Published in final edited form as:
Otolaryngol Head Neck Surg. 2013 April ; 148(4 0): E64–E89. doi:10.1177/0194599812459636.

Panel 5: Microbiology and Immunology Panel

Timothy F. Murphy, MD[1], **Tasnee Chonmaitree, MD**[2], **Stephen Barenkamp, MD**[3], **Jennelle Kyd, PhD**[4], **Johanna Nokso-Koivisto, MD, PhD**[5], **Janak A. Patel, MD**[2], **Terho Heikkinen, MD, PhD**[6], **Noboru Yamanaka, MD, PhD**[7], **Pearay Ogra, MD**[1], **W. Edward Swords, PhD**[8], **Tania Sih, MD, PhD**[9], and **Melinda M. Pettigrew, PhD**[10]

[1]University at Buffalo, the State University of New York, Buffalo, New York, USA

[2]University of Texas Medical Branch, Galveston, Texas, USA

[3]Saint Louis University School of Medicine, St Louis, Missouri, USA

[4]Central Queensland University, Rockhampton, Queensland, Australia, USA

[5]National Public Health Institute, Helsinki, Finland, USA

[6]Turku University Hospital, Turku, Finland, USA

[7]Wakayama Medical University, Wakayama, Japan

[8]Wake Forest University, Winston-Salem, North Carolina, USA

[9]University of São Paulo, São Paulo, Brazil

[10]Yale University, New Haven, Connecticut, USA

Abstract

Objective—The objective is to perform a comprehensive review of the literature from January 2007 through June 2011 on the virology, bacteriology, and immunology related to otitis media.

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

Corresponding Author: Timothy F. Murphy, MD, University at Buffalo, Clinical and Translational Research Center, 875 Ellicott Street, Buffalo, NY 14203, USA, murphyt@buffalo.edu.

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

Author Contributions

Timothy F. Murphy, assisted in coordination of postsymposium meeting, substantial contributions to writing and editing; **Tasnee Chonmaitree**, coordinated writing of virology section, substantial contributions to writing and editing; **Stephen Barenkamp**, coordinated writing of bacteriology section, substantial contributions to writing and editing; **Jennelle Kyd**, coordinated writing of immunology section, substantial contributions to writing and editing; **Johanna Nokso-Koivisto**, substantial contributions to writing and editing; **Janak A. Patel**, substantial contributions to writing and editing; **Terho Heikkinen**, substantial contributions to writing and editing; **Noboru Yamanaka**, substantial contributions to writing and editing; **Pearay Ogra**, substantial contributions to writing and editing; **W. Edward Swords**, substantial contributions to writing and editing; **Tania Sih**, substantial contributions to writing and editing; **Melinda M. Pettigrew**, substantial contributions to writing and editing.

Disclosures

Competing interests: Timothy F. Murphy is a consultant for GSK, Intercell, and Ancora and holds patents related to vaccines. Jennelle Kyd received funding from Intercell and holds patents relating to vaccines. W. Edward Swords receives research funding from AstraZeneca.

Review Methods—Three subpanels with co-chairs comprising experts in the virology, bacteriology, and immunology of otitis media were formed. Each of the panels reviewed the literature in their respective fields and wrote draft reviews. The reviews were shared with all panel members, and a second draft was created. The entire panel met at the 10th International Symposium on Recent Advances in Otitis Media in June 2011 and discussed the review and refined the content further. A final draft was created, circulated, and approved by the panel.

Conclusion—Excellent progress has been made in the past 4 years in advancing an understanding of the microbiology and immunology of otitis media. Advances include laboratory-based basic studies, cell-based assays, work in animal models, and clinical studies.

Implications for Practice—The advances of the past 4 years formed the basis of a series of short-term and long-term research goals in an effort to guide the field. Accomplishing these goals will provide opportunities for the development of novel interventions, including new ways to better treat and prevent otitis media.

Keywords

otitis media; virology; immunology; microbiology; bacteriology

Otitis media is caused by viral and/or bacterial infection of the middle ear space and the resulting host response to infection. The microbiology and immunology of otitis media have been the subject of tremendous research efforts over the past 4 years by a large number of researchers throughout the world. This work has resulted in advances in understanding mechanisms of microbial pathogenesis, molecular epidemiology, genomics, identification of new viruses, polymicrobial interactions, and other areas. Work on the immunology of otitis media has resulted in advances in understanding susceptibility to infection and also in elucidating the role of host responses in the pathogenesis of otitis media.

The goal of this panel report is to provide a comprehensive review of research in the virology, bacteriology, and immunology of otitis media over the past 4 years.

Methods

To review a broad and diverse discipline—actually, 3 disciplines (virology, bacteriology, and immunology) related to otitis media—3 subpanels were created with co-chairs. The members of each panel reviewed PubMed to identify relevant articles published between January 2007 and June 2011. All types of articles were included (original research articles, reviews, editorials) with the only restriction being English language.

Panel members reviewed assigned areas, wrote initial drafts summarizing the areas, and shared the drafts with members of the subpanel. Members of subpanels worked together to include additional relevant studies and minimize redundancy. A draft of the full document was circulated to all panel members in advance of the 10th International Symposium on Recent Advances in Otitis Media, where the panel met and reviewed the draft and discussed the literature. Based on these discussions, additional pertinent articles were identified for inclusion, and Research Goals for the next 4 years were developed. A revised draft of the

report was circulated to the panel for further comment and approval following the meeting at the symposium.

Discussion

Virology

Viral Pathogenesis—Viral upper respiratory tract infection (URI) usually precedes or coincides with acute otitis media (AOM). Recent research has focused on the establishment of the animal model to study viral pathogenesis related to OM, the role of new respiratory viruses in AOM development, and genetic factors that lead to variation of immunologic responses during viral URI and AOM.

Grieves et al[1] studied respiratory syncytial virus (RSV) pathogenesis in chinchillas to investigate how viral URI leads to AOM. After nasal RSV challenge, viral replication was seen from the site of inoculation to the pharyngeal orifice of the eustachian tube by 48 hours, and the virus could be detected in the distal part of the eustachian tube after 5 days. Although the virus was not detected in nasopharyngeal lavage fluids 14 days after infection, occasional clusters of immunopositive cells were present, which might explain viral persistence and polymerase chain reaction (PCR)–positive findings in asymptomatic subjects.

There have been further studies to investigate the immunologic responses to viral URI, specifically whether various cytokines or viruses have stronger impact on AOM development following URI. In a study of 151 children with 326 URI episodes, adenovirus and influenza virus infections induced higher interleukin (IL)–6 concentrations in nasopharyngeal secretions (NPS) compared with other viruses.[2] Concentrations of IL-1β were associated with AOM development following URI. When cytokines were measured from sera, high granulocyte colony-stimulating factor (G-CSF) concentration was associated with RSV-induced AOM, and high IL-13 concentration predicted early clinical failure of antibiotic treatment.[3] In a study of children with AOM and spontaneously perforated tympanic membrane, cytokine levels in migration inhibitory factor (MEF) were unrelated to the presence or absence of virus; however, the sample size and the rate of detected viruses were low in this study.[4]

There is increasing interest in genetic factors that lead to variation in immunologic responses to viral infections, which in turn may explain the differences in susceptibility to URI and AOM. Among 242 children followed for 1 year, children with IL-6^{-174} polymorphism had increased susceptibility to viral URI.[5] In addition, tumor necrosis factor (TNF)–α^{-308} polymorphism was associated with increased risk for AOM complicating URI. In another study, during RSV and rhinovirus infections, occurrence of a new otitis media (OM) episode was more frequent in patients with IL-$10^{-1082, -819, -592}$. In addition, during URI caused by rhinovirus, young age and IL-6^{-174} and TNF-α^{-308} polymorphisms predicted development of AOM.[6]

The pivotal role of viral infection in the pathogenesis of AOM was again confirmed by results from a recent randomized, double-blind controlled trial that assessed the efficacy of

early oseltamivir treatment of influenza in children 1 to 3 years of age.[7] In children for whom the antiviral treatment was started within 12 hours of the onset of symptoms, the incidence of AOM development during the influenza illness was decreased by 85% in children with any influenza and by 79% in children with influenza A.

Epidemiological and Clinical Aspects—Epidemiologic studies have shown a strong relationship between viral URI and AOM. Since the last report, progress has been made on studies of newly discovered viruses, viral transmission, and persistence.

Respiratory syncytial virus and adenoviruses are still among the most important viruses associated with AOM. In a prospective, longitudinal study of children younger than 4 years in the United States, 63% of 864 URI episodes were positive for respiratory viruses; rhinovirus and adenovirus were most frequently detected.[8] Of URI caused by a single virus, the rate of AOM complicating URI was highest in the episodes caused by adenovirus, coronavirus, and RSV. In a study from Japan, a respiratory virus was detected in 35% of 1092 children with AOM; the most common viruses were RSV, influenza virus, and adenovirus.[9] A study from Iceland reports that infants who have RSV infection early in life have increased risk for AOM during the following year compared with infants without RSV infection.[10] However, the rate of AOM following URI caused by various viruses may differ based on the local epidemiology of respiratory viral infections. It might be possible that different serotypes of the specific virus are associated with varying degrees of predisposition to AOM. For example, in Taiwan during an adenovirus type 3 outbreak in 2004 to 2005, the reported rate of AOM in children with adenovirus infection varied between 6.5% and 16.3%,[11] which is much lower than the 47% rate of AOM following adenovirus URI in a 4-year study performed in the United States.[8]

New respiratory viruses: Molecular technologies have made the detection of previously unknown or undiscovered viruses possible and have advanced studies of the relationship between these viruses and AOM.

Human metapneumoviruses (hMPVs) were discovered a decade ago, and they are now recognized as an important pathogen causing lower respiratory tract infection and URI in children. In a cohort of 1338 children with respiratory symptoms, hMPV was detected in 3.5% of the children, and 41% of infections were complicated by AOM.[12] The incidence of hMPV was highest in children younger than 2 years (7.6%); 61% of children <3 years of age had hMPV infections complicated by AOM.

Human bocavirus (hBoV) was discovered in 2005; to date, the significance of hBoV in causing symptomatic illness is still controversial. Human bocavirus occurs frequently in conjunction with other viruses and seems to persist for a long time in the respiratory tract. In asymptomatic children, hBoV has been detected from respiratory specimens at an alarmingly high rate (43%–44%).[13,14] In children with AOM, Beder et al[15] have reported an hBoV detection rate of 6.3% from nasopharyngeal secretions and 2.7% of middle ear fluids. The resolution time of AOM was longer and the rate of fever was higher in children with hBoV. The virus has also been detected from 3% of the middle ear fluids from young

children with otitis media with effusion.[16] The role of this virus in AOM and OME requires further investigation.

The new and old picornaviruses have also been studied in association with AOM. In young children with AOM, a new rhinovirus, HRV-C, was detected in almost half of the rhinovirus-positive NPS and MEF samples.[17] Another new picornavirus, enterovirus 104, was found in 8 children from different regions of Switzerland who had respiratory illnesses, including AOM.[18] Of 36 children who were seropositive for parechovirus 1, 50% had AOM, and parechovirus 1 RNA was detected from MEF and/or NPS in 15% of children with otitis media.[19]

In a study of 495 children with AOM in Japan, Yano et al[20] found 12 (2.4%) cases with cytomegalovirus (CMV) infection; 5 of these cases (3–25 months of age) were primary CMV infection or reactivation documented by IgM serology.[20] Four of these 5 had CMV or viral nucleic acids in the MEF; 2 of 5 had no bacteria cultured from the MEF. The investigators suggested the role of CMV in AOM etiology. Similar findings have previously been reported. Because CMV is a rare cause of viral URI in young children, it is likely that the contribution of this virus to AOM is limited although possible.

Viral persistence and transmission: Although symptoms of viral URI usually last for about 1 week, viral shedding from the nasopharynx may last up to 3 weeks or longer. The introduction of more sensitive detection methods for viral nucleic acids has made the interpretation of virus diagnostic results more difficult in terms of its relationship to the disease. Therefore, studies of viral persistence in the nasopharynx, viral transmission, and asymptomatic infections have become more important in understanding the pathogenesis of URI and AOM.

In a longitudinal study of young children followed for 1 year each for occurrences of URI and AOM, 76 children had ≥4 URI episodes in 6 months.[21] Of 581 URI episodes in these frequently infected children, 510 viruses were detected; 15% of the viruses, as detected by PCR, were also detected in the previous URI episodes. Viruses associated with repeated detection included adenovirus, rhinovirus, and enterovirus. By genetic sequencing of the repeatedly positive adenoviruses, the investigators detected the same viral serotype and strain continuously or intermittently for up to 203 days; they also detected different serotypes or strains sequentially.[21] Therefore, repeated virus-positive samples may represent a new serotype or strain as a new infection or persistence of the viral nucleic acids of the older infection. Martin et al[13] studied extended shedding of hBoV in nasal secretions taken 1 month apart in 3 large daycare centers; they observed viral shedding for up to 75 days. The authors suspected that hBoV shedding may increase the duration of respiratory symptoms caused by other pathogens.

Respiratory viruses also transmit between children very efficiently. In a study by Alper et al,[22] 2 siblings from 69 families were followed for 6 months; 27% of the URI episodes in 1 sibling occurred after respiratory infection onset in the other with a median interval of 3 days.[22] Sixty-two percent of newly diagnosed OM episodes occurred during a respiratory infection, and 27% of respiratory infections were complicated by OM. The same group also

showed that new OM was associated with the presence of virus in the nasopharynx irrespective of the presence or absence of symptomatic URI.[23] There was no significant difference between various respiratory viruses.

Viral-Bacterial Interactions—Pathogenesis of AOM involves complex interactions between viruses and bacteria; acute viral infection of the nasopharynx creates the environment that promotes the growth of pathogenic bacteria, which already colonize the nasopharynx and promote their adhesion to the epithelial cells and invasion into the middle ear. New data that further elucidate the detailed mechanisms are described below.

Respiratory syncytial virus nasal inoculation in chinchillas reduced the expression of the antimicrobial peptide, chinchilla β-defensin 1, and increased the load of *Haemophilus influenzae* in the nasopharynx.[24] Infection of the airway with a respiratory virus downregulates the expression of β-defensin, which increases the nasopharyngeal colonization with *H influenzae* and further promotes the development of AOM. In a mouse model, Sendai virus coinfection with *Streptococcus pneumoniae* and *Moraxella catarrhalis* increased the incidence rate, duration of AOM, and bacterial load.[25]

Viral-bacterial interactions have also been studied in humans. A community-based cohort study was conducted in Australia to investigate the high rates of AOM and OME in the Aboriginal population.[26] Relatively high rates of respiratory viruses were found from nasopharyngeal samples in asymptomatic children: 42% from Aboriginal and 32% from non-Aboriginal children. Rhinovirus was most frequently detected, with a significantly higher rate from Aboriginal children. The detection of rhinovirus or adenovirus in the nasopharynx was positively associated with the presence of *H influenzae* (Aboriginal children) and *M catarrhalis* (Aboriginal and non-Aboriginal children). However, adenovirus was negatively associated with *S pneumoniae* in Aboriginal children. In a study from Japan, 31% of hospitalized children with RSV had AOM.[27] The children with AOM more often seemed to have had β-lactamase nonproducing ampicillin-resistant *H influenzae* in nasopharyngeal culture compared with children without AOM, but the difference was not significant.

Viral Diagnostics—Molecular detection methods and diagnosis of viral infections have been rapidly evolving during the past decade. Discovery of new viral pathogens has also increased the demand for new and accurate detection methods. In viral diagnostics, important aspects are the tissue sample type, sample collection technique, detection method used, and interpretation of results.[28] Use of flocked swabs to obtain NPS sample seems to be as sensitive as nasal aspirates but easier to perform.[29] Also, a combined nose and throat swab specimen is nearly as sensitive as nasopharyngeal aspirate samples and yet less laborious.[30]

The use of nucleic acid amplification methods is continuously evolving; these methods provide fast and sensitive testing for respiratory viruses. In-house or commercial multiplex PCR techniques enable rapid testing for numerous viruses simultaneously. However, nucleic acid tests have made the interpretation of the positive results demanding. As discussed earlier, viral RNA/DNA can be detected from asymptomatic patients, over a prolonged

period, or more viruses may be detected simultaneously.[28] One way to determine the dominant virus in case of multiple virus detection or to associate the presence of the specific virus with clinical symptoms is by virus quantification. In the future, studies to associate viral load with URI outcome and development of AOM will be important. The role of different viruses and viral loads in viral-bacterial interactions also will need to be addressed.

Bacteriology

Streptococcus pneumoniae—Areas of advancement since 2007 include genomics, the role of biofilm formation in disease, mechanisms of pathogenesis, the development of novel animal models, molecular epidemiology, and insight into polymicrobial interactions with other co-colonizing species.

Genomics and population biology of *S pneumoniae*: Donati and colleagues[31] compared the genomes of 44 *S pneumoniae* and related commensals. These data confirm that *S pneumoniae* strains evolve primarily by homologous recombination, with *Streptococcus mitis* serving as the main genetic reservoir. With the exception of serotype 1, phylogeny was not associated with serotype and did not correlate with tissue-specific disease or geography. The *S pneumoniae* pan-genome, which is the total genome available to the species, contained 3221 genes. Approximately 52% of *S pneumoniae* genes were categorized as core, 48% as dispensable, and 12% as strain specific. The authors evaluated the distribution of 47 genes encoding virulence-associated and surface-exposed proteins, including several vaccine candidates. Core genes were often highly variable, and noncore genes were often acquired and lost. These data have important implications for vaccine design; subunit vaccines based on genetically variable or noncore genes might be subject to allelic replacement or discarded under immune selective pressure.

The theme of genetic plasticity in pneumococci was reinforced by efforts to reconstruct the natural history of the multidrug resistant Spain$^{23F\text{-}1}$ clonal lineage.[32] Comparative whole-genome sequencing of 240 PMEN1 isolates collected between 1984 and 2008 demonstrated that 74% of the reference genome had undergone recombination in at least one isolate. Recombination hotspots included capsule-encoding loci, antimicrobial resistance-encoding determinants, and potential protein vaccine targets. Ten capsule switches were identified, including a switch to PCV-7 vaccine-escape serotype 19A. Resistance to fluoroquinolones, rifampicin, and macrolides arose on several independent occasions. Hanage and colleagues[33] compared 6 loci in 1930 distinct *S pneumoniae* genotypes and 94 mitis group streptococci to identify instances of admixture between populations. They identified a subset of highly mosaic *S pneumoniae* strains with a history of hyper-recombination. Strains from this group were more likely to be resistant to several classes of antibiotics, perhaps because of their enhanced ability to acquire foreign DNA.

Hiller and colleagues[34] conducted whole-genome sequence analyses of *S pneumoniae* to demonstrate in vivo horizontal gene transfer. Six *S pneumoniae* nasopharyngeal isolates were collected during a 7-month period from a single child with chronic respiratory tract infection and OM. Three of the isolates were sequentially derived through multiple recombination events with a fourth donor strain. Recombination also occurred with an

unidentified donor. The authors estimated that 23 chromosomal segments, covering 7.8% of the genome, were exchanged. In vivo horizontal gene transfer may allow *S pneumoniae* to evade the host immune response during chronic colonization and OM.

