination for OM prevention. Future OM vaccine trials must include molecular diagnostics of middle ear effusion, for detection of viruses and bacteria that are persisting in biofilms and to enable accurate assessment of vaccine impact on OM etiology. Understanding population differences in natural and vaccine-induced immune responses to otopathogens is also important for development of the most effective OM vaccines. Improved understanding of the interaction between otopathogens will also advance development of effective therapies and encourage the assessment of the indirect benefits of vaccination.

Implications for Practice.—While NTHi and *M. catarrhalis* are the predominant otopathogens, funding opportunities to drive vaccine development for these species are limited due to a focus on prevention of childhood mortality rather than morbidity. Delivery of a comprehensive report on the high financial and social costs of OM, including the potential for OM vaccines to reduce antibiotic use and subsequent development of antimicrobial resistance (AMR), would likely assist in engaging stakeholders to recognize the value of prevention of OM and increase support for efforts on OM vaccine development. Vaccine trials with OM prevention as a clinical end-point are challenging, however a focus on developing assays that measure functional correlates of protection would facilitate OM vaccine development.

Keywords

otitis media AND: vaccine; adaptive immunity; innate immun*; clinical trial

1. INTRODUCTION

Otitis media (OM) remains a major cause of childhood infections and prescription of antibiotics, resulting in significant morbidity and contribution to the global increase in antibiotic resistance. The three primary bacterial pathogens that cause OM are nontypeable *Haemophilus influenzae* (NTHi), *Streptococcus pneumoniae*, and *Moraxella catarrhalis*, but only *S. pneumoniae* has currently licensed vaccines. Vaccination has the potential to have a major impact on OM, yet development efforts for vaccines against NTHi and *M. catarrhalis*

have not received adequate support from either funding bodies or pharmaceutical companies. The goal of this report is to update on the progress of OM vaccine development and OM immunology understanding over the past four years. The report will also discuss the challenges and identify gaps in research that could facilitate progression towards global OM prevention.

2. METHODS

The PubMed database was searched for articles published between 1st July 2015 and 30th June 2019 using the MeSH terms otitis media AND vaccine; otitis media AND adaptive immunity; otitis media AND innate immun*; otitis media AND clinical trial.

3. DISCUSSION

3.1 Etiology of OM

With the widespread use of pneumococcal conjugate vaccines (PCVs) that dramatically alter nasopharyngeal colonization patterns, the etiology of OM is undergoing continuing changes. Since PCV was first introduced in 2000, over 140 countries have implemented a PCV immunization program [1]. PCVs have led to a reduction of OM and nasopharyngeal colonization by pneumococcal vaccine serotypes but have also resulted in replacement by non-vaccine pneumococcal serotypes and possibly by NTHi and *M. catarrhalis* [2]. The increasing use of the 13- and 10-valent PCVs with *H. influenzae* Protein D as the conjugate protein will undoubtedly continue to affect nasopharyngeal colonization patterns and the distribution of pathogens causing OM, pneumonia, and invasive disease. These changes must be monitored carefully.

In clinical practice, OM is managed empirically, so the etiology of individual cases is rarely known. The most widely used method for determining OM etiology has been bacteriological culture of middle ear fluid (obtained by tympanocentesis). As knowledge of OM pathogenesis has advanced and the central role of biofilms in the course of the disease has become recognized, it is clear that middle ear fluid culture, long-considered gold standard for etiological diagnosis, only identifies etiology for a subset of OM. Effusions recovered from the middle ear are often negative by culture but contain abundant, viable bacterial pathogens present in biofilms [3–10]. Bacteria that persist in biofilms have a reduced growth rate, distinct transcriptome, increased resistance to effectors of innate and acquired immunity, and recalcitrance to the action of antimicrobial agents compared to planktonic bacteria.

Figure 1 shows results of studies published since 2012 that analyzed middle ear fluid obtained by tympanocentesis or spontaneous perforation of the tympanic membrane by culture and/or molecular analysis by PCR. As indicated by the difference in number of middle ear fluid samples analyzed since 2012, culture is still the most popular method used to determine OM etiology - despite ample evidence that PCR is more sensitive in detecting pathogens in middle ear fluid (particularly in the case of *M. catarrhalis*). The difference between culture and PCR results is likely due to the low sensitivity of culture in detecting bacteria in biofilms [4, 7, 11, 12]. Therefore, recognizing the role of biofilms in OM is

important for understanding the etiology of OM and in designing rational vaccine development strategies. For these reasons, trial designs to assess vaccine impact in preventing OM must take into account culture-negative OM and incorporate molecular diagnostic tools.

