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Vaccines for Nontypeable *Haemophilus influenzae*: the Future Is Now

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Infections due to nontypeable *Haemophilus influenzae* result in enormous global morbidity in two clinical settings: otitis media in children and respiratory tract infections in adults with chronic obstructive pulmonary disease (COPD). Recurrent otitis media affects up to 20% of children and results in hearing loss, delays in speech and language development and, in developing countries, chronic suppurative otitis media. Infections in people with COPD result in clinic and emergency room visits, hospital admissions, and respiratory failure. An effective vaccine would prevent morbidity, help control health care costs, and reduce antibiotic use, a major contributor to the global crisis in bacterial antibiotic resistance. The widespread use of the pneumococcal conjugate vaccines is causing a relative increase in *H. influenzae* otitis media. The partial protection against *H. influenzae* otitis media induced by the pneumococcal *H. influenzae* protein D conjugate vaccine represents a proof of principle of the feasibility of a vaccine for nontypeable *H. influenzae*. An ideal vaccine antigen should be conserved among strains, have abundant epitopes on the bacterial surface, be immunogenic, and induce protective immune responses. Several surface proteins of *H. influenzae* have been identified as potential vaccine candidates and are in various stages of development. With continued research, progress toward a broadly effective vaccine to prevent infections caused by nontypeable *H. influenzae* is expected over the next several years.

Vaccines composed of polysaccharide capsule conjugated to protein carriers have virtually eliminated infections caused by encapsulated *Haemophilus influenzae* type b, including meningitis and other systemic infections, in regions of the world where the vaccines are administered widely. However, these conjugate vaccines have no effect on infections caused by nontypeable (nonencapsulated) strains of *H. influenzae*. Common infections cause by nontypeable *H. influenzae* include otitis media in children and lower airway infections (exacerbations) of chronic obstructive pulmonary disease (COPD) in adults. Vaccine development for nontypeable strains of *H. influenzae* presents an entirely different challenge compared to vaccines for encapsulated type b strains for several reasons: (i) nontypeable *H. influenzae* lacks a polysaccharide capsule and thus will require the identification of alternative vaccine antigens; (ii) nontypeable strains demonstrate enormous genetic and antigenic heterogeneity among strains, whereas type b encapsulated strains are generally a clonal population; (iii) the pathogenesis of infections caused by nontypeable *H. influenzae* involves contiguous spread from mucosal surfaces, suggesting that a successful vaccine will require a different immune response from that required to protect from infections by type b strains, which occur through hematogenous dissemination.

This review will assess the current state of vaccine development for nontypeable *H. influenzae*, including the rationale for developing such vaccines, the populations who would benefit from a vaccine, its feasibility, some of the challenges, the antigens under consideration, and a discussion of what is needed to advance vaccine development to prevent infections by nontypeable *H. influenzae*.

RATIONALE FOR A VACCINE FOR NONTYPEABLE *H. INFLUENZAE*

Infections caused by nontypeable *H. influenzae* cause enormous morbidity in two clinical settings: (i) otitis media in children under the age of 6 years and (ii) adults with COPD. The bacterium also causes sinusitis and community-acquired pneumonia in children and adults (1). Nontypeable *H. influenzae* causes pneumonia in children in developing countries, but its role in this infection is not yet well defined (2, 3). Finally, in regions with *H. influenzae* type b vaccination programs, nontypeable strains are now the most common cause of invasive *H. influenzae* infection, although these are far less common than otitis media and exacerbations of COPD (4–7).

Vaccine development efforts are directed primarily toward otitis media and COPD; thus, the discussion of the rationale for nontypeable *H. influenzae* vaccines will focus on these two clinical settings.

OTITIS MEDIA

Otitis media is the most frequently diagnosed bacterial infection in young children requiring office or clinic visits in the United States. Approximately 70% of children will have at least one episode of otitis media by the age of 3 years (8, 9). Acute otitis media is an inflammation in the middle ear (the cavity between the ear drum and the inner ear) that is characterized by fever and ear pain. The pathogenesis of otitis media involves migration of pathogens from the nasopharynx to the middle ear through the Eustachian tube. Most episodes of bacterial otitis media are triggered by an initial viral upper respiratory tract infection.

