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Mission Statement

The mission of *Otolaryngology–Head and Neck Surgery* is to publish contemporary, ethical, clinically relevant information in otolaryngology, head and neck surgery (ear, nose, throat, head, and neck disorders) that can be used by otolaryngologists, scientists, clinicians, and related specialists to improve patient care and public health.

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Panel 1: Epidemiology, Natural History, and Risk Factors

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Abstract

Background. The First International Symposium on Recent Advances in Otitis Media (OM) with Effusion was held in Columbus, Ohio, in 1975. The symposium has been organized in the United States every 4 years since, followed by a research conference to (a) assess major research accomplishments, (b) identify important research questions and opportunities, (c) develop consensus on definitions and terminology, and (d) establish priorities with short- and long-term research goals. One of the principal areas reviewed quadrennially is Epidemiology, Natural History, and Risk Factors.

Objective. To provide a review of recent literature on the epidemiology, natural history, and risk factors for OM.

Data Sources and Review Methods. A search of OM articles in English published July 2007 to June 2011 was conducted using PubMed and related databases. Those with findings judged of importance for epidemiology, public health, and/or statistical methods were reviewed.

Results. The literature has continued to expand, increasing understanding of the worldwide burden of OM in childhood, complications from treatment failures, and comorbidities. Novel risk factors, including genetic factors, have been examined for OM susceptibility. Population-based studies in Canada, the United States, and other countries confirmed reductions in OM prevalence. Although most studies concentrated on acute OM (AOM) or OM with effusion (OME), a few examined severe chronic suppurative OM (CSOM), a major public health problem in developing countries and for certain indigenous populations around the world.

Conclusions and Implications for Practice. Recent publications have reinforced earlier epidemiological findings, while extending our knowledge in human population groups with high burden of OM.

Keywords

otitis media, epidemiology, natural history, risk factors, incidence, prevalence, disease burden, population-based study, cohort study, case-control study, hazard ratio, odds ratio, relative risk, logistic regression, randomized clinical trial, meta-analysis, quality of life

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The natural history and risk factors for otitis media (OM) have been well studied in population-based samples of young children over the past quarter century in developed countries and to a growing extent in developing countries. Several early epidemiological studies focused on indigenous populations of American Indians/Alaska Natives (AI/AN), Australian Aborigines, and Inuits in the Arctic (Greenland) in whom physicians had observed high rates of OM with complications.[1-9] Many significant aspects of OM epidemiology and natural history were first characterized during this quarter century, 1960 to 1985, of pioneering research. By the end of this era, most of the potential sociodemographic and parental lifestyle factors contributing to OM risk, such as child's age (at first and subsequent OM episodes), sex, race/ethnicity, low birth weight (LBW: <2500 g), preterm birth

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(<37 completed weeks of gestation), season of birth, breastfeeding vs bottle feeding, daycare attendance, number of siblings, parent's education, parent's occupation, household income, history of ear infections (plus other infectious illnesses or morbid conditions) in siblings or parents, health insurance coverage, and exposure to prenatal or postnatal cigarette smoking, had been evaluated.[10-15]

The risk factors established by these early, foundational studies continue to be investigated, often with refined study designs, improved methodology, and enhanced statistical analysis techniques. The scope of OM epidemiological studies has also expanded over the past decade. In recent years, the ability to perform detailed diagnostic evaluations of bacterial/viral infectious agents and to measure cytokines and other immunological responses has greatly increased. Advances in understanding of the human genome have led to sophisticated genotyping studies of children and families with high OM risk. These studies have led to discoveries that improved our understanding and may ultimately lead to interventions to prevent OM and its complications.

With the introduction of multivalent pneumococcal conjugate vaccines circa 2000, primarily for the purpose of controlling invasive pneumococcal disease (bacteremia and meningitis)[16] and secondarily for prevention of OM, modest reductions in OM prevalence have been observed in several developed countries.[17,18] The development of new vaccines against additional serotypes of pneumococcus or for the other 2 major acute otitis media (AOM) pathogens, nontypeable *Haemophilus influenza* (NTHi) and *Moraxella*, holds the potential for greatly decreasing the early childhood burden of OM.[19]

The panel convened June 9, 2011, as part of the post–Symposium Research Conference. The scope of the report and submission of draft sections were discussed. The panel agreed to review publications based on appropriateness of study design, analytic methodology, and potential significance of reported findings. The objective of this report is to present a compact survey of recent clinical, epidemiological, and public health findings of importance to the OM field.

Methods

Panel members were invited between January and March 2011 and, after agreeing to participate, were assigned particular sections or topics to divide the labor and produce an initial draft report prior to the OM 2011 symposium meeting. The review is focused on publications written in English within a 4-year time frame (July 2007–June 2011). Databases searched were PubMed (includes MEDLINE), Cochrane, Embase, Scopus, and Web of Science (Science Citation Index). Our aim was to identify original reports of observational cohort or case-control studies. However, we also examined review articles, systematic database reviews, randomized controlled trials (RCTs), and relevant statistical methodological papers. The primary search terms were *otitis media* and *epidemiology* in children (all stages of childhood). A further search was made using the terms *otitis media* and *statistical methods*. These search terms subsumed other critical ones, such as *acute OM, recurrent OM, OM with effusion, chronic suppurative OM, incidence, prevalence, risk factors*, and *sociodemographic factors*, and included studies that focused on "treatment" and "practice guidelines." The literature searches began in April 2011 and were completed in July 2011. A final check including all of the databases was performed in January 2012.

The search of databases identified 318 studies after discarding duplicates; another 53 studies were found by searching for *OM* and *statistical methods*. After retrieving abstracts of these 371 articles, we excluded 260 (70%) that were clinical or case series reports, narrative overviews, studies that were tutorial in nature, research studies performed using animal models, or studies whose main focus was outside the scope of epidemiology/natural history and recent statistical methodological developments with applications to OM research.

In addition to our broad systematic search of the literature, additional publications were identified by panel members who had more in-depth knowledge of the literature within their areas of interest and expertise. They performed searches on OM and *genetic susceptibility, prenatal and perinatal factors, allergy and atopy, knowledge of risk factors*, or *preventive strategies*. The search to characterize the early literature on OM epidemiology and natural history studies prior to 1985 was performed using PubMed or Google Scholar or identified by one of the panel members. Publications cited in the previous panel review were excluded from this review.[20]

Prevalence and Incidence

Trends in Prevalence. The annual prevalence of OM diagnoses in the United States has been tracked since 1997 as part of Healthy People 2010. The *Healthy People 2010: Final Review* reported that OM diagnoses in children and adolescents declined by 28% between 1997 and 2007, from 345 to 247 per 1000 children younger than 18 years in the United States. The 2007 prevalence estimate is lower than the target set at the beginning of the decade of 294 per 1000 children.[21] The youngest children (younger than 3 years) had the highest rates of OM diagnoses, as judged from surveys based on ambulatory care visits to physician offices, hospital outpatient departments (OPDs), and hospital emergency departments (EDs). In children younger than 3 years, OM diagnosis rates declined by 38% from 1160 per 1000 children in 1997 to 840 in 2006 and 724 in 2007.[22] These rates are derived from 2 large annual surveys conducted by the US National Center for Health Statistics (NCHS), Centers for Disease Control and Prevention (CDC): the National Ambulatory Medical Care Survey (NAMCS) and the National Hospital Ambulatory Medical Care Survey (NHAMCS). The survey data are combined to produce annual estimates of ambulatory medical care utilization.[23,24] In contrast to the youngest children (younger than 3 years), OM diagnosis rates among children aged 3 to 5 years and 6 to 17 years increased (275 to 316 and 70 to 107, respectively) between 2006 and 2007. This rate increase in short-term trends of OM diagnosis in older children may result from sampling variability or, perhaps, from infection with organisms whose serotypes were not included in the pneumococcal

conjugate vaccine or by replacement organisms (*H influenzae* and *Moraxella catarrhalis*). Males and non-Hispanic (NH) whites had higher reported OM visit rates in all age groups. Although frequent reference is made to the introduction of pneumococcal conjugate vaccines as probably an important factor in the decline in OM incidence and prevalence, another factor that may have contributed over the past decade is the guidelines encouraging primary care providers to use more stringent criteria in diagnosing AOM (eg, the 2004 American Academy of Family Physicians [AAFP]/American Academy of Pediatrics [AAP] guidelines).[25,26] Currently, there is no empirical evidence to support the suggestion that primary care providers are becoming more stringent in diagnosing AOM; however, this possibility is worth mentioning in connection with the declining AOM incidence. This topic merits further study.

Data from the Canadian National Longitudinal Survey of Children and Youth (NLSCY) were used to report trends in OM prevalence among 2- to 3-year-old children from 1994 to 2009; 26% experienced frequent OM (≥4 OM episodes) in 1994-1995, which decreased to 12.6% in 2008-2009, a highly significant reduction ($P < .001$).[27] The percentage of 2- to 3-year-old children with at least 1 ear infection also declined significantly over this time period from 67% in 1994-1995 to 50% in 2008-2009 ($P < .001$).

A statistical report from the US Agency for Healthcare Research and Quality (AHRQ) examined childhood ear infections using the Medical Expenditure Panel Survey (MEPS) 2006 Full Year Consolidated File.[28] The MEPS collects information from multiple sources: office-based medical provider visits, outpatient department visits, hospital in-patient stays, emergency room visits, home health file (parents'/caregivers' report), and a prescribed medicine file on reported OM episodes. Among children younger than 18 years in 2006, 8.8 million (11.8%) were reported to have OM as classified by the *International Classification of Diseases, 9th Revision, Clinical Modification* (*ICD-9-CM*) codes 381 or 382.[29] Boys (4.9 million) were 26% more likely to have reported cases of OM than girls (3.9 million), and about 90% of the children with reported OM episodes had a physician visit and/or were treated for OM. Expenditures for outpatient treatment and prescriptions totaled $2.8 billion in 2006.

Alpert and colleagues[30] reported on trends in exposure to secondhand smoke during a recent 13-year period when reported OM pediatric encounter visits for children ≤6 years old also declined. Smoking exposure data were derived from the 1993-2006 Tobacco Use Supplement to the Current Population Survey (CPS). Data on children ≤6 years with pneumococcal conjugate vaccine 7 (PCV7) coverage living in households during this time period were examined based on completely different surveys: the 2000-2003 National Health Interview Survey (NHIS) and the 2004-2006 National Immunization Survey (NIS). In this ecological study, the percentage of young children living in smoke-free homes increased 89% (from 45.5% in 1993 to 86.1% in 2006), whereas the nationally representative rates for primary outpatient diagnosis of OM derived from NAMCS and NHAMCS decreased 40% during this time period. In addition, annual hospital discharge rates for OM declined 73% as determined from the National Hospital Discharge Survey (NHDS), also conducted by NCHS. It is impossible to know whether the decline in reported secondary smoke exposure played a major role, given the potential for misleading results from ecological studies due to the ecological "fallacy."[31-33] The evidence is stronger that licensure of the pneumococcal conjugate vaccine for routine use in children in 2000 has had an impact on the decline of OM post-2000.[34] Whether there exists a causal association for the declining rates of OM from an increase in smoke-free homes is unclear.

Incidence or Initial Onset. All children born in Southwest British Columbia in 1999-2000 were followed until age 3 years as part of a birth cohort study linking multiple administrative databases to characterize the incidence (first diagnosis) of and risk factors for OM.[35] OM was defined by physician visits with diagnoses of *ICD-9* OM codes: 381, 381.0, 382, 382.0, and 382.9. In this cohort of over 50,000 births, 49% had 1 or more OM diagnoses during the 3-year period of follow-up, whereas 8% had recurrent otitis media (ROM), defined as 4 or more physician visits over 12 months or 3 or more visits during a 6-month period. Aboriginal status (native Canadian Indian), young maternal age (<20 years), male sex, and presence of older siblings were the strongest risk factors identified in the adjusted conditional logistic regression models for both incident OM and ROM.

Côté and colleagues[36] performed a prospective birth cohort study in Quebec, Canada. They conducted home interviews with mothers of the children from age 5 months annually until 8 years of age to determine the frequency of OM and other infections. In this cohort of 1238 families, children entering large group child care centers had increased OM incidence compared with those in home care before the age of 2.5 years (incidence rate ratio (IRR) = 1.62; 95% confidence interval [CI], 1.19-2.20). Large group child care centers were those in which professional educators provided care for up to 10 groups of 8 to 12 children in the same setting. Among 3 children's age groups (1.5-2.5, 3.5-4.5, and 5-8 years), adjusted IRRs were calculated for a variety of variables. Early entry into large group child care and low birth weight had the highest IRRs (1.61 and 1.62, respectively). The prevalences of risk factors were as follows: 48% were male children, 16% were mothers aged ≤21 years at first birth, 38% mothers smoked cigarettes, 18% mothers did not complete high school, and 25% had low-income households. Also, 71% (children aged 1.5-2.5 years) to 86% (children aged 5-8 years) had 1 or more siblings or other children in the household.

Recurrent Otitis Media. Wang et al[37] performed a study of Taiwan's pediatric population of children ≤12 years of age. They identified a cohort of 283,084 children treated for AOM using Taiwanese National Healthcare Insurance claims. The baseline (initial) AOM attack was stipulated to have occurred in 2006 and was defined based on either a primary or secondary diagnosis of AOM using *ICD-9-CM* code 381 or 382. The annual

incidence rate for AOM in 2006 was 65 cases per 1000 children aged ≤12 years. Based on the definition of person-years (or child-years) as the sum of the number of years that each member (children ≤12 years of age) of a population was diagnosed with a disease (AOM), the incidence density rate (IDR) per 100 child-years for ROM during a 1-year period following the baseline AOM attack was highest among children from birth to 2 years of age, with an IDR of 41.2 cases per 100 person-years, as compared with an IDR of 38.8 for 3- to 5-year-olds and an IDR of 26.7 for 6- to 12-year-olds. Boys had slightly higher IDRs than did girls (34.4 vs 32.5). The highest recurrence rates were from birth to age 2 years (40.6%) as compared with 3- to 5-year-olds (37.7%) and males (34.0%).

Miller et al[38] analyzed data from the NHIS to determine if the mean temperature between 1998 and 2006 influenced the likelihood of ROM (≥3 OM in a year). Prevalence was 6.3% in a group of 113,067 children with a mean age of 8 years. Mean temperature varied by 1.5 degrees over the period of the study and did not significantly influence OM rates.

Otitis Media with Effusion. Martines et al[39] studied a cohort of all school-aged (5-14 years) Sicilian children in the primary school district of Sciacca from September 2006 to June 2007 to determine the prevalence of and risk factors for otitis media with effusion (OME). In total, 2097 or 98.4% were examined with pneumatic otoscopy and tympanometry and also participated in skin-prick tests for 12 common perennial and seasonal allergens. The prevalence of OME was 6.8% for children overall and decreased with age from 12.9% in children 5 to 6 years old to 3% among those 13 to 14 years old. Smoke exposure and duration of breastfeeding were not significantly related to OME. Prevalence of OME was strongly associated with atopy in univariate analyses (odds ratio [OR] = 12.7; CI, 8.8-18.3). Multivariate analyses, stratified by atopy status, revealed 2 significant risk factors for the joint effect of atopy and OME: age (OR = 2.10; CI, 1.70-2.57) and history of upper respiratory tract infection (URI; OR = 2.71; CI, 1.81-3.98). Neither family history of atopy nor family history of middle ear disease was significantly related to OME.

The aim of a study by Umapathy and colleagues[40] was to investigate associations between OME, rhinitis, asthma, and atopy. The parents of an unselected population of 332 children at school entry (about age 5 years) in the East Berkshire district of the United Kingdom were sent postal questionnaires inquiring about various symptoms of OME, rhinitis, asthma, other atopic features, treatment for any of these problems, and possible family history of atopy. There were 254 (76.5% response rate) returned questionnaires with useful data that were then scored based on the number of symptoms or other outcomes reported using weights of 1, 2, or 3. A score of 1 to 5 was considered to be a possible diagnosis of the condition of interest, and ≥6 was considered to be a strong likelihood of the condition. About 33% had some otologic symptoms and 6% had a score of ≥6, indicating a high likelihood of OME. No significant correlations were found between scores for OME, eczema, urticaria, and food or drug allergies. Otologic and nasal symptoms for OME and rhinitis were highly correlated.

Chronic Otitis Media with Effusion. Gultekin and colleagues[41] reported that the prevalence of chronic otitis media with effusion (COME) was 8.7% in a cohort of 1740 Turkish children aged 5 to 12 years. *Chronic* was defined as lasting 12 weeks (3 months) or longer, which is the standard time interval for the definition of chronic illness. Univariate analyses were conducted, but the researchers did not provide multivariate models to assess the relative risks of these variables and confounders. Several risk factors were found to be significantly associated with COME in univariate analyses: center daycare, frequent AOM and/or URI in the past year, history of allergy, number of siblings, low level of parent's education, and maternal smoking. Other commonly identified risk factors for OM were not associated with COME (eg, child's sex, paternal smoking, low family income, breastfeeding, and duration of breastfeeding).