Despite widespread PCV implementation, a high burden of pneumococcal OM caused predominantly by non-vaccine serotypes still exists. In addition, NTHi and *M. catarrhalis* are detected in the middle ear fluid of approximately 70% of children with OM, with no current vaccines licensed to tackle this significant burden of disease. This review will assess the impact of existing pneumococcal vaccines on OM, advances made in development of novel OM vaccines, and how understanding innate and adaptive immunity to otopathogens may be used to inform vaccine development.

3.2 Impact of current vaccines on OM

The changing microbiological etiology of OM since the introduction of PCVs (specifically pneumococcal serotypes and antimicrobial susceptibility patterns) has been further explored over the last four years (2015–2019). Kaur *et al.* assessed the epidemiology of acute OM (AOM) in children in Rochester, New York, prospectively enrolling 615 children between 2006 and 2016 [13]. All AOM episodes were assessed for etiology by tympanocentesis; 23% of children had AOM in the first year of life, 60% had at least one AOM episode by three years of age and 24% had three or more AOM episodes by three years of age. Such high proportions of AOM in this PCV-vaccinated cohort demonstrate that PCV alone has a limited effect on all cause OM. Indirect herd protection from PCV13 introduction was observed in Israeli children with declines in AOM incidence prior to 4 months of age [14]. Fortanier *et al.* reported that time to first AOM was prolonged in children receiving PCV10 compared with PCV7 or no PCV, with children in the PCV10 group having a 21% lower risk of first AOM than those in the no PCV group [15]. Such declines in AOM have important implications for reducing the incidence of complex OM (see below). Indeed, Dagan *et al.* summarized evidence that prevention of early OM leads to reduction in complex OM [16].

Additional studies in the past four years have documented the impact of PCVs on pneumococcal OM. Post 7-valent PCV (PCV7) but prior to 13-valent PCV (PCV13) introduction, a multidrug resistant strain of pneumococcal serotype 19A (MLST 320) emerged as a dominant cause of AOM and mastoiditis and became associated with treatment failure. Recent studies in children with otorrhea [17] or in children with AOM undergoing tympanocentesis [18] identified predominantly non-PCV13 vaccine serotypes, suggesting PCV13 to be effective against serotype 19A. Pneumococcal serogroups 15 and 23, and serotypes 21, 11A, and 35B have become dominant causes of OM in both Italian and North American children [17, 18]. In addition, 24F has emerged in Italy as a prevalent serotype among cases of pneumococcal otorrhea. Among the vaccine serotypes, only serotype 3 remains as a prevalent cause of vaccine-type OM, with some additional cases due to serotype 19A. Residual disease caused by serotype 3 is most likely related to lower vaccine effectiveness against this serotype [19]. Decreases in the proportion of *S. pneumoniae* isolates recovered from the middle ear that were resistant to penicillin have been identified following PCV13 introduction [20].

Initial evidence documented a reduction in OM caused by NTHi following vaccination with 10-valent PCV (PCV10) [21], a vaccine that incorporates Protein D from NTHi as the carrier protein for most of the pneumococcal polysaccharides. Recent studies from New Zealand [22] and Australia [23], however, report contrasting data. Replacing PCV7 with PCV10 in New Zealand's national immunization program did not result in a decline in NTHi (or *S. pneumoniae*) prevalence [22]. Neither did it result in a decline in density [24] in the nasopharynx or middle ear of children undergoing tympanostomy tube insertion for recurrent AOM/OM with effusion (or in the nasopharynx of non-otitis-prone children). In contrast, a decreased prevalence of NTHi-infected ear discharge in PCV10-vaccinated Aboriginal children compared to PCV7-vaccinated Aboriginal children was observed when PCV10 was used in the Northern Territory of Australia from 2009 to 2011 [23].

Replacement of PCV10 with PCV13 on the Australian national immunization program in 2011 did not improve the prevalence of OM in the Northern Territory, with only 7% of children in the PCV13 era with healthy middle ears compared with 10% in the PCV10 era [25]. An increase in simultaneous detection of NTHi and *S. pneumoniae* in the middle ear was observed in the PCV13-vaccinated children in this study compared with the PCV10-vaccinated children (43% versus 12%, respectively), suggesting that the PCV10 vaccine may prevent NTHi OM in this population. Lower incidence of complex OM (children with severe or recurrent OM) has been reported in Denmark [26], Sweden [27], and Israel [28] following introduction of PCVs - adding to data reported at the 2015 ISOM meeting that demonstrated the initial impact of PCVs on vaccine serotype OM [29]. Further understanding of the mechanisms underlying the reduction in pneumococcal OM have been identified through the observation that the rate of serotype-specific progression from carriage to AOM was reduced for non-vaccine serotypes compared to vaccine serotypes [28].

Influenza vaccine clinical impact on OM.—A Cochrane report published in 2017 reviewed the effect of influenza vaccine on preventing AOM in infants and children and analyzed 11 trials involving 17,123 children [30]. The authors found a 4% reduction in AOM and an 11% reduction in the number of antibiotic prescriptions.