Recurrent otitis media is common, with up to 30% of children experiencing 3 or more episodes before the age of 3 years (10). Up to 20% of children experience 4 or more episodes of otitis media

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within a year, and these children are considered otitis prone (11). Otitis media with effusion refers to the presence of fluid in the middle ear without symptoms of acute otitis media. The persistent middle ear effusions associated with recurrent and chronic otitis media and otitis media with effusion cause conductive hearing loss and subsequent delay or impairment in speech and language development (12–14). Thus, preventing otitis media would have a potentially huge impact for the children and families who experience these disorders by preventing these important complications.

The burden of otitis media differs considerably between developing and developed countries. Chronic suppurative otitis media (defined by the World Health Organization as 2 weeks of persistent ear discharge) is a major cause of hearing loss in developing countries, affecting an estimated 65 million to 300 million people globally (15, 16). Socioeconomic conditions (overcrowding, reduced sanitation, and limited access to diagnosis and treatment) and genetic and cultural factors may all play a role in the high rate of early onset of acute otitis media and chronic suppurative otitis media in selected populations (17–19).

Etiology of otitis media. With the widespread use of pneumococcal conjugate vaccines, which dramatically alter nasopharyngeal colonization patterns, the etiology of otitis media is undergoing continuing changes. The 3 primary bacterial pathogens that cause otitis media are nontypeable *H. influenzae*, *Streptococcus pneumoniae*, and *Moraxella catarrhalis*. Since 2000, most children in the United States have received the 7-valent pneumococcal conjugate vaccine, and this has led to a reduction of otitis media and nasopharyngeal colonization by the 7 vaccine serotypes, resulting in "replacement" by nonvaccine pneumococcal serotypes, nontypeable *H. influenzae*, and *M. catarrhalis*. These changes in nasopharyngeal colonization patterns are resulting in changes in the distribution of pathogens that cause otitis media and in an increasing role for nontypeable *H. influenzae*, which is now the most common cause of acute otitis media and recurrent otitis media according to several recent studies (20–24). The introduction of the 13-valent pneumococcal conjugate vaccine and the increasing use of the 10-valent pneumococcal vaccine with *H. influenzae* protein D as the carrier molecule in ~40 countries where it is licensed will undoubtedly cause additional changes in nasopharyngeal colonization patterns and the distribution of pathogens causing otitis media (25). These patterns must be monitored carefully.

In clinical practice, otitis media is managed empirically, and so the etiology of individual cases of otitis media is rarely known by those clinicians managing such patients. The gold standard for determining the etiology of acute otitis media has been culture of middle ear fluid obtained by tympanocentesis. While studies vary, reasonable current estimates of etiology of acute otitis media based on culture report that nontypeable *H. influenzae* and pneumococcus each cause 25 to 35% of episodes and *M. catarrhalis* causes 10 to 20%.

Implications of biofilms in etiology and clinical trial design. As knowledge of the pathogenesis of otitis media has advanced and the central role of biofilms in the course of the disease has become apparent, it is clear that culture of middle ear fluid does not tell the whole story regarding the etiology of otitis media (26). A biofilm is a community of bacteria encased in a matrix. Biofilms show a reduced growth rate, a distinct transcriptome, and increased resistance to effectors of innate and acquired immunity and to the action of antimicrobial agents, compared to planktonic bacteria. Effusions recovered from the middle ear are often sterile by culture but contain abundant pathogens in the form of a biofilm. Hall-Stoodley et al. (27) showed that sterile effusions (by culture) from children with chronic otitis media and otitis media with effusion contained bacterial biofilms based on fluorescent *in situ* hybridization and confocal scanning laser microscopy. Furthermore, PCR demonstrated the presence of DNA, and live-dead fluorescent stain showed abundant viable bacteria despite negative culture. Another important observation from this study was the presence of a mixed microbial etiology for some of the effusions from children with chronic otitis media or otitis media with effusion.

Studies from several centers have demonstrated by PCR the presence of DNA from *H. influenzae*, *S. pneumoniae*, and *M. catarrhalis* in sterile middle ear fluids (28–30). Rayner et al. (31) further demonstrated the presence of mRNA, indicating the presence of viable bacteria, in effusions that were PCR positive but sterile by culture. A recent PCR study that examined middle ear effusions from 207 consecutive children who were undergoing tympanostomy placement demonstrated the presence of DNA from otitis media pathogens in 87% of children with acute otitis media and in 51% of children with otitis media with effusion (32).