Sociodemographic Risk Factors: Age, Sex, Race/Ethnicity, Nationality, Socioeconomic Status

Vakharia and colleagues[42] examined demographic disparities among children with ROM based on parental reports for an annualized population of more than 72.6 million children (<18 years old; mean age 8.6 years) using data from the annual NHIS. These data, collected from 1997 to 2006, were used to determine ROM prevalence, defined as 3 or more ear infections reported by a parent/guardian during the past 12 months. The average annual prevalence of ROM in children <18 years was 6.6% over the 10-year time period. Recurrent OM occurred more often in children who were white (7.0%) or living below the poverty level (8.0%) but was reported less often for uninsured (5.4%) children. Those with insurance had a slightly higher rate of ROM (6.4%), probably because they had better access to care, increasing the likelihood of diagnosis for ear disease and other conditions. With multivariate analyses, those living below the poverty level (OR = 1.32; CI, 1.19-1.46) were at increased risk of ROM, whereas black, Hispanic, and other racial groups were at lower risk (ORs < 1.0—namely, 0.63, 0.76, and 0.60, respectively) and were less likely to have ROM than white children, possibly due to less access to care, resulting in undiagnosed OM episodes.

In a companion article, the same authors analyzed the influence of race and ethnicity in the United States on access to care among children with ROM (3 or more ear infections in the past year).[43] Children with ROM were slightly more likely to be male (52.5%). Multivariate analysis revealed that Hispanic and NH black children with ROM had increased odds ratios relative to NH white children for not being able to afford prescription medications (OR = 1.47 and OR = 1.76, respectively; $P < .002$), for not being able to see a specialist (OR = 1.86 and OR = 1.62, respectively; $P < .001$), and also for visiting the emergency department (ED) (OR = 1.32 and OR = 2.50, respectively; $P < .001$).

To better understand the role of demographic and AOM risk factors, researchers conducted a cross-sectional study of 594 children, 1 to 6 years of age, attending 18 daycare centers in Finland.[44] Recurring AOM (RAOM), defined as ≥4 OM episodes in the preceding 12 months, was more common in

children 1 to 3 years old (15%) than in children 4 to 6 years old (2.5%). Variables significantly associated with decreased risk of RAOM based on multivariable stepwise logistic regression analyses were age 24 to 35 months (OR = 0.11; CI, 0.03-0.47) and age 36 to 47 months (OR = 0.05; CI, 0.01-0.23), both referred (compared) to children 1 year of age (12-23 months), and breastfeeding and bottle feeding ≥6 months (OR = 0.20; CI, 0.07-0.56), compared with breastfeeding and bottle feeding <6 months. Variables in the multivariable model that increased the risk of RAOM were attending a daycare center for 13 to 23 months vs ≤12 months (OR = 3.34; CI, 1.03-10.78), mother's higher (academic) education (OR = 5.02; CI, 1.73-14.56), and having had recurrent acute upper respiratory tract infections (RURI) in the past 12 months (OR = 3.96; CI, 1.44-10.86).

To assess the perceived multinational disease burden and management of OM, an international panel of experienced physicians was selected for a cross-sectional survey in 9 countries (n = 1800 physicians, 200 from each country). The physicians were asked to estimate how many children younger than 5 years they had seen in the past year with various infectious diseases, including OM, pneumonia, sinusitis, and meningitis.[45] Pediatricians and family practitioners from the 9 countries participating in the survey indicated that OM was the most common diagnosis in this age range. The estimated average annual caseload for OM across all 9 countries was 375; however, the mean number of children diagnosed with OM varied from a low of 128 in Thailand and 156 in Mexico to a high of 1003 in South Korea. Germany, Saudi Arabia, and Spain had intermediate mean numbers of children diagnosed with OM of 544, 463, and 389, respectively. The authors estimated that 54% of children younger than 5 years had an initial episode, 38% had ROM, and 15% had OM with complications (eg, treatment failures, ROM/COM, or hearing problems). These results were based on physicians' estimates within each country of the previous year's OM morbidity, rather than from documented chart review of cases for diagnoses of OM or other infectious illnesses.

Daycare, Exposure to Other Children, Crowding, Allergy, and Respiratory Infections

A study conducted among 618 children ≤7 years old from rural Aboriginal communities in Australia found that children who attended daycare had more than twice the odds of having an ear infection in the previous week (OR = 2.35; CI, 1.32-4.20).[46] The child's primary caregiver reported on recent ear infections and other common childhood illnesses during a structured interview. None of the measures of crowding was associated with having an ear infection in the past week, including number of children ≤7 years old in the dwelling, number of adults in the dwelling, and number of residents per bedroom. In a subsequent report, Bailie and colleagues[47] concluded that after adjustment for a range of potential confounding variables, there was no consistent reduction in a caregiver's reporting of common childhood illnesses in association with improvements in household infrastructure, either for specific illnesses or for illnesses in general. Although the authors reported a strong association between improvement in household infrastructure and improvement of hygienic condition of the homes, there were only marginal improvements in crowding or the underlying social, economic, and other environmental conditions in the Australian indigenous communities that were studied.

A hospital/clinic-based, case-control study conducted in Nigeria measured daycare attendance and number of people in the household.[48] Neither factor was associated with chronic suppurative otitis media (CSOM). The authors' method of using the median number of persons in the household to distinguish the exposed (>10 persons) from nonexposed (≤10 persons) may have exceeded the threshold of increased risk in a West African context, which may have biased the results toward the null hypothesis.

The remaining 4 studies employed community-based samples and investigated different types of OM. A study conducted in Istanbul, Turkey, found daycare attendance and >2 siblings in the household associated with OM diagnosed with otoscopy; however, this study did not adjust for potential confounders.[41,49] A community-based study among inner-city children attending Head Start in New York City collected serum from a subgroup of 494 children with a mean age of 4 years, which was analyzed for specific IgE responses to dust mite, cockroach, mouse, and cat allergens.[50] After stratifying by atopy status, Perzanowski and colleagues[50] reported an increase in respiratory infections at age 4 years that was associated with increasing birth order and ≥4 ear infections in the past year (RAOM), which was significant only among children with negative responses to IgE specific to dust mites, mouse, cockroach, and cats. A population-based study conducted among 926 infants followed from birth to 12 months of age on Crete, Greece, found an almost 2-fold odds of an AOM episode (OR = 1.95; CI, 1.46-2.61) among children with siblings. Those who attended daycare outside the home had a greater than 4-fold increased odds of AOM (OR = 4.74; CI, 2.16-10.40).[51] Strong significant associations were also observed for ROM, defined as ≥3 episodes in the past year. In another population-based study conducted in Sciacca, Italy, among children 5 to 14 years of age, family size was not associated with OME.[39,52]

MacIntyre and coworkers[35] studied the incidence of physician-diagnosed OM throughout the first 2 years of life. The incidence of atopic disease was also assessed in this German cohort study. Children diagnosed with OM during infancy were at greater risk for developing late-onset allergic eczema and asthma during school age, and associations were stronger for frequent OM.

Kvaerner and collaborators[53] reported on the relationship between ROM, OM surgery, and allergy in a 60-year perspective in the general population among 40,000 randomly selected Norwegians and found that ROM and OM surgery were more common in respondents with allergy. On the other hand, Souter and coworkers[54] assessed the prevalence of allergic symptoms and nasal symptoms in children aged 6 or 7 years with OME confirmed intraoperatively during ventilation tube insertion between 2001 and 2005 (n = 89) but found no

difference in the prevalence of allergic symptoms suggesting rhino-conjunctivitis, asthma, or eczema between the OME and reference group.

Upper respiratory tract infections are generally believed to be the most frequent risk factor for OM, especially AOM. However, studies conducted to confirm this finding are not abundant, even though it is frequently stated that URI is the strongest risk factor for OM. Most studies to date have dealt with URI and AOM or OME, but surprisingly few studies have dealt with URI and COM with or without suppuration.

Karevold et al[55] assessed the infectious susceptibility in a population-based sample of 3406 ten-year-old children living in Oslo, Norway. In this cohort, 345 children (10%) had prior surgery for OM, which included adenoidectomy, myringotomy or tympanostomy tube insertion, or any combination of these procedures excluding combinations with tonsillectomy. The authors found that OM in 10-year-old children was associated with previous surgical interventions and that parental reports of surgeries in early childhood were reliable.

Patel et al[56] published a study involving 566 children with AOM between 2 months and 7 years of age enrolled between 1989 and 1998 in whom middle ear fluid (MEF), nasal wash, and serology were obtained. Respiratory syncytial virus (RSV), which causes both lower and upper respiratory tract infections, was found in 16% of all children and in 38% of children who were virus positive. In total, 42% of the 566 children had concurrent virus detected in the MEF, nasal wash, or serologically. Nasal wash specimens showed the highest detection rate (68%) compared with 44% in MEF. The enrollment procedure may not have been optimal since it was based on generally healthy children attending primary care clinics over a 10-year time period. Children who received antibiotics during the preceding week or who had underlying disease or comorbidity other than URI were excluded.

Winther et al[57] followed 60 children from 24 families from October through April with daily parental recording of illness signs in their children, weekly pneumatic otoscopic examinations, and periodic polymerase chain reaction assay of collected nasal fluids for common viruses. In 73% of cold-like illnesses, 1 or more viruses could be detected, most frequently rhinoviruses, whereas this rate was 18% in non-cold-like illnesses. In 93 diagnosed OM episodes, 65 (70%) occurred during a cold-like illness. In 79 OM episodes with available nasal samples, 61 (77%) had a virus detected. The overall OM complication rate for a cold-like illness was 33%, meaning that one-third of all URIs resulted in OM.

Chonmaitree et al[58] performed a prospective cohort study involving 294 children between 6 months and 3 years of age. The observation period was 1 year. Illness onset (ie, symptoms of a cold or URI, eg, nasal congestion, rhinorrhea, cough, sore throat, or fever) was reported in a timely manner by parents, and the children were examined as soon as possible by a study physician. Follow-up home visits were performed. Tympanometry was performed and respiratory specimens and nasal swabs were collected. The URI incidence was 5.06 per child-year, with 1.72 AOM episodes per child-year. Samples were obtained from 864 URI episodes, and 63% were virus positive. Rhino- or adenoviruses were most frequently detected. Overall, OM was a complication of URI in 61%; of these, 37% were classified as AOM and 24% were OME. Young age was a predictor of OM complication for URI. AOM occurred more frequently in URI with detection of adeno-, corona-, or RSV viruses compared with those with rhino-, entero-, influenza, or parainfluenza viruses. This report was followed by a letter to the editor from Elliot and Fleming,[59] which showed high coincidences of fluctuations in annual cold and OM incidences. Their findings were based on a sentinel general practitioner surveillance network in England and Wales that reports new episodes on a weekly basis.

Salomon et al[60] performed a controlled study involving 2456 children between 3 and 6 years of age in France. About 20% (505 children) had consultations for nasopharyngitis treated with or without antibiotics. Only 37 of the children developed AOM from 2 days to 2 weeks after the first consultation for nasopharyngitis. Of these, 24 occurred in children not treated with antibiotics. The hazard ratio for AOM after nasopharyngitis treated with antibiotics was 0.2 (P = .002), which was significantly lower than for those who were not treated with antibiotics. In biostatistical usage, *hazard* is defined as the rate at which events happen (hazard means *chance* in French) over a short period.

Mandel et al[61] studied 148 unselected children from 1 to 8 years of age from November through the end of April by daily parental reports and weekly pneumatic otoscopy. The incidence during this time period was 317 for OME, 74 for AOM, and 456 cold-like illnesses per 27,232 child-days. Seasonal variations in AOM and OME incidence were correlated with cold-like illnesses: the calculated correlation coefficient was r = 0.27 for OME (P = .09) and r = 0.23 for AOM (P < .01).

Alper et al[62] conducted a 6-month longitudinal study of 176 children from 102 families, with OM diagnosed by pneumatic otoscopy. Coincidence rates between cold-like illnesses (URI) and OM were 44% when rhinovirus was detected in nasal secretions, 56% for RSV detection, 50% for adenovirus, 39% for coronavirus, 36% for parainfluenza virus, and 20% for influenza viruses A and B. The coincidence rates between the different viruses were not significant, probably due to the small numbers of detected viruses.

Patel et al[63] studied acute phase cytokines (interleukin [IL]–1β, IL-6, and tumor necrosis factor [TNF]–α) in nasopharyngeal secretions from 151 virus-positive children aged 3 to 36 months with URI. The relation between AOM and IL-1β concentrations was found to be significantly higher in children with URI who developed AOM than in those not developing AOM. The importance of this finding is not known.

Hatakka et al[44] performed a cross-sectional questionnaire study of 594 children aged 1 to 6 years from 18 daycare centers in Helsinki, Finland. Recurrent URI, defined as 4 or more episodes of URIs per year, was present in 44% of the children 1 to 3 years old and 23% of those 4 to 6 years old. Recurring AOM was present in 15% and 2.5%, respectively. For RURI, atopic disease in parents (OR = 1.53; P = .033), mother's high academic education (OR = 1.77; P = .008), and a medium length of daycare attendance, 13 to 23 months, compared with

a shorter period (OR = 1.67; P = .049) increased the risk of RURI, whereas furry pets (OR = 0.44; P = .003) and an older child (OR = 0.38; P = .001) reduced the risk. For RAOM, RURI (OR = 3.96; P = .008), mother's high academic education (OR = 5.02; P = .003), and a medium length of daycare attendance compared with a shorter period (OR = 3.34; P = .044) increased the risk, whereas partial breastfeeding >6 months (OR = 0.20; P = .002) and an older child (OR = 0.05; P = .001) reduced the risk of RAOM. Thus, several risk factors are common for both RURI and RAOM.

Kalu et al[64] prospectively studied otoscopic findings between 1 and 7 days after URI onset in 294 children between 6 months and 3 years of age. Acute OM was diagnosed in 22% and myringitis (inflamed tympanic membrane without fluid) in 7%. When AOM episodes were followed up for 28 days after URI onset, researchers found a wide range of tympanic membrane appearances graded on a scale. These findings were interpreted as evidence of different clinical phenotypes.

Koch et al[65] published a rarely seen prospective population-based cohort study on the incidence of COM, with and without suppuration, and associated risk factors, including URI, in a high-risk population of 465 Greenlandic children followed clinically for 2 years. Cumulative risk of CSOM (defined as ≥14 days of suppuration) was 14% at 4 years of age with a median debut age of 336 days. Risk factors were attending childcare centers (hazard ratio [HR] = 3.18; CI, 1.53-6.61), smokers in the household (HR = 4.56; CI, 1.07-19.4), maternal history of purulent ear discharge (HR = 3.27; CI, 1.74-6.13), a high burden of URI (HR = 1.19; CI, 1.03-1.37), and being of Inuit descent (HR = 5.56; CI, 0.78-50). Investigators concluded that URI strongly increased the risk of CSOM, but whether URI is an independent risk factor or an intermediate step in the pathogenesis of CSOM is unknown.

In conclusion, all of these cited studies found an association between URI and OM. Several risk factors are shared between URI and OM. Future studies may wish to consider URI more explicitly. Thus, instead of considering OM and URI independently, it is probably more correct to consider first URI and treat OM as a complication, since it is more likely that URI leads to OM rather than that OM leads to URI.

Breastfeeding, Passive Smoke Exposure, and Pollutants

Breastfeeding. Sabirov and colleagues[66] aimed at explaining the mechanisms behind the lower incidence of AOM found in breast- vs formula-fed children. Levels of specific serum IgG antibody to NTHi and P6 were highest in breastfed, intermediate in breast/formula-fed, and lowest in formula-fed infants. Breastfeeding shows an association with higher levels of antibodies to NTHi and P6, suggesting that breastfeeding modulates the serum immune response to NTHi and P6. Higher serum IgG might facilitate protection against AOM and nasopharyngeal (NP) colonization in breastfed children.

In a cross-sectional study of 254 infants 6 to 7 months old, the aim was to determine the effect of passive tobacco smoking on growth and infection rate of infants and to evaluate whether breastfeeding might be protective against harmful effects of cigarette smoke.[67] Multivariate analysis of factors related to OM found that smoking mothers and fathers increased their infant's OM risk 9.4-fold and 6.2-fold, respectively, and breastfeeding decreased the risk by a factor of 5.4 (one-fifth the risk), indicating that breastfeeding protected the infants who were passively exposed to tobacco smoke against infections.