3.3. Vaccine development for OM prevention

Clinical trials of licensed vaccines against OM.—Clinical trials with combinations of existing pneumococcal vaccines have been reported in the last four years. A cluster randomized controlled study (FinIP) of PCV10 effectiveness on respiratory tract infections (RTI) in Finnish children under two years of age revealed a reduction in RTI with AOM in PCV10-vaccinated children compared with non-PCV10-vaccinated children [31]. PCV10 vaccine efficacy (VE) was 23% (95% CI, 0–40%) against RTIs with AOM. In Australia, the PneuMUM study investigated the impact of maternal immunization among Indigenous women with the 23-valent pneumococcal polysaccharide vaccine (PPV23) on infant OM [32]. The PneuMUM study revealed that maternal immunization with PPV23 was not efficacious against all-cause infant OM (VE 12% [95% CI –12% to 31%]) or PPV23-type nasopharyngeal carriage (VE 30% [95% CI –34% to 64%]) at 7 months of age [32]. Post-hoc analysis, however, revealed a more specific outcome of maternal PPV23 efficacy against infant OM concurrent with vaccine-related serotype carriage (VE 51% [95% CI –2% to 76%]), suggesting that maternal immunization with PPV23 in pregnancy may complement

infant pneumococcal vaccination programs. Also, in Australia, the PREVIX-COMBO trial is assessing the combination of PCV13 and PCV10 in comparison with either PCV13 or PCV10 alone in Indigenous children at a high-risk of developing chronic OM ([NCT01174849](#); [33]). As the pneumococcus is not the predominant cause of OM in Australia and many other countries, and OM from vaccine serotypes is even rarer in the PCV-era, the development of vaccines that impact on other OM etiologies is essential to reduce the burden of OM.

Clinical trials of novel vaccine candidates against OM.—Protein antigens that are conserved across pneumococcal serotypes offer an alternative to serotype-restricted vaccination strategies. Two such pneumococcal proteins are pneumolysin (Ply) and the pneumococcal histidine triad protein D (PhtD). Alone or in combination, Ply and PhtD have demonstrated protection against pneumococcal disease or carriage in *in vitro* and animal studies and were selected by GlaxoSmithKline for clinical development. An investigational vaccine (PhiD-CV/dPLY/PhtD) containing 10 pneumococcal serotype-specific conjugates and detoxified pneumolysin (dPLY) and PhtD was studied in Gambian children ([NCT01262872](#)) and separately dPLY/PhtD was assessed in combination with PCV13 in Native American infants ([NCT01545375](#)) for immunogenicity and impact on nasopharyngeal colonization and/or clinical OM. The polysaccharide and protein components of this vaccine were immunogenic and well-tolerated in both trials; however, efficacy against pneumococcal nasopharyngeal carriage prevalence, density, acquisition, or clearance was not observed in either study [34–39]. Clinical OM episodes were not assessed in the Gambian study. In the Native American study, significant VE against acute OM was not observed. VE against all AOM episodes (as defined by the American Academy of Pediatrics) was 3.8% (95% CI: –11.4, 16.9). VE against all episodes of draining AOM, pneumococcal draining AOM, and non-pneumococcal draining AOM ranged from –32.2% (–298.3, 56.1) to 17.8% (–57.0, 57.0). VE against first episodes of clinical AOM tended to be higher compared to VE against all episodes. This finding was more pronounced in a post-hoc analysis restricted to first OM episodes within the first year of life. Exploratory analyses of VE against various AOM endpoints among participants with low (<27,498 EL.U/mL for Ply; <3739.5 EL.U/mL for PhtD) and high (>27,498 EL.U/mL for Ply; >3739.5 EL.U/mL for PhtD) post-primary anti-protein antibody concentrations revealed a trend for higher efficacy against AOM endpoints with higher post-primary anti-Ply antibody levels [39]. These results indicate that failure to impact on pneumococcal colonization with a protein-based vaccine does not preclude an impact on disease as observed with the meningococcal serogroup B vaccine [40].

Other pneumococcal protein vaccines with the potential to impact OM have undergone clinical evaluation over the past four years. A pneumococcal whole cell vaccine (wSp) advanced into Phase 2 studies in toddlers in Kenya ([NCT02097472](#)). Another multi-antigen pneumococcal protein vaccine candidate, PnuBioVax™, underwent a Phase 1 trial that demonstrated safety and immunogenicity [41]. Impact on pediatric OM has not been assessed with these vaccine formulations; however, a recent study in mice demonstrated that a single subcutaneous dose of wSp reduced pneumococcal density in the middle ear but did not prevent pneumococcal OM [42].