Comparative genome sequencing analyses were used to identify antimicrobial resistance mutations and *S pneumoniae* bacteriophage. Novel modes of resistance to linezolid were identified using in vivo selection of resistance followed by whole-genome sequencing of isolates.[35] Mutations were identified in ABC transporters and in an rRNA methyltransferase. The majority of *S pneumoniae* clinical isolates contain bacteriophage, but their precise role in pathogenesis is unknown. Bacteriophage genomes were sequenced from 10 different *S pneumoniae* strains.[36] The phage genomes were grouped into 3 main classes. Additional findings included the identification of genes homologous to known phage-encoded virulence genes from other bacteria species and the presence of a toxin-antitoxin system. A PCR-based typing system was developed to identify and distinguish each of the 3 *S pneumoniae* bacteriophage groups.[37] The sequences of these pneumococcal phage genomes will facilitate understanding of the role of *S pneumoniae* bacteriophage in OM pathogenesis.

These newer genomic studies of *S pneumoniae* demonstrate (1) a high degree of genomic plasticity in *S pneumoniae*, which enhances their ability to adapt to clinical and public health interventions on a global scale. (2) In vivo horizontal gene transfer occurs and likely allows pneumococci to rapidly adapt to immune selection pressures encountered during colonization and infection. (3) Comparative genome analyses will continue to reveal novel modes of resistance to antibiotics and facilitate greater understanding of the biology of *S pneumoniae*.

Mechanisms of pathogenesis: Biofilm formation: Biofilms play an important role in OM pathogenesis. *Streptococcus pneumoniae* formed biofilms in vivo in the experimental chinchilla model of OM.[38] Viable *S pneumoniae* were present 12 days after infection, host cells were observed throughout the biofilm, and biofilm development was associated with the formation of neutrophil extracellular traps.

Additional work by William Swords's research group has provided valuable insight into the role of coinfections in *S pneumoniae* biofilm formation.[39] Compared with *S pneumoniae* alone, *S pneumoniae* biofilms are larger and form at a higher frequency in the presence of *H influenzae*. Intriguingly, chinchillas were more likely to develop invasive disease when *S pneumoniae* was inoculated alone compared with *H influenzae*. Thus, coinfections may actually alter the course of infection. Recently, members of this same research group conducted coinfection studies with *S pneumoniae* and a β-lactamase–producing *H influenzae* strain or its β-lactamase–deficient isogenic mutant. Susceptible *S pneumoniae* obtain protection from antibiotics through the production of *H influenzae* β-lactamases and in biofilms.[40]

Investigators are beginning to untangle the roles of neuraminidase and sialic acid in biofilm formation. Neuraminidase cleaves sialic acid from glycoconjugates in the upper airways. Neuraminidase A (NanA) is important for *S pneumoniae* biofilm formation.[41] Small-molecule inhibitors of NanA disrupt biofilm formation in vitro; the greatest effect has been

observed using the lead compound XX1. Trappetti and colleagues[42] also demonstrated the importance of sialic acid in *S pneumoniae* biofilm formation, suggesting that sialic acid serves as a signaling molecule that stimulates increased *S pneumoniae* biofilm formation and bacterial load, thereby facilitating the spread of *S pneumoniae* to other tissue sites.

Thus, significant advances in understanding biofilms of *S pneumoniae* include the following: (1) *S pneumoniae* biofilms form in vivo and are accompanied by the formation of neutrophil extracellular traps. (2) The presence of other bacterial pathogens (ie, *H influenzae*) in *S pneumoniae* biofilms may alter the effectiveness of antimicrobials and the outcome of infection. (3) Sialic acid and *S pneumoniae* encoded neuraminidases play a critical role in colonization and biofilm formation. (4) Novel strategies to prevent pneumococcal OM may arise through additional research on biofilms.

Mechanisms of pathogenesis: Tissue-specific virulence: Results from the first signature-tagged mutagenesis (STM) screen for OM showed that of 5280 *S pneumoniae* STM mutants inoculated directly into the middle ear, 248 were attenuated for OM in the chinchilla model.[43] These mutations were mapped to 169 different genes. The OM-attenuated mutants included pneumococcal surface protein A (PspA), choline binding protein A (CbpA), and RlrA, which is a transcriptional activator for the pilus encoding the *rlrA* pathogenicity islet, and others mapped to genes encoding transport, cellular processing, and transcriptional functions. However, the majority of mutations were identified in genes of unknown function (n = 66, 39%). Only 31% of the genes identified in the OM screen were critical for colonization in a mouse colonization model.

Serotype 19A was a major cause of replacement disease following introduction of PCV-7.[44–46] Thomas et al[47] studied the genetic diversity and virulence of strains of similar genetic background (clonal complex 199) expressing 2 different serotypes (19A and 15B/C). The CC199 phylogeny split into a predominantly carriage isolate clade and a disease isolate clade. The ability to colonize and cause acute OM in chinchillas did not differ by serotype. A screen of a large panel of clinical isolates resulted in the identification of 4 genetic regions that were at higher prevalence in middle ear isolates, including SP0463 (*rrgB*), which is on the *rlrA* pathogenicity islet. Earlier observations from the same group indicated similar fitness for OM in the chinchilla model when serotype 19A and 15B/C isolates were inoculated together in competition.[48] Serotype 15B/C is not included in second-generation conjugate vaccines.

Forbes et al[49] studied 14 *S pneumoniae* strains in the chinchilla model through direct inoculation into the tympanic bullae and demonstrated that strains of the same *S pneumoniae* serotype differ in their ability to cause OM and invasive disease.

Taken as a whole, these data indicate the following: (1) although the polysaccharide capsule is a critical virulence determinant, additional genetic loci influence tissue-specific virulence potential in *S pneumoniae*. (2) Additional research is needed to define the role of the many unknown and hypothetical proteins in *S pneumoniae* pathogenesis.

Mechanisms of pathogenesis: Complement: The *S pneumoniae* serotype 6A isolates were compared in their ability to bind complement C3.[50] There were no significant differences between high- and low-complement binding strains in the level of nasopharyngeal colonization in the chinchilla model. In contrast, high-complement binding strains were less capable of causing OM. Tong and colleagues[51] inoculated 2 different pneumococcal serotypes into the middle ears of a series of mice deficient in complement C1qa, factor B, or factor B and C2. Both the classical and alternative pathways were critical for protecting the host against pneumococcal OM. In vitro data support that the *S pneumoniae* capsule inhibits complement deposition by both the classical and alternative pathway.[52] The *S pneumoniae* serotypes differ in their susceptibility to complement deposition and in their resistance to killing by opsonophagocytosis.[53,54] Therefore, virulence factors, in addition to the polysaccharide capsule, are important in limiting complement deposition.[50,54] Virulence factors of importance include NanA, which was shown to work together with the β-galactosidase, BgaA, and an *N*-acetylglucosaminidase, StrH, to facilitate *S pneumoniae* resistance to complement and killing by neutrophils.[55]

Mechanisms of pathogenesis: Glycosidases: The importance of glycosidases in *S pneumoniae* pathogenesis is multifactorial.[56] A surface-associated O-glycosidase, encoded by SP0368, cleaves sialylated core-1 O-linked glycans in the upper airways. Deletion mutants exhibit reduced adherence to human epithelial cell lines and reduced colonization in mice.[57] Mucins protect mucosal epithelial cells by trapping bacteria and viruses for mucociliary clearance. NanA expression is upregulated in the presence of mucin.[58] Mucins can also provide a source of nutrients for *S pneumoniae*. Terra and colleagues[59] characterized a newly identified *S pneumoniae* galactosidase encoded by SPD_0065. Expression was induced when glycoconjugates or mucin were provided in vitro. Deletion mutants grew more slowly in mucin-containing media and were less capable of cleaving galactose. Galactosidase activity was critical for colonization in a murine model but not for bacteremia or pneumonia.[59] The overproduction of mucin is associated with OM in children. MUC5AC is a mucin-encoding gene that plays an important role in the pathogenesis of OM. *Streptococcus pneumoniae* and *H influenzae* were shown to synergistically induce transcription of MUC5AC in human epithelial cell lines.[60]

Mechanisms of pathogenesis: Lysozyme: Lysozyme serves as a host innate immune antimicrobial by degrading peptidoglycan in bacterial cell walls. PgdA and Adr modify the structure of *S pneumoniae* peptidoglycan and have been implicated in resistance to the antimicrobial properties of lysozyme. Davis et al[61] studied wild-type *S pneumoniae* and single or double mutants in *pgd*A and *adr* in competition in a nasal colonization model using lysozyme-sufficient and lysozyme-deficient mice. These studies demonstrate that: (1) both PgdA and Adr are required for *S pneumoniae* resistance to lysozyme, and (2) PgdA- and Adr-mediated peptidoglycan modifications are associated with reduced fitness of *S pneumoniae*. However, this reduced fitness is outweighed by the beneficial effect of resistance to lysozyme in vivo. Members of David Lim's group established the importance of lysozyme for defense against pneumococcal OM.[62] Lysozyme M–deficient mice were more susceptible to pneumococcal OM and experienced more inflammation.

Mechanisms of pathogenesis: *S pneumoniae* pilus: Since the discovery of the *S pneumoniae* pilus, major advances in the understanding of its structure, function, and antigenic diversity have been made. The *S pneumoniae* type 1 pilus is composed of 3 structural subunit proteins, RrgA, RrgB, and RrgC, which are encoded in the *rlr*A pathogenicity islet. RrgA is required for pilus-mediated adherence.[63,64] RrgA mutants, but not RrgB or RrgC mutants, exhibit defects in biofilm formation.[65] High variability in RrgB makes this protein less attractive as a vaccine candidate in comparison to RrgA and RrgC.[66] There are 2 clades of RrgA; these variants have similar adhesive properties and elicit cross-protection upon passive immunization in mice.[67] Several research groups have elucidated the critical role of sortases in pilus assembly.[68–71] Type 1 pili are regulated by complex 2-component regulatory systems, and their expression is dependent on phase of growth.[72,73]

The *rlr*A pathogenicity islet is present in approximately 30% of *S pneumoniae* isolates and half of antibiotic-resistant strains.[66] A second pilus-encoding locus is present in approximately 16% of isolates.[63] The prevalence of *S pneumoniae* strains carrying both type 1 pilus and type 2 pilus has increased in recent years, corresponding with increases in the prevalence of non-vaccine-covered serotypes.[74,75]

Mechanisms of pathogenesis: Additional papers of interest: Neutrophils are central to defense against *S pneumoniae*.[56] A new role in pathogenesis has been identified for the cytolytic pore-forming toxin, pneumolysin (Ply).[76] Upon autolysis, Ply activates NADPH oxidase, thereby generating the release of reactive oxygen species into intracellular vesicular compartments within neutrophils.

In summary, recent work on the pathogenesis of *S pneumoniae* indicates that (1) *S pneumoniae* strains that limit complement deposition are more pathogenic for OM. Additional research is needed to (a) clarify the respective roles of capsule and other virulence determinants in complement binding and (b) define the respective roles of the classical and alternative complement pathway. (2) *Streptococcus pneumoniae* express a number of glycosidases that are important for colonization of the respiratory tract. (3) Lysozyme is critical for host defense against pneumococci. (4) The *S pneumoniae* pilus is present in a subset of strains and is being studied as a potential vaccine candidate. Genetic variability among pilus subunits will likely present challenges for vaccine design.

Animal models of disease: Progress has been made in the development of animal models of pneumococcal OM.[77–79] Experimental OM models often involve the direct inoculation of pathogens into the middle ear. A noninvasive mouse model has been developed to study pneumococcal OM.[80] The model involves intranasal inoculation of mice with *S pneumoniae* and a pressure cabin to facilitate the translocation of *S pneumoniae* from the nasopharynx into the middle ear space. A similar noninvasive method has also been developed to study *S pneumoniae* biofilm formation in rats.[81]

A ferret model has been developed to study *S pneumoniae* transmission.[82] McCullers and colleagues[82] used sets of infected and uninfected ferrets to show that prior infection with influenza increases the level of *S pneumoniae* colonization; the proportion infected; the severity of diseases, including OM; and the transmission of *S pneumoniae* to other animals.

This model also indicated that prior influenza infection increases susceptibility to acquiring *S pneumoniae*.

The chinchilla model has been used to elucidate the impact of *S pneumoniae*–mediated inner ear damage and hearing loss. Steven Juhn's group demonstrated that *S pneumoniae* mutants lacking pneumococcal surface protein A (PspA) and pneumococcal surface antigen A (PsaA) could not pass through the round window membrane into the inner ear.[83] The *S pneumoniae* strains induced pathologic changes in the inner ears of chinchillas and hearing loss 28 days after infection.[84]

In summary, (1) new noninvasive rodent models of pneumococcal OM have been developed. (2) A novel model has been developed to study *S pneumoniae* transmission. (3) Progress has been made in understanding hearing loss associated with *S pneumoniae*.

Molecular epidemiology of *S pneumoniae*: Otitis media is one of the most common infections in infants and young children and is associated with excess antibiotic use.[85–87] The incidence of OM decreased in the United States following introduction of the 7-valent pneumococcal conjugate vaccine (PCV-7) in 2001.[85–87] Further declines in OM incidence may be achieved with PCV-13, which was introduced in 2010. However, concerns remain regarding the lack of PCV coverage in many developing countries and the potential for increases in OM due to nonvaccine serotypes and antimicrobial-resistant *S pneumoniae*.[85,86,88]

Several new serotypes of *S pneumoniae* have been described since the last panel report. Serotype 6C was identified in 2007 in a subset of *S pneumoniae* classified as 6A by the Quelling reaction.[89] The prevalence of serotype 6C isolates has increased in the United States over recent years.[90,91] Serotype 6C has been identified in middle ear fluid isolates.[92] An experimentally induced alteration in the capsule encoding the operon of 6B resulted in the creation of related serotype 6D.[93] Naturally occurring carriage isolates of serotype 6D were first identified in Fijian children.[94]

Over the past decade, serotype 19A emerged as a major cause of acute OM, recurrent OM, and severe mastoiditis.[44–46] The increase in 19A was often attributed to introduction of PCV-7. However, Dagan and colleagues[95] described the emergence of serotype 19A as a cause of OM prior to introduction of PCV-7 in Israel. Analysis of antibiotic administration patterns suggests that antibiotic use may contribute to the emergence of certain lineages of *S pneumoniae*.[96]

In summary, molecular epidemiologic studies have indicated that (1) new *S pneumoniae* serotypes continue to be discovered, and more are likely to evolve. (2) PCV-7 may not be the sole reason for observed increases in serotype 19A. (3) Antibiotic pressure may contribute to the emergence of multidrug-resistant strains. (4) Serotype replacement continues to be a concern.

Polymicrobial interactions: Krishnamurthy and colleagues[25] used a murine model of nasal colonization and acute OM to study relationships among various combinations of bacterial OM pathogens (*S pneumoniae*, *H influenzae*, and *M catarrhalis*) and Sendai virus, which is

the murine equivalent of human parainfluenza virus. As expected, viral infection significantly increased the incidence of acute OM. Coinfections with *S pneumoniae* and *M catarrhalis* increased the incidence and duration of pneumococcal OM compared with *S pneumoniae* alone and *S pneumoniae* and *H influenzae* together.

Pettigrew and colleagues[97] showed that the risk of *S pneumoniae* colonization in children during upper respiratory tract infection differed based on whether *H influenzae* and *M catarrhalis* also co-colonized. Colonization by *S pneumoniae* was negatively associated with colonization by *H influenzae* when *M catarrhalis* was absent. However, when *M catarrhalis* was present, *S pneumoniae* was positively associated with colonization by *H influenzae*. Negative associations were also identified between *S pneumoniae* and *Staphylococcus aureus* and between *H influenzae* and *S aureus*. High-throughput 454-based pyrosequencing of 16S rRNA genes was used to compare microbial communities in the upper respiratory tract of children experiencing upper respiratory tract infection with and without concurrent OM.[98] Commensals such as *Corynebacterium* and *Dolosigranulum* were protective for both *S pneumoniae* colonization and OM. Commensals of the genera *Actinomyces, Rothia, Neisseria*, and *Veillonella*, which are not considered OM pathogens, were associated with an increased risk of OM. These data support the contention that vaccination and treatment strategies that target individual bacterial species could alter competitive interactions, the nasopharyngeal flora, and disease outcome.

Selva and colleagues[99] have elucidated the underlying mechanism, which involves a novel *S pneumoniae* interspecies competition strategy that selectively kills lysogenic *S aureus. Streptococcus pneumoniae* release H_2O_2, which activates the *S aureus* SOS DNA repair stress response. In turn, the SOS response triggers the lytic cycle of *S aureus* prophage, thereby killing *S aureus*. Even though *S pneumoniae* also carry lysogenic prophage, their SOS response and phage are not activated upon exposure to H_2O_2.

Host competition may also affect the selection of virulence characteristics in *S pneumoniae*.[100] A combination of theoretical models and in vivo nasopharyngeal colonization experiments was used to demonstrate that competition with *H influenzae* may select for more virulent strains of *S pneumoniae*.

Taken as a whole, these studies indicated that (1) the specific combination of colonizing bacteria and respiratory viruses can alter the incidence and duration of OM. (2) Research is needed to identify the combinations associated with the highest risk of disease. (3) Pneumococci have several methods to compete with co-colonizing and coinfecting species.

Haemophilus influenzae—Significant progress has been made in our understanding of the genomics and population biology of *H influenzae* strains, defining virulence factors and their role(s) in carriage and disease, defining genetic regulatory networks important to bacterial persistence and virulence, delineating bacterial determinants of resistance to immune clearance, and understanding the genetics and biochemistry of bacterial surface moieties.

Genomics and population biology: Analysis of the genome of 12 strains shows a high degree of genomic diversity among different nontypeable *H influenzae* strains.[101] Juhas et al[102] showed the presence of discrete genomic islands that are differentially distributed among strains and some degree of clustering of sets of strains within the larger body of *H influenzae* lineages.[103] These data are consistent with Garth Ehrlich's distributed genome hypothesis, which holds that populations of opportunistic pathogens have a core set of genes accompanied by a differentially distributed set of accessory genes that are horizontally exchanged between individual strains.[104]

Murphy et al[105] made the unexpected observation that some strains of *H haemolyticus* are nonhemolytic. This observation has important implications because the sole characteristic that is used in clinical microbiology laboratories throughout the world to distinguish *H influenzae* and *Haemophilus haemolyticus* is hemolysis on blood plates. Analysis of 490 respiratory tract isolates identified as *H influenzae* by currently accepted methods demonstrated that 40% of sputum isolates and 27% of nasopharyngeal isolates were in fact *H haemolyticus*. This conclusion was based on 4 independent methods, including (1) analysis of 16SrDNA sequences, (2) multilocus sequence analysis, (3) DNA-DNA hybridization with genomic DNA, and (4) sequence analysis of the highly conserved P6 gene. This observation has been reproduced in simultaneous work in Dr Janet Gilsdorf's laboratory.[106,107] *Haemophilus influenzae* causes otitis media, whereas *H haemolyticus* is an upper respiratory tract commensal.[105]

Thus, with regard to population genetics of *H influenzae*, the following themes are apparent: (1) existing paradigms regarding the clonality of overt pathogens may not be applicable for nontypeable *H influenzae* populations, which have a significant host commensal niche. (2) Simultaneous infection with multiple strains/clones is probably more the rule than the exception for this species, especially in the context of an opportunistic infection such as otitis media. (3) Genetic exchange between subpopulations of *H influenzae* is likely to be frequent and may be an important driver of dissemination and emergence of persistence determinants. (4) Currently accepted methods used in clinical microbiology laboratories throughout the world do not accurately distinguish between *H influenzae* and *H haemolyticus*. This observation has important implications in the design of future studies and in the interpretation of the literature.