Thus, the recognition of the role of biofilms in multiple forms of otitis media has important implications in understanding the etiology of otitis media and in designing rational vaccine development strategies. Relying solely on cultures of middle ear fluids as the endpoint in clinical trials of otitis media will assess only a subset of otitis media, i.e., those that are culture positive. The design of trials to assess the impact of vaccines in preventing otitis media should take into account the role of biofilms and culture-negative otitis media. Table 1 summarizes potential benefits resulting from a vaccine that will prevent otitis media caused by nontypeable *H. inflluenzae*.

TABLE 1 Rationale for vaccine development for nontypeable *Haemophilus influenzae*

Infection	Population to vaccinate	What vaccine will accomplish
Otitis media	All children beginning at 2 mo of age	Prevention of: Acute otitis media Recurrent otitis media Chronic otitis media Otitis media with effusion related to bacterial biofilm formation Chronic suppurative otitis media Reductions in: Excess antibiotic use Health care costs
Exacerbation of COPD	Adults with COPD	Prevention of: Acute exacerbations of COPD Chronic airway colonization Reductions in: Excess antibiotic use Health care costs

INFECTIONS IN COPD

COPD afflicts ~24 million Americans and is the third most common cause of death in the United States and the world (33, 34). The course of the disease is characterized by intermittent exacerbations that result in enormous morbidity, including lost work

time, doctor's office and clinic visits, emergency room visits, hospital admissions, and respiratory failure requiring mechanical ventilation. Bacteria cause approximately half of the exacerbations, with nontypeable *H. influenzae* as the most common bacterial cause (35). Antibiotic therapy results in faster recovery and fewer complications in selected exacerbations, and so many exacerbations are treated with antibiotics. A vaccine to prevent exacerbations would result in a major benefit in reducing morbidity and reducing the use of antibiotics in this population.

H. influenzae also plays a more subtle role in the course and pathogenesis of COPD. The organism is absent in healthy airways but colonizes the lower airways of adults with COPD, even during clinically stable periods. The bacteria release highly inflammatory antigens (e.g., lipooligosaccharide, outer membrane protein P6, peptidoglycan fragments, and other antigens) into the airways, contributing to airway inflammation, which is a hallmark of COPD (36). Symptoms of COPD parallel airway inflammation, suggesting that eradication of nontypeable *H. influenzae* in the airways, for example, by a vaccine, could reduce airway inflammation and thus have a beneficial effect on the course of COPD (37). Table 1 summarizes the potential benefits of a vaccine that would prevent exacerbations and eradicate airway colonization by nontypeable *H. influenzae* in adults with COPD.

VACCINES TO REDUCE ANTIMICROBIAL RESISTANCE

Widespread use of antimicrobial agents is causing a global crisis in antibiotic resistance (38, 39). Otitis media is the most common reason for children in industrialized countries to receive antimicrobial therapy, and children play an important role in transmission of resistant isolates into the community (40). Infections in COPD are one of the most common indications for antimicrobial therapy in adults. Thus, antimicrobial therapy for infections in these two clinical settings (otitis media in children and exacerbations of COPD in adults) plays an important role in driving global antimicrobial resistance. Because up to 70% of all children experience at least one episode of otitis media and up to 20% of children experience recurrent otitis media, the microbiome of a substantial proportion of the population is exposed to the antimicrobial agents used to treat otitis media.

The availability of a vaccine to prevent otitis media and exacerbations of COPD will reduce the need to treat these infections with antimicrobial agents. Indeed, the rate of antibiotic-resistant invasive pneumococcal infections has decreased in young children and older adults with the widespread use of pneumococcal conjugate vaccines (41). Palmu et al. (42) performed an innovative cluster randomized controlled trial using data from a national registry of antibiotic purchases to show that vaccination of every 5 children with the 10-valent pneumococcal *H. influenzae* protein D conjugate vaccine prevented one antibiotic purchase. A growing body of evidence supports the concept that vaccines to prevent otitis media and exacerbations of COPD will have a broad impact in reducing antimicrobial use, an important contributor to the global burden of antimicrobial resistance (43, 44).