Higher valency PCVs (15- to 20+-valent) are also currently being assessed in various phases of clinical development, and two or more are anticipated to be licensed in the next four years ([NCT02531373](#), [NCT03835975](#), [NCT03803202](#)). These vaccines have the potential to prevent OM caused by additional pneumococcal vaccine serotypes but present a high likelihood of further serotype replacement and, therefore, limited impact on residual pneumococcal disease burden. While pneumococcal vaccines have progressed to trials in the last four years, the majority of OM (especially complicated OM) still has no preventative therapy. Bacterial OM prevention by immunization requires vaccines that are directed against all of the primary otopathogens, including NTHi and *M. catarrhalis*. While NTHi (and *M. catarrhalis*) vaccine development for adults with chronic lung disease has generated some interest (Table 1), no clinical trials with new vaccine formulations have been assessed for OM prevention in the last four years.

3.4. Preclinical studies of new vaccines against OM

Pneumococcal protein vaccines.—Pneumococcal proteins that are conserved and universally present are potential candidates as vaccines for serotype-independent pneumococcal disease prevention. Many potential antigens have been identified and studied in animal models, including those of OM [29]. An emerging strategy is the use of multiple proteins in a vaccine formulation. A multiple-antigen vaccine (MAV) prepared from *S. pneumoniae* TIGR4 lysates contains heat shock proteins, which act as immune adjuvants. MAV also contains several well-characterized protein antigens such as pneumococcal surface protein A (PspA) and Ply [43]. In animal studies, active or passive immunization with MAV induced functional antibody responses to multiple pneumococcal serotypes, including non-vaccine serotypes [44]. In addition, passive transfer of immune sera from MAV-vaccinated mice protected against sepsis due to both homologous and heterologous serotypes [44].

NTHi vaccines.—Globally, NTHi is the dominant pathogen detected in middle ear effusion, particularly in children with chronic or recurrent OM [45]. Since the 2015 ISOM report, NTHi antigen discovery and refinement has predominated the literature with several novel observations and new candidate antigens [46–49]. Recombinant Protein D, truncated Protein E, and a Protein E-D fusion protein showed that immunogenicity in mice and antibodies were bactericidal and opsonophagocytic *in vitro* [50–53]. Among surface-exposed proteins identified as essential for NTHi survival, antibodies against Hel1 and Hel2 prevented bacteremia in a rat model [54]. A monoclonal antibody against ketodeoxyoctanoate (KDO) was bactericidal against NTHi *in vitro* and KDO decoration of lipooligosaccharide within NTHi biofilms from the chinchilla middle ear was shown *ex vivo* [55]. Immunization of chinchillas with outer membrane vesicles isolated from HMW1/ HMW2- and Hia- expressing NTHi prevented experimental OM [56]. Therapeutic immunization with the outer membrane protein (OMP) P5- and Type IV pilus-directed antigen, chimV4, delivered by band-aid, resolved NTHi-induced OM in a chinchilla model [57] and additionally prevented OM in a viral- bacterial model of experimental disease [58]. Finally, intracellular elongation factor thermal-unstable (EF-Tu) was recently identified as a novel NTHi surface protein [59]; and antibodies to EF-Tu can promote complement-dependent killing of NTHi [60], indicating that this antigen should be included among

surface proteins under consideration as vaccine antigens for NTHi diseases. NTHi colonization prevention and OM development has recently been demonstrated in mice using intranasal delivery of a closely related Pasteurellaceae species [61]. Due to the expansive heterogeneity of NTHi strains [62], demonstrating protection against multiple NTHi strains is important for any new NTHi-targeted therapy.

M. catarrhalis vaccines.—Several surface proteins of *M. catarrhalis* are in various stages of preclinical development as vaccine antigens, reviewed recently by several groups [9, 63, 64] and summarized in the 2015 ISOM report [29]. Since the 2015 ISOM report, three new potential antigens have been identified, including two substrate binding proteins of ABC transporters (CysP, AfeA) and a peptide of lactoferrin binding protein A [65–67]. Each antigen expresses epitopes on the bacterial surface, is conserved among strains, and induces enhanced clearance in the mouse pulmonary clearance model following immunization. In addition to the identification of new antigens, important advances have been made in developing existing *M. catarrhalis* vaccine antigens [68–71], Table 1.

A challenge to vaccine development for *M. catarrhalis* is the lack of suitable animal models and a reliable correlate of protection. In experimental models, animals rapidly clear the organism, which is consistent with the characteristic of *M. catarrhalis* as an exclusively human pathogen. The most widely used model for assessing *M. catarrhalis* vaccine antigens has been the mouse pulmonary clearance model. While the chinchilla OM model has been useful in assessing *S. pneumoniae* and NTHi vaccine antigens, *M. catarrhalis* is cleared readily from the middle ear of chinchillas [72]. More recently, researchers have taken the approach of studying nasopharyngeal colonization and co-infection of chinchillas with either NTHi and/or viruses to study *M. catarrhalis* infections and putative vaccines [73–76]. Human challenge models have become a more widely accepted approach to assess human-specific pathogens [77]. The pneumococcal human challenge model is now well established with approximately 1,000 participants [78] and provides a suitable model to test the impact of new vaccine formulations on pneumococcal colonization prevention. An NTHi human challenge model has also been successfully undertaken in 15 adult volunteers [79], providing a model to test putative vaccine antigens and other novel therapies. Developing a human challenge model with *M. catarrhalis* may also be warranted, not only to have a relevant model to test vaccine formulations but also to better understand the role of *M. catarrhalis* in OM.