Mechanisms of pathogenesis: Adherence: Like most mucosal pathogens, *H influenzae* has multiple redundant mechanisms for adhering to host tissues,[108] including a variety of proteinaceous adhesins. There has been considerable recent progress in defining the mechanisms for secretion and proteolytic processing of the autotransporter family of adhesins, of which HMW-1 and HMW-2 proteins of *H influenzae* are the paradigmatic example.[109–113]

Jurcisek and colleagues[114,115] demonstrated that type IV pili promote *H influenzae* adherence to epithelial cells, formation of biofilms, and persistence in the chinchilla model of otitis media. Moreover, as will be discussed in the update on vaccines, antibodies against these pili are protective.[116] *Haemophilus influenzae* also use the P5 adhesin to bind to ICAM-1 in the chinchilla model of otitis media.[117]

Mechanisms of pathogenesis: Intracellular entry, persistence, and growth: Although *H influenzae* has been traditionally thought of as an extracellular pathogen, it has long been recognized that one can observe *H influenzae* bacteria within a variety of host cells in patient tissues. Persistence of bacteria within cells could provide a protected niche from antibiotics and immune defenses. Morey and colleagues[118] showed that *H influenzae* bacteria enter epithelial cells by a macropinocytic route that involves PI-3 kinase activation. It is notable that a significant percentage of *H influenzae* bacteria observed in tissues from patients with otitis media were found within host cells, particularly within adenoid tissues.[119] Defining the significance of the intracellular niche in nontypeable *H influenzae* disease remains an important area for additional work.

From these studies, the following points become clear: (1) *H influenzae* have multiple means for adhering to host epithelia and mucus, some of which may be upregulated during chronic infection or coinfection with other species. (2) Although it is clear that *H influenzae* are found often within various host cells, there is still a pressing need for definition of the role of this process in the context of disease. Internalization may be a means for bacterial clearance by host epithelial cells, or alternatively, this may be a niche for persistent infection.

Mechanisms of pathogenesis: Biofilm formation: Like many pathogens residing on mucosal surfaces, *H influenzae* forms multicellular biofilm communities. Although the relevance of biofilms has been questioned by some,[120] it is now clear that biofilms are a significant contributing factor in chronic and recurrent *H influenzae* disease (particularly otitis media). Biofilms are present in the middle ears of patients with recurrent acute OM disease but less so in acute OM.[121] Other work from this group shows that biofilms are formed by *H influenzae* and other otopathogens on or within adenoid tissue,[119,122] which may serve as a reservoir for recurrent infection.

The composition of the *H influenzae* biofilm includes extracellular DNA,[114,123] pilus protein,[115] and discrete subsets of the lipooligosaccharides on the bacterial surface.[124,125] Shifts in lipooligosaccharide populations during biofilm formation and growth are coordinated by autoinducer-2 quorum signals.[126] Notably, these quorum signals can also affect biofilm formation and persistence of other otopathogens.[127]

There is also a growing appreciation that the *H influenzae* “biofilm” includes host cellular components and in many ways fits the definition for an exudate or neutrophil extracellular trap (NET).[128] *Haemophilus influenzae* activate neutrophils to form NETs via recognition of bacterial components by host pattern recognition receptors.[129] However, rather than being killed, *H influenzae* survive in multicellular clusters within NETs, and some of the surface moieties that promote biofilms are important to resistance to bactericidal effects of the NET[128] and killing by additional incoming neutrophils.[129]

The following points are clear from recent work on *H influenzae* biofilms: (1) a paradigmatic carbohydrate matrix for the *H influenzae* biofilm has yet to be discovered. For some, this raises questions regarding whether this organism can be thought of as a biofilm pathogen. However, it now appears clear that chronic and recurrent *H influenzae* infections,

particularly in the context of otitis media disease, fit the well-established profiles for a biofilm infection. (2) Quorum signaling contributes significantly to the formation and maturation of *H influenzae* biofilms and to its coordinate formation of polymicrobial biofilms with other otopathogens. (3) Extracellular DNA makes up a substantial part of the *H influenzae* biofilm matrix, and thus study of so-called bacterial apoptosis may have merit. (4) The *H influenzae* biofilms have a significant host component, mainly provided by incoming neutrophils that die to form NETs. Small multicellular communities of *H influenzae* bacteria that fit all of the defining characteristics of *H influenzae* biofilms survive within these NETs.

Mechanisms of pathogenesis: Lipooligosaccharides: *Haemophilus influenzae* have on their outer leaflet a diverse assortment of lipooligosaccharide (LOS) glycolipids. Many *H influenzae* strains produce sialylated LOS forms, which promote both resistance to complement-mediated killing and formation of biofilms. Sialic acid is taken up by a tripartite, adenosine triphosphate (ATP)–dependent transporter that is localized to the periplasmic space, and uptake is essential for both assimilation of sialic acid into the lipooligosaccharide and its catabolism as a nutrient source.[130–133]

The presence of sialic acid serves as a metabolic cue that is an important determinant of virulence.[134] Notably, elegant biochemical work profiling lipooligosaccharide populations from bacteria obtained directly from the chinchilla middle ear space revealed that the glycoform pools change during the course of infection, with less sialylated forms predominating later in infection.[135]

There has been significant progress on the definition of the genetics and biochemistry of LOS biosynthesis and assembly in the past 4 years. A number of studies have defined genes involved in addition to a number of specific oligosaccharide moieties to the carbohydrate portion of the LOS.[135–139]

The following points can be made regarding recent advances in understanding the *H influenzae* LOS: (1) sialylation offers an important potential therapeutic target because of the distinct biochemistry involved in biosynthesis and assembly of sialylated glycoforms. It is also significant to note that the uptake of sialic acid has dramatic metabolic effects that are important to persistence of the organism in vivo. (2) The biochemical methodology has now advanced sufficiently to permit detailed characterization of LOS glycoforms from populations in vivo. This is an important advance that can provide significant insight not only into what variants persist but at what stage particular variants predominate.

Mechanisms of pathogenesis: Bacterial stress-response: Work from Harrison and colleagues[140] delineated the OxyR regulon, which is an important regulatory network in bacterial resistance to oxidant. Additional work from Wong et al[141,142] has shown that the ArcA/B regulon has parallel function in resistance to oxidant and other stresses, in addition to conferring resistance to complement-mediated killing.

Mechanisms of pathogenesis: Virulence: The *sap* locus, which is upregulated in the chinchilla infection model, was shown by Mason et al[143] to be required for acquisition of heme.

Using a deep sequencing approach to differentiate between inocula and persisting populations of *H influenzae* transposon mutants, Gawronski and colleagues[144] have identified a number of genes required for virulence in a mouse pulmonary challenge model.

The *H influenzae* isolates from carriage within the upper airway were compared with isolates from patients with pulmonary infections, including exacerbations of chronic obstructive pulmonary disease in a recent study by Nakamura et al.[145] The results showed a significantly increased resistance of the disease isolates to complement-mediated killing, which subsequent genetic studies correlated with *vacJ* and *yrb*, which function in other species to modulate phospholipid content of the outer membrane and, as a consequence, hydrophobicity of the bacterial surface.

With reference to otitis media disease, the role of sialylation in resistance of *H influenzae* to complement-mediated clearance was clarified in a study in which an asialylated *siaB* mutant strain was shown to survive in chinchillas in which complement was depleted by treatment with cobra venom.[146] Work by Steven Juhn's group showed that chronic *H influenzae* otitis media infections can cause pathology in the inner ear, with associated impact on auditory function in the chinchilla.[147]

From evaluation of the recent work on the pathogenesis of *H influenzae* otitis media infections, the following points are clear: (1) nutrient acquisition and resistance to environmental stress are important determinants of *H influenzae* persistence. (2) Modulation of complement efficacy is likely to be an important determinant of host susceptibility to *H influenzae*. (3) On the basis of work in the chinchilla model, sequelae of otitis media may be more wide-ranging than is typically appreciated and could include neurological deficits in the inner ear. (4) Polymicrobial infection is common and may represent the majority of cases of otitis media. There is a pressing need for additional insights into how combinations of bacterial (and viral) agents affect the course and treatability of otitis media.

Moraxella catarrhalis—Progress has been made in characterizing the *Moraxella catarrhalis* genome, elucidating mechanisms of pathogenesis, understanding interactions with the human host, further defining the role of *M catarrhalis* as a pathogen in otitis media, and characterizing the molecular epidemiology of *M catarrhalis*.

Genomic studies of *M catarrhalis*: For a long time, investigators have relied on the unannotated, partial genome sequence of a single reference strain of *M catarrhalis*. An important recent advance has been the first completely assembled and annotated genome sequence of a bloodstream isolate of *M catarrhalis*.[148] Shortly thereafter, the genome sequences of 11 additional isolates, including 4 middle ear fluid isolates from children with otitis media, were reported.[149] Overall, the *M catarrhalis* genome shows similar chromosome organization and modest genomic diversity among the 12 strains. The

availability of genome sequences of clinical isolates of *M catarrhalis* represents a critical advance that will facilitate research on the organism considerably.

Prior to the availability of the genome sequence of these 12 strains, the genome sequence of ATCC strain 43617 was annotated and used to create a microarray of the predicted open reading frames in the *M catarrhalis* genome.[150] The microarray was used to perform transcriptional profiling studies by Dr Eric Hansen's group, and these approaches led to a series of papers elucidating metabolic pathways of *M catarrhalis*.[151–155]

Ruckdeschel et al[156] also used the genome sequence of ATCC strain 43617 in a genome mining approach to identify a set of novel vaccine antigens and proteins that are targets of the human immune response.[157,158] This is discussed in the report by the Vaccine Panel.

Mechanisms of pathogenesis: Adhesins: As an exclusively human pathogen, *M catarrhalis* has a restricted ecological niche: the human respiratory tract. The expression of multiple adhesins, each with its own binding specificity to host molecules in the human respiratory tract, reveals the importance of adherence in survival of *M catarrhalis*. Over the past 4 years, 3 novel adhesins (Mch or Mha, McmA, and type 4 pili) and a putative adhesin (OlpA) have been identified and characterized. In addition, elegant studies have further characterized host interactions with previously identified adhesins (MID/Hag, UspA1, McaP, OMP CD). These new observations are summarized briefly in Table 1.

In a study that investigated the effect of temperature on pathogenesis, Spaniol et al[159] showed that at 26°C, a temperature approximating that of the human nasopharynx, *M catarrhalis* upregulates the expression of the UspA1 adhesin, which is accompanied by an increase in binding of fibronectin and IgA. Thus, exposure to a physiologically relevant temperature affects the host pathogen interaction and may contribute to pathogenesis.

Mechanisms of pathogenesis: Lipooligosaccharide: The LOS of *M catarrhalis* has 3 serotypes, A, B, and C, that are based on the composition and linkage of oligosaccharide chains. Construction of LOS mutants and biochemical analysis of structures contributed new data on the biosynthetic pathways of LOS, which are now characterized for all 3 serotypes.[160–164] Various mutants were used to show that the oligosaccharide is important in adherence to epithelial cells and in mediating serum resistance.[165] In addition, human serum antibodies are directed at both core and side chain structures of the LOS molecule.[166]

Mechanisms of pathogenesis: Biofilm formation: In a study that has implications in understanding a potential role of biofilms in bacterial persistence in otitis-prone children, Hoa et al[121] studied adenoids of otitis-prone children for the presence of bacterial biofilms. All 6 adenoids studied had biofilms, and 3 of the 6 had *M catarrhalis* biofilms. This observation, in combination with the study of Heiniger et al[167] showing that *M catarrhalis* resides intracellularly in the adenoid, indicates that *M catarrhalis* is present in the adenoid far more commonly than is indicated by surface cultures.

Wang et al[150] compared the transcriptional profile of *M catarrhalis* during planktonic growth with that during growth as a biofilm. Growth as a biofilm results in increased

expression of many gene products, especially those that can function in energy generation and in resisting innate immune responses. Pearson and Hansen[168] used random transposon mutagenesis to show that the surface protein UspA2H plays a role in biofilm formation.

Mechanisms of pathogenesis: Outer membrane vesicles: Gram-negative bacteria shed outer membrane vesicles during growth. In a series of innovative studies, Schaar and colleagues[169] characterized the proteome of outer membrane vesicles secreted by *M catarrhalis* and showed that vesicles are complex structures that contain multiple outer membrane components. They further demonstrated that vesicles are internalized by human epithelial cells; induce inflammatory responses, including triggering TLR2 responses; and activate B cells.[169,170] Thus, vesicles represent a mechanism whereby *M catarrhalis* delivers antigens to host cells and induces and modulates host inflammation. *Moraxella catarrhalis* vesicles also inhibit complement-dependent killing of *H influenzae*, suggesting that pathogens collaborate to evade innate immunity and survive in the respiratory tract.[171]

Mechanisms of pathogenesis: Host responses: Work in the past 4 years involving human cell lines and primary cells has shed new light on host responses and signaling pathways triggered by *M catarrhalis*.[172–177] Additional studies included the observation that *M catarrhalis* activates tonsillar B cells to secrete nonspecific IgM and the observation that the UspAs neutralize α1-antichymotrypsin.[178,179] *Moraxella catarrhalis* is the only otitis media pathogen to demonstrate an interaction with α1-antichymotrypsin, suggesting a unique virulence mechanism that requires additional exploration.

Most pathogenic isolates of *M catarrhalis* belong to a seroresistant lineage.[180] New studies have advanced our understanding of complement evasion strategies, a prominent feature of *M catarrhalis*. UspAs block complement activation by binding C3, and new work has furthered the understanding of the UspA2-vitronectin interaction in serum resistance.[181,182] The complement resistance phenotype is mediated by multiple gene products.[183]

Mechanisms of pathogenesis: Other progress: Easton et al[184] identified a general porin in *M catarrhalis* and demonstrated that it functions in nutrient uptake and is essential for nasal colonization of mice. Attia et al[185] identified the first bacteriocin and its immunity factor in *M catarrhalis* and showed that strains with the bacteriocin inhibited growth of other strains.

Role of *M catarrhalis* in otitis media: The gold standard in determining the etiology of bacterial otitis media is culture of middle ear fluid. Several studies that employed culture of middle ear fluid recovered by tympanocentesis, drainage from tympanostomy tubes, or spontaneous otorrhea have been reported in the past 4 years.[44,95,186–193] Such studies are important to track changes in the distribution of pathogens that cause otitis media, particularly with anticipated changes in patterns of vaccine use for otitis media pathogens. Several themes are apparent from these studies: (1) *M catarrhalis* continues to be an important cause of otitis media, being the third most common cause after *S pneumoniae* and *H influenzae* in many centers. (2) Substantial geographic variability is observed in the proportion of otitis media caused by *M catarrhalis*. For example, the rate of *M catarrhalis* in Beer-Sheva, Israel, is low, whereas *M catarrhalis* is the most common bacterial cause of recurrent otitis media in children with tympanostomy tubes in Turku, Finland.[186,193] (3) As

the distribution of pathogens changes with widespread use of pneumococcal conjugate vaccines, the relative proportion of otitis media due to *M catarrhalis* is increasing in some studies.[188,189]

Broides et al[186] reviewed the clinical and epidemiological characteristics of 501 episodes of otitis media in children whose middle ear fluid grew *M catarrhalis*. Compared with acute otitis media caused by other pathogens, acute otitis media caused by *M catarrhalis* was characterized by (a) a higher proportion of mixed infection, (b) younger age at diagnosis, (c) lower proportion of spontaneous tympanic membrane perforation, and (d) absence of mastoiditis.

Nasopharyngeal colonization and molecular epidemiology: A number of studies that contributed to the body of knowledge on nasopharyngeal colonization patterns and the molecular epidemiology of colonizing isolates have been performed over the past 4 years.[194–201] Several themes regarding colonization by *M catarrhalis* are apparent from these and previous studies: (1) *M catarrhalis* is a common colonizer of infants and children, often being the most common colonizer among otitis media pathogens. (2) The rate of *M catarrhalis* colonization decreases with age. (3) Geographic variability is seen in colonization and infection rates by *M catarrhalis*. (4) Co-colonization with *H influenzae* was observed in 1 study.[201] (5) Colonizing strains of *M catarrhalis* show genotypic and phenotypic diversity.[201–205] (6) In an interesting set of experiments, Krishnamurthy et al[25] showed that polymicrobial nasal colonization with otitis media pathogens affected the incidence rate, duration, and bacterial load in a mouse model.

Antimicrobial susceptibility: Surveillance studies of *M catarrhalis* indicate that most clinical isolates produce β-lactamase and are thus resistant to penicillins, including amoxicillin. The newly identified outer membrane porin M35 also mediates susceptibility to penicillins.[206,207] *Moraxella catarrhalis* is susceptible to most other classes of antibiotics used for the treatment of otitis media, and resistance patterns appear stable over the past 4 years worldwide.[208]

Bell et al[209] developed zone diameter criteria for 19 antimicrobial agents using current minimum inhibitory concentration (MIC) interpretive criteria and examined 318 strains of *M catarrhalis*. Because no Clinical and Laboratory Standards Institute (CSLI) method exists currently, the availability of this new method will facilitate antimicrobial susceptibility testing of *M catarrhalis* enormously.

Immunology

General Immunology—Major advances have been made in understanding immune mechanisms and the relationship between the microbe, host innate responses, and development of acquired immunity. Our increased knowledge has served to reinforce the complexity associated with the precise mechanisms of host defenses associated with OM and the need for the pursuit of knowledge of the immune mechanism in the upper respiratory tract.

The airway epithelium is the first line of defense against respiratory viruses and bacteria. It has a range of defenses that include mechanical (eg, mucociliary apparatus), innate (eg, defensins, inflammatory mediators), and acquired/adaptive (eg, antigen specific and immune memory). When respiratory viruses and bacteria interact with airway epithelial cells, antimicrobial agents such as interferons (IFN), lactoferrin, β-defensins, and nitric oxide (NO) and chemical signaling agents such as cytokines and chemokines are induced as part of the innate immune response and influence the adaptive immune system.[225,226] Although these defense mechanisms are intended to facilitate rapid microbial clearance, bacteria and viruses have developed elaborate strategies to evade a range of antimicrobial mechanisms, as well as innate and adaptive immune responses.