To determine whether cumulative weekly breastfeeding duration for at least 13 weeks was associated with infant OM, respiratory and gastrointestinal illness, or total illness (sick-baby) visits through 12 months of age, Freeman et al[68] analyzed data from a randomized clinical trial of low-income, primarily Hispanic and black women and children, enrolled from 2 medical center–affiliated clinics. Outpatient and emergency room visit data for OM were obtained for 255 mothers and infants. The authors concluded that the intervention group's increased breastfeeding intensity (defined on a 7-point scale ranging from 100% breast milk to 100% artificial milk or solids, ie, weaned) was not associated with a reduction in OM-related visits or with any of the other illness measures. The authors concluded that the low exclusive breastfeeding rates and lack of coverage for health visits may have been the reasons for their negative findings.

Vogazianos and colleagues[69] assessed the effect of breastfeeding and its duration on AOM in children to determine the period of breastfeeding necessary to achieve optimal preventive results against AOM. They found that for an optimal preventive effect to be achieved, a child should be breastfed for at least the first 11 months of life. Continuing breastfeeding for up to 18 months showed some additional preventive effect, although not statistically significant in their study.

A Finnish study investigated the relationship between child characteristics, parental and environmental factors, and the occurrence of acute URI and AOM among children attending daycare centers (DCCs). The study was conducted using a cross-sectional questionnaire completed by parents of 594 children aged 1 to 6 years from 18 DCCs in Helsinki, Finland, showing that partial breastfeeding ≥6 months (OR = 0.20; P = .002) reduced the risk of ROM.[44]

Ladomenou and colleagues[51] studied a representative sample of 1049 mother-infant pairs in Crete, Greece, conducting interviews 1, 3, 6, 9, and 12 months after birth. Acute OM episodes were recorded by parental reports of diagnosis made by a physician. Feeding during the first 6 months of life was defined as exclusive breastfeeding, combined breastfeeding and formula feeding, and exclusive formula feeding. Suboptimal or nonexclusive breastfeeding was found to predispose to AOM, but the study did not confirm any relationship between parental tobacco use and risk for AOM.

To describe the incidence and prevalence of AOM and OME, the contribution of cold-like infections, and other traditional risk factors to the incidence and burden of OM, Mandel et al[61] studied 148 children (74 male; 131 white), aged 1.0 to 8.6 years, and followed them from November 1 to April 30 with weekly pneumatic otoscopy to diagnose OM and by daily parental diary to record cold-like infection episodes. The lack

of breastfeeding, cold-like infections, and prior OM were significant predictors of OME and AOM incidence.

Inadequate breastfeeding was not identified as a significant risk factor for OM in a case-control study of 800 preschool-aged children in rural India.[70] In this study by Sophia et al,[70] passive smoking was found to be a significant risk factor (OR = 3.29; CI, 1.05-10.33).

Bartick and Reinhold[71] used data from a 2007 breastfeeding report from the AHRQ to estimate excess costs associated with current below-optimal breastfeeding rates in the United States. These costs were calculated as projected costs if more parents complied with the medical recommendation to breastfeed exclusively for 6 months. Otitis media–related excess costs attributed to current breastfeeding rates, compared with 90% compliance to the medical recommendation of 6 months exclusive breastfeeding, were estimated to be $9.1 million.

Passive Smoke Exposure. A population-based birth cohort of 465 children aged between 0 and 4 years from Sisimiut, Greenland, was followed for a 2-year period (1996-1998). Cases of CSOM were registered based on medical history and clinical examinations. Cumulative risk of CSOM at 4 years of age was 14%, and having smokers in the household was found to be a significant risk factor (HR = 4.56; CI, 1.07-19.4).[65]

Data from the Norwegian Mother and Child Cohort Study used questionnaires to collect data on tobacco smoke exposure and AOM up to 18 months of age from 32,077 children born between 2000 and 2005.[72] Acute OM was reported to be slightly more common in children exposed to parental smoking. Incidence from birth to 6 months of age was 4.7% in unexposed children and 6.0% in children exposed both pre- and postnatally to smoking. Estimates of relative risk were computed using a generalized linear model for binary data, controlling for parental postnatal smoking exposure and adjusting for child's sex, mother's age, atopy, parity, educational level, season of birth, birth weight, preterm delivery, and breastfeeding at 6 months of age. The relative risk estimate for AOM during the first 6 months of life when exposed to maternal smoking in pregnancy was 1.34 (CI, 1.06-1.69). Maternal smoking in pregnancy was associated with AOM up to 12 months of age. Compared with nonexposed children, there was a slightly increased risk of ROM for children exposed to smoking both pre- and postnatally with an adjusted relative risk (RR) of 1.24 (CI, 1.01-1.52). Recurrent OM was defined as a mother's report of 4 or more AOM episodes during the 12-month period when the child was 6 to 18 months old.

A total of 1800 children attending 4 primary schools in the Sisli and Beyoglu districts of Istanbul were screened, and 1740 children who met the inclusion criteria were enrolled into a study to determine the impact of environmental, epidemiologic, and familial factors in the development of persistent OME (COME). Differences in smoking history of both parents ($\chi^2 = 6.5$; $P < .01$) and smoking history of the mothers ($\chi^2 = 33.5$; $P < .0001$) were found between the 2 groups (persistent OME or COME vs healthy children).[39]

Brook et al[73] investigated the frequency of isolating potential pathogens in the nasopharynx of healthy and OM-prone children and their parents. Posterior nasopharynx cultures were taken from 40 healthy and 40 OM-prone children and one of their parents. Twenty parents in each group were smokers. Parents who smoked were more often colonized with pathogens than those who did not smoke. The nasopharynx of healthy children of smokers harbored a high number of pathogens similar to the flora found in their parents and OM-prone children. Pathogenic organisms were found more often in OM-prone children of both smoking and nonsmoking parents, compared with healthy children whose parents were nonsmokers. Concordance with pathogens in the parent was high among the OM-prone children of smoking parents, but this was not observed in the OM-prone children of nonsmokers.

To determine the risk of OM associated with passive smoking in young children in Western Australia, a prospective cohort study of 100 Aboriginal and 180 non-Aboriginal children was performed, including clinical examinations by an ear, nose, and throat (ENT) specialist up to 3 times before the age of 2 years and tympanometry at routine field follow-up visits from the age of 4 months.[74] Exposure to environmental tobacco smoke increased the risk of specialist-diagnosed OM in Aboriginal children (OR = 3.54; CI, 1.68-7.47). Non-Aboriginal children exposed to environmental tobacco smoke but not attending childcare were at increased risk of OM (OR = 1.91; CI, 1.07-3.42), whereas those attending childcare had no increased smoking-related risk.

Hammaren-Malmi et al[75] evaluated the effect of parental smoking on the risk of ROM in children treated with tympanostomy tubes. They enrolled 217 children, ages 1 to 4 years, who underwent insertion of tympanostomy tubes because of middle ear disease. The children were followed up for 12 months. Maternal smoking was associated with a highly increased risk of ROM (OR = 4.15; CI, 1.45-11.9) after the insertion of tympanostomy tubes. Although the mechanism is unclear, the authors speculated that since tobacco smoke contains substances that irritate mucosal membranes, this could interfere in the function of ciliated cells in the eustachian tube and induce expression of activation molecules on the epithelial cells. If this occurs, then it may facilitate the adhesion and invasion of pathogenic microbes that benefit from impairments in ciliary function and also lead to the use of the activation molecules as coreceptors, with the result of an increased likelihood of ROM.

Pollutants. The impact of ambient air pollution on outpatient physician visits for OM was evaluated using data from a population-based birth cohort of children born in Southwest British Columbia during 1999-2000 and followed until 2 years of age.[35] Residential air pollution exposures were estimated for the first 24 months of life. Complete exposure and risk factor data were available for 45,513 children (76% of all births). A total of 42% of the children had 1 or more physician visits for OM during the 24 months of follow-up after birth. Modest but consistent associations were found between some measures of air pollution and physician diagnoses of OM in a large birth cohort exposed to relatively low levels of ambient air pollution.

To investigate the potential association between ambient air pollution exposure and ED visits for OM, 10 years of ED data were obtained from Edmonton, Alberta, Canada, and linked to levels of air pollution as measured by carbon monoxide (CO), nitrogen dioxide (NO_2), ozone (O_3), sulfur dioxide (SO_2), and particulate matter (PM) of median aerometric diameter ≤10 and 2.5 μm (PM10 and PM2.5, respectively). Positive associations between ED visits for OM and interquartile increases in CO and NO_2 levels were observed even after adjusting for ambient temperature and relative humidity.[76]

Genetic Susceptibility

A genome-wide linkage scan using the 10K Affymetrix SNP panel (Affymetrix, Santa Clara, California) was performed to map possible OM susceptibility genes.[77] Full siblings who had tympanostomy tubes inserted for persistent/recurrent OM (affected), their parents, and other full siblings without a history of tympanostomy tube insertion (unaffected) were eligible for the study. Genotyping was performed for 403 white families containing 1431 individuals and 377 affected sibling pairs, as well as 26 African American families with 75 genotyped individuals and 27 genotyped affected sibling pairs. The most significant linkage peaks were on chromosome 17q12 regions and 10q22.3, which may contain susceptibility genes that influence the risk for recurrent/persistent OM. Plausible candidates in 17q12 include AP2B1, CCL5, and a cluster of other CCL genes and in 10q22.3 surfactant protein A (SFTPA).

The mouse has been used as a model of human disease since comparable physiology between the human and the mouse can result in analogous pathological symptoms.[78] In addition, the mouse has a short gestation time (9 weeks), and the availability of both the murine and human genome sequences, combined with over 90% conserved synteny between the genomes, greatly facilitates the identification and manipulation of disease-modifying genes. Single-gene, mouse mutant studies of OM have identified a number of genes as potential and biologically relevant candidates for human disease. Five genes originally identified in mouse models of OM have been investigated in genetic association replication studies of human OM with significant association reported between polymorphism at the *TLR4, PAI1*, and *FBXO11* genes and disease in humans. The human-to-mouse approach can also be applied to undertake an in-depth functional analysis of loci with a significant association in human genome-wide association studies of OM.

Jeff and Jumbo, 2 mouse mutants, are implicated in severe OM in murine models. The Jeff mutation maps to the *FBXO11* gene and the Jumbo mutation to the *EVI1* gene.[79,80] These genes interact with the transforming growth factor–β (TGF-β). Rye and colleagues investigated these genes as well as genes of the TGF-β pathway as candidate ROM/COME susceptibility genes in 2 predominantly white populations in Australia (cohort I: 561 cases and cohort II: 253 cases and 866 controls).[81] Single-nucleotide polymorphisms (SNPs) within *FBXO11* using family-based association testing were associated with severe OM, whereas there was no association with the *EVI1* gene. These data provide strong evidence for *FBXO11* as a susceptibility gene for severe OM.

Acute OM severity has been associated with cytokine gene polymorphisms and other risk factors.[82] Proinflammatory cytokine gene polymorphisms and other risk factors influence the severity of AOM following URI. A total of 295 AOM episodes in 128 subjects were included. More severe AOM symptoms were associated with young age ($P = .01$), family history of AOM ($P = 0.001$), tobacco smoke exposure ($P = 0.008$), and early diagnosis after onset of URI ($P = 0.02$). Among children with bulging or perforated ear drum (206 episodes, 104 subjects), those who had IL-1β^{+3953} polymorphism experienced higher symptom scores ($P < .02$). This study suggests that risk factors such as family history and tobacco smoke exposure are likely to have a stronger association with AOM severity than proinflammatory gene polymorphisms.

A review of recent research evaluating the role of the innate immunity in the middle ear and OM considered whether Toll-like receptor (TLR) signaling to activate the innate immune system is critical for the timely resolution of bacterial OM.[83] Expression of proinflammatory cytokines and the activation of macrophages to phagocytose and kill bacteria are severely disabled in mice lacking key TLR signaling molecules, which leads to failure of bacterial clearance and to OM persistence. The entire TLR signaling network must work to effectively ensure recovery.

Mucins control the viscoelastic properties of the secretion in the middle ear and can thereby affect the mucociliary clearance; some mucin gene polymorphisms may promote clearance, whereas others can impede clearance. In a study by Ubell et al,[84] gene polymorphisms were explored in children 6 months to 14 years of age scheduled for tube treatment: 20 with OME and 20 with ROM were compared with 40 children without a significant history of OM (≤1 episode/y). Blood was collected and DNA extracted and analyzed by Southern blot assays and polymerase chain reaction; gene-specific probes were used to determine sizes of *MUC2, MUC5AC*, and *MUC5B* genes. There were no statistically significant differences in polymorphism expression identified between the 3 groups of children for *MUC2* and *MUC5B* genes. However, the OME children were more likely than controls or ROM children to carry the longer *MUC5AC-b* allele. Since *MUC5AC* is a primary innate defense mechanism for middle ear (ME) epithelium, alterations in protein structure have potential to affect that defense and predispose to disease. The young age (6 months) of some of the children is a short period in which to classify children as controls. If those without significant OM history developed ROM or COM after 6 months of age, they would have been misclassified. No information was provided on the number of 6-month-old controls who participated in the study, who were later found to be genetically susceptible to ROM or COM. However, the median age of controls, 67.9 months, was considerably higher than for those with ROM and OME, 24.7 and 46.6 months, respectively.

Ubell and colleagues[85] biopsied middle ear epithelium in children 6 months to 15 years of age who were being treated with tympanostomy tubes for recurrent or chronic OM (27 OM children, 8 controls). They evaluated *MUC2* expression using reverse transcription polymerase chain reaction (RT-PCR) and found a 6-fold increase in *MUC2* expression in samples from

patients with ROM or persistent middle ear fluid (MEF) compared with controls. A 5-fold increase was noted for those with ROM and an 8-fold increase for participants with an OME history (COME).

Middle ear fluid from 6 COME patients undergoing surgery for tympanostomy tubes was studied to determine protein content via proteonomics with mass spectrophotometry.[86] This approach identified *MUC5B* in all 6 of the samples; *MUC5AC* was identified in only 1 effusion. Additional analyses revealed a 6-fold higher signal from *MUC5B* than *MUC5AC* ($P < .001$). *MUC5B* may be the most common or predominant mucin involved in COME.

Researchers followed 23 children who were 9 months to 7 years old with ROM and/or COME and 5 control patients (with cochlear implants) and compared them for expression of *MUC5AC* in middle ear epithelium.[87] Compared with controls, children with ROM had 26-fold higher levels of *MUC5AC*, and those with COME had 155-fold higher levels compared with controls.

Alper and colleagues[88] have shown that cytokine polymorphisms predict the prevalence of OM as a complication of rhinovirus and RSV infections in children. Previous studies showed that distributions of cytokine polymorphisms (eg, IL-6, TNF-α) varied between cases and controls, whereas IL-1α, IL-1β, and IL-Ira did not show between-group differences. Two hundred thirty children were followed prospectively during the URI season to determine the relationship between genetic factors and the role of RSV and rhinovirus in OM. Logistic regression modeling showed that young age was protective against new OM episodes during the first rhinovirus infection. Younger age, history of OM, daycare outside the home, longer breastfeeding, high production of IL-10 and TNF-α, and low production of IL-6 predicted new OM episodes. Among children with RSV, IL-10 was a significant predictor of OM.

Revai and colleagues[89] have shown an association between cytokine gene polymorphisms and risk for URI and AOM. Children aged 6 to 35 months were evaluated to determine factors associated with development of AOM as a complication of URI. In 32% of URI episodes, children were AOM "susceptible," defined as having had ≥4 AOM/y, ≥3 AOM in 6 months, 6 AOM by 6 years, or AOM onset by 6 months. Those with ≤2 AOM by age 2 years were classified as nonsusceptible. Children with IL-6^{-174} polymorphism were significantly more likely to be susceptible to AOM than those with the normal IL-6 genotype.

Twin studies, identification of polymorphisms involved in genetic susceptibility, and genome linkage studies support the important role of candidate genes and gene polymorphisms in the development of OM.[90] Chromosomal regions associated with COME have been identified, including 3p, 10q, 10q22.3, and 19q. However, there are no current recommendations to use genetic testing to determine a child's susceptibility to OM.