Protein-based NTHi and *M. catarrhalis* vaccines are in clinical trials (Table 1) [80]. While none of these trials are assessing impact on OM, results from healthy adults and adults with chronic obstructive pulmonary disease (COPD) will inform age de-escalation studies to eventually test these formulations for OM prevention. In view of the availability of well characterized and promising vaccine antigens and the urgent need for a vaccine to prevent OM, we anticipate and support continued investment into clinical trials to evaluate vaccine formulations that include NTHi and *M. catarrhalis* vaccine antigens, along with the multiple ongoing approaches to prevent pneumococcal OM.

Vaccination against the major bacterial otopathogens may result in replacement disease from other, previously under-appreciated, microorganisms. It is therefore essential that with the development of OM-targeted vaccines that comprehensive surveillance studies are

conducted using molecular tools to monitor vaccine impact on the respiratory tract microbiome and etiology of OM.

3.5. Novel vaccine development approaches against OM

While parenteral immunization routes (subcutaneous and intramuscular) are the mainstay approach to assess the immunogenicity and efficacy of most preclinical and clinical vaccine candidates [50–56, 81], greater emphasis has more recently been placed on alternative immunization regimens for preventing otopathogen colonization or OM. Herein we provide an update on alternative approaches being explored for delivery of novel OM vaccines.

Intranasal immunization.—Madhi *et al.* immunized mice via the intranasal (IN) route with several *S. pneumoniae*-directed antigens, including alpha-glycerophosphate oxidase (GlpO), each formulated with *Escherichia coli* heat-labile toxin (LT) as an adjuvant [82]. Subsequent IN challenge with pneumococcal serotypes 6A or 4 showed a significant reduction in nasopharyngeal bacterial load, compared to mice administered LT only. Further, Kye *et al.* administered PspA admixed with the nanoparticle-forming adjuvant, polysorbitol transporter to mice via the IN route and showed immunogen-specific immunoglobulin G (IgG) in serum maintained for 15 weeks; production of PspA-specific IgG and IgA in bronchoalveolar fluids; and complete protection against a lethal challenge dose of *S. pneumoniae* strain WU2 [83]. Additionally, Iwasaki *et al.* focused on the utility of monophosphoryl lipid A (MPL) as an adjuvant to deliver NTHi strain 76 OMP preparations to mice via the IN route [84]. The greatest NTHi OMP-specific serum IgG and nasopharyngeal immunoglobulin A (IgA) titers were achieved with delivery of NTHi OMP plus 20 μ g monophosphoryl lipid A (MPL), compared to lesser doses of adjuvant and a reduction in nasopharyngeal NTHi burden in cohorts administered NTHi OMP admixed with 10 or 20 μ g MPL was achieved, compared to controls.

Sublingual immunization.—Maseda *et al.* examined sublingual immunization (SL) of mice with phosphorylcholine conjugated to keyhole limpet hemocyanin admixed with cholera toxin (CT) [85]. This regimen was compared to IN immunization with the same formulation and contrasted to administration of CT alone. Both SL and IN approaches induced the production of phosphorylcholine- specific serum immunoglobulin M (IgM), IgA, and IgG in addition to IgA within saliva, the nasopharynx, and the vagina of mice. Additionally, antibodies in nasopharyngeal lavage fluids recognized cell membrane lysates from a panel of NTHi and pneumococcal isolates.

Transcutaneous immunization.—Novotny *et al.* examined transcutaneous immunization (TCI) to deliver an NTHi dual adhesin-targeted immunogen ('chimV4') admixed with a double mutant of *E. coli* heat-labile enterotoxin (dmLT) as an adjuvant to chinchillas via band-aid placed at the post-auricular region [57, 58]. This approach provided significant therapeutic efficacy to resolve active NTHi-induced OM. It also provided significant protection from development of NTHi-induced OM in an adenovirus-NTHi polymicrobial model of disease.

Maternal immunization.—As a strategy to protect young infants from pneumococcal OM beginning at birth, maternal immunization has been proposed with the premise that IgG antibodies induced by immunization of pregnant women are transferred across the placenta to the developing fetus. As mentioned above, Binks *et al.* demonstrated that infants from mothers that received maternal PPV23 had a 51% reduction in risk of OM due to nasopharyngeal carriage of PPV23 pneumococcal serotypes compared to infants from non-PPV23-vaccinated mothers [32]. Maternal immunization with vaccines that also impact nasopharyngeal carriage of otopathogens may further reduce OM risk in infants due to preventing mother-to-newborn transmission.