FEASIBILITY OF A VACCINE FOR NONTYPEABLE *H. INFLUENZAE*

Pneumococcal conjugate vaccines, particularly the 10-valent pneumococcal *H. influenzae* protein D conjugate vaccine, represent a proof of principle of the feasibility of a vaccine to prevent otitis media caused by pneumococcus and by *H. influenzae* (25, 45). In a prospective clinical trial, 4,968 infants were randomized to receive the protein D conjugate vaccine or hepatitis A vaccine as a control. The vaccines were administered at the ages of 3, 4, 5, and 12 to 15 months. Children were followed for episodes of otitis media and underwent tympanocentesis and culture of middle ear fluid for clinically documented otitis media. The primary endpoint of the study was protective efficacy against the first episode of acute otitis media caused by pneumococcal vaccine serotypes. A secondary endpoint was protective efficacy against a first episode of otitis media caused by nontypeable *H. influenzae*. During the follow-up period, an efficacy of 36% for preventing a first episode of *H. influenzae* otitis media was observed (45). While improvement in this level of efficacy is needed, the observation that immunization with a surface protein of *H. influenzae* induces protection from otitis media in children is important in establishing the feasibility of vaccines to prevent otitis media caused by *H. influenzae*.

An important mechanism by which pneumococcal conjugate vaccines induce protection is through reduction of nasopharyngeal colonization by the pneumococcus (46–48). The question of whether the protein D pneumococcal conjugate vaccine also reduces nasopharyngeal colonization by *H. influenzae* was addressed by van den Bergh et al. (49), who performed a randomized controlled trial comparing the effect of the 7-valent pneumococcal conjugate vaccine (which does not contain an *H. influenzae* antigen) with the effect of the protein D pneumococcal conjugate vaccine on nasopharyngeal colonization by *H. influenzae*. The protein D pneumococcal conjugate vaccine had no differential effect on *H. influenzae* nasopharyngeal colonization compared to the 7-valent vaccine in children up to 2 years of age. The strengths of this study included the randomized controlled study design, high follow-up rate, assessment of colonization at multiple time points, use of molecular methods in addition to culture to detect bacterial density, and the inclusion of a 7-valent pneumococcal vaccine control group. Based on this study, "herd immunity" to *H. influenzae* infection is not likely to be observed with widespread use of the protein D pneumococcal conjugate vaccine, but further studies are needed.

CORRELATES OF PROTECTION

For a vaccine antigen to be effective, it must induce a protective immune response in the human host. Thus, an important consideration in vaccine development is identifying a correlate of protection. Several animal models have contributed important information on immune responses to *H. influenzae*, including pulmonary clearance and nasopharyngeal colonization models in rats and mice. The chinchilla model of otitis media is widely regarded as the best animal model for studying otitis media. The model is used extensively by multiple research groups and has played a key role in elucidating mechanisms of pathogenesis of *H. influenzae* otitis media and in assessing and prioritizing *H. influenzae* vaccine antigens (10, 50–55).

Novotny et al. (56) demonstrated that passive immunization of chinchillas with serum from children who were immunized with protein D pneumococcal conjugate vaccine conferred 34% protection against otitis media, closely paralleling the level of protection (36%) observed in the clinical trial described above (45). This important study provided a line of evidence that protection from otitis media in the chinchilla model predicts protection from otitis

TABLE 2 *Haemophilus influenzae* vaccine antigens under study

Antigen	Molecular mass (kDa)	Function	Reference(s)
Haemophilus adhesin protein (Hap)	~155	Adhesin	71–73
HMW1, HMW2	120–125	Adhesins	74–76
H. influenzae adhesin (Hia)	~115	Adhesin	77, 78
D15 protein	~80	Predicted nucleotidyltransferase	79, 80
HtrA	~46	Heat shock protein	81
P2 porin	36–42	Porin protein	65, 66, 82
Lipoprotein D	~42	Glycerophosphodiester phosphodiesterase	45, 56, 83
P5 fimbrin	27–35	Adhesin, OMP A-like protein	51, 84–86
P4 protein	~30	Acid phosphatase	87–89
Protein F	~30	Adhesin, ABC transporter	90–92
OMP 26	~26	Skp family of translocation proteins	85, 93–95
P6 protein	~16	Peptidoglycan-associated lipoprotein	96–102
Protein E	~16	Adhesin, binds IgD	103–106
PilA (type IV pilus)	~14	Adhesin, transformation	51, 107–109
Detoxified lipooligosaccharide	3–5	Endotoxin	110–113

media in humans. The study also provided evidence that protection is at least partially antibody mediated.