Many of the functions of innate immunity in the mucosal surfaces are mediated by host-specific microbial–pathogen recognition receptors (PRRs), which can recognize unique pathogen-associated molecular patterns (PAMPs) that are integral to the structure of most microorganisms. Recently, Ogra[226] reviewed the important elements of neonatal mucosal adaptive immunity. Mucosal tissues contain lymphoid cells derived by the homing of antigen-activated cells from the inductive sites, with mostly IgA-activated B cells (up to 80%) detected shortly after birth and IgA-producing plasma cells detected at approximately 7 to 10 days of age. Environmental antigenic stimulation, including the acquisition and nature of mucosal microflora, is critical to the development of the immune system and corresponds with the expansion of activated cells within the mucosal sites. Ogra reinforces the evidence that mucosal immune responses may also be pathologic and foster the induction of immunologically mediated disease states and autoimmunity. The early and appropriate development of the mucosal immune system is essential for maintaining mucosal homeostasis and prevention of disease.

Key lymphoid tissues in the upper respiratory tract mucosa include the adenoids, tonsils, and nasopharynx-associated lymphoid tissue (NALT). The mechanism of NALT organogenesis differs from that of other lymphoid tissues, and NALT is important for the generation of T helper (Th) 1 and Th2 cells and IgA-committed B cells, and unlike other lymphoid organs, NALT develops postnatally. Krege et al,[227] using a rodent model, identified at least 2 different pathways associated with NALT development.

Cellular Immunology—Children with adenoid hypertrophy and acute OM were found to have lower $CD4^+Bcl\text{-}2^+$, $CD8^+Bcl\text{-}2^+$, and $CD19^+Bcl\text{-}2^+$ lymphocytes (Bcl-2 is an antiapoptotic protein) but higher percentages of $CD4^+$, $CD8^+$, and $CD19^+$ cells with the $CD95^+$ antigen than children with adenoid hypertrophy unrelated to OM.[228] According to Zelazowska-Rutkowska and colleagues,[228] reduced proportions of T and B lymphocytes with Bcl-2 expression but elevated percentages expressing $CD95^+$ may reflect local immunity disorders. In another study, the populations of dendritic cells and lymphocyte subpopulations of adenoid and peripheral blood in patients with adenoid hypertrophy and otitis media with effusion (OME) found differences between patients with adenoid hypertrophy with coexisting OME and children without OME in the adenoids but not in blood.[229] Local induction of inducible nitric oxide synthase (iNOS) in adenoids has also been suggested to be of importance for preventing development of OME following evidence that children with OME exhibited lower levels of iNOS than controls.[230] Inducible nitric

oxide synthase is one of the enzymes that regulates production of nitric oxide, a key mediator in the local immune response of human airways.

The immune system must be tightly regulated to balance antimicrobial inflammatory responses to prevent immune-mediated tissue destruction. Regulatory T cells (Treg) are critical to this process. Tregs can be $CD4^+$ or $CD8^+$ and are distinguished by expression of the transcription factor FoxP3. There are 2 functional types of Treg cells: naturally occurring and induced. Tonsillar $FoxP3^+CD8^+$ T cells are mostly $CD25^-$, with some cells expressing the proinflammatory cytokines TNF-α, IFN-γ, or IL-17A, and suppress the proliferation of $CD4^+$ T cells in co-cultures.[231] Induced Tregs are generated from naive T cells in the periphery after an encounter with antigen presented by dendritic cells (DCs) that have been conditioned by epithelial cells in contact with microorganisms.[232] Cytokines such as IL-10 and TGF-β produced by Tregs are involved in suppression of T-cell responses.[233]

The recent discovery of Th17 cells as key inflammatory mediators of the mucosa is opening new insights into mucosal immunity and its regulation. It now appears that Th17 inflammatory cells can differentiate into Tregs and back, depending on the cytokine milieu.[231,234,235] Termed *plasticity*, this ability means that the balance between effective immunity, which results in clearance of bacteria, and the associated inflammatory tissue damage can be quickly and efficiently managed. The switch of a T cell from an inflammatory (Th1, Th2, Th17) to suppressive (Treg) phenotype occurs in both mice and humans and in $CD4^+$ as well as $CD8^+$ Tregs.[231,235] Further evidence that all T cells are transiently Treg during activation suggests that Treg plasticity may be an important regulatory mechanism for the immune system in general.[236]

There is a growing realization that bacteria can control the mammalian immune system.[237] Some bacterial species appear to promote survival by actively inducing Tregs via Toll-like receptor (TLR) signaling in epithelial cells or mucosally conditioned DCs. To date, most studies have been associated with gut and oral microbes showing functionally distinct receptor-signaling pathways that direct the Th17/Treg balance. It is not known whether the same applies in the upper airway, with opportunistic commensals such as *S pneumoniae*, nontypable *H influenzae* (NTHi), and *M catarrhalis*. $CD4^+$ and $CD8^+$ Tregs are found in nasal mucosa[238] and tonsils,[231,235] and although a role has been implicated in allergen-specific immunotherapy,[239] a role in promoting bacterial survival in the nasopharynx has yet to be demonstrated. Depletion of $CD25^+$ cells from palatine tonsils resulted in suppression of the effector $CD4^+$ T-cell response restricted to the mucosa and was most marked in children at greatest risk of meningococcal disease.[240] These studies concluded that proinflammatory, anti-meningococcal T-cell responses may limit invasive disease at the mucosa but that Treg induction may restrict the effectiveness of the protective response. It is possible that commensal bacteria within the nasopharynx induce a tolerance through induction of Treg responses that suppress effector T-cell responses, contributing to immune-failure in individuals who are susceptible to or suffer from chronic respiratory infections such as OM. However, such a hypothesis has yet to be tested.

Innate Immunology: The Role of Innate Cell Receptors and Signaling—Toll-like receptor signaling is involved in both the innate immune responses to infection and the

development of acquired immune responses. Hirano et al[241] found that the mucosal immune response in wild-type (WT) mice is superior to that in TLR4-mutant mice, indicating that TLR4 may play an important role in enhancing mucosal and systemic immune responses. They showed that immune responses against the outer membrane protein (OMP) from NTHi were elicited in both TLR4-mutant and WT mice but that the mucosal IgA, systemic IgG, and Th1 cell responses were superior in WT mice than in TLR4-mutant mice. This suggests that TLR4 plays an important role in relation to Th1 function for optimal development of acquired immune responses. Activated TLRs can signal through either MyD88 to primarily induce interleukin expression or TRIF for type I IFN expression. Leichtle et al[242] reported that expression of TRIF mRNA was only modestly enhanced during OM but that both type I IFN signaling genes and type I IFN-inducible genes were significantly upregulated in WT mice. In response to NTHi infection, TRIF-deficient mice had reduced but persistent mucosal hyperplasia and less leukocyte infiltration into the middle ear than did WT animals. Their results demonstrate that activation of TRIF/type I IFN response has a role in both the response to and resolution of NTHi OM.

The role of TLR2 in defense against *S pneumoniae* middle ear infection was investigated using WT (C57BL/6) and TLR2-deficient ($TLR2^{-/-}$) mice, and the study found that the $TLR2^{-/-}$ mice had an approximately 50% mortality rate due to bacteremia within 3 days after challenge compared with 12.5% in WT mice.[243] The levels of proinflammatory cytokines were significantly lower in the ears of $TLR2^{-/-}$ mice than in WT mice, which correlated with poorer clearance of bacteria from the middle ear and increased sepsis, demonstrating the importance of TLR2 to host responses to otitis media. Examination of the rat mucosa for TLR2 and TLR4 expression in the tubotympanum, nasopharynx, and oral cavity showed differences in the expression of these in different parts of the tubotympanum and upper aerodigestive tract, suggesting that there may be region-specific functional modulation of the innate immune system and pathophysiology of otitis media.[244]

Expression in middle ear effusion of TLR9, nucleotide-binding oligomerization domain (Nod)–1, Nod-2, and retinoic acid–inducible gene (RIG)–I mRNA found that levels of TLR-9, Nod-1, and RIG I mRNAs were significantly lower in the otitis-prone group than in the non–otitis-prone group.[245] In these same children, the concentrations of IgG, IgA, and IgM in effusion fluid did not differ, nor did they correlate with the expression of PRRs, suggesting that expression of these PRRs may have a role in susceptibility to OME. A role for DNA sensing via TLR9 in OM pathogenesis and recovery has been identified using a murine model of NTHi OM and $TLR9^{-/-}$ mice.[246]

Not only is local stimulation important, but there are possible mechanisms whereby systemic immunomodulation by the microbiota at distant sites can operate through the PRR Nod-1 to enhance bacterial killing. Local recognition of peptidoglycan from a gram-negative bacterium, such as NTHi, induces signaling through the Nod-1 that enhanced the killing of complement-opsonized *S pneumoniae* by neutrophils.[247] Peptidoglycan from the gut translocates to neutrophils in the bone marrow and influences neutrophil function.[248] The absence of Nod-1 in mice has made them more susceptible to early pneumococcal sepsis, indicating that Nod-1 is involved in priming innate defenses, with these studies providing strong evidence for the role of normal biota in this priming.

The epithelial cells lining the human upper respiratory tract may also be influenced by environmental agents, including cigarette smoke (CS). Examination of the effect of CS condensate (CSC) or extract (CSE) on signal transduction and cytokine production in primary and immortalized epithelial cells of human or murine origin in response to NTHi and *S aureus* found that IL-8 and IL-6, but not β-interferon (IFN-β), was significantly inhibited in the presence of CS and by either CSC or CSE.[249] Cigarette smoke extract also affected cell signaling and decreased nuclear factor (NF)–κB activation and highlights a possible contributing mechanism in children who are exposed to CS and have higher incidences of OM.

Innate Immunology—Defense Molecules—Mason et al[143] showed that immune evasion can supersede important iron acquisition functions. The Sap translocator function is necessary for NTHi mediation of diseases of the human airway.[143] The study also showed that the antimicrobial peptides human β-defensins 2 and 3, human cathelicidin LL-37, human neutrophil protein 1, and melittin could displace heme bound to SapA, demonstrating a hierarchy wherein immune evasion was able to supersede important iron acquisition functions.

Shimada et al[62] aimed to assess the muramidase activity and the antimicrobial property of lysozyme in the eustachian tube of lysozyme $M^{-/-}$ mice to evaluate the role of lysozyme in OM pathogenesis. They showed that depletion of lysozyme results in delayed clearance of *S pneumoniae* from the middle ear cavity.

Lee et al[250] investigated NTHi-induced β-defensin expression in airway mucosa, including the middle ear, and showed that the major NTHi-specific receptor in human middle ear epithelial cells–1 was TLR2, which activated the Toll/IL-1 receptor-MyD88-IRAK1-TRAF6-MKK3/6-p38 MAPK signal transduction pathway. This induced β-defensin 2, which was highest in response to NTHi lysate, suggesting that the ligand stimulus may be soluble macromolecules. They suggest that this provides an evolutionary advantage to the cells in dealing with infections and initiating an innate immune response.

The antimicrobial host defense peptide SPLUNC1 is believed to aid in maintaining airway health through both bactericidal and nonbactericidal mechanisms. Knockdown of cSPLUNC1 expression did not affect survival of NTHi in the chinchilla middle ear under the conditions tested, whereas expression of cSPLUNC1 was essential for maintenance of middle ear pressure and efficient mucociliary clearance,[251] indicating that cSPLUNC1 functions to maintain homeostasis and is important for protection of the middle ear.

Human middle ear epithelial cells were used to investigate the relationship between the inflammatory response and microRNA (miRNA; short, noncoding RNA thought to regulate gene expression through sequence-specific base pairing).[252] The study found 15 differentially expressed genes: 5 miRNAs upregulated and 10 miRNAs downregulated in response to lipopolysaccharide (LPS), suggesting that miRNA may play an important role in the pathogenesis of OM.

Role of Cytokines and Chemokines—Evaluation of lymphocytes from peripheral blood and adenoids of children with recurrent otitis found these children had a significantly lower proportion of $CD8^+$-producing IFNγ cells in adenoids than children with <3 otitis per year, suggesting that a reduced capability to produce INFγ may contribute to the susceptibility to the recurrent OM in this cohort.[253] Patel and coworkers[3] investigated systemic levels for 17 cytokines during AOM in the sera from 145 children and correlated these with viral etiology and clinical outcome. Their results indicated that higher G-CSF concentrations produced an 87.6% accuracy to predict RSV-induced AOM, and elevated IL-13 concentrations produced an 84.2% accuracy to predict early clinical failure of antibiotic treatment.

In addition to the ability to minimize phagocytosis, *S pneumoniae* undergoes autolysis in the stationary phase through activation of the cell wall–bound amidase LytA. Clinical isolates of *S pneumoniae* exhibited significantly reduced induction of TNF, IFNγ, and IL-12 in peripheral blood mononuclear cells compared with other closely related *Streptococcus* species, but levels of IL-6, IL-8, and IL-10 production were similar.[254] Martner et al[254] demonstrated that components associated with the autolysed pneumococcus can affect the inflammatory response of mononuclear cells and interfere with phagocyte-mediated elimination of live pneumococci.

The role of allergy and the Th1/Th2 balance by expression of GATA3, T-bet, IL-4, and IFN-γ mRNA in OME patients was investigated in fluid collected from 46 OME patients having ventilating tubes inserted.[255] The study showed that although levels of GATA3 and T-bet mRNA in effusion fluid correlated positively with the levels of IL-4 and IFN-γ mRNA, respectively, there was no difference between the allergy and nonallergy groups, thus questioning that OME with allergy is related to a Th2-driven immune response.

IL-22 is expressed at barrier surfaces, and it is suggested that it plays a critical role in the maintenance of normal barrier homeostasis through signaling by IL-22 through the IL-22 receptor (IL-22R) to promote antimicrobial immunity, inflammation, and tissue repair at barrier surfaces (reviewed in Sonnenberg et al[256]). Although this has not been the subject of specific studies associated with OM, it has been investigated within the respiratory tract, and a proinflammatory/pathological role has been identified for IL-22 in airway inflammation.[257] These studies also found that IL-17A regulated the expression and/or proinflammatory properties of IL-22. The presence or absence of IL-17A appeared to govern the proinflammatory vs tissue-protective properties of IL-22.

Adaptive Immunology—Individual antibody levels in otitis-prone individuals do not appear to have an age-dependent rise. Lebon et al[258] have reported that in the first year of life, no association between maternal IgG levels and colonization was seen, nor was there an association between the IgG and IgA levels in the child vs colonization status. It is believed that the failure to develop a good antibody response to common bacterial antigens, such as PspA and P6, may be associated with persistent or recurrent disease.[259] Another supporting study reported that pneumococcal acute otitis media, when present with pneumonia, affects pneumococcal serology, whereas nasopharyngeal carriage has little effect except if associated with the acquisition of a new serotype.[260] Further analysis of the relationship

between antibody levels and the presence of bacteria in effusion fluid (detected by standard bacterial culture and PCR) found there was no correlation between immunoglobulin concentrations in effusion fluid and the presence of bacteria.[261] In contrast, serum immunoglobulin concentration was related to the presence of bacteria in the effusion, with serum IgG, IgA, and IgM in patients with OME being lower than in control patients.

The work of Hyams et al[52] of the role of complement in immune protection to pneumococcal OM was discussed above.

Colonization in mice elicits cross-reactive antibodies to PspA, putative proteinase maturation protein A (PpmA), and pneumococcal surface adhesin A (PsaA), with PspA being the major target of surface-bound cross-reactive IgG in sera.[262] However, human sera differed, with PpmA seeming to be the main target of surface IgG. This study demonstrated that PspA, PpmA, and PsaA were not essential for cross-protection induced by carriage and have suggested that a whole-organism approach may be needed to broadly diminish carriage. Immune responses induced by mucosal vaccines composed of PspA and PspC as recombinant proteins or delivered by *Lactobacillus casei* resulted in PspC vaccines not protecting mice against an invasive challenge with pneumococcus, but protection was observed for immunization with vaccines composed of PspA from clade 5 delivered intranasally.[263]

Protection conferred against fatal pneumococcal infections during infancy by maternal immunity was evaluated in mice immunized with PspA with, or without, cholera toxin B (CTB) delivered intranasally prior to pregnancy.[264] Anti-PspA-specific IgG antibody was induced in sera and breast milk at birth and maintained for 14 days during nursing periods in the PspA-immunized mother mice, and offspring delivered from PspA-immunized mothers had levels of anti-PspA-specific IgG antibody in sera similar to those in their mothers on the day of birth. The induction of specific immune responses in the sera and colostrum of mother mice was transferred to neonate mice by maternal intranasal immunization with PspA and contributes to the ability of the neonate's ability to fight infection.

The development of antibodies to PspA families 1 and 2 present in the serum and saliva of children with a history of culture-proven pneumococcal colonization and/or acute otitis media and in the serum and saliva of adults was investigated.[53] The majority of the children had high serum and salivary anti-PspA concentrations to the PspA family they had encountered and low concentrations to the other, whereas adults had high antibody concentrations to both PspA families, both in serum and in saliva. The results suggest that a PspA vaccine for children should contain members of both major PspA families.

Cao et al[265] found that immunizing mice intranasally with a mixture of ClpP (the caseinolytic protease) and CbpA (choline binding protein A) elicited better protection than immunizing with either singly, with the combination providing an additive effect in inhibiting adherence to A549 cells and increased complement-dependent killing by neutrophils. Antisera to both antigens could also kill *S pneumoniae* by neutrophils in a complement-dependent way. Depletion of $CD4^+$ T lymphocytes abrogated the induction of the mucosally induced antibody, indicating a critical role for these cells in developing

mucosal protein-based vaccines against invasive pneumococcal infection. Both these studies are encouraging for mucosal vaccine development.

Major histocompatibility complex class II– and DM-dependent retrograde transport from lysosomes to the cell surface is required to present polysaccharides to $CD4^+$ T cells. The zwitterionic capsular polysaccharide Sp1 of *S pneumoniae* caused an accumulation of Th1- and Th17-polarized $CD4^+$ $CD44^{(high)}$ $CD62^{(low)}$ $CD25^-$ memory T cells in an experimental mouse model of cellular immunity.[266] The study showed that these polysaccharides can induce clonal expansion of $CD4^+$ T cells and increase serum immunoglobulin.

The leucine zipper transcription factor Nrf2 is important for protection against oxidant-induced injury. $Nrf2^{-/-}$ mice were found to have increased lymphocytic airway inflammation compared with WT mice following NTHi lung challenge but also generated significantly enhanced and persistent levels of serum antibodies against P6,[267] suggesting a role for Nrf2 in regulating NTHi-induced airway inflammation.

OMP P2, the major outer membrane porin of NTHi, was evaluated as a recombinant protein immunogen and found to induce both mucosal and systemic immune responses with mucosal immunization inducing antibodies to epitopes on the bacterial surface of both homologous and several heterologous strains.[268] However, systemic immunization induced antibodies to non-surface-exposed epitopes.