Respiratory syncytial virus (RSV) is the most common cause of lower respiratory tract infection (LRTI) in neonates and young infants and is observed in middle ear effusion as a coinfection with *S. pneumoniae*, NTHi, or *M. catarrhalis*. Maternal immunization strategies are being developed for RSV and the World Health Organization (WHO) recently published a roadmap for priority activities for the development, evaluation, licensure, and global use of RSV vaccines [86]. A Phase 3 clinical trial of an aluminum adjuvanted RSV fusion (F) protein recombinant nanoparticle vaccine in 4,636 pregnant women was recently completed. Although the study failed to meet the primary objective of medically attended significant RSV LRTI, the vaccine did show efficacy against the secondary objective of RSV LRTI hospitalization [87]. The impact of RSV immunization, particularly maternal RSV immunization, on OM outcomes in infants has yet to be assessed

3.6. New insights into innate and adaptive immunity in OM

Understanding the natural immune response to putative vaccine antigens is important to validate their inclusion in the next generation of vaccines for OM. Exposure to an otopathogen, either from colonization alone or from an episode of OM, induces measurable systemic and mucosal immune responses. Over the last four years, natural antibody titers and cell-mediated immune responses to antigens from *S. pneumoniae*, NTHi, and *M. catarrhalis* have been assessed in different cross-sectional and longitudinal cohorts of healthy and otitis-prone children to inform vaccine development.

S. pneumoniae.—While serotype-specific vaccines are licensed for use against infection with *S. pneumoniae*, these are generally targeted against the serotypes that most commonly cause invasive disease and do not provide protection against all pneumococcal serotypes. A well-researched longitudinal stringently defined otitis prone (sOP) cohort of North American children 6 to 24 months of age has provided several avenues of immune dysfunction to be considered. Including more frequent colonization with *S. pneumoniae*, lower nasopharyngeal mucosal IgG and IgA antibody titers to pneumococcal proteins PhtD, CbpA and PlyD1, and lower antibody to pneumococcal proteins and polysaccharides in comparison with non-otitis-prone (NOP) children [88–91]. The sOP children were found to have fewer memory B-cells, switched memory B-cells, and plasma cells than age-matched NOP controls [91, 92]. When assessing the relationship of specific cell types to otitis-proneness, the percentages of T-helper (Th) 1 and Th17 cells in sOP children were less than in NOP children, suggesting a role for Th17 in the sOP condition. Interestingly, reduced Th17 responses to *S. pneumoniae* could be ameliorated through the addition of Th17-

promoting cytokines [93]. The importance of Th17 responses for protection against OM was further supported by a mouse study demonstrating that a mucosal Th17 response to PspA-vaccination protects mice from experimental pneumococcal colonization and development of OM [94].

A cross-sectional study assessing IgG and IgA antibody titers to pneumococcal proteins PspA1 and 2, CbpA and Ply in serum and saliva from Australian Aboriginal and non-Aboriginal children 2 to 16 years old with a history of recurrent AOM (rAOM) revealed no reduction in antibody titers in comparison with NOP non-Aboriginal children [95]. Indeed, salivary antibody responses to these proteins were higher in Aboriginal children with a history of rAOM indicating that this may be a marker of exposure rather than protection from infection. In another Australian study of non-Aboriginal children 6 to 36 months of age with or without a history of rAOM, functional antibody responses to pneumococcal polysaccharides and Ply were determined to be similar between groups [96], demonstrating that otitis-prone children can produce functional opsonizing antibodies to pneumococcal polysaccharides and neutralizing antibodies to Ply. Together these data suggest that either antibodies may not be protective against infection and disease or, possibly, that responses to different antigens are required for protection against the OP condition.

NTHi.—With NTHi now the leading OM pathogen in many countries [45], NTHi-targeted vaccine development is essential. Several studies have investigated natural immunity to Protein D (PD) (an NTHi carrier protein in PCV10), and to PilA (an NTHi protein that is currently in a vaccine being trialed for COPD). In a Chinese population, the ontogeny of PD and P6 antibody titers was assessed in 605 subjects. Antibody to these proteins peaked between seven months and three years of age and declined to their lowest titers between 21 and 30 years of age before increasing in the elderly [97]. Importantly, in the American cohort of sOP children and NOP children, higher serum anti-PD IgG correlated with reduced risk for future AOM [98]. These data also fit with data showing that population differences in natural antibody development exist in high-risk Australian Aboriginal children and non-Aboriginal children undergoing surgery for OM, with decreased anti-PD IgG titers when compared to healthy controls [95]. Together these data suggest that PCV10-induced PD antibodies may indeed be able to impact on OM development, but also that population and age-related differences are important to assess when evaluating potential vaccine effects. Seppanen *et al.* compared the differences of innate and cell-mediated immune responses between otitis-prone and NOP children, and found that innate and T cell-mediated responses to NTHi infection were similar despite differences in PBMC composition [99].