Prenatal and Perinatal Factors

Recurrent OME (ROME) is a leading cause of acquired hearing loss in childhood. Since histological chorioamnionitis (HCA) is an important cause of preterm delivery and neonatal morbidity and mortality, De Felice and colleagues[91] have studied the association between ROME during the first 3 years of life and HCA in very low birth weight (VLBW: <1500 g) infants. The majority of infants in the study were not only VLBW but also extremely low birth weight (ELBW: <1000 g), and they were not only preterm (<37 completed weeks of gestation), but nearly all were also very preterm (≤32 completed weeks of gestation). One hundred ten randomly selected ELBW and very preterm newborns with HCA and 135 gestational age- and sex-matched, HCA-negative ELBW and very preterm infants were evaluated prospectively during the first 3 years of life for the presence of OME, as diagnosed on the basis of otoscopy, type B or C tympanogram, ipsilateral absence of transient evoked otoacoustic emission responses, and ipsilaterally increased threshold at diagnostic auditory brain response evaluation. Potential risk factors for OME were also examined in the 2 groups. The HCA-positive infants had approximately a 6 times higher frequency of ROME ($P < .0001$), increased frequency (>5 per year) of clinical OM episodes ($P = .0001$), approximately 5 times higher frequency of adenoid hypertrophy ($P < .0001$), a significant seasonal pattern of birth with autumn predominance ($P < .0001$), and an earlier occurrence of the first OME ($P < .0001$) as compared with the HCA-negative controls. On the basis of these findings, the authors concluded that HCA is a previously unrecognized risk factor for the development of bilateral ROME in VLBW preterm infants during the first 3 years of life.

Bentdal and colleagues[92] assessed whether preterm birth and low birth weight were associated with single and recurrent episodes of AOM during the first 18 months of life using a population of 33,192 children enrolled in the Norwegian Mother and Child Cohort, conducted by the Norwegian Institute of Public Health (NIPH). They reported a modest increased risk of AOM associated with very preterm birth (≤32 weeks' gestation), which appeared more important than VLBW (<1500 g), when both these measures were included in the analyses.

Other Risk and/or Protective Factors

Ladomenou and colleagues[51] studied a sample of 1049 mother-infant pairs from Crete, Greece (29% of the births in the fall 2004 and spring 2005). A total of 926 infants were followed for 1 year; 28.6% had ≥1 AOM during this period. Seven variables were significantly related to AOM, but data were not provided on the prevalence of these factors. The factors were ≥1 sibling in the family (OR = 1.95; CI, 1.46-2.61), out-of-home daycare (OR = 4.74; CI, 2.16-10.4), mother ill during pregnancy (OR = 1.61; CI, 1.18-2.19), birth in spring or summer (OR = 1.63; CI, 1.23-2.18), rural residence (OR = 1.40; CI, 1.05-1.87), and admission to a neonatal ward (OR = 1.64; CI, 1.07-2.54).

A study investigating the possible relationship between use of chewing gum and OME in children has been performed in the Netherlands.[93] Chewing obviously activates jaw movements and increases salivary flow, the rate of swallowing, and

the rate of activations of peritubal muscles and tubal openings. Chewing also requires nasal respiration, thus preventing mouth breathing. In this cross-sectional study of 1756 apparently healthy children aged 2 to 6 years enrolled in Dutch child health centers in the region of Utrecht between September 1999 and April 2002, OME was diagnosed by tympanometry and otoscopy. The criterion for OME was unilateral or bilateral type B tympanogram, according to Jerger's classification.[94] The parents filled out a questionnaire that included a question pertaining to the chewing habits of their child. Logistic regression analysis showed that age, season, and chewing gum–consuming habits significantly influenced the prevalence of OME. The strongest effects were age (OME becomes less frequent with increasing age) and season (OME occurs less often when climate is more favorable/warmer). In addition, children chewing gum daily or at least weekly showed significantly lower risk of OME for each year of age compared with children who seldom or did not chew gum ($P = .023$). With regular use of chewing gum, the probability of having OME was reduced by 40%. Children with immature oral motor development may refuse to chew gum, which limits applicability. The American Academy of Pediatric Dentistry (AAPD) advises that children only chew sugar-free gum and also has noted that zylitol chewing gum may have a benefit of decreasing a child's caries rate.[95]

Otitis Media as a Risk Factor Affecting Body Size/Weight

Seven studies were found that specifically examined the relationship between OM and measures of body size among children. All but one of these studies were cross-sectional in nature, and so the evidence is subject to interpretation in terms of the true directionality of the relationship. Three cross-sectional reports came from the same South Korean clinical sample. In the first, Kim et al[96] compared 155 children aged 2 to 7 years who received unilateral or bilateral tympanostomy tubes as treatment for OME with 118 children with no OM history but who underwent surgical procedures for conditions other than ear disease. The authors reported higher mean body mass index (BMI) and mean total cholesterol among the group receiving tympanostomy tubes. The follow-up study by Kim et al[97] used the same criteria to compare 140 children aged 2 to 7 years who received tympanostomy tubes with 190 children with no OM history who underwent surgery for conditions unrelated to OM. Each group was stratified by weight status, which was defined with BMI cut points (BMI below normal: BMI ≤5th; normal weight: 5th < BMI < 85th; overweight: 85th ≤ BMI < 95th; obese: BMI ≥95th percentiles) of the age- and sex-specific distributions. A greater prevalence of OME was observed in the obese group but not in the overweight group.

The third study from the South Korean clinical sample included a taste protocol among 42 children aged 3 to 7 years receiving tympanostomy tubes for COME and 42 control children undergoing tonsillectomy for conditions other than COME.[98] Higher electro-gustometric taste thresholds were found on the anterior but not posterior tongue for children with COME (tubes) as compared with control children (tonsillectomy). Mean thresholds for whole-mouth sweet and salty solutions were also higher for COME children (compared with control children with tonsillectomies), but there were no differences in thresholds determined for whole-mouth sour or bitter solutions.

Shibli et al[99] found fewer than expected cases of AOM among overweight infants and children younger than 2 years among 2139 children admitted to a hospital in Haifa, Israel. Similarly, fewer than expected cases of URI and asthma were also observed within the overweight stratum. Seaberg et al[100] found no relationship between number of AOM episodes and either electro-gustometric threshold or elevated BMI among a clinical sample of 142 children aged 5 to 18 years in Toronto, Canada.

The only longitudinal study was reported by Nelson et al,[101] who followed a cohort of 430 children recruited from 1991 to 1996 through a health maintenance system in the Minneapolis–St Paul metropolitan area. The authors observed that ROM, defined as ≥4 episodes in a 12-month period by age 2 years, was not associated with weight status. However, tympanostomy tube insertion, which may have been a better indicator of OM severity, was associated with obesity (BMI ≥95th percentile) at 2 years of age (OR = 3.32; CI, 1.43-7.72), even after controlling for overweight/obese at 2 months, birth weight, maternal prenatal smoking, maternal education, and family income. The alternative hypothesis, which was that children with larger BMI at 2 months of age would have a greater number of OM episodes by 2 years of age, was not significant.

Natural History, Complications, and Sequelae

Kvaerner and coworkers[102] performed a national registry-based study with complete data on hospitalization for acute mastoiditis and cortical mastoidectomy in Norway during 1999 to 2005, which included 339 Norwegian children younger than 17 years. The incidence of acute mastoiditis in children younger than 2 years ranged from 13.5 to 16.8 per 100,000 during the study period. The age-specific incidence rose to a peak during the second and third year of life, and acute mastoiditis was more common in boys. No increase in incidence was found during the study time period.

Mostafa and colleagues[103] performed a retrospective study of all 3364 cases, with both acute and chronic suppurative OM (SOM), who presented at a tertiary referral university hospital in Cairo, Egypt, during a 10-year period. They reported that 422 (12.5%) patients had complications, with a ratio nearly 1:1 of extracranial vs intracranial complications, and that both types coexisted often in the same patient. Patients ranged in age from 3 to 65 years, and the median age was 21.5 years. Types of OM were 71% CSOM without cholesteatoma, 28% CSOM with cholesteatoma, and 1% with acute suppurative otitis media (ASOM). By far the most common complication was mastoiditis, which occurred in 91.6% of patients; other common extracranial complications were facial palsy (29%), extratemporal abscess (29%), and brain abscess (21%). The most common intracranial complication was lateral sinus

thrombophlebitis (29%). In this study, there were 6 deaths (1.4%) and additional morbidity in 16 patients (3.8%). Complications tended to occur in young patients from lower socioeconomic circumstances without sex preponderance. The authors emphasized the need for better education of primary care physicians to manage and follow-up OM more diligently, particularly among marginalized and illiterate communities, given the high rate of continuing incidence and the subtleness of onset of complications.

Effective interventions for children suffering adverse sequelae from CSOM should convert a bad and perhaps worsening quality of life (QoL) into immediate and long-term improvements. Nadol and colleagues[104] noted that up to 2% of the population in developed countries will have a serious health impact from CSOM, which they defined as a disease of the middle ear or mastoid with irreversible mucosal change with infection lasting more than 3 months. Chronic suppurative OM is frequently associated with symptoms of inflammation, including discharge from the ear or pain. It is also associated with hearing loss as well as the functional limitations of hearing impairment, such as communication problems that may impede social interaction and school- or job-related performance. When CSOM is accompanied by cholesteatoma, complications such as facial nerve paralysis, meningitis, or encephalitis may develop and potentially threaten a patient's life. Baumann and colleagues[105] collected prospective audiological data and data from validated questionnaires on general and disease-specific QoL assessments to evaluate the health-related QoL for 121 adult (range, 18-75 years; median age was 48 years) patients with CSOM, with and without cholesteatoma, treated at the University of Heidelberg, Germany. Clinical symptoms associated with CSOM included hearing loss, otorrhea, fullness of the ears, ear pain, headaches, and often tinnitus. Tympanoplasty—surgical reconstruction of the tympanic membrane and establishment of ossicular continuity from the tympanic membrane to the oval window—was performed in all patients; audiological and QoL data were collected preoperatively and 6 months following surgery. Tympanoplasty resulted in significant mean reduction in the air-bone gap by 7.9 decibels (dB) hearing level (HL) as well as a significant 9.7-dB HL improvement in mean air conduction thresholds—that is, the pure-tone average (PTA) of thresholds at 0.5, 1, 2, and 4 kHz fell from 51.2 dB HL preoperatively to 41.5 dB HL 6 months after surgery; the mean bone conduction thresholds were stable, 24.3 dB HL and 22.0 dB HL, pre- and postsurgery. Their results showed patients with CSOM benefit from tympanoplasty in both subjective evaluations and in air conduction audiometric threshold measures, confirming earlier results by Nadol and colleagues.[104] The QoL measures also confirmed that disease-specific questionnaires are superior to general QoL questionnaires when the disease burden is lower than the threshold measured by the general instruments.[106] The authors also noted that only moderate correlations existed between the audiologically measured outcomes and the subjectively evaluated hearing function. The type of CSOM, with or without cholesteatoma, had no influence on the evaluation of the disease-specific QoL. The authors attributed this finding to the early recognition and treatment of both types of CSOM in their hospital. Revision surgery, as compared with primary tympanoplasty surgery, resulted in a poorer evaluation of QoL, which may be explained by the worse course and associated higher burden of the disease.

Vlastos and colleagues[107] reported a QoL study of 45 children, aged 4 to 14 years, diagnosed and treated because of CSOM at Aghia Sophia Children's Hospital in Athens, Greece. Children were offered either tympanoplasty or radical mastoidectomy surgery, and caregivers were administered a 5-item QoL survey (COM-5), a modified version of OM-6, twice before surgery, then 6 months and 1 year postoperatively. The authors found good test-retest reliability, intraclass correlation coefficient (ICC) = 0.73, and a significant correlation, $r = -0.49$, for construct validity between COM-5 score and global ear-related QoL. The median COM-5 score was 2.6 (based on a 7-point ordinal scale with higher scores indicating a poorer QoL). After successful tympanoplasty, the mean COM-5 score was reduced by 1.1, which correlated well ($r = -0.55$) with the change in global ear-related QoL. The mean standardized improvement in response was 1.3 points better after tympanoplasty, suggesting responsiveness of the COM-5 instrument to clinical change after the procedure, but was 0.7 points poorer after radical mastoidectomy.

Prevalence and Knowledge of Risk Factors

Oberg et al[108] conducted a global study of the burden of secondhand tobacco smoke exposure on children younger than 16 years and nonsmoking adults from 192 countries using 2004 data. Rate of smoke exposure for children worldwide was 40%, with 33% of male and 35% of female nonsmoking children exposed to secondhand smoke. Rates varied considerably by region, with lowest smoke exposure in African men (4%-7%) and highest in European men and women (66% for both). Seventy-one deaths occurred in children younger than 3 years (range, 0-23 months) with OM in 2004. Worldwide, there were 24,900 disability-adjusted life years (DALYs) for children younger than 3 years with OM and exposure to secondhand smoke.

Smith and Boss[109] conducted a systematic review using MEDLINE, identifying 15 (of 428) articles on OM and tympanostomy tube treatment meeting study criteria—namely, inclusion of US minority children, PubMed abstract, and health disparities addressed. The articles spanned 40 years. The most common risk factor identified was socioeconomic status (SES). There was extensive variability in racial/ethnic disparities in disease prevalence: Hispanic and NH black children were considerably less likely to be treated with tympanostomy tubes for COM/ROM; 1 study showed that NH black children had a higher prevalence of OM, 6 studies reported a lower prevalence in NH black children, and 2 studies showed no difference in prevalence between NH black and NH white children. Four of 5 studies revealed that Hispanic children had a lower prevalence of OM (comparison group not identified), and 1 study did not show a significant difference in prevalence between Hispanic and NH white children. Studies comparing

children from lower and higher SES were not definitive: 2 showed higher OM prevalence and 2 showed lower OM prevalence in NH black children, whereas the fifth study showed no difference between the 2 groups.

Srikanth and colleagues[110] used a cluster design to select 300 caregivers whose children attended preschool in the Vellore district of India. Researchers used a questionnaire to collect data on knowledge, attitudes, and practices related to risk factors for OM and to explore relationships between these factors and parental education, SES, and family type. Participants were more likely to be female (55%), be in a nuclear family (76%), and live in a *pucca* (a brick and cement house) indicating higher SES (43%). Five questions were administered to the caregiver focusing on knowledge about (1) RURI and otorrhea, (2) breastfeeding >6 months and OM prevention, (3) parental smoking in the home and OM, (4) smoke from cooking and OM, and (5) measles and DPT vaccines and reduction of OM. Knowledge of risk factors did not vary significantly between illiterate and educated fathers, but mothers were slightly more likely to know that parental smoking in the house was related to OM (30% vs 25%). Those in a nuclear family were less likely to know that the vaccines reduced frequent OM. Exploration of attitudes about risk factors resulted in one significant association: educated mothers were significantly more likely than illiterate mothers to believe that immersing the head leads to ear infections (83.7% vs 61.1%; $P = .005$), and those with lower SES had a borderline association with this belief (84.8% vs 79.6%; $P = .06$).

In a study of 800 preschool Asian Indian children living in rural areas, Sophia et al[70] collected data in a case-control study of OM (69 cases, 731 controls). Prevalence of OM (cases) in the whole group was 8.6%. Variables significant in univariate analyses were entered into a multivariate model, which revealed several factors significantly related to OM after adjusting for other factors. These factors included persistent rhinorrhea (cases, 76.8%; controls, 34.8%), adjusted OR = 7.56 (CI, 2.73-20.92); snoring (cases, 29.8%; controls, 7.4%), adjusted OR = 4.89 (CI, 1.32-18.17); passive smoke exposure (cases, 31.6%; controls, 23.5%), adjusted OR = 3.29 (CI, 1.05-10.33); and seasonal rhinitis (cases, 91.2%; controls, 68.7%), adjusted OR = 5.93 (CI, 1.33-26.51).

Preventive Strategies

Research into preventive strategies for AOM has advanced in 4 relevant areas by studies examining the use of xylitol, probiotics, zinc supplementation, and vaccination.

Previous research demonstrated that xylitol, a sugar alcohol with known antibacterial properties, could reduce the occurrence of AOM when given in a dose of 1.7 to 2 grams per dose 5 times daily as a gum or syrup without the occurrence of osmotic diarrhea, a known side effect of xylitol that occurs at higher doses. However, this therapy has not had widespread use, presumably because of impracticality of the effective dosing regimen. No new efficacy trials have been published since the previous report, but new mechanistic studies have further elucidated the molecular mechanisms by which xylitol impedes bacterial growth.[111-113] A National Institutes of Health (NIH)–sponsored, practice-based clinical trial of the efficacy of a viscous xylitol solution for AOM prophylaxis in otitis-prone children is currently under way.