A recent study by Sabirov et al[269] comparing children with AOM and healthy children according to feeding status found an association between breastfeeding and higher levels of antibodies to NTHi and P6 and suggested that breastfeeding might modulate the serum immune response to NTHi and P6. Nasal vaccination provides an ideal route for delivery for vaccines aimed at preventing otitis media. Appropriate adjuvants and formulation remain an important subject for investigation. The efficacy of fms-like tyrosine kinase receptor–3 ligand (Flt3L) as a mucosal adjuvant formulated with the NTHi P6 protein was demonstrated.[270] A surface-exposed portion of the NTHi Hia protein expressed as a recombinant GEMEX-Hia was used to generate antisera that mediated opsonophagocytic killing.[271]

Nontypable *H influenzae* also has mechanisms that involve attracting specific host complement regulators directly to the bacterial surface, as well as LOS and several outer membrane proteins that confer resistance against complement-mediated attacks.[272,273]

To better understand the human immune response to *M catarrhalis* infection in vivo, a specific LOS-based enzyme-linked immunosorbent assay (ELISA) containing the 3 major *M catarrhalis* serotypes and a complete series of truncated LOS mutants was used to detect the development of new antibodies to specific regions of the oligosaccharide molecule.[166] The study found variability in the antibody response to LOS from serotype-specific antibodies, antibodies to the LOS of each serotype, broadly cross-reactive antibodies, to no new antibodies. *Moraxella catarrhalis* secretes outer membrane vesicles (OMVs) that interact with host cells during infection. The composition of these OMVs was recently analyzed in detail and found to contain 57 proteins that included known surface proteins such as ubiquitous surface proteins (Usp) A1/A2 and *Moraxella* IgD-binding protein (MID).[169]

Many of the proteins were adhesins/virulence factors, some of which are known to aid bacteria to evade the host defense. TLR2 was found to be involved in internalization, with the OMVs able to modulate epithelial proinflammatory responses and UspA1-bearing OMVs specifically downregulating the reaction, indicating these OMVs may be highly biologically active bacterial virulence factors. The Usp proteins are also known to be involved in complement resistance, and recently, the ability of *M catarrhalis* to bind C3 was found to correlate with UspA expression, and this contributed to serum resistance in a large number of clinical isolates.[181] The study determined that the binding of C3 to UspAs was an efficient way to block the activation of complement and to inhibit C3a-mediated inflammation.

Implications for Practice

Short-term Research Goals

- The role of various inflammatory mediators and their mechanisms of action in the pathogenesis of AOM following viral URI need to be further studied.
- Studies should examine the impact of virus quantity (viral load) in the nasopharynx on generation of local inflammatory mediators and cytokines, local leukocyte migration and function, quantitative bacterial count, and risk for development of AOM. Similarly, the impact of viral load in the middle ear on disease severity and outcome needs to be studied.
- The clinical relevance of positive findings and prolonged presence of viral nucleic acids in the MEF and nasopharynx needs to be further elucidated to better assess the significance of asymptomatic viral infections on the pathogenesis of OM.
- The role of host genetics in URI susceptibility and AOM development following URI needs to be further explored.
- Further research should be performed to evaluate if specific viruses interact or promote the colonization of specific bacteria and to elucidate mechanisms of viral-bacterial interaction on the mucosal level.
- Further studies on the prevention of AOM by means of prevention and/or early treatment of viral URI should be performed.
- Exploit the rapid advances in bacterial genomics to understand mechanisms of pathogenesis, molecular epidemiology, and emerging antimicrobial resistance patterns of otitis media pathogens.
- Apply genomic technology to understand the dynamics of nasopharyngeal colonization and interaction of pathogens and commensals.
- Elucidate molecular mechanisms of pathogenesis by the 3 major bacterial pathogens of otitis media, *S pneumoniae*, *H influenzae*, and *M catarrhalis*. Such studies will provide opportunities for the development of novel interventions.
- Study trafficking of immune cells to the nasopharynx and the middle ear.
- Characterize how the middle ear interacts in the common mucosal immune system.

- Study how viral and bacterial pathogens alter pathways of innate immunity and the role of these alterations in pathogenesis.
- Study the role of cigarette smoke in infection by otitis media pathogens.
- Continue to perform tympanocentesis as part of studies at specialized research centers to accurately monitor the etiology of otitis media and changes in etiology as new vaccine programs are implemented.

Long-term Research Goals

- Standardize viral diagnostics.
- Elucidate the significance of new vs persistent viral infections.
- Elucidate the precise role of newly identified viruses in otitis media.
- Understand mechanisms of virus-bacteria interactions.
- Characterize the microbial ecology of the nasopharynx and middle ear to reveal the role of these complex environments in otitis media.
- Clarify the role of biofilms in otitis media by further studying their role in pathogenesis and assessing therapeutic approaches.
- Continue to exploit the expanding databases and knowledge related to bacterial genomes of otitis media pathogens.
- Perform research to understand how otitis media pathogens interact with one another and with commensals in the nasopharynx and the middle ear.
- Focus efforts on global approaches to understanding the fundamental immunology of otitis media.
- Characterize pathways of innate immunity as they relate to otitis media.
- Create an overall integrated map of the cytokines, chemokines, mediators, and signaling pathways relevant to the host response in otitis media.
- Elucidate the role of Tregs and the T17 axis in the host response and in protection from otitis media.
- Study the role of allergy in otitis media.
- Continue a global effort for better surveillance and monitoring of the etiology and mechanisms of otitis media in the developing world.

Acknowledgments

Sponsorships: The National Institutes of Health provided funding for the 1-day postsymposium meeting to bring experts together to review progress in the microbiology and immunology of otitis media.

Funding source: National Institutes of Health funded the 1-day postsymposium meeting.

References

1. Grieves JL, Jurcisek JA, Quist B, et al. Mapping the anatomy of respiratory syncytial virus infection of the upper airways in chinchillas (*Chinchilla lanigera*). Comp Med. 2010; 60:225–232. [PubMed: 20579438]
2. Patel JA, Nair S, Revai K, Grady J, Chonmaitree T. Nasopharyngeal acute phase cytokines in viral upper respiratory infection: impact on acute otitis media in children. Pediatr Infect Dis J. 2009; 28:1002–1007. [PubMed: 19859015]
3. Patel JA, Nair S, Grady J, et al. Systemic cytokine response profiles associated with respiratory virus–induced acute otitis media. Pediatr Infect Dis J. 2009; 28:407–411. [PubMed: 19352211]
4. Skovbjerg S, Roos K, Nowrouzian F, et al. High cytokine levels in perforated acute otitis media exudates containing live bacteria. Clin Microbiol Infect. 2010; 16:1382–1388. [PubMed: 19832705]
5. Revai K, Patel JA, Grady JJ, Nair S, Matalon R, Chonmaitree T. Association between cytokine gene polymorphisms and risk for upper respiratory tract infection and acute otitis media. Clin Infect Dis. 2009; 49:257–261. [PubMed: 19522649]
6. Alper CM, Winther B, Hendley JO, Doyle WJ. Cytokine polymorphisms predict the frequency of otitis media as a complication of rhinovirus and RSV infections in children. Eur Arch Otorhinolaryngol. 2009; 266:199–205. [PubMed: 18560870]
7. Heinonen S, Silvennoinen H, Lehtinen P, et al. Early oseltamivir treatment of influenza in children 1–3 years of age: a randomized controlled trial. Clin Infect Dis. 2010; 51:887–894. [PubMed: 20815736]
8. Chonmaitree T, Revai K, Grady JJ, et al. Viral upper respiratory tract infection and otitis media complication in young children. Clin Infect Dis. 2008; 46:815–823. [PubMed: 18279042]
9. Yano H, Okitsu N, Hori T, et al. Detection of respiratory viruses in nasopharyngeal secretions and middle ear fluid from children with acute otitis media. Acta Otolaryngol. 2009; 129:19–24. [PubMed: 18607974]
10. Kristjansson S, Skuladottir HE, Sturludottir M, Wennergren G. Increased prevalence of otitis media following respiratory syncytial virus infection. Acta Paediatrica. 2010; 99:867–870. [PubMed: 20002623]
11. Cheng CC, Huang LM, Kao CL, et al. Molecular and clinical characteristics of adenoviral infections in Taiwanese children in 2004–2005. Eur J Pediatr. 2008; 167:633–640. [PubMed: 17876605]
12. Heikkinen T, Osterback R, Peltola V, Jartti T, Vainionpaa R. Human metapneumovirus infections in children. Emerg Infect Dis. 2008; 14:101–106. [PubMed: 18258088]
13. Martin ET, Fairchok MP, Kuypers J, et al. Frequent and prolonged shedding of bocavirus in young children attending day-care. J Infect Dis. 2010; 201:1625–1632. [PubMed: 20415535]
14. Longtin J, Bastien M, Gilca R, et al. Human bocavirus infections in hospitalized children and adults. Emerg Infect Dis. 2008; 14:217–221. [PubMed: 18258113]
15. Beder LB, Hotomi M, Ogami M, et al. Clinical and microbiological impact of human bocavirus on children with acute otitis media. Eur J Pediatr. 2009; 168:1365–1372. [PubMed: 19221788]
16. Rezes S, Soderlund-Venermo M, Roivainen M, et al. Human bocavirus and rhino-enteroviruses in childhood otitis media with effusion. J Clin Virol. 2009; 46:234–237. [PubMed: 19736042]
17. Savolainen-Kopra C, Blomqvist S, Kilpi T, Roivainen M, Hovi T. Novel species of human rhinoviruses in acute otitis media. Pediatr Infect Dis J. 2009; 28:59–61. [PubMed: 19057460]
18. Tapparel C, Junier T, Gerlach D, et al. New respiratory enterovirus and recombinant rhinoviruses among circulating picornaviruses. Emerg Infect Dis. 2009; 15:719–726. [PubMed: 19402957]
19. Tauriainen S, Oikarinen S, Taimen K, et al. Temporal relationship between human parechovirus 1 infection and otitis media in young children. J Infect Dis. 2008; 198:35–40. [PubMed: 18462136]
20. Yano H, Okitsu N, Watanabe O, et al. Acute otitis media associated with cytomegalovirus infection in infants and children. Int J Pediatr Otorhinolaryngol. 2007; 71:1443–1447. [PubMed: 17618694]
21. Kalu SU, Loeffelholz M, Beck E, et al. Persistence of adenovirus nucleic acids in nasopharyngeal secretions: a diagnostic conundrum. Pediatr Infect Dis J. 2010; 29:746–750. [PubMed: 20308936]

22. Alper CM, Winther B, Mandel EM, Doyle WJ. Temporal relationships for cold-like illnesses and otitis media in sibling pairs. Pediatr Infect Dis J. 2007; 26:778–781. [PubMed: 17721370]
23. Alper CM, Winther B, Mandel EM, Hendley JO, Doyle WJ. Rate of concurrent otitis media in upper respiratory tract infections with specific viruses. Arch Otolaryngol Head Neck Surg. 2009; 135:17–21. [PubMed: 19153302]
24. McGillivary G, Mason KM, Jurcisek JA, Peeples ME, Bakaletz LO. Respiratory syncytial virus–induced dysregulation of expression of a mucosal beta-defensin augments colonization of the upper airway by non-typeable *Haemophilus influenzae*. Cell Microbiol. 2009; 11:1399–1408. [PubMed: 19500108]
25. Krishnamurthy A, McGrath J, Cripps AW, Kyd JM. The incidence of *Streptococcus pneumoniae* otitis media is affected by the polymicrobial environment particularly *Moraxella catarrhalis* in a mouse nasal colonisation model. Microbes Infect. 2009; 11:545–553. [PubMed: 19306940]
26. Moore HC, Jacoby P, Taylor A, et al. The interaction between respiratory viruses and pathogenic bacteria in the upper respiratory tract of asymptomatic Aboriginal and non-Aboriginal children. Pediatr Infect Dis J. 2010; 29:540–545. [PubMed: 20134359]
27. Tomochika K, Ichiyama T, Shimogori H, Sugahara K, Yamashita H, Furukawa S. Clinical characteristics of respiratory syncytial virus infection–associated acute otitis media. Pediatr Int. 2009; 51:484–487. [PubMed: 19674360]
28. Loeffelholz M, Chonmaitree T. Advances in diagnosis of respiratory virus infections. Int J Microbiol. 2010; 2010:126049. [PubMed: 20981303]
29. Abu-Diab A, Azzeh M, Ghneim R, et al. Comparison between pernasal flocked swabs and nasopharyngeal aspirates for detection of common respiratory viruses in samples from children. J Clin Microbiol. 2008; 46:2414–2417. [PubMed: 18480225]
30. Lambert SB, Whiley DM, O'Neill NT, et al. Comparing nose-throat swabs and nasopharyngeal aspirates collected from children with symptoms for respiratory virus identification using real-time polymerase chain reaction. Pediatrics. 2008; 122:e615–e620. [PubMed: 18725388]
31. Donati C, Hiller NL, Tettelin H, et al. Structure and dynamics of the pan-genome of *Streptococcus pneumoniae* and closely related species. Genome Biol. 2010; 11:R107. [PubMed: 21034474]
32. Croucher NJ, Harris SR, Fraser C, et al. Rapid pneumococcal evolution in response to clinical interventions. Science. 2011; 331:430–434. [PubMed: 21273480]
33. Hanage WP, Fraser C, Tang J, Connor TR, Corander J. Hyper-recombination, diversity, and antibiotic resistance in pneumococcus. Science. 2009; 324:1454–1457. [PubMed: 19520963]
34. Hiller NL, Ahmed A, Powell E, et al. Generation of genic diversity among *Streptococcus pneumoniae* strains via horizontal gene transfer during a chronic polyclonal pediatric infection. PLoS Pathog. 2010; 6:e1001108. [PubMed: 20862314]
35. Feng J, Lupien A, Gingras H, et al. Genome sequencing of linezolid-resistant *Streptococcus pneumoniae* mutants reveals novel mechanisms of resistance. Genome Res. 2009; 19:1214–1223. [PubMed: 19351617]
36. Romero P, Croucher NJ, Hiller NL, et al. Comparative genomic analysis of ten *Streptococcus pneumoniae* temperate bacteriophages. J Bacteriol. 2009; 191:4854–4862. [PubMed: 19502408]
37. Romero P, Garcia E, Mitchell TJ. Development of a prophage typing system and analysis of prophage carriage *in Streptococcus pneumoniae*. Appl Environ Microbiol. 2009; 75:1642–1649. [PubMed: 19168661]
38. Reid SD, Hong W, Dew KE, et al. *Streptococcus pneumoniae* forms surface-attached communities in the middle ear of experimentally infected chinchillas. J Infect Dis. 2009; 199:786–794. [PubMed: 19434911]
39. Weimer KE, Armbruster CE, Juneau RA, Hong W, Pang B, Swords WE. Coinfection with *Haemophilus influenzae* promotes pneumococcal biofilm formation during experimental otitis media and impedes the progression of pneumococcal disease. J Infect Dis. 2010; 202:1068–1075. [PubMed: 20715928]
40. Weimer KE, Juneau RA, Murrah KA, et al. Divergent mechanisms for passive pneumococcal resistance to beta-lactam antibiotics in the presence of *Haemophilus influenzae*. J Infect Dis. 2011; 203:549–555. [PubMed: 21220774]

41. Parker D, Soong G, Planet P, Brower J, Ratner AJ, Prince A. The NanA neuraminidase of *Streptococcus pneumoniae* is involved in biofilm formation. Infect Immun. 2009; 77:3722–3730. [PubMed: 19564377]
42. Trappetti C, Kadioglu A, Carter M, et al. Sialic acid: a preventable signal for pneumococcal biofilm formation, colonization, and invasion of the host. J Infect Dis. 2009; 199:1497–1505. [PubMed: 19392624]
43. Chen H, Ma Y, Yang J, et al. Genetic requirement for pneumococcal ear infection. PLoS One. 2008; 3:e2950. [PubMed: 18670623]
44. Casey JR, Adlowitz DG, Pichichero ME. New patterns in the otopathogens causing acute otitis media six to eight years after introduction of pneumococcal conjugate vaccine. Pediatr Infect Dis J. 2010; 29:304–309. [PubMed: 19935445]
45. Ongkasuwan J, Valdez TA, Hulten KG, Mason EO Jr, Kaplan SL. Pneumococcal mastoiditis in children and the emergence of multidrug-resistant serotype 19A isolates. Pediatrics. 2008; 122:34–39. [PubMed: 18595984]
46. Xu Q, Pichichero ME, Casey JR, Zeng M. Novel type of *Streptococcus pneumoniae* causing multidrug-resistant acute otitis media in children. Emerg Infect Dis. 2009; 15:547–551. [PubMed: 19331730]
47. Thomas JC, Figueira M, Fennie KP, et al. *Streptococcus pneumoniae* clonal complex 199: genetic diversity and tissue-specific virulence. PLoS One. 2011; 6:e18649. [PubMed: 21533186]
48. Laufer AS, Thomas JC, Figueira M, Gent JF, Pelton SI, Pettigrew MM. Capacity of serotype 19A and 15B/C *Streptococcus pneumoniae* isolates for experimental otitis media: implications for the conjugate vaccine. Vaccine. 2010; 28:2450–2457. [PubMed: 20067753]
49. Forbes ML, Horsey E, Hiller NL, et al. Strain-specific virulence phenotypes of *Streptococcus pneumoniae* assessed using the *Chinchilla laniger* model of otitis media. PLoS One. 2008; 3:e1969. [PubMed: 18398481]
50. Sabharwal V, Ram S, Figueira M, Park IH, Pelton SI. Role of complement in host defense against pneumococcal otitis media. Infect Immun. 2009; 77:1121–1127. [PubMed: 19139190]
51. Tong HH, Li YX, Stahl GL, Thurman JM. Enhanced susceptibility to acute pneumococcal otitis media in mice deficient in complement C1qa, factor B, and factor B/C2. Infect Immun. 2010; 78:976–983. [PubMed: 20065024]
52. Hyams C, Camberlein E, Cohen JM, Bax K, Brown JS. The *Streptococcus pneumoniae* capsule inhibits complement activity and neutrophil phagocytosis by multiple mechanisms. Infect Immun. 2010; 78:704–715. [PubMed: 19948837]
53. Melin M, Jarva H, Siira L, Meri S, Kayhty H, Vakevainen M. *Streptococcus pneumoniae* capsular serotype 19F is more resistant to C3 deposition and less sensitive to opsonophagocytosis than serotype 6B. Infect Immun. 2009; 77:676–684. [PubMed: 19047408]
54. Melin M, Trzcinski K, Antonio M, et al. Serotype-related variation in susceptibility to complement deposition and opsonophagocytosis among clinical isolates of *Streptococcus pneumoniae*. Infect Immun. 2010; 78:5252–5261. [PubMed: 20855517]
55. Dalia AB, Standish AJ, Weiser JN. Three surface exoglycosidases from *Streptococcus pneumoniae*, NanA, BgaA, and StrH, promote resistance to opsonophagocytic killing by human neutrophils. Infect Immun. 2010; 78:2108–2116. [PubMed: 20160017]
56. Kadioglu A, Weiser JN, Paton JC, Andrew PW. The role of *Streptococcus pneumoniae* virulence factors in host respiratory colonization and disease. Nat Rev Microbiol. 2008; 6:288–301. [PubMed: 18340341]
57. Marion C, Limoli DH, Bobulsky GS, Abraham JL, Burnaugh AM, King SJ. Identification of a pneumococcal glycosidase that modifies O-linked glycans. Infect Immun. 2009; 77:1389–1396. [PubMed: 19139197]
58. Yesilkaya H, Manco S, Kadioglu A, Terra VS, Andrew PW. The ability to utilize mucin affects the regulation of virulence gene expression in *Streptococcus pneumoniae*. FEMS Microbiol Lett. 2008; 278:231–235. [PubMed: 18053067]
59. Terra VS, Homer KA, Rao SG, Andrew PW, Yesilkaya H. Characterization of novel beta-galactosidase activity that contributes to glycoprotein degradation and virulence in *Streptococcus pneumoniae*. Infect Immun. 2010; 78:348–357. [PubMed: 19841081]