The first NTHi-targeted vaccine to be assessed in the clinic (NTHi-10-AS01E, Table 1) contains three NTHi proteins: PilA, Protein E, and PD. This vaccine has been shown to be immunogenic in healthy adults [100] and has recently completed a Phase 2 assessment in adults with COPD [101]. Antiserum from animals vaccinated with PilA can disrupt and prevent polymicrobial NTHi and *M. catarrhalis* biofilm formation *in vitro* [102], which is a desirable property for an OM vaccine. Natural IgG titers to the putative NTHi vaccine antigen EF-Tu have been shown to increase with age and upon development of NTHi OM [60]. Antibody to NTHi proteins HMW1 and HMW2 in convalescent sera from children with NTHi OM was found to kill the infecting NTHi strain but not heterologous NTHi

strains [103], while sera from healthy adults could kill heterologous NTHi indicative of a more expansive repertoire of anti-HMW antibodies in adults (presumably from multiple exposures to different NTHi strains). The ability to elicit cross-protective antibody against multiple NTHi strains is an essential property for future NTHi vaccines.

M. catarrhalis.—*M. catarrhalis* is still a frequently observed otopathogen that plays a role in the establishment and persistence of OM. Ren *et al.* demonstrated that serum IgG to the proteins OMP CD, OppA, Msp22, Hag, and PilA2 increase between 6 and 36 months of age [104]. Interestingly noncolonized children had higher antibody titers to OppA, Hag, and Msp22 [104]. In sOP children, lower titers of mucosal and serum antibody to putative vaccine antigens were observed in comparison to NOP children [105] suggesting that these antigens warrant further investigation as potential vaccine candidates.

Overall, these data suggest that differences occur in some, but not all, cellular compartments of the immune response between otitis-prone and NOP children, which warrants further investigation. The work in this area suggests that systemic and mucosal antibody responses to antigens from the three major bacterial otopathogens can protect against development of AOM but may also represent a marker of exposure in children with recurrent AOM. Clinical trials with these antigens will reveal their true potential in preventing OM and having functional assays to measure correlates of protection for these antigens is essential for effective protein-based multi-species vaccine development.

Several studies have been carried out to investigate the role of host innate immune molecules in the pathogenesis of OM using *in vitro* and *in vivo* approaches. Cho *et al.* demonstrated that NF- κ B signaling plays an important role in mucosal hyperplasia during OM by using inhibitors of either classical or non-canonical NF- κ B activation [106]. Hafrén *et al.* [107] and Toivonen *et al.* [108] found that polymorphisms in Toll-like receptors (TLR4 and TLR2) gene locus influences the genetic predisposition to childhood OM implicating that host genetics can contribute to OM susceptibility. Kaur *et al.* examined innate immune gene expression using middle ear fluids from the American sOP cohort and found that levels of IL-8, CC chemokine ligand 3 (CCL3), and secretory leukocyte protease inhibitor were significantly upregulated, whereas levels of interferon regulatory factor 7, IFN- α , Z-DNA binding protein 1, CCL5, MAPK8, and TICAM2 were downregulated [109, 110]. Earlier studies from the same group have showed that serum concentrations of S100A12 are significantly increased in children at onset of AOM caused by *S. pneumoniae* or NTHi [111]. Interestingly, the expression of S100A12 and other S100 proteins (S100A8, S100A9) in the middle ear mucosa is also elevated in recurrent OM and chronic OM patients [112]. Consistent with these results, S100A8, S100A9, and S100A12 gene expression was also found to be elevated in human middle ear epithelial cells after pneumococcal infection.

The transcriptome profile of the middle ear mucosa isolated from mice during a complete episode of acute OM event was evaluated by microarray analysis [113]. This study identified the major cellular pathways including TLRs, NOD-like receptors, and inflammasome genes that are activated during OM to control the host immune response. Using *ccl3*^{-/-} mice, Deniffel *et al.* found that CCL3 plays a critical role in the bacterial clearance, inflammation resolution, and mucosal host defense [114]. Kurabi *et al.* analyzed the phenotypes in *Asc*^{-/-}

mice and revealed the contribution of the inflammasome and IL-1 β maturation in OM [115]. These data demonstrate that the innate immune system is critical for recovery from bacterial OM. Zielnik-Jurkiewicz *et al.* found that anti-inflammatory cytokines (IL-10 and IL-1Ra) play an important role in the pathological processes during OM [116]. Konduru *et al.* showed that MKP-1 acts as a negative regulator for NTHi-induced CXCL5 expression [117]. In addition, Lee *et al.* demonstrated that the anti-stroke drug, Vinpocetine, inhibits pneumococcal-induced MUC5AC production via upregulating MKP-1 expression [118]. These studies may lead to the development of new OM therapeutics by targeting key negative regulators of the innate immune response.