Probiotics have also been identified as a promising strategy for AOM prevention. Recent developments in this area have included 3 new trials. In a double-blind randomized trial of once-daily infant formula supplementation with 10^{10} colony-forming units of both *Lactobacillus rhamnosus* GG and *Bifidobaterium lactis* Bb-12, a 56% reduction in clinically diagnosed AOM and a 48% reduction in antibiotic use among probiotic-treated infants was observed.[114] In a blinded cluster-randomized dental study examining supplementation of milk once daily with 10^7 colony-forming units per milliliter of *L rhamnosus* LB21 and fluoride, treated subjects experienced a 50% reduction in days with clinically diagnosed AOM and a 60% reduction in days of antibiotic treatment.[115] However, in a small double-blind randomized trial of infant supplementation with *Bifidobacterium animalis* subsp. *lactis* BB-12 at a dose of 5×10^9 colony-forming units twice daily, no reduction in clinically diagnosed AOM was observed, although the power of the study to detect a meaningful difference was quite small.[116] None of the probiotic studies reported an increased risk of adverse events in treated subjects compared with controls, but the sample sizes were not large enough to detect small increases in potential adverse effects of treatment or rare serious adverse effects.

Zinc supplementation has been hypothesized to reduce the incidence of a number of acute pediatric infectious diseases, especially among children in the developing world where zinc deficiency is common. A recent blinded randomized trial of zinc (and propolis) supplementation conducted in Italy reported a 32% reduction in AOM incidence among treated children.[117] A Cochrane review of zinc supplementation for AOM prevention published in 2010 concluded that evidence of the effect of zinc on AOM is mixed and further research will be needed to reach any definitive conclusions.[118]

A recent study from Canada documented an overall 13% reduction in clinically diagnosed AOM episodes after widespread implementation of pneumococcal conjugate vaccine (PCV).[119] A study from Greece found a 38% reduction in ED visits for otorrhea after widespread use of the vaccine.[120] A randomized trial conducted in Sweden among children with a history of RAOM demonstrated a 26% reduction in AOM among the PCV-vaccinated group (although parents were not blinded to treatment assignment).[121] Further advances in vaccination against AOM are focusing on the effect of vaccination against additional pneumococcal serotypes and on development of vaccines against the other 2 predominant AOM pathogens, NTHi and *M catarrhalis*.[19]

Methodologic and Analytic Strategies

Meta-analyses are based on systematic reviews of findings from all available studies relevant to a particular question. One method proposed as a major improvement in meta-analytic methods for the study of subgroup effects is to use individual patient data (IPD). The key element is combining the results across all the studies in a quantitative summary that has greater

statistical power to detect significant results. Typically, the quantitative tool used has been to combine the various published summary statistics from each study using weighted averages (where weighting may be proportional to the individual study sample sizes).[122] An alternative, when data-sharing conditions allow, is to reanalyze all individual patients' data from multiple studies as if they were ascertained from one large, pooled study. This approach has many advantages, such as being able to examine the underlying data in much greater detail and the ability to use consistent analytic techniques across studies. However, there are often major distinctions between studies, and these distinctions can be examined by stratified analyses of the pooled data. Most IPD analyses will have both prognostic and outcome information available on individuals, which permits the examination of treatment effects by baseline covariates. This additional information can be explored for subgroup analyses and to compare interaction effects across studies. Without IDP data, detailed meta-analytic subgroup comparisons are severely hampered or impossible.

Koopman and colleagues[123] reported their findings on subgroup effects from conventional and IPD meta-analyses using identical data sets derived from 6 trials (n = 1643) on the effectiveness of antibiotics in children with AOM. Only 2 of the 6 studies could be included for the conventional analyses because they were the only ones to report on relevant subgroup effects. The conventional meta-analysis showed both larger and smaller subgroup effects by age (<2 years vs ≥2 years) and wider CIs than both IPD meta-analyses. The authors indicated the most important lesson learned was that the 2 studies included in the conventional meta-analysis reported outcomes that were different from each other and from the IPD meta-analyses. The empirical example in this study suggests that conventional meta-analyses may not allow proper subgroup analyses, whereas IPD meta-analyses can yield accurate estimation of subgroup effects.

In a study comparing different methods of handling missing data in IPD meta-analyses, Koopman and colleagues[124] compared imputation techniques over (across) trials and within trials to an approach often used: no imputation, just restrict the analysis to the situation where complete information is available. *Imputation* is a term used by statisticians for procedures whereby "missing" data are replaced by new values using statistical prediction methods of varying degrees of likelihood or precision. For purposes of this study, the authors used 2 methods to replace missing values. For variables with a single missing value, they used conditional means, that is a prediction model was fit for each variable with a missing value (the variable with a missing value is the outcome), and all other variables (measured covariates) are included as predictors in the model. The missing values are then replaced by estimates resulting from the prediction model. The imputation procedure used when variables had multiple missing values was more complicated. It was based on a combination of advanced statistical methods, including additive regression, bootstrapping, and predictive mean matching for multiple imputations.[124] The authors noted that the method of imputation for missing data over trials could lead to bias since associations for covariates are likely to differ across the selected studies. Therefore, despite the gain in statistical power, they recommended against imputation over (across) trials. Instead, based on their empirical example, they recommended that the most appropriate approach to handling missing data in IPD meta-analyses was imputation *within* trials.

Special Populations

American Indians/Native Alaskans (AI/AN). Tobacco use is common in AI communities, and smoking predisposes users to respiratory problems and has been associated with OM in young children.[125] They have higher smoking rates than other racial/ethnic groups in the United States. Among adults, 34% of AI and 49% to 58% of Northern Plains Indians smoke tobacco, compared with 23% of NH African Americans and 21.9% of NH whites.

Forster et al[126] conducted a study to learn about patterns of tobacco use of American Indians in Minnesota, and 300 participants ≥18 years old were interviewed. Age and sex quotas consistent with the AI population in Minnesota were used to reflect community practices. Smoking was prevalent: 62% were current smokers and only 12% reported smoking <100 cigarettes. Current smokers reported wanting to quit (68%), with 53% of all smokers reporting unsuccessful attempts at quitting in the past 12 months.

In studies conducted from 2002 to 2004 by the Centers for Disease Control and Prevention (CDC) and Substance Abuse and Mental Health Services Administration (SAMHSA), 23% of 12- to 17-year-old AI/AN youth smoked cigarettes, which is higher than smoking rates for NH white (14.9%) and NH African American (6.5%) youth in the United States.[127] During a similar time period, this difference was also true for those aged 18 years or older in the United States; 37% of AI/AN adults smoked cigarettes in the past 30 days compared with 28% of NH white or African American adults.[128] Based on the 2007 California Health Interview Survey (CHIS), commercial tobacco use by the AI population (35.3%) was more than twice the prevalence rate of the general Californian population (13.8%).[129,130] In 2007, 5.5 million (7.6%) US children were exposed to environmental tobacco smoke (ETS) or secondhand smoke in their homes, and children in low-income (below poverty level) and low-education (<12 years) households had 7.3 and 10.6 times higher odds of ETS exposure compared with children in high-income (>400% of the poverty level) or high-education (16+ years) households.[131]

Rhodes and colleagues[132] interviewed women in AI health clinics (1 urban, 3 on Minnesota AI reservations) during the prenatal period and when their infants were between 2 weeks and 6 months old. Fifty-nine percent initiated breastfeeding postpartum, whereas at 6 months, 20% breastfed exclusively or in combination with bottle feeding. Factors related to breastfeeding at birth included positive attitudes about and social support for breastfeeding. At 6 months, use of traditional American Indian medicines and lack of maternal smoking were related to breastfeeding.

Data on outpatient visits and hospitalizations for OM in 2003 to 2005 for AI children <5 years of age were analyzed

using 3 large national databases.[133] Rates of OM outpatient visits for AI/AN varied by region and demographic factors. Children younger than 1 year had 184.8 visits per 100, and those aged 1 to 4 years had 71.3 visits per 100. Males had more visits than females (93.6 vs 84.3). Alaska Native children had rates that ranged from 1.8 times higher (compared with Northern Plains Indians) to 2.6 times higher (compared with AI children in the western US region). Otitis media outpatient visit and hospitalization rates declined considerably between 1994-1996 and 2003-2005: from 138/100 to 89/100 for outpatient visits and 1542/100 to 675/100 hospitalizations, perhaps due to the introduction of pneumococcal conjugate vaccines in 2000.

Arctic Inuits (and Danes) in Greenland. Risk factors for COM, with or without suppuration, have not been frequently dealt with in the literature, probably because the disease is relatively rarely seen in industrialized countries where research funds are much higher than in areas inhabited by special populations or in developing countries where COM and CSOM are far more frequent. Koch et al[65] conducted a prospective population-based cohort study of 465 Greenlandic children followed clinically for 2 years. The study aims were to determine incidence, median age at debut, risk factors, and associated population attributable risks for CSOM in young children in Sisimiut, the second largest town of Greenland (population 5400), where living conditions are relatively Western and approximately 90% are Inuits. The cumulative risk of CSOM, using World Health Organization (WHO) definitions[134] with ≥14 days of suppuration, was 14% at 4 years of age with a median debut age of 336 days (11 months old). They identified risk factors and associated population attributable risks, which indicated that preventive measures regarding use of childcare centers and passive smoking could reduce the burden of CSOM in this high-risk population.

Australian Aborigines. Three to 5 children die each year in Australia because of otitis media complications, and 15 children will suffer permanent hearing loss each year as a result of otitis media.[135] The contrasting rates of childhood otitis media among indigenous and nonindigenous children have implications for the frequency and types of complications occurring in both groups. Otitis media with effusion and acute otitis media predominate among nonindigenous children, whereas CSOM occurs most commonly among indigenous children. Each year in Australia, 300,000 children are estimated to experience mild to moderate temporary hearing impairment as a result of OM, and over 60,000 children suffer from CSOM. The prevalence rates of OM among Aboriginal children in Australia are among the highest in the world.[135] Middle ear disease has been reported to be as high as 91% in indigenous children in rural communities, and 50% have signs of CSOM. The rates of OM among nonindigenous children in Australia are similar to that found in other developed countries.

Nigerians/West Africa. A study by Lasisi and colleagues[136] examined the onset of early OM in the first few months of life as a potential factor in the increasing prevalence of later CSOM. They implemented a questionnaire and examination study conducted in 5 sites located in 2 states of Nigeria. In this study of age at onset of otorrhea and associated risk factors among 189 children with CSOM, early OM was found in 136 (70%) young children with CSOM; mean age for CSOM children was 5 years. Among the 85 (45%) CSOM children with hearing loss, early OM was found for 58% (49/85), which was significantly associated with CSOM with hearing loss but not with otorrhea. Bottle feeding, adenoiditis/adenoid hypertrophy, indoor cooking, and URI were not found related to early vs later onset of OM. However, early onset OM was associated with CSOM with hearing loss, identified allergy, low social status, and chronic exposure to overcrowding through increased number of children in the household.

Olatoke and colleagues[137] conducted a cross-sectional study to determine the prevalence of hearing loss among 1500 Nigerian schoolchildren aged 9 to 15 years who had CSOM. The sample was drawn from 3 schools representing 3 different levels of SES. Overall, CSOM was present in 2.3% of children: 4.0% among students attending the low-SES school, 2.8% of children at the medium-SES school, and 1.5% of children at the high-SES school ($P = .04$). The PTA hearing threshold measures were significantly elevated for the 52 ears (35 children) with CSOM compared with 160 control ears without CSOM. Of the children having ears with CSOM, 35% had PTAs within normal limits, 39% had mild conductive hearing loss, and 27% had a moderate hearing loss. Except for 2 of the 160 control subjects, PTA hearing was within normal limits. Academic performance was assessed and found to be significantly lower ($P < .002$) in CSOM patients vs controls. Hence, hearing loss was found to be a significant sequela of CSOM in this population, and it was associated with an adverse effect on academic performance; children in the low-SES group were most at risk.

Implications for Practice

During the past 4 years, we have witnessed the emergence of new research tools coupled with traditional epidemiological principles that can be embedded in large-scale, population-based cohort studies. The scope of the panel's report now embraces a much broader, more complicated spectrum of intermediate variables and end points for use in exploring the complicated web of risk associations for OM, as well as its complications and comorbidities. These developments have occurred recently and are far from being fully exploited in current epidemiologic research studies.

Epidemiologic research continues to expand with more sophisticated research designs being implemented in diverse communities. In many instances, the true nature of underlying risk factors has been revealed. This research on risk factors and natural history, when done thoroughly, can then be acted upon to implement more effective interventions. The panel has identified several short- and long-term research goals below.

Short-Term Goals

1. *Pre- and perinatal influences*. Research studies need to be conducted to increase our understanding of pre- and perinatal influences on early OM onset.

Comment: Early onset OM is a risk factor for ROM. Currently, several large epidemiologic data sets from national cohort studies (eg, in Denmark, Norway, and the United States) can be used to expand our knowledge in this area.

2. *Tracking and monitoring of the impact of OM vaccines*. These vaccines may have a significant impact on effectively reducing the incidence of OM. In particular, studies are needed to determine whether 13-valent pneumococcal vaccine and the 10-valent vaccine coupled with protein D will continue to reduce OM incidence.

Comment: Studies in many countries have reported declining OM incidence. Several studies have investigated changes in the microbiology of OM pathogens following the introduction of 7-valent pneumococcal conjugate vaccine; these studies continue to be necessary. The role of seasonal influenza virus vaccines in possibly reducing OM incidence also needs investigation.

3. *Epidemiologic and natural history studies to assess hearing loss as a consequence of OM*. Additional research is needed to comprehend the hearing loss consequences of AOM, ROM, and especially CSOM.

Comment: A recent draft of a WHO Global Burden of Disease report on OM has been reviewed by selected experts. When available, the findings of this report should be evaluated to determine where the needs for prevention efforts and intervention studies are greatest from a worldwide perspective.

4. *Acute OM prophylaxis*. Additional research needs to be conducted to understand how putative prophylactic agents can reduce the incidence of AOM.

Comment: We now have a greater understanding of the molecular mechanisms by which xylitol impedes bacterial growth, and research is continuing into the efficacy of xylitol solution for prevention of AOM. Further investigation is also needed on the usefulness of probiotics.

5. Q*uality-of-life measures in OM epidemiological studies*. Inclusion of QoL measures to determine the satisfaction of parents and family or other caregivers following treatments for OM will provide valuable new information.

Comment: Otitis media (disease-specific) QoL measures should be used when comparing OM treatments or interventions, whereas global QoL measures are appropriate in comparing the experience of OM with other childhood illnesses or conditions. Insights gleaned from these measures will be important for planning and implementing future prevention or intervention efforts.

6. *Sharing epidemiological data across studies*. The importance of sharing data across studies has been demonstrated recently in conjunction with genetic studies that require large samples. However, lack of uniform collection of information and inconsistent rules across institutional review boards (IRBs) at local, national, and international levels are a hindrance, delaying and even preventing the sharing of data.

Comment: Two suggestions for consideration were proposed. First, strive to incorporate data-sharing permission prospectively in the development of study protocols. Second, form working groups to expedite the comparison of epidemiological data in joint or coordinated analyses and reports of findings.

7. *Improve epidemiological data quality and consistency across studies*. Failure to use similar definitions and consistent methods across epidemiologic studies was identified as a major concern for later combining of data across studies to achieve consensus on important findings.

Comment: Efforts should be made to identify resources to promote the standardization of methods and definitions in epidemiological studies to better quantify the magnitude of effects and improve comparisons across studies.

8. *Improve statistical methods of analysis in OM publications*. Multivariate methods of analysis that take into account the complexity of the data and account for correlated outcomes are encouraged. Otitis media researchers have made much progress recently in this area, as nearly all epidemiological studies control for other variables in estimating the "independent" effects of the variables of interest. In addition, most studies now report confidence intervals in place of, or in addition to, *P* values (significance levels).

Comment: In addition to standard statistical summary measures such as odds ratios and confidence intervals, studies should consider calculating and presenting the "number" of children needed to treat and other useful measures. Some multivariate methods of potential use include general estimating equations, structured equations, and time event modeling for longitudinal studies. Bayesian analyses may be useful for a variety of applied statistical problems encountered in clinical and epidemiologic studies. Consider power when planning studies and the use of confidence intervals to provide information on the magnitude of risk or degree of uncertainty.

Long-Term Goals

1. *Include OM research objectives during the early planning phases of large prospective studies*. Otitis media experts should participate in planning large, prospective cohort studies to incorporate questions and measurements of OM, other ear diseases, and

their complications within cohorts of pediatric populations. Particular attention should be given to the nearly universal association between URIs and OM when performing epidemiological studies.

Comment: There is a need for large, well-planned, prospective studies to research OM etiology and pathogenesis. In the United States, the National Children's Study and other large prospective cohorts could be a valuable resource in meeting this goal. These studies should include the elements needed to make scientific conclusions about OM (eg, eustachian tube dysfunction and microbiological, immunological, genetic, and environmental measures) as well as interactions between these factors. Including clinical measures in large studies is expensive and must typically be funded by governmental health agencies.