60. Shen H, Yoshida H, Yan F, et al. Synergistic induction of MUC5AC mucin by nontypeable *Haemophilus influenzae* and *Streptococcus pneumoniae*. Biochem Biophys Res Commun. 2008; 365:795–800. [PubMed: 18037371]
61. Davis KM, Akinbi HT, Standish AJ, Weiser JN. Resistance to mucosal lysozyme compensates for the fitness deficit of peptidoglycan modifications by *Streptococcus pneumoniae*. PLoS Pathog. 2008; 4:e1000241. [PubMed: 19079576]
62. Shimada J, Moon SK, Lee HY, et al. Lysozyme M deficiency leads to an increased susceptibility to *Streptococcus pneumoniae*–induced otitis media. BMC Infect Dis. 2008; 8:134. [PubMed: 18842154]
63. Bagnoli F, Moschioni M, Donati C, et al. A second pilus type in *Streptococcus pneumoniae* is prevalent in emerging serotypes and mediates adhesion to host cells. J Bacteriol. 2008; 190:5480–5492. [PubMed: 18515415]
64. Hilleringmann M, Giusti F, Baudner BC, et al. Pneumococcal pili are composed of protofilaments exposing adhesive clusters of Rrg A. PLoS Pathog. 2008; 4:e1000026. [PubMed: 18369475]
65. Munoz-Elias EJ, Marcano J, Camilli A. Isolation of *Streptococcus pneumoniae* biofilm mutants and their characterization during nasopharyngeal colonization. Infect Immun. 2008; 76:5049–5061. [PubMed: 18794289]
66. Moschioni M, Donati C, Muzzi A, et al. *Streptococcus pneumoniae* contains 3 rlrA pilus variants that are clonally related. J Infect Dis. 2008; 197:888–896. [PubMed: 18269316]
67. Moschioni M, Emolo C, Biagini M, et al. The two variants of the *Streptococcus pneumoniae* pilus 1 RrgA adhesin retain the same function and elicit cross-protection in vivo. Infect Immun. 2010; 78:5033–5042. [PubMed: 20823200]
68. Falker S, Nelson AL, Morfeldt E, et al. Sortase-mediated assembly and surface topology of adhesive pneumococcal pili. Mol Microbiol. 2008; 70:595–607. [PubMed: 18761697]
69. LeMieux J, Woody S, Camilli A. Roles of the sortases of *Streptococcus pneumoniae* in assembly of the RlrA pilus. J Bacteriol. 2008; 190:6002–6013. [PubMed: 18606733]
70. Manzano C, Contreras-Martel C, El Mortaji L, et al. Sortase-mediated pilus fiber biogenesis in *Streptococcus pneumoniae*. Structure. 2008; 16:1838–1848. [PubMed: 19081060]
71. Neiers F, Madhurantakam C, Falker S, et al. Two crystal structures of pneumococcal pilus sortase C provide novel insights into catalysis and substrate specificity. J Mol Biol. 2009; 393:704–716. [PubMed: 19729023]
72. Rosch JW, Mann B, Thornton J, Sublett J, Tuomanen E. Convergence of regulatory networks on the pilus locus of *Streptococcus pneumoniae*. Infect Immun. 2008; 76:3187–3196. [PubMed: 18443093]
73. Song XM, Connor W, Hokamp K, Babiuk LA, Potter AA. The growth phase–dependent regulation of the pilus locus genes by two-component system TCS08 in *Streptococcus pneumoniae*. Microb Pathogen. 2009; 46:28–35. [PubMed: 18983906]
74. Regev-Yochay G, Hanage WP, Trzcinski K, et al. Re-emergence of the type 1 pilus among *Streptococcus pneumoniae* isolates in Massachusetts, USA. Vaccine. 2010; 28:4842–4846. [PubMed: 20434550]
75. Zahner D, Gudlavalleti A, Stephens DS. Increase in pilus islet 2-encoded pili among *Streptococcus pneumoniae* isolates, Atlanta, Georgia, USA. Emerg Infect Dis. 2010; 16:955–962. [PubMed: 20507746]
76. Martner A, Dahlgren C, Paton JC, Wold AE. Pneumolysin released during *Streptococcus pneumoniae* autolysis is a potent activator of intracellular oxygen radical production in neutrophils. Infect Immun. 2008; 76:4079–4087. [PubMed: 18559434]
77. Chiavolini D, Pozzi G, Ricci S. Animal models of *Streptococcus pneumoniae* disease. Clin Microbiol Rev. 2008; 21:666–685. [PubMed: 18854486]
78. Sabirov A, Metzger DW. Mouse models for the study of mucosal vaccination against otitis media. Vaccine. 2008; 26:1501–1524. [PubMed: 18295938]
79. Trune DR, Zheng QY. Mouse models for human otitis media. Brain Res. 2009; 1277:90–103. [PubMed: 19272362]
80. Stol K, van Selm S, van den Berg S, et al. Development of a non-invasive murine infection model for acute otitis media. Microbiology. 2009; 155:4135–4144. [PubMed: 19762437]

81. Chaney EJ, Nguyen CT, Boppart SA. Novel method for non-invasive induction of a middle-ear biofilm in the rat. Vaccine. 2011; 29:1628–1633. [PubMed: 21211589]
82. McCullers JA, McAuley JL, Browall S, Iverson AR, Boyd KL, Henriques Normark B. Influenza enhances susceptibility to natural acquisition of and disease due to *Streptococcus pneumoniae* in ferrets. J Infect Dis. 2010; 202:1287–1295. [PubMed: 20822454]
83. Schachern P, Tsuprun V, Cureoglu S, et al. The round window membrane in otitis media: effect of pneumococcal proteins. Arch Otolaryngol Head Neck Surg. 2008; 134:658–662. [PubMed: 18559736]
84. Tsuprun V, Cureoglu S, Schachern PA, et al. Role of pneumococcal proteins in sensorineural hearing loss due to otitis media. Otol Neurotol. 2008; 29:1056–1060. [PubMed: 18833010]
85. Grijalva CG, Nuorti JP, Griffin MR. Antibiotic prescription rates for acute respiratory tract infections in US ambulatory settings. JAMA. 2009; 302:758–766. [PubMed: 19690308]
86. Vergison A, Dagan R, Arguedas A, et al. Otitis media and its consequences: beyond the earache. Lancet Infect Dis. 2010; 10:195–203. [PubMed: 20185098]
87. Zhou F, Shefer A, Kong Y, Nuorti JP. Trends in acute otitis media-related health care utilization by privately insured young children in the United States, 1997–2004. Pediatrics. 2008; 121:253–260. [PubMed: 18245415]
88. Mera RM, Miller LA, Amrine-Madsen H, Sahm DF. The impact of the pneumococcal conjugate vaccine on antimicrobial resistance in the United States since 1996: evidence for a significant rebound by 2007 in many classes of antibiotics. Microb Drug Resist. 2009; 15:261–268. [PubMed: 19857132]
89. Park IH, Park S, Hollingshead SK, Nahm MH. Genetic basis for the new pneumococcal serotype, 6C. Infect Immun. 2007; 75:4482–4489. [PubMed: 17576753]
90. Nahm MH, Lin J, Finkelstein JA, Pelton SI. Increase in the prevalence of the newly discovered pneumococcal serotype 6C in the nasopharynx after introduction of pneumococcal conjugate vaccine. J Infect Dis. 2009; 199:320–325. [PubMed: 19099489]
91. Nunes S, Valente C, Sa-Leao R, de Lencastre H. Temporal trends and molecular epidemiology of recently described serotype 6C of *Streptococcus pneumoniae*. J Clin Microbiol. 2009; 47(2):472–474. [PubMed: 19073873]
92. Porat N, Park IH, Nahm MH, Dagan R. Differential circulation of *Streptococcus pneumoniae* serotype 6C clones in two Israeli pediatric populations. J Clin Microbiol. 2010; 48:4649–4651. [PubMed: 20943862]
93. Bratcher PE, Park IH, Hollingshead SK, Nahm MH. Production of a unique pneumococcal capsule serotype belonging to serogroup 6. Microbiology. 2009; 155:576–583. [PubMed: 19202106]
94. Jin P, Kong F, Xiao M, et al. First report of putative *Streptococcus pneumoniae* serotype 6D among nasopharyngeal isolates from Fijian children. J Infect Dis. 2009; 200:1375–1380. [PubMed: 19803727]
95. Dagan R, Givon-Lavi N, Leibovitz E, Greenberg D, Porat N. Introduction and proliferation of multidrug-resistant *Streptococcus pneumoniae* serotype 19A clones that cause acute otitis media in an unvaccinated population. J Infect Dis. 2009; 199:776–785. [PubMed: 19434927]
96. Dagan R, Klugman KP. Impact of conjugate pneumococcal vaccines on antibiotic resistance. Lancet Infect Dis. 2008; 8:785–795. [PubMed: 19022193]
97. Pettigrew MM, Gent JF, Revai K, Patel JA, Chonmaitree T. Microbial interactions during upper respiratory tract infections. Emerg Infect Dis. 2008; 14:1584–1591. [PubMed: 18826823]
98. Laufer AS, Metlay JP, Gent JF, Fennie KP, Kong Y, Pettigrew MM. Microbial communities of the upper respiratory tract and otitis media in children. MBio. 2011; 2:e00245–00210. [PubMed: 21285435]
99. Selva L, Viana D, Regev-Yochay G, et al. Killing niche competitors by remote-control bacteriophage induction. Proc Natl Acad Sci USA. 2009; 106:1234–1238. [PubMed: 19141630]
100. Lysenko ES, Lijek RS, Brown SP, Weiser JN. Within-host competition drives selection for the capsule virulence determinant of *Streptococcus pneumoniae*. Curr Biol. 2010; 20:1222–1226. [PubMed: 20619820]

101. Hogg JS, Hu FZ, Janto B, et al. Characterization and modeling of the *Haemophilus influenzae* core and supragenomes based on the complete genomic sequences of Rd and 12 clinical nontypeable strains. Genome Biol. 2007; 8:R103. [PubMed: 17550610]
102. Juhas M, Power PM, Harding RM, et al. Sequence and functional analyses of *Haemophilus* spp. genomic islands. Genome Biol. 2007; 8:R237. [PubMed: 17996041]
103. Erwin AL, Sandstedt SA, Bonthuis PJ, et al. Analysis of genetic relatedness of *Haemophilus influenzae* isolates by multilocus sequence typing. J Bacteriol. 2008; 190:1473–1483. [PubMed: 18065541]
104. Boissy R, Ahmed A, Janto B, et al. Comparative supragenomic analyses among the pathogens *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Haemophilus influenzae* using a modification of the finite supragenome model. BMC Genomics. 2011; 12:187. [PubMed: 21489287]
105. Murphy TF, Brauer AL, Sethi S, Kilian M, Cai X, Lesse AJ. *Haemophilus haemolyticus*: a human respiratory tract commensal to be distinguished from *Haemophilus influenzae*. J Infect Dis. 2007; 195:81–89. [PubMed: 17152011]
106. McCrea KW, Xie J, LaCross N, et al. Relationships of non-typeable *Haemophilus influenzae* strains to hemolytic and nonhemolytic *Haemophilus haemolyticus* strains. J Clin Microbiol. 2008; 46:406–416. [PubMed: 18039799]
107. Sandstedt SA, Zhang L, Patel M, et al. Comparison of laboratory-based and phylogenetic methods to distinguish between *Haemophilus influenzae* and *H. haemolyticus*. J Microbiol Methods. 2008; 75:369–371. [PubMed: 18652852]
108. Erwin AL, Smith AL. Nontypeable *Haemophilus influenzae*: understanding virulence and commensal behavior. Trends Microbiol. 2007; 15:355–362. [PubMed: 17600718]
109. Gross J, Grass S, Davis AE, Gilmore-Erdmann P, Townsend RR, St Geme JW III. The *Haemophilus influenzae* HMW1 adhesin is a glycoprotein with an unusual N-linked carbohydrate modification. J Biol Chem. 2008; 283:26010–26015. [PubMed: 18621734]
110. St Geme JW III, Yeo HJ. A prototype two-partner secretion pathway: the *Haemophilus influenzae* HMW1 and HMW2 adhesin systems. Trends Microbiol. 2009; 17:355–360. [PubMed: 19660953]
111. Yeo HJ, Yokoyama T, Walkiewicz K, Kim Y, Grass S, Geme JW III. The structure of the *Haemophilus influenzae* HMW1 pro-piece reveals a structural domain essential for bacterial two-partner secretion. J Biol Chem. 2007; 282:31076–31084. [PubMed: 17699157]
112. Grass S, Lichti CF, Townsend RR, Gross J, St Geme JW III. The *Haemophilus influenzae* HMW1C protein is a glycosyl-transferase that transfers hexose residues to asparagine sites in the HMW1 adhesin. PLoS Pathog. 2010; 6:e1000919. [PubMed: 20523900]
113. Li H, Grass S, Wang T, Liu T, St Geme JW III. Structure of the *Haemophilus influenzae* HMW1B translocator protein: evidence for a twin pore. J Bacteriol. 2007; 189:7497–7502. [PubMed: 17693509]
114. Jurcisek JA, Bakaletz LO. Biofilms formed by nontypeable *Haemophilus influenzae* in vivo contain both dsDNA as well as type IV pilin protein. J Bacteriol. 2007; 189:3868–3875. [PubMed: 17322318]
115. Jurcisek JA, Bookwalter JE, Baker BD, et al. The PilA protein of non-typeable *Haemophilus influenzae* plays a role in biofilm formation, adherence to epithelial cells and colonization of the mammalian upper respiratory tract. Mol Microbiol. 2007; 65:1288–1299. [PubMed: 17645732]
116. Novotny LA, Clements JD, Bakaletz LO. Transcutaneous immunization as preventative and therapeutic regimens to protect against experimental otitis media due to nontypeable *Haemophilus influenzae*. Mucosal Immunol. 2011; 4:456–467. [PubMed: 21326197]
117. Bookwalter JE, Jurcisek JA, Gray-Owen SD, Fernandez S, McGillivary G, Bakaletz LO. A carcinoembryonic antigen-related cell adhesion molecule 1 homologue plays a pivotal role in nontypeable *Haemophilus influenzae* colonization of the chinchilla nasopharynx via the outer membrane protein P5-homologous adhesin. Infect Immun. 2008; 76:48–55. [PubMed: 17938212]
118. Morey P, Cano V, Marti-Lliteras P, et al. Evidence for a non-replicative intracellular stage of nontypable *Haemophilus influenzae* in epithelial cells. Microbiology. 2011; 157:234–250. [PubMed: 20929955]

119. Nistico L, Kreft R, Gieseke A, et al. Adenoid reservoir for pathogenic biofilm bacteria. J Clin Microbiol. 2011; 49:1411–1420. [PubMed: 21307211]
120. Moxon ER, Sweetman WA, Deadman ME, Ferguson DJ, Hood DW. *Haemophilus influenzae* biofilms: hypothesis or fact? Trends Microbiol. 2008; 16:95–100. [PubMed: 18280163]
121. Hoa M, Tomovic S, Nistico L, et al. Identification of adenoid biofilms with middle ear pathogens in otitis-prone children utilizing SEM and FISH. Int J Pediatr Otorhinolaryngol. 2009; 73:1242–1248. [PubMed: 19525016]
122. Hoa M, Syamal M, Schaeffer MA, Sachdeva L, Berk R, Coticchia J. Biofilms and chronic otitis media: an initial exploration into the role of biofilms in the pathogenesis of chronic otitis media. Am J Otolaryngol. 2010; 31:241–245. [PubMed: 20015753]
123. Izano EA, Shah SM, Kaplan JB. Intercellular adhesion and biocide resistance in nontypeable *Haemophilus influenzae* biofilms. Microb Pathogen. 2009; 46:207–213. [PubMed: 19490830]
124. Hong W, Mason K, Jurcisek J, Novotny L, Bakaletz LO, Swords WE. Phosphorylcholine decreases early inflammation and promotes the establishment of stable biofilm communities of nontypeable *Haemophilus influenzae* strain 86-028NP in a chinchilla model of otitis media. Infect Immun. 2007; 75:958–965. [PubMed: 17130253]
125. Hong W, Pang B, West-Barnette S, Swords WE. Phosphorylcholine expression by nontypeable *Haemophilus influenzae* correlates with maturation of biofilm communities in vitro and in vivo. J Bacteriol. 2007; 189:8300–8307. [PubMed: 17573475]
126. Armbruster CE, Hong W, Pang B, et al. LuxS promotes biofilm maturation and persistence of nontypeable *Haemophilus influenzae* in vivo via modulation of lipooligosaccharides on the bacterial surface. Infect Immun. 2009; 77:4081–4091. [PubMed: 19564381]
127. Armbruster CE, Hong W, Pang B, et al. Indirect pathogenicity of *Haemophilus influenzae* and *Moraxella catarrhalis* in polymicrobial otitis media occurs via interspecies quorum signaling. MBio. 2010; 1:e00102–10. [PubMed: 20802829]
128. Hong W, Juneau RA, Pang B, Swords WE. Survival of bacterial biofilms within neutrophil extracellular traps promotes nontypeable *Haemophilus influenzae* persistence in the chinchilla model for otitis media. J Innate Immun. 2009; 1:215–224. [PubMed: 20375579]
129. Juneau RA, Pang B, Weimer KE, Armbruster CE, Swords WE. Nontypeable *Haemophilus influenzae* initiates formation of neutrophil extracellular traps. Infect Immun. 2011; 79:431–438. [PubMed: 20956567]
130. Muller A, Severi E, Mulligan C, et al. Conservation of structure and mechanism in primary and secondary transporters exemplified by SiaP, a sialic acid binding virulence factor from *Haemophilus influenzae*. J Biol Chem. 2006; 281:22212–22222. [PubMed: 16702222]
131. Severi E, Randle G, Kivlin P, et al. Sialic acid transport in *Haemophilus influenzae* is essential for lipopolysaccharide sialylation and serum resistance and is dependent on a novel tripartite ATP-independent periplasmic transporter. Mol Microbiol. 2005; 58:1173–1185. [PubMed: 16262798]
132. Johnston JW, Coussens NP, Allen S, et al. Characterization of the N-acetyl-5-neuraminic acid-binding site of the extracytoplasmic solute receptor (SiaP) of nontypeable *Haemophilus influenzae* strain 2019. J Biol Chem. 2008; 283:855–865. [PubMed: 17947229]
133. Johnston JW, Zaleski A, Allen S, et al. Regulation of sialic acid transport and catabolism in *Haemophilus influenzae*. Mol Microbiol. 2007; 66:26–39. [PubMed: 17880422]
134. Jenkins GA, Figueira M, Kumar GA, et al. Sialic acid mediated transcriptional modulation of a highly conserved sialometabolism gene cluster in *Haemophilus influenzae* and its effect on virulence. BMC Microbiol. 2010; 10:48. [PubMed: 20158882]
135. Lundstrom SL, Li J, Deadman ME, Hood DW, Moxon ER, Schweda EK. Structural analysis of the lipopolysaccharide from nontypeable *Haemophilus influenzae* strain R2846. Biochemistry. 2008; 47:6025–6038. [PubMed: 18465844]
136. Lundstrom SL, Twelkmeyer B, Sagemark MK, et al. Novel globoside-like oligosaccharide expression patterns in non-typeable *Haemophilus influenzae* lipopolysaccharide. FEBS J. 2007; 274:4886–4903. [PubMed: 17725645]
137. Deadman ME, Hermant P, Engskog M, et al. Lex2B, a phase-variable glycosyltransferase, adds either a glucose or a galactose to *Haemophilus influenzae* lipopolysaccharide. Infect Immun. 2009; 77:2376–2384. [PubMed: 19289512]

138. Engskog MK, Yildirim HH, Li J, et al. A dual role for the lex2 locus: identification of galactosyltransferase activity in non-typeable *Haemophilus influenzae* strains 1124 and 2019. Carbohydrate Res. 2009; 344:632–641.