In addition to the chinchilla model, serum bactericidal assays are potentially useful as guides in identifying potentially protective antigens. The presence of serum bactericidal antibodies to a strain of nontypeable *H. influenzae* is associated with protection from otitis media due to that strain (57, 58). Therefore, antigens that induce bactericidal antibodies are potentially promising vaccine candidates.

APPROACH TO VACCINE DEVELOPMENT FOR NONTYPEABLE *H. INFLUENZAE*

Many research groups are taking the approach of identifying surface proteins that are conserved among strains as vaccine antigens (10, 59). A challenge to this approach is the enormous genetic heterogeneity among strains of nontypeable *H. influenzae*, resulting in sequence heterogeneity of many surface antigens (60–64). For example, the P2 porin protein, the most abundant protein on the bacterial surface, contains several surface loops that show sequence differences among strains, raising the possibility that P2 may not induce broadly reactive immune responses.

Characteristics of an ideal vaccine antigen for nontypeable *H. influenzae*. (i) Surface exposure. As noted above, *in vitro* and *in vivo* evidence indicates that humoral immunity is important for protection from nontypeable *H. influenzae* infection (56, 57). Antibodies induce protective responses by blocking adherence, directing complement-mediated killing, or opsonizing for killing, all of which require binding to the bacterial surface.

(ii) Sequence conservation among strains. A conserved antigen will induce a response that protects against all or most strains. A related strategy to identifying conserved surface proteins is identification of conserved regions of abundantly expressed surface molecules. For example the conserved regions of the P2 porin protein may represent an effective approach (65, 66). Similarly, the lipooligosaccharide molecule is a prominent surface antigen that displays antigenic variability among strains. However, a detoxified form of lipooligosaccharide utilizing relatively conserved regions of the molecule has shown promise in animal models (67, 68).

(iii) Phase variation. A vaccine antigen must be expressed by the bacterium during infection or colonization in the human host. A surface molecule that is expressed when grown *in vitro* but whose expression is shut off under *in vivo* conditions would not be an effective vaccine antigen.

(iv) Immunogenicity of the antigen. In order for an antigen to be effective, it must be capable of inducing an immune response in the target population. Otitis-prone children will benefit most from a vaccine to prevent otitis media; however, it is not yet possible to predict which children are otitis prone. Thus, a vaccine for otitis media would need to be immunogenic in infants, because an episode of otitis media in the first year of life is a risk factor for recurrent otitis media. Therefore, many authorities recommend universal vaccination for otitis media beginning in the first 2 months of life once effective vaccines are available (69, 70). Multiple protein vaccines used in routine immunization of infants reliably induce protective immune responses, providing evidence that surface proteins of *H. influenzae* will be immunogenic.

(v) Induction of protective responses. As discussed above, serum bactericidal antibodies and protection in the chinchilla model are reasonable correlates of protection. Each vaccine antigen will require testing in rigorous clinical trials as the definitive test of efficacy.

CANDIDATE VACCINE ANTIGENS

Table 2 lists candidate vaccine antigens that have many of the characteristics of vaccines noted above and are in various stages of development. This list reflects data that are available from publications in peer-reviewed journals and in the public domain. Additional antigens may be under consideration, and additional data on the antigens in Table 2 may be known but not yet widely available.

Vaccines for nontypeable *H. influenzae* are an active area of research. A search of clinicaltrials.gov using the search terms "vaccine" and "*Haemophilus influenzae*" revealed 226 registered clinical trials. A total of 25 of those involve administration of noncapsular *H. influenzae* antigens in vaccine formulations designed for preventing infections cause by nontypeable *H. influenzae*. Of those 25, 3 are actively recruiting, 2 are registered but not yet recruiting, 18 have been completed, and 2 were terminated. Many of the completed trials involve(d) testing of protein D formulations.

SUMMARY AND FUTURE DIRECTIONS

This is a dynamic and exciting time for the development of vaccines to prevent infections caused by nontypeable *H. influenzae*, in particular, otitis media in children and exacerbations in adults with COPD. A vaccine to prevent infections in these clinical settings would result in prevention of an enormous global morbidity, reduced mortality related to COPD, and billions of dollars in health care savings. The early success of protein D in preventing episodes of otitis media provides a path forward to assess additional *H. influenzae* antigens as vaccines. Several conserved surface proteins are in various stages of development as vaccine antigens. Several of these antigens are ready for preclinical and early clinical testing as vaccines for nontypeable *H. influenzae*. Investment in such studies is needed to advance the development of these important vaccines.

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