2. *Need to assess new measures for use in epidemiological studies of OM*. For example, new measures for use in epidemiologic studies of OM risk may be derived from current imaging tools and other methods of assessing anatomic features and functional measures to explain population differences (eg, quantitative measurements of the eustachian tube and nasopharyngeal space).

Comment: As more large population-based studies are conducted, coordinated standardization of methods and definitions will facilitate future meta-analyses. Standardization across studies should lead to better quantification of the magnitude (size of effect) of risk factors for OM.

3. *Perform genetic studies, including family linkage and large-scale population association studies*. Large cohort studies in many countries should be used to examine genetic loci and alleles that predispose to OM.

Comment: There has been considerable progress in exploring the genetics of OM. This panel report includes several genetic studies reporting on heritability, linkage, fine mapping, and candidate gene studies of OM and related conditions. There is more work to be done in this area. To date, there are no genetic studies of OM in high-risk populations, gene-gene/gene-environment interactions, or large-scale association studies.

4. *Use large cohort studies from various countries to examine genetic loci and alleles that predispose to OM*. Encourage collaboration and sharing data to increase analytical power of the available genetic material.
5. *Examine registries and other sources of outcomes data for OM-related complications and sequelae*. Encourage collection of population-based registry and outcome data to monitor complications from otitis media.
6. *Prospectively monitor the impact of new guidelines on adverse OM events*. Guidelines to reduce antibiotic use in OM treatment have been proposed. Examine rates of severe complications (eg, mastoiditis and hospitalizations for AOM) as a benchmark to evaluate and compare current and evolving guidelines.
7. *Compare effectiveness of OM treatment guidelines in different countries on clinical practice*. Compare data from population-based studies to assess how well guidelines for OM treatment are followed in various countries, while allowing for diverse cultural norms. Rates of mastoiditis and other intratemporal and intracranial complications should be tracked to determine the population effect of guidelines for reducing antibiotic use in OM treatment. Prospective monitoring will determine the effect of guidelines on actual clinical practice. As guidelines evolve, longitudinal tracking will be needed and, where possible, national-level data should be used.

Comment: Although there have been several reports examining adherence to OM treatment guidelines, there is a continuing need for further research and monitoring to detect changes over time and elucidate trends.

8. *Investigate the relationship between atopy, allergy, and OM*. The relationship between atopy and allergy (as etiologic factors or comorbidity) and OM remains unclear. This potential relationship may have important clinical implications for diagnosis and treatment.
9. *Encourage OM studies in high-risk populations and developing countries*. Otitis media is a challenge in high-risk populations and developing countries. More research on the etiology, including genetic, immunologic, and socioeconomic factors, is needed. Intracranial and other severe complications are common, but prevalence rates are unknown. Researchers from developing countries should be invited to attend OM research meetings.

Comment: Conduct genetic studies in high-risk populations especially prone to OM (American and Canadian Indians, Alaskan and Greenland Inuits, and Australian Aboriginals). Gene-gene and gene-environment interactions should also be explored.

10. *Encourage research on the role of prenatal and perinatal factors*. Most children experience OM onset very early in life. Although several studies have focused on perinatal factors, few have investigated prenatal influence on OM onset. More epidemiologic studies should be conducted to increase understanding of the role of prenatal and perinatal factors in early OM onset.
11. *Conduct intervention studies to determine the impact of risk factor reduction on OM incidence and prevalence*. Intervention studies designed to determine the impact of risk factor reduction on

OM incidence and prevalence will enhance knowledge about OM prevention strategies. However, because of the high heritability of OM, intervention studies designed to determine the impact of modifiable environmental risk factors will need to have realistic expectations.

12. *Identify useful clinical prediction rules to guide treatments for OM*. Develop valid and accurate clinical prediction rules. The goal of an accurate prediction rule is to help clinicians distinguish children with a poor natural course (those who probably will benefit most from intervention) from those expected to have a favorable natural course. Predictors are developed based on subgroups of patients to guide management decisions for individual children.
13. *Ensure proper measures of study size.* This will improve the ability to gauge the magnitude of effect sizes and, hence, the impact of risk reduction on end points in intervention studies.
14. *Develop proper and consistent measures for URI using the same terminology and definitions.* This will allow for comparisons among epidemiological research studies.
15. *Encourage studies on intra- and inter-country differences in diagnosis, treatment, and outcome*. Such studies should identify regions having the greatest need for intervention programs.
16. *Conduct studies on the impact of diagnostics in OM prevalence and incidence figures*. Monitoring the quality of OM diagnoses remains a continuing high-priority topic with important implications for research and public health practice.

Discussion

The studies reviewed here have improved in quality compared with earlier periods. However, there is still a need to stress the importance of using uniform definitions, sound methodology, and appropriate, innovative statistical analyses to obtain the maximum useful amount of information from expensive and often difficult to implement epidemiological field studies. Careful planning at the beginning of large-scale epidemiological studies is still critical to making better informed conclusions at the end. Given the current high cost of epidemiological research and serious financial constraints on research funds, it is especially important to design new studies with the expectation of comparing results across multiple studies when finished. Careful planning should also include a commitment to cooperate afterward in joint analyses based on IPD meta-analyses. These meta-analyses may shed light on subgroup effects to the substantial benefit of both scientific understanding and improvement in the design of preventive interventions.

The consistent decrease in the incidence and prevalence of OM, which was suggested in the prior review[20] of epidemiological studies in 2003 through 2007, has now become evident in data from multiple countries. If we can identify the reasons for this decline, it will be of potentially great interest and use in aiding further reductions in the disease burden of OM worldwide.

One of the pitfalls of performing these quadrennial, periodic reviews of only the most recent OM epidemiologic literature—embracing just the past 4 years—is that the reader may assume everything of importance has been discussed. Of course, this is not the case. By focusing only on the recent past, it is difficult to retain perspective on several issues that have temporarily fallen by the wayside. Moreover, there is the need for a still wider view of what studies are worth contemplating and, therefore, should have been addressed. It may be worth noting some outstanding questions in the OM epidemiological field that have not yet been resolved. Many of these we included in our short- and long-term goals of the preceding section.

One feature noticeably lacking from our review was reference to any publication in the current 4-year time frame that represented a serious overview of the present knowledge of "preventable" or currently modifiable OM risk factors. Since it was not included in the prior review[20] for the years 2003 to 2007, we call attention to a publication in 2006 by Brazilian researchers Lubianca et al,[138] who published a systematic literature review of modifiable risk factors for ROM in children ≤18 years old. Using MEDLINE with no language restrictions, they searched the literature from January 1966 to July 2005 and found 257 articles using descriptors "acute OM" and "risk factors." From these articles, they selected studies that analyzed modifiable risk factors for the development of RAOM as the main objective and identified 9 risk factors linked to the host and 8 linked to the environment. Although one may object to how feasible it would be to modify many of the risk factors they identified, we believe their effort deserved to receive more notice and credit for the valuable resource they provided. Among the modifiable host risk factors mentioned were allergy, craniofacial anomalies, gastroesophageal reflux, and presence of adenoids. Environmental factors listed were URIs, daycare center attendance, presence of siblings/family size, passive smoking, breastfeeding, and use of pacifiers. On the basis of their classification in terms of levels of evidence, they concluded the "established" modifiable risk factors for ROM were use of pacifiers and care in daycare centers. The remaining ones were classified as "probable" risk factors. None of the risk factors were labeled as "unlikely," although several were described as requiring further study.

Such efforts to categorize the "important" modifiable OM risk factors usually invite controversy and disputation. Many large-scale observational studies have reported weak or nonexistent associations between passive smoking and risk for OM. Hence, exposure to ETS has remained a controversial risk factor for OM. On the other hand, we could reach the opposite conclusion based on well-conducted studies in the literature. Stenstrom and colleagues[139] evaluated the association between exposure to ETS and RAOM in 85 cases and 85 age- and sex-matched controls younger than 5 years. Cases were defined as having 4 or more physician-documented AOM episodes in the preceding 12 months and controls were otitis-free in the prior 12 months. The authors used conditional logistic regression to control for other risk factors, such as daycare attendance, socioeconomic status, prematurity, and family history of otitis media, and were able to demonstrate a

significant association between ETS and RAOM (OR = 2.68; CI, 1.27-5.65). They also found a significant exposure-response relationship between increasing level of exposure to ETS and increased risk of RAOM. On the basis of the population attributable fraction (PAF) formula, they calculated that up to 34% of RAOM cases may be accounted for by ETS exposure. The PAF (also referred to as attributable risk or etiologic fraction) is a measure of the proportional reduction in population disease (eg, RAOM) that would occur if exposure to the risk factor (eg, ETS) were eliminated. They concluded that exposure to ETS is an important and modifiable risk factor for RAOM in children younger than 5 years. A similar conclusion was reached in a systematic review by Strachan and Cook,[140] who performed a quantitative meta-analysis using random effects modeling to pool odds ratios from different studies. They found the evidence for middle ear disease to be remarkably consistent, with pooled ORs if either parent smoked of 1.48 (CI, 1.08-2.04) for ROM and 1.38 (CI, 1.23-1.55) for MEF. They were unable to use their modeling approach for AOM, although they reported ORs for AOM in the range of 1.0 to 1.6. They concluded there is a likely causal relationship between parental smoking and both acute and chronic middle ear disease in children.

Hanafin and Griffiths[141] examined whether pacifier use causes ear infections in children who are younger than 2 years. They found a clear increase in the risk of OM associated with use of a pacifier (dummy) that could well be causal, yet they worried that the relationship may have been confounded by sociodemographic factors. This is a particularly interesting example, since numerous carefully conducted infant mortality studies have reported that use of pacifiers in young infants is protective against sudden infant death syndrome (SIDS).[142,143] Despite the evidence for a dose-response relationship between pacifier use and OM, Hanafin and Griffiths decided not to claim their analysis revealed a causal relationship. Instead, the authors suggested, "Rather than advising a parent not to use a pacifier for fear of causing OM, advice in relation to this issue might best be restricted to pacifier users suffering from the problem in order to reduce the chances of recurrence." This example provides a valuable lesson in the proper use of caution and the frequent need for a more thorough understanding of underlying trade-offs. Of course, this was an instance when an immediate recommendation advising against use of pacifiers could have led to unintended consequences, a possible increase of infant deaths due to SIDS.

The advice to inform parents about the possible negative effects of using a pacifier once their child has been diagnosed with AOM to avoid recurrent episodes has been strengthened by findings from a recent study conducted by Rovers and colleagues.[144] Several studies have shown both risks and benefits of pacifiers, including, for example, the use of pacifiers for very preterm infants born less than or equal to 32 weeks' gestation who are fed through a tube (gavage feeding) before they can effectively feed from the breast or bottle. Sucking on a pacifier (nonnutritive sucking) during gavage feeding is recommended to encourage the development of sucking behavior and improve digestion of the feeding; it may also have a calming effect on infants, although there is concern it could interfere with breastfeeding later.[145] Sexton and Natale[146] have written a balanced summary of the pros and cons of pacifier use. They note that the AAP and the AAFP recommend weaning children from pacifiers in the second 6 months of life to reduce the risk of otitis media; however, pacifier use should not be actively discouraged and may be especially beneficial in the first 6 months of life—most SIDS deaths occur in the first 6 months of life.[147] Evidence for the effect of pacifier use on early childhood caries has been inconsistent (ie, there is no proven correlation). In fact, the AAPD recommends pacifiers over "thumbs" to comfort babies.[95] The AAPD notes that sucking on a thumb, finger, or pacifier is normal for infants and young children and that most children will stop on their own. If a child does not stop, then the AAPD recommends discouraging the habit (pacifier use or thumb/finger sucking) after the age of 3 years. Concerns about dental malocclusion have also been raised, with some evidence that adverse dental effects may occur after 3 years of age.[148]

Many potentially modifiable risk factors may remain beyond our ability to implement appropriately and effectively. As a final example, we cite a well-controlled study of temporal factors on 2512 randomly selected children in Finland who were monitored until the age of 2 years. Alho and colleagues[149] considered risk factors for AOM, taking into account both time dependency and confounding factors, while keeping in mind the importance of biological interpretability of each risk factor. They determined the major risk factors were the existence of a previous episode of AOM in general (OR = 2.03; CI, 1.81-2.25) or, in particular, during the preceding three months (OR = 3.74; CI, 3.40-4.10) and attendance at a daycare nursery or center (OR = 2.06; CI, 1.81-2.34). Their conclusion was the logical one that only the form of daycare was modifiable and that infants should be cared for at home, particularly after they have already experienced an episode of AOM. Although this advice may be practical in Scandinavia, it is unlikely to be feasible in many countries of the world.

The above studies were all drawn from an earlier time frame but are discussed as examples of some of the superb work performed in the past that continues to inform present clinical advice and practice. We hope this discussion also serves as a useful reminder that the aim of the panel review was not to replace the knowledge from past epidemiologic research studies conducted prior to the past 4 years. Our primary goal was to review the literature in an organized, systematic fashion that could lead to quicker assimilation to inform clinical practice and future OM research.

Appendix

Abbreviations

Classifications of diseases/symptoms. AOM, acute otitis media; ASOM, acute suppurative otitis media; COM, chronic otitis media, *chronic* is 3 months or longer; COME, chronic otitis media with effusion; CSOM, chronic suppurative otitis media; HCA, histological chorioamnionitis; *ICD-9-CM,*

International Classification of Diseases, 9th Revision, Clinical Modification; ME, middle ear; MEF, middle ear fluid; OM, otitis media; OME, otitis media with effusion; RAOM, recurrent acute otitis media, typically defined as 3 or more, or 4 or more, AOM diagnoses over a 12-month (1-year) time period; ROM, recurrent otitis media; ROME, recurrent otitis media with effusion; RSV, respiratory syncytial virus; RURI, recurrent upper respiratory tract infection; SIDS, sudden infant death syndrome; SOM, suppurative otitis media; URI, upper respiratory tract infection.

Bacterial/viral, biochemical, clinical, or genetic terms. *AP2B1*, gene located on the long arm of chromosome 17, which may be associated with OM susceptibility; CO, carbon monoxide; 3p, 10q, 17q12, 19q, chromosomal markers (eg, 3p refers to the short arm of chromosome 3, 10q refers to the long arm of chromosome 10, 17q12 refers to position 12 on the long arm of chromosome 17, and 19q refers to the long arm of chromosome 19); *EVI1* (Evi1), gene associated with OM; *FBXO11* (Fbxo11), gene/protein that regulates the TGF-β pathway; Jeff, a deaf mouse mutant strain whose mutation maps to the *FBXO11* gene; Jumbo, a hearing-impaired mouse mutant strain whose mutation maps to the *EVI1* gene; IL-1α, IL-1β, IL-6, IL-10, IL-Ira, polymorphisms of interleukin inflammatory cytokines; ILF, interleukin enhancer binding factor; IG, immunoglobulin; IgG, immunoglobulin G; *MUC, MUC2, MUC5B, MUC5AC*, mucin genes/proteins; NO_2, nitrogen oxide; NP, nasopharyngeal (eg, NP bacterial colonization); NTHi, nontypeable *Haemophilus influenzae*; O_3, ozone; PCV, pneumococcal conjugate vaccine; P6, vaccine candidate outer membrane protein P6 antigen; RSV, respiratory syncytial virus; RT-PCR, reverse transcription polymerase chain reaction; SFTPA, surfactant protein A; SNP, single-nucleotide polymorphism; TGF-β, transforming growth factor–β, a signaling pathway involved in many cellular processes; TLR, Toll-like receptors are a class of proteins that play a key role in activating the innate immune system; TNF-α, tumor necrosis factor–α.