139. Hood DW, Deadman ME, Engskog MK, et al. Genes required for the synthesis of heptose-containing oligosaccharide outer core extensions in *Haemophilus influenzae* lipopolysaccharide. Microbiology. 2010; 156:3421–3431. [PubMed: 20688825]

140. Harrison A, Ray WC, Baker BD, Armbruster DW, Bakaletz LO, Munson RS Jr. The OxyR regulon in nontypeable *Haemophilus influenzae*. J Bacteriol. 2007; 189:1004–1012. [PubMed: 17142400]

141. Wong SM, Alugupalli KR, Ram S, Akerley BJ. The ArcA regulon and oxidative stress resistance *in Haemophilus influenzae*. Mol Microbiol. 2007; 64:1375–1390. [PubMed: 17542927]

142. Wong SM, St Michael F, Cox A, Ram S, Akerley BJ. ArcA-regulated glycosyltransferase lic2B promotes complement evasion and pathogenesis of nontypeable *Haemophilus influenzae*. Infect Immun. 2011; 79:1971–1983. [PubMed: 21357723]

143. Mason KM, Raffel FK, Ray WC, Bakaletz LO. Heme utilization by nontypeable *Haemophilus influenzae* is essential and dependent on Sap transporter function. J Bacteriol. 2011; 193:2527–2535. [PubMed: 21441512]

144. Gawronski JD, Wong SM, Giannoukos G, Ward DV, Akerley BJ. Tracking insertion mutants within libraries by deep sequencing and a genome-wide screen for *Haemophilus* genes required in the lung. Proc Natl Acad Sci U S A. 2009; 106:16422–16427. [PubMed: 19805314]

145. Nakamura S, Shchepetov M, Dalia AB, et al. Molecular basis of increased serum resistance among pulmonary isolates of non-typeable *Haemophilus influenzae*. PLoS Pathog. 2011; 7:e1001247. [PubMed: 21253576]

146. Figueira MA, Ram S, Goldstein R, Hood DW, Moxon ER, Pelton SI. Role of complement in defense of the middle ear revealed by restoring the virulence of nontypeable *Haemophilus influenzae* siaB mutants. Infect Immun. 2007; 75:325–333. [PubMed: 17088344]

147. Schachern PA, Tsuprun V, Wang B, et al. Effect of lipooligosaccharide mutations of *Haemophilus influenzae* on the middle and inner ears. Int J Pediatr Otorhinolaryngol. 2009; 73:1757–1760. [PubMed: 19853312]

148. de Vries SP, van Hijum SA, Schueler W, et al. Genome analysis of *Moraxella catarrhalis* strain RH4, a human respiratory tract pathogen. J Bacteriol. 2010; 192:3574–3583. [PubMed: 20453089]

149. Davie JJ, Earl J, de Vries SP, et al. Comparative analysis and supragenome modeling of twelve *Moraxella catarrhalis* clinical isolates. BMC Genomics. 2011; 12:70. [PubMed: 21269504]

150. Wang W, Reitzer L, Rasko DA, et al. Metabolic analysis of *Moraxella catarrhalis* and the effect of selected in vitro growth conditions on global gene expression. Infect Immun. 2007; 75:4959–4971. [PubMed: 17620351]

151. Attia AS, Sedillo JL, Wang W, et al. *Moraxella catarrhalis* expresses an unusual Hfq protein. Infect Immun. 2008; 76:2520–2530. [PubMed: 18362134]

152. Hoopman TC, Wang W, Brautigam CA, Sedillo JL, Reilly TJ, Hansen EJ. *Moraxella catarrhalis* synthesizes an auto-transporter that is an acid phosphatase. J Bacteriol. 2008; 190:1459–1472. [PubMed: 18065547]

153. Wang W, Kinkel T, Martens-Habbena W, Stahl DA, Fang FC, Hansen EJ. The *Moraxella catarrhalis* nitric oxide reductase is essential for nitric oxide detoxification. J Bacteriol. 2011; 193:2804–2813. [PubMed: 21441505]

154. Wang W, Richardson AR, Martens-Habbena W, Stahl DA, Fang FC, Hansen EJ. Identification of a repressor of a truncated denitrification pathway in *Moraxella catarrhalis*. J Bacteriol. 2008; 190:7762–7772. [PubMed: 18820017]

155. Hoopman TC, Liu W, Joslin SN, Pybus C, Brautigam CA, Hansen EJ. Identification of gene products involved in the oxidative stress response of *Moraxella catarrhalis*. Infect Immun. 2011; 79:745–755. [PubMed: 21098105]

156. Ruckdeschel EA, Kirkham C, Lesse AJ, Hu Z, Murphy TF. Mining the *Moraxella catarrhalis* genome: identification of potential vaccine antigens expressed during human infection. Infect Immun. 2008; 76:1599–1607. [PubMed: 18227159]

157. Ruckdeschel EA, Brauer AL, Johnson A, Murphy TF. Characterization of proteins Msp22 and Msp75 as vaccine antigens of *Moraxella catarrhalis*. Vaccine. 2009; 27:7065–7072. [PubMed: 19786139]

158. Yang M, Johnson A, Murphy TF. Characterization and evaluation of the *Moraxella catarrhalis* oligopeptide permease A as a mucosal vaccine antigen. Infect Immun. 2011; 79:846–857. [PubMed: 21134967]

159. Spaniol V, Troller R, Aebi C. Physiologic cold shock increases adherence of *Moraxella catarrhalis* to and secretion of interleukin 8 in human upper respiratory tract epithelial cells. J Infect Dis. 2009; 200:1593–1601. [PubMed: 19835476]

160. Faglin I, Wilson JC, Tiralongo J, Peak IR. *Moraxella catarrhalis* Lgt2, a galactosyltransferase with broad acceptor substrate specificity. Carbohydr Res. 2010; 345:2151–2156. [PubMed: 20832776]

161. Faglin I, Tiralongo J, Wilson JC, Collins PM, Peak IR. Biochemical analysis of Lgt3, a glycosyltransferase of the bacterium *Moraxella catarrhalis*. Biochem Biophys Res Commun. 2010; 393:609–613. [PubMed: 20153730]

162. Peak IR, Grice ID, Faglin I, et al. Towards understanding the functional role of the glycosyltransferases involved in the biosynthesis of *Moraxella catarrhalis* lipooligosaccharide. FEBS J. 2007; 274:2024–2037. [PubMed: 17388814]

163. Gao S, Peng D, Zhang W, Muszynski A, Carlson RW, Gu XX. Identification of two late acyltransferase genes responsible for lipid A biosynthesis in *Moraxella catarrhalis*. FEBS J. 2008; 275:5201–5214. [PubMed: 18795947]

164. Schwingel JM, St Michael F, Cox AD, Masoud H, Richards JC, Campagnari AA. A unique glycosyltransferase involved in the initial assembly of *Moraxella catarrhalis* lipooligosaccharides. Glycobiology. 2008; 18:447–455. [PubMed: 18337458]

165. Peng D, Hu WG, Choudhury BP, Muszynski A, Carlson RW, Gu XX. Role of different moieties from the lipooligosaccharide molecule in biological activities of the *Moraxella catarrhalis* outer membrane. FEBS J. 2007; 274:5350–5359. [PubMed: 17892485]

166. Schwingel JM, Edwards KJ, Cox AD, et al. Use of *Moraxella catarrhalis* lipooligosaccharide mutants to identify specific oligosaccharide epitopes recognized by human serum antibodies. Infect Immun. 2009; 77:4548–4558. [PubMed: 19651870]

167. Heiniger N, Spaniol V, Troller R, Vischer M, Aebi C. A reservoir of *Moraxella catarrhalis* in human pharyngeal lymphoid tissue. J Infect Dis. 2007; 196:1080–1087. [PubMed: 17763332]

168. Pearson MM, Hansen EJ. Identification of gene products involved in biofilm production by *Moraxella catarrhalis* ETSU-9 in vitro. Infect Immun. 2007; 75:4316–4325. [PubMed: 17562762]

169. Schaar V, de Vries SP, Perez Vidakovics ML, et al. Multicomponent *Moraxella catarrhalis* outer membrane vesicles induce an inflammatory response and are internalized by human epithelial cells. Cell Microbiol. 2011; 13:432–449. [PubMed: 21044239]

170. Vidakovics ML, Jendholm J, Morgelin M, et al. B cell activation by outer membrane vesicles—a novel virulence mechanism. PLoS Pathog. 2010; 6:e1000724. [PubMed: 20090836]

171. Tan TT, Morgelin M, Forsgren A, Riesbeck K. *Haemophilus influenzae* survival during complement-mediated attacks is promoted by *Moraxella catarrhalis* outer membrane vesicles. J Infect Dis. 2007; 195:1661–1670. [PubMed: 17471436]

172. N'Guessan PD, Temmesfeld-Wollbruck B, Zahlten J, et al. *Moraxella catarrhalis* induces ERK- and NF-kappaB-dependent COX-2 and prostaglandin E2 in lung epithelium. Eur Respir J. 2007; 30:443–451. [PubMed: 17537778]

173. N'Guessan PD, Vigelahn M, Bachmann S, et al. The UspA1 protein of *Moraxella catarrhalis* induces CEACAM-1-dependent apoptosis in alveolar epithelial cells. J Infect Dis. 2007; 195:1651–1660. [PubMed: 17471435]

174. Slevogt H, Maqami L, Vardarowa K, et al. Differential regulation of *Moraxella catarrhalis*–induced interleukin-8 response by protein kinase C isoforms. Eur Respir J. 2008; 31:725–735. [PubMed: 18184679]

175. Slevogt H, Zabel S, Opitz B, et al. CEACAM1 inhibits Toll-like receptor 2-triggered antibacterial responses of human pulmonary epithelial cells. Nat Immunol. 2008; 9:1270–1278. [PubMed: 18836450]
176. Slevogt H, Seybold J, Tiwari KN, et al. *Moraxella catarrhalis* is internalized in respiratory epithelial cells by a trigger-like mechanism and initiates a TLR2- and partly NOD1-dependent inflammatory immune response. Cell Microbiol. 2007; 9:694–707. [PubMed: 17054439]
177. Xie H, Gu XX. *Moraxella catarrhalis* lipooligosaccharide selectively upregulates ICAM-1 expression on human monocytes and stimulates adjacent naive monocytes to produce TNF-alpha through cellular cross-talk. Cell Microbiol. 2008; 10:1453–1467. [PubMed: 18363879]
178. Jendholm J, Samuelsson M, Cardell LO, Forsgren A, Riesbeck K. *Moraxella catarrhalis*–dependent tonsillar B cell activation does not lead to apoptosis but to vigorous proliferation resulting in nonspecific IgM production. J Leukoc Biol. 2008; 83:1370–1378. [PubMed: 18372337]
179. Manolov T, Tan TT, Forsgren A, Riesbeck K. *Moraxella*-dependent alpha 1-antichymotrypsin neutralization: a unique virulence mechanism. Am J Respir Cell Mol Biol. 2008; 38:609–617. [PubMed: 18096871]
180. Wirth T, Morelli G, Kusecek B, et al. The rise and spread of a new pathogen: seroresistant *Moraxella catarrhalis*. Genome Res. 2007; 17:1647–1656. [PubMed: 17895425]
181. Hallstrom T, Nordstrom T, Tan TT, et al. Immune evasion of *Moraxella catarrhalis* involves ubiquitous surface protein A–dependent C3d binding. J Immunol. 2011; 186:3120–3129. [PubMed: 21270401]
182. Singh B, Blom AM, Unal C, Nilson B, Morgelin M, Riesbeck K. Vitronectin binds to the head region of *Moraxella catarrhalis* ubiquitous surface protein A2 and confers complement-inhibitory activity. Mol Microbiol. 2010; 75:1426–1444. [PubMed: 20199596]
183. Hays JP, Gorkink R, Simons G, et al. High-throughput amplification fragment length polymorphism (htAFLP) analysis identifies genetic lineage markers but not complement phenotype-specific markers in *Moraxella catarrhalis*. Clin Microbiol Infect. 2007; 13:55–62. [PubMed: 17184288]
184. Easton DM, Maier E, Benz R, Foxwell AR, Cripps AW, Kyd JM. *Moraxella catarrhalis* M35 is a general porin that is important for growth under nutrient-limiting conditions and in the nasopharynges of mice. J Bacteriol. 2008; 190:7994–8002. [PubMed: 18931134]
185. Attia AS, Sedillo JL, Hoopman TC, et al. Identification of a bacteriocin and its cognate immunity factor expressed by *Moraxella catarrhalis*. BMC Microbiol. 2009; 9:207. [PubMed: 19781080]
186. Broides A, Dagan R, Greenberg D, Givon-Lavi N, Leibovitz E. Acute otitis media caused by *Moraxella catarrhalis*: epidemiologic and clinical characteristics. Clin Infect Dis. 2009; 49:1641–1647. [PubMed: 19886799]
187. Leibovitz E, Serebro M, Givon-Lavi N, et al. Epidemiologic and microbiologic characteristics of culture-positive spontaneous otorrhea in children with acute otitis media. Pediatr Infect Dis J. 2009; 28:381–384. [PubMed: 19319018]
188. Aguilar L, Alvarado O, Soley C, Abdelnour A, Dagan R, Arguedas A. Microbiology of the middle ear fluid in Costa Rican children between 2002 and 2007. Int J Pediatr Otorhinolaryngol. 2009; 73:1407–1411. [PubMed: 19683349]
189. Guevara S, Soley C, Arguedas A, Porat N, Dagan R. Seasonal distribution of otitis media pathogens among Costa Rican children. Pediatr Infect Dis J. 2008; 27:12–16. [PubMed: 18162931]
190. Sierra A, Lopez P, Zapata MA, et al. Non-typeable *Haemophilus influenzae* and *Streptococcus pneumoniae* as primary causes of acute otitis media in Colombian children: a prospective study. BMC Infect Dis. 2011; 11:4. [PubMed: 21208431]
191. Brook I, Gober AE. Bacteriology of spontaneously draining acute otitis media in children before and after the introduction of pneumococcal vaccination. Pediatr Infect Dis J. 2009; 28:640–642. [PubMed: 19561428]
192. Stamboulidis K, Chatzaki D, Poulakou G, et al. The impact of the heptavalent pneumococcal conjugate vaccine on the epidemiology of acute otitis media complicated by otorrhea. Pediatr Infect Dis J. 2011; 30:551–555. [PubMed: 21297521]

193. Ruohola A, Meurman O, Nikkari S, Skottman T, Heikkinen T, Ruuskanen O. The dynamics of bacteria in the middle ear during the course of acute otitis media with tympanostomy tube otorrhea. Pediatr Infect Dis J. 2007; 26:892–896. [PubMed: 17901793]

194. Smith-Vaughan H, Byun R, Halpin S, et al. Interventions for prevention of otitis media may be most effective if implemented in the first weeks of life. Int J Pediatr Otorhinolaryngol. 2008; 72:57–61. [PubMed: 18006084]

195. De Baere T, Vaneechoutte M, Deschaght P, Huyghe J, Dhooge I. The prevalence of middle ear pathogens in the outer ear canal and the nasopharyngeal cavity of healthy young adults. Clin Microbiol Infect. 2010; 16:1031–1035. [PubMed: 19895585]

196. Labout JA, Duijts L, Lebon A, et al. Risk factors for otitis media in children with special emphasis on the role of colonization with bacterial airway pathogens: the Generation R study. Eur J Epidemiol. 2011; 26:61–66. [PubMed: 20821039]

197. Jourdain S, Smeesters PR, Denis O, et al. Differences in nasopharyngeal bacterial carriage in preschool children from different socio-economic origins. Clin Microbiol Infect. 2010; 17:907–914. [PubMed: 20977542]

198. Mackenzie GA, Leach AJ, Carapetis JR, Fisher J, Morris PS. Epidemiology of nasopharyngeal carriage of respiratory bacterial pathogens in children and adults: cross-sectional surveys in a population with high rates of pneumococcal disease. BMC Infect Dis. 2010; 10:304. [PubMed: 20969800]

199. Brook I, Gober AE. Recovery of potential pathogens in the nasopharynx of healthy and otitis media–prone children and their smoking and nonsmoking parents. Ann Otol Rhinol Laryngol. 2008; 117:727–730. [PubMed: 18998498]

200. Konno M, Baba S, Mikawa H, et al. Study of nasopharyngeal bacterial flora: variations in nasopharyngeal bacterial flora in schoolchildren and adults when administered antimicrobial agents. J Infect Chemother. 2007; 13:235–254. [PubMed: 17721687]

201. Verhaegh SJ, Snippe ML, Levy F, et al. Colonization of healthy children by *Moraxella catarrhalis* is characterized by genotype heterogeneity, virulence gene diversity and co-colonization with *Haemophilus influenzae*. Microbiology. 2011; 157:169–178. [PubMed: 20847012]

202. Mitov IG, Gergova RT, Ouzounova-Raykova VV. Distribution of genes encoding virulence factors ompB2, ompCD, ompE, beta-lactamase and serotype in pathogenic and colonizing strains of *Moraxella catarrhalis*. Arch Med Res. 2010; 41:530–535. [PubMed: 21167392]

203. Thomas JC, Pettigrew MM. Multilocus sequence typing and pulsed field gel electrophoresis of otitis media causing pathogens. Methods Mol Biol. 2009; 493:179–190. [PubMed: 18839348]

204. Pingault NM, Lehmann D, Bowman J, Riley TV. A comparison of molecular typing methods for *Moraxella catarrhalis*. J Appl Microbiol. 2007; 103:2489–2495. [PubMed: 17850316]