4. RECOMMENDATIONS AND IMPLICATIONS FOR CLINICAL PRACTICE

4.1. Future direction for OM vaccine and immunology research

1. Understanding population differences in natural and vaccine induced immune responses to otopathogens is important to developing effective vaccines.
2. Understanding the middle ear immune system in children may provide insight into OM susceptibility.
3. Understanding the interaction between ototropic viruses and bacteria will advance development of effective therapies.
4. Measuring the indirect effects of anti-viral or anti-bacterial vaccines on OM is important in future vaccine trials.
5. Future trials to assess vaccine impact on preventing OM should use molecular tools for otopathogen detection and surveillance to account for the role of biofilms and culture-negative OM.
6. New greater valency PCVs (15 - to 20-valent) currently in Phase 2/3 development are expected to be licensed and introduced in the next few years and replacement carriage and disease (including OM) should be monitored carefully.
7. Current pneumococcal vaccines have a limited but significant impact on OM and developing multi-species OM vaccines is essential.

4.2. Challenges with developing OM-targeted therapies

1. Funding opportunities have been limited because many funding agencies focus on high mortality diseases rather than diseases with high morbidity such as OM.
2. The increasing concern regarding rising AMR may represent an opportunity for funding further vaccine development to prevent OM.
3. Challenges with clinical trials for OM are related to either non-specific clinical endpoints or ethical issues regarding tympanocentesis in some countries.
4. Challenges exist with obtaining new vaccine regulatory approvals for NTHi and *M. catarrhalis* that are likely to require large clinical efficacy studies, which is in contrast with PCVs that have defined correlates of protection.

5. COPD has been prioritized over OM for NTHi vaccine clinical development, which may be due to COPD having a more favorable benefit-to-risk ratio in adults with existing disease versus the benefit-to-risk ratio of OM prevention in healthy infants.
6. The value proposition for OM vaccines that could entice greater investment in OM vaccines has not been fully determined. This includes direct and indirect healthcare costs, understanding the social impact (increased education and incarceration costs), and the role of treatment in driving the global AMR crisis. A large-scale health economic analysis is required to fully realize the economic and social burden of OM.

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Figure 1.

Etiologies of acute and "complicated" otitis media from studies from 2012 to present.

Top: Etiology of AOM based on results of culture and polymerase chain reaction (PCR) analysis of middle ear fluids from 11 studies from 2012 to present [13, 119–128].

Bottom: Etiology of "complicated" OM based on results of culture and PCR analysis of middle ear fluids from 9 studies from 2012 to present [119–123, 125, 127–129].

Complicated OM includes recurrent OM, treatment failure, chronic OM, and OM with effusion. Numbers at the bottom of the bars indicate the number of samples upon which results are based.

Abbreviations: *S.pn*, *Streptococcus pneumoniae*; *NTHi*, *non-typeable Haemophilus influenzae*; *Mcat*, *Moraxella catarrhalis*.

Table 1.

New vaccine formulations with antigens from NTHi and *Moraxella catarrhalis* antigens tested in humans in the last four years[†]

Clinical Trial Number (clinicaltrials.gov)	Vaccine Antigens	Phase	Study Endpoint	Status
NCT01657526	NTHi: PD, PE, PilA	2	Safety and immunogenicity (healthy adults)	completed
NCT01678677	NTHi: PD, PE, PilA	1	Safety, reactogenicity, and immunogenicity (adults: current and former smokers)	completed
NCT02075541	NTHi: PD, PE, PilA	2	Safety, reactogenicity, and immunogenicity (COPD patients)	completed
NCT03894969	NTHi: PD, PE, PilA Mcat: UspA2	2	Safety and immunogenicity (adults: current and former smokers)	planned
NCT03281876	NTHi: PD, PE, PilA Mcat: UspA2	2B	COPD symptoms (adults)	active
NCT03443427	NTHi: PD, PE, PilA Mcat: UspA2	2	Safety, reactogenicity, and immunogenicity	active
NCT03201211	NTHi: PD, PE, PilA Mcat: UspA2	1	Safety, reactogenicity, and immunogenicity (4-year follow up)	active

Abbreviations: NTHi, nontypeable *Haemophilus influenzae*; PD, Protein D; PE, Protein E; PilA, pilus A; GSK, GlaxoSmithKline; COPD, chronic obstructive pulmonary disease; Mcat, *Moraxella catarrhalis*; UspA2, ubiquitous surface protein A.

[†]None of the vaccine formulations listed in this table have been tested for impact on OM and all studies listed are sponsored by GlaxoSmithKline.