Sociodemographic, environmental, public health, and statistical terms or measures. AI/AN, American Indian/Alaska Native; BMI, body mass index; case-control study, a type of epidemiological observational study of persons with the disease (other outcome) of interest and a selected group of people (reference group or comparison group) without the disease (other outcome); CI, 95% confidence interval; cohort study, a longitudinal observational study of a group of people ("cohort") who are studied until they develop a disease or outcome of interest—the cohort is defined by having a common characteristic or experience within a defined period (eg, the US 2010 birth cohort consists of all infants born in the United States in 2010); COM-5, a 5-item quality-of-life survey, a modified version of the OM-6; DALYs, disability-adjusted life years; dB, decibels, a measure of sound intensity such that 1 dB is the just noticeable difference in sound intensity for the normal human ear; DCC, daycare center; ELBW, extremely low birth weight (<1000 g); extremely preterm birth, less than or equal to 32 completed weeks of gestation; ETS, environmental tobacco smoke; HR, hazard ratio (ie, a ratio of event rates since *hazard* is defined as the rate at which events happen over a short period of time); HL, hearing level is quantified relative to "normal" hearing in decibels (dB), with higher numbers of dB indicating worse hearing; incidence, the number of new cases of disease (other outcome) during a specified time period, such as a year; IDR, incidence density rate, the number of new cases of disease (other outcome) per 100 person-years (child-years); ICC, intraclass correlation coefficient; IPD, individual patient data (eg, for use in meta-analyses); IRR, incidence rate ratio; LBW, low birth weight, <2500 g; NH, non-Hispanic; OM-6, quality-of-life outcomes questionnaire for use with otitis media treatments; OR, odds ratio; *P* (or *P* value), probability of occurrence by chance (ie, level of statistical significance); PAF, population attributable fraction—namely, the proportional reduction in disease if exposure to a risk factor is eliminated; PM, particulate matter; prevalence, the percentage of a population affected with a particular disease (other outcome) at a given time or during a specified time period, such as a year; person-years (child-years), the sum of the number of years that each member of a population has been diagnosed or followed up for a disease (other condition); preterm birth, less than 37 completed weeks of gestation; PTA, pure-tone average of hearing thresholds, typically for frequencies of 500, 1000, 2000, and 4000 Hertz, usually expressed in dB HL units; QoL, quality of life; *r*, correlation coefficient; RCT, randomized controlled trial, that is, a clinical experiment with random allocation of patients to treatments (interventions) to examine the efficacy of the treatments compared with no treatment (placebo control) or alternative treatments; RR, relative risk; SES, socioeconomic status; VLBW, very low birth weight (<1500 g).

Professional terms/organizations. AAFP, American Academy of Family Physicians; AAP, American Academy of Pediatrics; AAPD, American Academy of Pediatric Dentistry; AHRQ, Agency for Healthcare Research and Quality; CDC, Centers for Disease Control and Prevention; CHIS, California Health Interview Survey; CPS, Current Population Survey; ED, emergency department; ENT, ear, nose, and throat specialist or doctor; MEPS, Medical Expenditure Panel Survey; NAMCS, National Ambulatory Medical Care Survey; NCHS, National Center for Health Statistics; NHAMCS, National Hospital Ambulatory Care Survey; NHDS, National Hospital Discharge Survey; NHIS, National Health Interview Survey; NIDCD, National Institute on Deafness and Other Communication Disorders; NIH, National Institutes of Health; NIPH, Norwegian Institute of Public Health (Folkehelsa); NIS, National Immunization Survey; NLSCY, Canadian National Longitudinal Survey of Children and Youth; OPD, outpatient department; MoBa, Norwegian Mother and Child Cohort Study of over 100,000 mothers and babies; SAMHSA, Substance Abuse and Mental Health Services Administration; WHO, World Health Organization.

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Author Contributions

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Panel 2: Eustachian Tube, Middle Ear, and Mastoid—Anatomy, Physiology, Pathophysiology, and Pathogenesis

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Abstract

Objective. This report reviews the literature to identify the advances in our understanding of the middle ear (ME)–Eustachian tube (ET) system during the past 4 years and, on that basis, to determine whether the short-term goals elaborated in the last report were achieved and propose updated goals to guide future otitis media (OM) research.

Data Sources. Databases searched included PubMed, Web of Science (1945-present), Medline (1950 to present), Biosis Previews (1969-present), and the Zoological Record (1978 to present). The initial literature search covered the time interval from January 2007 to June 2011, with a supplementary search completed in February 2012.

Review Methods. The panel topic was subdivided; each contributor performed a literature search and provided a preliminary report. Those reports were consolidated and discussed when the panel met on June 9, 2011. At that meeting, the progress was evaluated and new short-term goals proposed.

Conclusions. Progress was made on 16 of the 19 short-term goals proposed in 2007. Significant advances were made in the characterization of ME gas exchange pathways, modeling ET function, and preliminary testing of treatments for ET dysfunction.

Implications for Practice. In the future, imaging technologies should be developed to noninvasively assess ME/ET structure and physiology with respect to their role in OM pathogenesis. The new data derived from form/function experiments should be integrated into the finite element models and used to develop specific hypotheses concerning OM pathogenesis and persistence. Finally, rigorous studies of treatments, medical or surgical, of ET dysfunction should be undertaken.

Keywords

middle ear, Eustachian tube, imaging, otitis media, human, animal

The middle ear (ME) is a relatively noncollapsible cavity within the temporal bone that can be subdivided into the osseous orifice of the Eustachian tube (ET), the tympanum, and the multiply partitioned, mastoid air cell system (MACS), all continuous in the air phase. The bony portion of the ET couples via the cartilaginous portion to the nasopharynx, and the tympanum couples the tympanic membrane (TM) to the oval window via the ossicles and is essential for sound transmission. Otitis media (OM) is an emergent property of this system; it is at least in part a consequence of 2 anatomic features, an essentially noncollapsible air pocket connected to the environment by the normally collapsed cartilaginous ET. These features are unique to this diverticulum of the respiratory system.

The purpose of this report is the following: (1) summarize the new contributions to our understanding of the anatomy, physiology, and pathophysiology of the ME-ET system relevant to OM and related diseases and disorders, published since the Ninth International Symposium on Recent Advances in Otitis Media in June 2007; (2) based on these results, determine whether the short- and long-term research goals

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identified at the previous meeting have been met; (3) identify deficiencies remaining in our understanding of the anatomy, physiology, and pathophysiology of the ME-ET system to formulate and prioritize research goals, which will be addressed during the next 4 years; and (4) define the short- and long-term goals and methods by which they can be accomplished.

Methods

The panel topic was subdivided, and each contributor performed a literature search and provided a preliminary report. Among the literature bases searched were PubMed, Web of Science (1945-present), Medline (1950 to present), Biosis Previews (1969-present), and the Zoological Record (1978 to present). The primary literature search covered the time interval from January 2007 to June 2011, with a supplementary search completed in February 2012. Keywords searched (number of articles retrieved) included *middle ear* or *Eustachian tube*, with *otitis media*, for their intersection with *anatomy* (ME = 351, ET = 77), *physiology* (ME = 510, ET = 114), *pathophysiology* (ME = 176, ET = 76), and *pathology* (ME = 312, ET = 59), with the search being limited to the last 5 years. The panel met on June 9, 2011. The preliminary reports were consolidated and circulated.

Discussion

Middle Ear

In the past, much research has focused on the ME as a sound transducer linking vibrations of the TM to those of the round window membrane. This remains a research focus[1-6] and includes computer-based finite element modeling (FEM) of the various structures and functions.[2,7-9] Characterization of the components of the ME sound transduction system[10,11] and appropriately sized animal models[12] are especially important in the face of developments of internal hearing amplification systems.[12-15] However, from the perspective of this panel, the more important concern is defining the mechanism(s) by which the normal ME is maintained near ambient pressures.

The TM is the first component of the sound transduction pathway; as the only truly deformable component of the system, its status reflects the state of the ME and affects the efficiency of sound transmission. With the advent of novel sensing technologies such as laser vibrometry,[16] optoelectronic holography,[17] and tensometry,[18] animal models and cadaveric materials have been used to model modal TM function and the resulting ossicular chain movements. In a series of TM studies employing these techniques, degradation of transduction efficiency due to transtympanic pressure gradients,[19,20] ME effusions,[16,21] TM incisions and their repairs,[22] and simulated TM calcifications[17] were reported. Volandri and colleagues[23] evaluated 8 TM FEMs and concluded that there was good agreement with respect to modal frequencies and umbo displacement but that the amount of variability in the measurements suggested that further refinements are in order. Studies to establish boundary conditions for these FEMs have included characterization of ex vivo human TM tissue properties.[24] In vivo quantitative characterization of TM status was explored using optical coherence tomography to noninvasively image the trilamellar TM structure and to distinguish among sclerotic, atrophic, and hyperkeratotic areas.[25]

The central projections of the TM afferent innervation were examined in vivo using noninvasive technologies. Subcortical components arising in response to static pressure changes on the TM were evaluated using a 64 surface electrode standard electroencephalogram cap. The induced evoked responses were repeatable and localized to the brain stem and cerebellum.[26] Using 3-Tesla functional magnetic resonance imaging (MRI), central neurologic responses were localized in response to rhythmic unilateral pressure applications to the TM. The response was bilateral in the caudal part of the postcentral gyrus in Brodmann area 43, which has been implicated in oral intake.[27] These studies extend the findings from histological studies to the in vivo state, opening up the opportunity to ascertain not only whether OM can be due to issues of innervation but also whether degradation of the peripheral nervous system affects hearing.

The second stage in the transduction pathway, the ME ossicles, has also received attention with respect to their anatomical and functional response to acute OM (AOM). A rat model of AOM was used to evaluate the histopathologic effects of the disease on ossicular bone remodeling. Initially, as with other ME osseous structures, there was bony erosion followed by neo-osteogenesis, but a temporal delay in the ossiclar response was evident.[28] The impact of excessive osteological remodeling on ME function was evaluated in osteopetrotic knockout mice (RANKL, c-Fos), which lack osteoclasts. Doppler vibrometry indicated a static malleus, and the auditory brain stem response showed corresponding increases in hearing thresholds. These physiologic measures correlated with histological increases in bone volume, resulting in larger ossicles and a smaller tympanic cavity.[29] Noninvasive evaluation of ossicular bone morphology with computed tomography (CT) has been refined[30]; unfortunately, soft-tissue discrimination remains poor. Since soft-tissue morphology is critical to accurate ME modeling, Buytaert and Dirckx[31] used the results of high-resolution orthogonal-plane fluorescence combined with micro-CT to build an FEM of the gerbil ME. Refinements to computer models of sound transduction, including accounting for the ossicular articulations, ligaments, and muscular components, demonstrated that pressures developed at the stapes foot plate accord with audiological data.[32,33]

Gas Exchange. For adequate sound transmission and prevention of pathology, the total gas pressure in the ME must approach ambient. It has been suggested that 3 routes of gas exchange control ME pressure: bolus gas exchange during periodic opening of the ET[34-39] (reviewed elsewhere); transtympanic membrane gas exchange, which has recently been dismissed for the normal and abnormal atrophic, sclerotic TM[40,41]; and gas exchange/production by the ME mucosa and MACS.[42,43] However, the last can be questioned given that a number of early studies showed that the CO_2, O_2, and

H_2O partial pressures in the ME approximate those of blood, but that of N_2 is much greater. Because diffusion drives gas exchange been blood and the ME, the mechanism responsible for the higher N_2 concentration, if real, must involve active transport.

Of note is the fact the MACS is significantly smaller in ears with a past history of disease, but it is unclear if this results from a genetically programmed small mastoid or an effect of disease on the MACS growth, although the latter is better supported.[44,45] For example, Sade and colleagues[44] reported smaller MACS in ears with recurrent AOM, and Csakanyi et al,[45] in a cross-sectional study of infants, children, and adolescents with and without ear disease, found that the diseased groups had significantly smaller MACS.

The geometry of the MACS has been studied using CT scans, and over a wide range of volumes, the left-right MACS surface area and volumes were linearly related; that is, the surface area is a linear function of volume, and the surface area/volume ratio is independent of volume.[46,47] Identical results were reported for MACS during growth and development.[45] This geometry does not support the hypothesis that the normal MACS serves as a gas transfer reserve. While a number of studies showed preferential areas of ME gas reserve or changes in the mucosal thickness during pathology, these effects can be expected only to increase or decrease the rate of transmucosal gas exchange[48,49] but will not solve the problem of the partial-pressure difference between blood and ME. Similarly, in a set of studies of questionable design, the transmucosal rate of all physiological gas was reported to be perfusion limited,[50] but these results were not replicated in other experiments[51] and again do not affect the passive ME to blood exchange properties of ME mucosa.

One model to explain the effect of a smaller MACS volume on OM depends on both the ET and MACS gas transfers and proposes that a larger MACS reduces the frequency and efficacy of ET openings during transient obstruction of that structure; experimental data supporting this explanation have been published.[52,53]

However, once an effusion develops, its persistence may be controlled by physiological processes associated with fluid balance. Using a physiologic sodium chloride solution, Li and colleagues[54] quantified ME fluid volume changes in the rat model and then modulated these rates using amiloride (sodium channel anagonist), gluconate (chloride substitute), and a chloride channel blocker. Petrova and colleagues[55] employed solutions of various osmolarities, in the context of an obstructed ET in guinea pigs, to demonstrate fluid absorption (hypotonic) and production (hypertonic) through the ME mucosa. They also demonstrated an effect of noise exposure on fluid resorption.

Eustachian Tube

Anatomy. Although less frequently than in the past, human temporal bone ET specimens continue to be studied. For example, Ozturk and colleagues[56] compared mucosal folds of the ET lumen of children and adults, concluding that children have more folds than adults and that they might function as ''microturbinates'' for protection and clearance. Doyle and Swarts[57] studied the vector relationships between the ET, tensor veli palatine muscle, and the cranial base in skulls of children and adults. They reported sexual dimorphism and that the significant differences between children and adults could account for the observed poorer ET function in children.

Despite the extensive knowledge gained from studying the ET anatomy of cadaveric specimens, the focus of anatomical studies has shifted to noninvasive imaging of living subjects. Oshima and colleagues[58] employed MRI to compare the cartilage morphology of normal subjects to those with patulous ETs. They confirmed its hook-shaped configuration but found diverse morphologies uncorrelated to the ET physiologic status.[58] Another MRI study based on 35 human cadaveric heads showed that Ostmann's fat pad was the only anatomic structure evidencing age-dependent changes, decreasing in size with advancing age.[59] In a study using CT scanning and multiplanar reconstruction of children and adults, Takasaki and colleagues[60] showed that the angle and length of the ET is more horizontal and shorter in infants. Using this same method, Yoshioka and coworkers[61] compared the ET of children with adults and showed that the ET lengthens with age and that the angle between the osseous and cartilaginous ET was more acute in children, as documented originally in histopathological temporal bone specimens. Most recently, this group employed a 320-row CT scanner and sophisticated postprocessing to capture ET dilation and torus tubarius movements during swallowing.[62] Bergin and coworkers[63] reviewed 200 CT scans to assess the distance between the ET and the internal carotid artery and reported a decrease with age and that aberrant carotid arteries are closer to the ET; thus, CT scanning, with contrast, prior to skull-base surgery is recommended.[63]

Physiology. Sonotubometry continues to be refined and used as a noninvasive test of ET function. Using an updated sonotubometry instrument, van der Avoort and colleagues[64] reported that 82% of 61 healthy children had ET openings on at least 1 of 2 testing opportunities, and those results were highly correlated between sessions (Spearman r = 0.89). In a subsequent study,[33] children with otitis media with effusion (OME) and poor ET function, tested before and 1 and 3 weeks after TT insertion resolved to the control group levels.[65] Among the technical refinements proposed and tested was the use of ''perfect sequences'' as the probe tone. Using that modification, all 40 ETs (20 subjects) of otologically healthy adults and 35 of 40 pathological ears (32 subjects) had ET openings.[66] Handzel and colleagues[67] employed simultaneous sonotubometry and nasopharyngeal (NP) endoscopy to evaluate ET function in a cohort of 18 healthy adults; one-third were unable to dilate their ET. The other two-thirds successfully dilated their ETs on 75% of their attempts, and the dilations averaged 440 $\pm$ 240 milliseconds in duration. There was 100% congruence between swallowing and yawning as methods for dilation.[67]

From Korea, Shim and coworkers[68] compared the maximum osseous ET cross-sectional area from coronal temporal bone CT images of 80 (80 ears) patients with chronic OM to those of 50 (100 ears) controls. Chronic OM subjects with poor outcomes, evaluated 1 year after ME surgery, had smaller cross-sectional areas than the subjects with normal otoscopy and type A tympanograms and the controls.[68] Mathew and colleagues[69] used dynamic slow-motion video endoscopy and a detailed classification scheme to visually evaluate ET function in a prospective, case-controlled study. They reported that the protocol was a useful tool for diagnosing ET dysfunction, especially, for example, patulous ET.