205. Verhaegh SJ, Streefland A, Dewnarain JK, Farrell DJ, van Belkum A, Hays JP. Age-related genotypic and phenotypic differences in *Moraxella catarrhalis* isolates from children and adults presenting with respiratory disease in 2001–2002. Microbiology. 2008; 154:1178–1184. [PubMed: 18375810]

206. Jetter M, Heiniger N, Spaniol V, Troller R, Schaller A, Aebi C. Outer membrane porin M35 of *Moraxella catarrhalis* mediates susceptibility to aminopenicillins. BMC Microbiol. 2009; 9:188. [PubMed: 19732412]

207. Jetter M, Spaniol V, Troller R, Aebi C. Down-regulation of porin M35 in *Moraxella catarrhalis* by aminopenicillins and environmental factors and its potential contribution to the mechanism of resistance to aminopenicillins. J Antimicrob Chemother. 2010; 65:2089–2096. [PubMed: 20801781]

208. Sahm DF, Brown NP, Thornsberry C, Jones ME. Antimicrobial susceptibility profiles among common respiratory tract pathogens: a GLOBAL perspective. Postgrad Med. 2008; 120(3 suppl 1):16–24. [PubMed: 18931467]

209. Bell JM, Turnidge JD, Jones RN. Development of a disk diffusion method for testing *Moraxella catarrhalis* susceptibility using clinical and laboratory standards institute methods: a SENTRY antimicrobial surveillance program report. J Clin Microbiol. 2009; 47:2187–2193. [PubMed: 19458179]

210. Hallstrom T, Muller SA, Morgelin M, Mollenkvist A, Forsgren A, Riesbeck K. The *Moraxella* IgD-binding protein MID/Hag is an oligomeric autotransporter. Microbes Infect. 2008; 10:374–381. [PubMed: 18400547]

211. Bullard B, Lipski S, Lafontaine ER. Regions important for the adhesin activity of *Moraxella catarrhalis* Hag. BMC Microbiol. 2007; 7:65. [PubMed: 17608944]

212. Balder R, Krunkosky TM, Nguyen CQ, Feezel L, Lafontaine ER. Hag mediates adherence of *Moraxella catarrhalis* to ciliated human airway cells. Infect Immun. 2009; 77:4597–4608. [PubMed: 19667048]

213. Balder R, Hassel J, Lipski S, Lafontaine ER. *Moraxella catarrhalis* strain O35E expresses two filamentous hemagglutinin-like proteins that mediate adherence to human epithelial cells. Infect Immun. 2007; 75:2765–2775. [PubMed: 17371858]

214. Plamondon P, Luke NR, Campagnari AA. Identification of a novel two-partner secretion locus in *Moraxella catarrhalis*. Infect Immun. 2007; 75:2929–2936. [PubMed: 17420235]

215. Lipski SL, Holm MM, Lafontaine ER. Identification of a *Moraxella catarrhalis* gene that confers adherence to various human epithelial cell lines in vitro. FEMS Microbiol Lett. 2007; 267:207–213. [PubMed: 17166229]

216. Lipski SL, Akimana C, Timpe JM, Wooten RM, Lafontaine ER. The *Moraxella catarrhalis* autotransporter McaP is a conserved surface protein that mediates adherence to human epithelial cells through its N-terminal passenger domain. Infect Immun. 2007; 75:314–324. [PubMed: 17088358]

217. Conners R, Hill DJ, Borodina E, et al. The *Moraxella* adhesin UspA1 binds to its human CEACAM1 receptor by a deformable trimeric coiled-coil. EMBO J. 2008; 27:1779–1789. [PubMed: 18497748]

218. Spaniol V, Heiniger N, Troller R, Aebi C. Outer membrane protein UspA1 and lipooligosaccharide are involved in invasion of human epithelial cells by *Moraxella catarrhalis*. Microbes Infect. 2008; 10:3–11. [PubMed: 18069032]

219. Brooks MJ, Sedillo JL, Wagner N, et al. *Moraxella catarrhalis* binding to host cellular receptors is mediated by sequence-specific determinants not conserved among all UspA1 protein variants. Infect Immun. 2008; 76:5322–5329. [PubMed: 18678656]

220. Qiu H, Kumita W, Sato K, et al. uspA1 of *Moraxella catarrhalis* clinical isolates in Japan and its relationship with adherence to HEp-2 cells. J Med Dent Sci. 2009; 56:61–67. [PubMed: 19697520]

221. Akimana C, Lafontaine ER. The *Moraxella catarrhalis* outer membrane protein CD contains two distinct domains specifying adherence to human lung cells. FEMS Microbiol Lett. 2007; 271:12–19. [PubMed: 17391370]

222. Brooks MJ, Laurence CA, Hansen EJ, Gray-Owen SD. Characterization of the *Moraxella catarrhalis* opa-like protein, OlpA, reveals a phylogenetically conserved family of outer membrane proteins. J Bacteriol. 2007; 189:76–82. [PubMed: 17041038]

223. Luke NR, Jurcisek JA, Bakaletz LO, Campagnari AA. Contribution of *Moraxella catarrhalis* type IV pili to nasopharyngeal colonization and biofilm formation. Infect Immun. 2007; 75:5559–5564. [PubMed: 17908808]

224. Luke-Marshall NR, Sauberan SL, Campagnari AA. Comparative analyses of the *Moraxella catarrhalis* type-IV pilus structural subunit PilA. Gene. 2011; 477:19–23. [PubMed: 21256201]

225. Vareille M, Kieninger E, Edwards MR, Regamey N. The airway epithelium: soldier in the fight against respiratory viruses. Clin Microbiol Rev. 2011; 24:210–229. [PubMed: 21233513]

226. Ogra, Pl. Mucosal immune system in neonatal period and early infancy. Pediatr Health. 2010; 4:637–647.

227. Krege J, Seth S, Hardtke S, Davalos-Misslitz AC, Forster R. Antigen-dependent rescue of nose-associated lymphoid tissue (NALT) development independent of LTbetaR and CXCR5 signaling. Eur J Immunol. 2009; 39:2765–2778. [PubMed: 19757439]

228. Zelazowska-Rutkowska B, Wysocka J, Skotnicka B. Chosen factors of T and B cell apoptosis in hypertrophic adenoid in children with otitis media with effusion. Int J Pediatr Otorhinolaryngol. 2010; 74:698–700. [PubMed: 20338643]

229. Kotowski M, Niedzielski A, Niedzielska G, Lachowska-Kotowska P. Dendritic cells and lymphocyte subpopulations of the adenoid in the pathogenesis of otitis media with effusion. Int J Pediatr Otorhinolaryngol. 2011; 75:265–269. [PubMed: 21144597]
230. Granath A, Norrby-Teglund A, Uddman R, Cardell LO. Reduced iNOS expression in adenoids from children with otitis media with effusion. Pediatr Allergy Immunol. 2010; 21:1151–1156. [PubMed: 21073541]
231. Siegmund K, Ruckert B, Ouaked N, et al. Unique phenotype of human tonsillar and in vitro–induced FOXP3+CD8+ T cells. J Immunol. 2009; 182:2124–2130. [PubMed: 19201865]
232. Iliev ID, Spadoni I, Mileti E, et al. Human intestinal epithelial cells promote the differentiation of tolerogenic dendritic cells. Gut. 2009; 58:1481–1489. [PubMed: 19570762]
233. Mills KHG. Regulatory T cells: friend or foe in immunity to infection? Nat Rev Immunol. 2004; 4:841–855. [PubMed: 15516964]
234. Lee YK, Mukasa R, Hatton RD, Weaver CT. Developmental plasticity of Th17 and Treg cells. Curr Opin Immunol. 2009; 21:274–280. [PubMed: 19524429]
235. Voo KS, Wang YH, Santori FR, et al. Identification of IL-17-producing FOXP3+ regulatory T cells in humans. Proc Natl Acad Sci U S A. 2009; 106:4793–4798. [PubMed: 19273860]
236. Pillai V, Ortega SB, Wang CK, Karandikar NJ. Transient regulatory T-cells: a state attained by all activated human T-cells. Clin Immunol. 2007; 123:18–29. [PubMed: 17185041]
237. Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. Nat Rev Immunol. 2009; 9:313–323. [PubMed: 19343057]
238. Malmhall C, Bossios A, Pullerits T, Lotvall J. Effects of pollen and nasal glucocorticoid on FOXP3+, GATA-3+ and T-bet+ cells in allergic rhinitis. Allergy. 2007; 62:1007–1013. [PubMed: 17686103]
239. Radulovic S, Jacobson MR, Durham SR, Nouri-Aria KT. Grass pollen immunotherapy induces Foxp3-expressing CD4+ CD25+ cells in the nasal mucosa. J Allergy Clin Immunol. 2008; 121:1467–1472. [PubMed: 18423565]
240. Davenport V, Groves E, Hobbs CG, Williams NA, Heyderman RS. Regulation of Th-1 T cell–dominated immunity to *Neisseria meningitidis* within the human mucosa. Cell Microbiol. 2007; 9:1050–1061. [PubMed: 17166235]
241. Hirano T, Kodama S, Moriyama M, Kawano T, Suzuki M. The role of Toll-like receptor 4 in eliciting acquired immune responses against nontypeable *Haemophilus influenzae* following intranasal immunization with outer membrane protein. Int J Pediatr Otorhinolaryngol. 2009; 73:1657–1665. [PubMed: 19765832]
242. Leichtle A, Hernandez M, Pak K, Webster NJ, Wasserman SI, Ryan AF. The Toll-like receptor adaptor TRIF contributes to otitis media pathogenesis and recovery. BMC Immunol. 2009; 10:45. [PubMed: 19656404]
243. Han F, Yu H, Tian C, et al. Role for Toll-like receptor 2 in the immune response to *Streptococcus pneumoniae* infection in mouse otitis media. Infect Immun. 2009; 77:3100–3108. [PubMed: 19414550]
244. Song JJ, Cho JG, Woo JS, Lee HM, Hwang SJ, Chae SW. Differential expression of Toll-like receptors 2 and 4 in rat middle ear. Int J Pediatr Otorhinolaryngol. 2009; 73:821–824. [PubMed: 19303147]
245. Kim MG, Park DC, Shim JS, et al. TLR-9, NOD-1, NOD-2, RIG-I and immunoglobulins in recurrent otitis media with effusion. Int J Pediatr Otorhinolaryngol. 2010; 74:1425–1429. [PubMed: 20980062]
246. Leichtle A, Hernandez M, Lee J, et al. The role of DNA sensing and innate immune receptor TLR9 in otitis media. Innate Immun. 2012; 18:3–13. [PubMed: 21239460]
247. Lysenko ES, Clarke TB, Shchepetov M, et al. Nod1 signaling overcomes resistance of *S. pneumoniae* to opsonophagocytic killing. PLoS Pathog. 2007; 3:e118. [PubMed: 17722978]
248. Clarke TB, Davis KM, Lysenko ES, Zhou AY, Yu Y, Weiser JN. Recognition of peptidoglycan from the microbiota by Nod1 enhances systemic innate immunity. Nat Med. 2010; 16:228–231. [PubMed: 20081863]

249. Kulkarni R, Rampersaud R, Aguilar JL, Randis TM, Kreindler JL, Ratner AJ. Cigarette smoke inhibits airway epithelial cell innate immune responses to bacteria. Infect Immun. 2010; 78:2146–2152. [PubMed: 20194598]
250. Lee HY, Takeshita T, Shimada J, et al. Induction of beta defensin 2 by NTHi requires TLR2 mediated MyD88 and IRAK-TRAF6-p38MAPK signaling pathway in human middle ear epithelial cells. BMC Infect Dis. 2008; 8:87. [PubMed: 18578886]
251. McGillivary G, Bakaletz LO. The multifunctional host defense peptide SPLUNC1 is critical for homeostasis of the mammalian upper airway. PLoS One. 2010; 5:e13224. [PubMed: 20949060]
252. Song JJ, Kwon SK, Cho CG, Park SW, Chae SW. Microarray analysis of microRNA expression in LPS induced inflammation of human middle ear epithelial cells (HMEECs) [published online March 4, 2011]. Int J Pediatr Otorhinolaryngol.
253. Avanzini AM, Castellazzi AM, Marconi M, et al. Children with recurrent otitis show defective IFN gamma-producing cells in adenoids. Pediatr Allergy Immunol. 2008; 19:523–526. [PubMed: 18266836]
254. Martner A, Skovbjerg S, Paton JC, Wold AE. *Streptococcus pneumoniae* autolysis prevents phagocytosis and production of phagocyte-activating cytokines. Infect Immun. 2009; 77:3826–3837. [PubMed: 19528220]
255. Shim HJ, Park DC, Lee YC, Eun YG, Yeo SG. Expression of GATA3, T-bet, IL-4, and IFN-gamma mRNA in the effusion of OME patients. Int J Pediatr Otorhinolaryngol. 2009; 73:1119–1123. [PubMed: 19481821]
256. Sonnenberg GF, Fouser LA, Artis D. Border patrol: regulation of immunity, inflammation and tissue homeostasis at barrier surfaces by IL-22. Nat Immunol. 2011; 12:383–390. [PubMed: 21502992]
257. Sonnenberg GF, Nair MG, Kirn TJ, Zaph C, Fouser LA, Artis D. Pathological versus protective functions of IL-22 in airway inflammation are regulated by IL-17A. J Exp Med. 2010; 207:1293–1305. [PubMed: 20498020]
258. Lebon A, Verkaik NJ, de Vogel CP, et al. The inverse correlation between *Staphylococcus aureus* and *Streptococcus pneumoniae* colonization in infants is not explained by differences in serum antibody levels in the Generation R Study. Clin Vaccine Immunol. 2011; 18:180–183. [PubMed: 21084460]
259. Yamanaka N, Hotomi M, Billal D. Clinical bacteriology and immunology in acute otitis media in children. J Infect Chemother. 2008; 14:180–187. [PubMed: 18574652]
260. Korppi M, Leinonen M, Ruuskanen O. Pneumococcal serology in children's respiratory infections. Eur J Clin Microbiol Infect Dis. 2008; 27:167–175. [PubMed: 18087733]
261. Yeo SG, Park DC, Lee SK, Cha CI. Relationship between effusion bacteria and concentrations of immunoglobulin in serum and effusion fluid in otitis media with effusion patients. Int J Pediatr Otorhinolaryngol. 2008; 72:337–342. [PubMed: 18242717]
262. Roche AM, Weiser JN. Identification of the targets of cross-reactive antibodies induced by *Streptococcus pneumoniae* colonization. Infect Immun. 2010; 78:2231–2239. [PubMed: 20231407]
263. Ferreira DM, Darrieux M, Silva DA, et al. Characterization of protective mucosal and systemic immune responses elicited by pneumococcal surface protein PspA and PspC nasal vaccines against a respiratory pneumococcal challenge in mice. Clin Vaccine Immunol. 2009; 16:636–645. [PubMed: 19279169]
264. Katsurahara T, Hotomi M, Yamauchi K, Billal DS, Yamanaka N. Protection against systemic fatal pneumococcal infection by maternal intranasal immunization with pneumococcal surface protein A (PspA). J Infect Chemother. 2008; 14:393–398. [PubMed: 19089550]
265. Cao J, Gong Y, Li D, et al. CD4(+) T lymphocytes mediated protection against invasive pneumococcal infection induced by mucosal immunization with ClpP and CbpA. Vaccine. 2009; 27:2838–2844. [PubMed: 19366577]
266. Groneck L, Schrama D, Fabri M, et al. Oligoclonal CD4+ T cells promote host memory immune responses to zwitterionic polysaccharide of *Streptococcus pneumoniae*. Infect Immun. 2009; 77:3705–3712. [PubMed: 19546196]

267. Lugade AA, Vethanayagam RR, Nasirikenari M, Bogner PN, Segal BH, Thanavala Y. Nrf2 regulates chronic lung inflammation and B cell responses to non-typeable *Haemophilus influenzae*. Am J Resp Cell Mol Biology. 2011; 45:557–565.

268. Ostberg KL, Russell MW, Murphy TF. Mucosal immunization of mice with recombinant OMP P2 induces antibodies that bind to surface epitopes of multiple strains of nontypeable *Haemophilus influenzae*. Mucosal Immunol. 2009; 2:63–73. [PubMed: 19079335]

269. Sabirov A, Casey JR, Murphy TF, Pichichero ME. Breast-feeding is associated with a reduced frequency of acute otitis media and high serum antibody levels against NTHi and outer membrane protein vaccine antigen candidate P6. Pediatr Res. 2009; 66:565–570. [PubMed: 19581824]

270. Kodama S, Hirano T, Noda K, Abe N, Suzuki M. A single nasal dose of fms-like tyrosine kinase receptor-3 ligand, but not peritoneal application, enhances nontypeable *Haemophilus influenzae*–specific long-term mucosal immune responses in the nasopharynx. Vaccine. 2010; 28:2510–2516. [PubMed: 20117272]

271. Winter LE, Barenkamp SJ. Antibodies specific for the Hia adhesion proteins of nontypeable *Haemophilus influenzae* mediate opsonophagocytic activity. Clin Vaccine Immunol. 2009; 16:1040–1046. [PubMed: 19474261]

272. Hallstrom T, Resman F, Ristovski M, Riesbeck K. Binding of complement regulators to invasive nontypeable *Haemophilus influenzae* isolates is not increased compared to nasopharyngeal isolates, but serum resistance is linked to disease severity. J Clin Microbiol. 2010; 48:921–927. [PubMed: 20089757]

273. Hallstrom T, Riesbeck K. *Haemophilus influenzae* and the complement system. Trends Microbiol. 2010; 18:258–265. [PubMed: 20399102]

Table 1

Adhesins of *Moraxella catarrhalis* and New Observations over the Past 4 Years

Adhesin	Putative Function	New Observation	Reference
MID/Hag	Adhesin, binds IgD, hemagglutinin	• Distinct regions of MID/Hag mediate binding to epithelial cells and collagen • MID/Hag is an oligomeric autotransporter • MID/Hag mediates adherence to ciliated human bronchial epithelial cells	210–212
MchA1, MchA2 (MhaB1, MhaB2)	Filamentous hemagglutinin-like adhesin	• Identification of 2-partner secretion locus that encodes a newly identified adhesin	213, 214
McmA	Metallopeptidase-like adhesin	• Identification of new adhesin	215, 216
UspA1	Adhesin	• CEACAM1 binding region is a trimeric coiled-coil • UspA1 facilitates invasion of epithelial cells • UspA1 of some strains shows variability in selected binding domains • UspA1 induces apoptosis of pulmonary epithelial cells • Expression of UspA1 is upregulated at 26°C	159, 173, 217–220
McaP	Adhesin and phospholipase B	• An N-terminal passenger domain mediates adherence to host cells	216
OMP CD	OMP A–like protein, binds mucin, adhesin	• OMP CD has 2 distinct cell binding domains	221
OlpA	Homologous with *Neisseria* Opa adhesins	• Newly identified genes that belong to a conserved family of adhesins (OlpA has not yet been identified as an adhesin) • Sequence is conserved among strains	222
Type IV pili	Adhesin, transformation, biofilm formation	• Mediate adherence to eukaryotic cells • Enhance biofilm formation • Contribute to nasopharyngeal colonization in the chinchilla model	223, 224