Neural control of ET function remains a lacuna. Since the ET innervation studies of Eden and Gannon[70,71] 20 years ago, only a few studies have been performed examining the neural control of ME aeration. Following unilateral sectioning of the Jacobson nerve and sham surgery contralaterally in rabbits, Ceylan and colleagues[72] found significantly more retraction pockets, ME effusion, and goblet cells in the experimental ears. These differences were attributed to disruption of tympanic glomus cells, which function as ME chemoreceptors. Songu and colleagues[73] attempted to discriminate between baroreceptor and mechanoreceptor control mechanisms for middle ear pressure (MEP) using 95 (95 ears) volunteers. They topically anesthetized ME and ET baroreceptors and mechanoreceptors in various combinations and assessed ET function using the Toynbee or Williams test. Anesthesia of the baroreceptors of the tympanic plexus elicited more frequent ET dysfunction, in contrast to the low frequency found in subjects with blockade of TM mechanoreceptors.[73]

Pathophysiology. In 2 articles, Bluestone and colleagues offered an evolutionary biological perspective on OM. Specifically, they posited that OM is a side effect of morphological adaptations in the midface for speech and/or diet, which modify ET morphology and secondarily reduce the efficiency of the ET/NP muscles with respect to ME pressure equilibration.[37,74]

Recently, an analogous situation has been identified in dogs. *Primary secretory OM* is the term veterinarians use to describe chronic OM with effusion in canines. It has been reported to occur almost exclusively in short-faced canines, and its prevalence in the Cavalier King Charles spaniel breed is estimated at about 40%.[75] The effusion is mucoid and fills the entire ME. Diagnosis is either by microscopic examination, CT scanning, or MRI and has been confirmed at the time of myringotomy. Myringotomy and tympanostomy tube placement has been recommended for treatment.[76] Artificial selection of this breed has, until recently, focused on producing a shortened front-to-back diameter of the skull, a result of premature fusion of the coronal sutures. These breeds are characterized as brachyephalic and neotenic (retention of juvenile characteristics into adulthood).[77] Bluestone and Swarts[74] have speculated that the Cavalier King Charles is a natural animal model, induced by artificial selection for those breed characteristics, which secondarily elicit chronic OM with effusion due to ET dysfunction.[74]

Poe and Pyykko[78] quantified endoscopically visible NP orifice movements in healthy subjects with OME or with patulous ETs. They found that the lateral excursion of the anterolateral wall of the ET lumen was less in the two groups with ET dysfunction in comparison with healthy individuals.[78] In a longitudinal study of ET function in 3-year-old children with either chronic OME or recurrent AOM, tested within 3 months of tympanostomy tube placement with the forced response test (FRT), passive resistance and one measure of active function were higher in the rAOM group.[79] Ghadiali and colleagues,[80] using an FEM, reported that the timing and magnitude of the forces produced by the tensor and levator veli palatini muscles simulate the ET resistance patterns found during swallowing in the FRT.[80] From the same team, again using an FEM of cleft palate infant ET function, they found that the tensor veli palatini muscle force was directly proportional to and mucosal/cartilage compliance was indirectly proportional to ET dilation. ET function was independent of hamular position and the magnitude of levator veli palatini muscle force.[36]

The role of gastroesophageal reflux in the pathogenesis of ET dysfunction and OM continues to be a subject of investigation. Pepsin, identified in the ME in several human studies,[81] correlated with reflux episodes documented by dual-probe pH monitoring,[82] although one study[83] found a lower prevalence than previously reported. Sudhoff et al[84] found India ink, a tracer, in the larynx, ET, and ME of guinea pigs in response to gastric pressure when the lower esophageal sphincter was relaxed using Aquo-trinitrosan. From Turkey, Yazici and coworkers[85] found that rats whose NP was exposed to gastroesophageal reflux demonstrated ET mucosal histopathological changes consistent with ET dysfunction that induces OM. Further studies using animal models suggested that it is the acid pH, not the pepsin, that initiates Muc5b[86] upregulation and that *Helicobacter pylori* whole-cell protein extracts can elicit cytokines and an ME inflammatory response.[87]

Other NP inflammatory stimuli continue to receive attention with respect to ET dysfunction and its associated OM. The role of allergic rhinitis in the pathogenesis of ET dysfunction was investigated in a comparison of 123 children with allergic rhinitis to 141 control subjects; significant differences in ET function, total eosinophils, and serum and ME effusion IgE concentrations were not evident.[88] However, in a rat model of allergen-induced allergic rhinitis, Ebert and colleagues[89] reported that immune modulatory oligonucleotides prevented nasal allergen-induced ET dysfunction. The pathological effect of cigarette smoke on the ET has been demonstrated in the rat. Kong and colleagues[90] exposed rats to cigarette smoke, which resulted in ET histologic changes including goblet cell proliferation and excessive mucus secretion. The protective effect of pulmonary surfactant on ET cilia was documented by Ma and his colleagues[91] in a guinea pig model of pneumococcal-induced OM using auditory brain stem response and histopathology.

Coldlike illnesses (CLI), documented by daily tympanometry and symptom diary, corroborated by in-home visits, identified 566 CLI episodes in 169 children over the course of 7 months (16% CLI/child burden), with MEE resulting as a complication in 37% of the episodes.[92] Using the same methodology, daily tympanometry and symptom diary, 249 children from 123 families were evaluated to ascertain whether constitutional ET function could be abstracted from that data. The investigators found that mapping of MEP standard deviation on average MEP allows interpretations of ET function.[93]

Patulous ET. The patulous ET continues to be a commonly heard patient complaint; thus, it is a concern of clinicians and scientists. There have been several reports of the patulous ET co-occurring with a variety of diseases and disorders. Two reports identified this condition in patients with amyotrophic lateral sclerosis,[94,95] in 2 patients following bariatric surgery,[96,97] associated with hemodialysis,[98] in spontaneous intracranial hypotension syndrome,[99,100] and as a complication of anorexia nervosa.[101]

A variety of treatments have been recommended. Poe[102] found that the insertion of a submucosal cartilage graft at the NP end of the ET restored the normal convexity to the ET wall and provided relatively long-term relief in 14 patients. Although the condition is thought to be rare in children, it was diagnosed in a 4-year-old child with a history of chronic unilateral tympanostomy tube otorrhea. It was successfully managed by the endoscopic transoral injection of calcium hydroxylapitite.[103] Another strategy for treating the symptoms of a patulous ET, without manipulating the ET, was a simple mass loading of the TM with Blu Tack, a claylike, nontoxic substance. This technique successfully improved the symptoms of 14 patients.[104] In another apparently new method of treatment, Olthoff and colleagues[105] injected botulinum toxin into the ET in 1 patient with long-standing symptoms; the problem was relieved for 9 months. Another method, used by Takano and coworkers,[106] involved ligating the NP end of the ET of patients with intractable symptoms, which produced relief in some but not all of the patients.

ET Management

There have been advances in medical therapy and in the development of surgical procedures to improve ET function. However, to accelerate this rate, there is a need to define ET dysfunction, to develop validated disease-specific quality-of-life indicators, and to develop rating scales for pathology contributing to ET dysfunction in order to evaluate and compare the benefits of novel treatments.

Medical Treatments. There is increasing evidence that surfactants may be efficacious in the treatment of OM and ET dysfunction. As noted above, Ma et al[91] demonstrated a protective effect of porcine phospholipid given by intratympanic injection into guinea pigs with established OME induced by heat-killed pneumococci. Surfactant-treated animals demonstrated a significantly faster recovery of ME and ET cilia and resolution of effusion versus the placebo treatment. Johnson et al[107] found that an aerosolized surfactant, synthetic preparation of dipalmitoylphosphatidylcholine in a 200:1 ratio with cholesterol palmitate, introduced as a nasal spray into gerbils with induced OME significantly reduced time to resolution of the effusion relative to the placebo. In addition, they found that combining surfactant with phenylephrine significantly prolonged the time to effusion resolution, hypothesizing that the decongestant-drying effect inhibits surfactant distribution, which is dependent on wet surfaces. Thus, the common clinical practice of treating ET dysfunction with decongestants may be counterproductive.

In a cohort of 8 Navy divers, Duplessis and colleagues[108] assessed the effects of oxymetazoline, acetylcysteine, pseudoephedrine, and pulmonary surfactant on ET function as measured by the 9-step test and sonotubometry prior to and after simulated dives in a water tank within a hyperbaric chamber. ET function was degraded by repeated dives when the treatment was the saline placebo. The active treatments all produced decreases in ET opening pressure on the post dive tests, but only those elicited by oxymetazoline were significant.[108]

Delivering medications to the ME through the ET has been proposed, and a novel endoscopic approach was introduced by Todt et al.[109] Under local anesthesia, supine and with the head turned laterally and dependently, a 1.6-mm-diameter flexible endoscope was passed into the NP orifice of the ET, and topical nasal decongestant was injected through the working channel. The fluid was observed by otoscopy in the ME cavity.

Surgical Treatments. Eustachian tuboplasty to debulk soft tissue or cartilage from the posteromedial wall of the cartilaginous ET lumen was first performed in 1997[110]; modifications of the techniques have been reported recently. Metson and colleagues[111] performed a prospective study on 20 adult patients with ET dysfunction in which Eustachian tuboplasty using a microdebrider technique was performed concurrently with nasal sinus surgery. Seventy percent of the patients showed significant improvement; the failures correlated with elevated tissue eosinophil count and advanced sinus CT stage. There were no complications. The study was limited by a lack of a control group and possible confounding effects due to simultaneous surgical treatments (sinus and tuboplasty). These results were consistent with earlier reports of Eustachian tuboplasty in which failures are associated with advanced mucosal disease or ongoing inflammatory disease.

A follow-up study of a cohort of patients who underwent diode or Argon laser Eustachian tuboplasty was reported by Poe et al.[112] Of the original 13 adults with long-term refractory OME who had undergone tuboplasty, 8 were reevaluated 2 years after surgery; of these, 3 were free of OME. Failures correlated with the presence of laryngopharyngeal reflux and allergic disease, supporting the role of mucosal inflammation for ET dysfunction and the etiology of OME. Sedlmaier et al[113] performed diode laser Eustachian

tuboplasties in 38 adult patients via a transnasal approach primarily using local anesthesia for a variety of indications including chronic OM with TM perforation, OME, chronic atelectasis, and difficulty during flying or scuba diving. Outcome measures included opening pressures, in those with perforations; the Valsalva maneuver; and tympanograms. Sixty-four percent showed improved tubal function, similar to earlier reports, and the results remained stable over the 1-year follow-up period.[113] Caffier and colleagues,[114] using the same testing and treatment protocols, reported that laser ET surgery of the hyperplastic mucosa of the epipharyngeal dorsal ostium was successful in 62% of 31 adults who had been enrolled for chronic ET dysfunction.

A novel technique for laser Eustachian tuboplasty, introduced by Yañez et al,[115] employed a KTP laser to create vertical full-thickness cross-hatches through the luminal mucosa into the cartilage. The goal was to weaken the spring of the medial cartilaginous lamina within the torus tubarius, expanding the diameter of the lumen. Debulking of mucosa other than within the cross-hatches was not performed, but a substantial amount of mucosa was debulked in the course of creating the cross-hatches. Patients were adults with unspecified ET dysfunction, symptoms of ear blockage, and abnormal tympanograms. Thirty-five ETs of 25 patients were treated under general anesthesia; improvement as evaluated by tympanometry was reported in 92% over a mean 15-month follow-up. There were no complications.[115] The early results from Eustachian tuboplasty to debulk the tissues of the posteromedial luminal wall of the ET suggest that the procedure may improve tubal function and that the benefits can be durable.

A new approach for Eustachian tuboplasty using a balloon catheter for dilation of the tubal lumen was introduced in 2010. Ockermann and colleagues worked with a manufacturer to develop a balloon catheter designed for dilation of the bony and cartilaginous portions of the ET. The catheter was evaluated in a cadaver study[116,117] and subsequently employed on 13 ETs of 8 patients (age range, 21-81 years) having nonspecified manifestations of ET dysfunction.[112] The outcome measure, a nonvalidated score, was the sum of the subjective perception of tubal opening as a consequence of swallows or Valsalva maneuvers and tubal opening as assessed by tubomanometry. There were no complications and no temporal bone fractures seen on postoperative CT scans. ET function scores increased significantly from baseline and continued to improve throughout the follow-up 8-week period. Weaknesses of this study include no clear of definition of ET dysfunction, lack of description of preoperative or postoperative findings, and a short duration of follow-up. In addition, because the study attempted to dilate the ET osseous portion, there was an unacceptable increased risk of injury to the internal carotid artery.[118-120]

Dilation of the cartilaginous ET using sinuplasty balloons was first investigated in a cadaver study,[121] and a pilot study was then performed unilaterally in 11 adult patients with refractory OME (>5 years).[122] Under general anesthesia, sinuplasty balloons (6 or 7 mm × 16 mm) were passed transnasally with endoscopic guidance into the cartilaginous ET, where they were inflated for 1 minute to a maximum of 12 atmospheres. There were no significant complications. Outcome measures included tympanograms for intact TMs, Valsalva maneuvers, and a nonvalidated ET mucosal inflammation severity score. After the procedure, all 11 patients successfully changed their ME pressure using the Valsalva maneuver, but by 6 months, that number decreased to 7. OME, atelectasis, and ET pathology severity scores all improved. The study was limited by lack of a control group and lack of objective tubal function testing in patients with a TM perforation.

Implications for Practice

Progress on 2007 Short- and Medium-Term Goals. In this section, we outline the progress that has been made since the last conference on the short-term goals (STG # indicates the short-term goal from the 2007 report).

Significant progress has been made in a number of areas identified in the previous report. Pathways of ME gas exchange have received focused attention, including TM permeability to the physiologic gases both in its normal and pathologic state and mucosal gas exchange via both isopressure and isovolume experiments, although much less information has been developed concerning bolus ET dilatory pressure equilibration (STG 5, 12). Several estimates of ME and mastoid volume and surface area, with respect to age and OM history, have been developed, although the capillary architecture has not been elucidated (STG 19). ET anatomy, physiology, and modeling continued as a focus of development. That focus has shifted from histopathologic studies to studies employing noninvasive imaging techniques, including endoscopy, CT, MRI, and optical coherence (STG 3). Previously obtained histology specimens have been used as the substrate for ET FEM to identify the structures, specifically the lateral membraneous wall compliance and tensor veli palatini muscle force, which are significant for effective tubal dilation and ME-NP pressure equilibration. These FEMs have been applied to the analysis of cleft palate ET dynamics as well as to the physiology of subjects with persistent OM (STG 4). In the face of persistent ET dysfunction into adulthood, new surgical methods, including mucosal debulking, cartilage cross-hatching, and balloon tuboplasty, have been developed and piloted (STG 3). The role of viruses in the pathogenesis has also received significant effort, including refinement of monitoring methodologies, assessment of NP inflammatory status, and the temporal dynamics of the infection within families (STG 10). Significant efforts have been made to ascertain the relationship of gastroesophageal reflux disease to OM, including the prevalence of ME pepsin concentrations, identification of the causal inflammatory component of refluxant, and quantifying the causal link between episodes for gastroesophageal reflux and OM (STG 11). Lastly, the pathogenesis of the patulous ET and potential management options were extensively explored (STG 13).

Some progress was reported on a series of short-term goals. Two reports examined ME fluid homeostasis via manipulation of the osmolarity and by targeting the sodium and chloride receptors of the epithelial cells (STG 6). Efforts continue on the goal of delineating the neural control of ET function, during this study period focusing primarily on the afferent branch of the pathway (STG 14). Initial efforts to discriminate between ET function in subjects with rAOM and those with OME showed significant differences in passive resistance and active function, supporting differences in their pathogenesis (STG 16).

No studies were reported for 3 short-term goals. No progress has been made on the anatomy and function of the ET lymphatic system over the past 10 years (STG 1), so the committee recommends this goal be eliminated. Further characterization of the ET histopathology of special populations with a high incidence of OM, such as aborigines of Australia and specific Native American groups, did not advance in the intervening time (STG 2). In addition, no studies examining the impact of normal and pathologic ME pressures and gas composition on biofilm formation, or the converse, were performed (STG 18).

Future Directions

Short to mid-term goals.

1. Perform studies of OM-prone populations, especially the cleft palate population, to establish the relationship between ET anatomy, as defined by noninvasive imaging modalities, and physiology.
2. Develop and employ various imaging modalities, especially those with low risk, to discriminate among OM categories, examine pathogenic mechanisms, and evaluate the efficacy of treatments.
3. Develop physical ET models to study structure/function correlates and the effect of interventions on these relationships.
4. Investigate, in appropriate animal models, the disease processes underlying abnormalities in the ET/ME/mastoid system.
5. Demonstrate the safety and efficacy of ET interventions, medical or surgical, using state-of-the-art scientific methodologies.
6. Develop experimental and computational tools to evaluate the relative importance of the various ET structure-function relationships, including the effects of inflammation, especially with respect to luminal surface properties.
7. Determine, quantitatively, the actual values and relative contributions of ET bolus gas transfer and the ME mucosal gas exchange to the homeostasis of ME gas composition and pressure under normal and inflammatory conditions discriminating between the OM etiologies. Expand these studies into human subjects.
8. Study the physiology of ion and fluid transport in ET/ME epithelium.
9. Determine the response of ME epithelial cell cultures to pathologic underpressures and gas composition and apply the mechanotransduction concept to investigate the effect of underpressures on cell function. Evaluate this mechanism with respect to the transduction of deficient ME pressure regulation.
10. Evaluate those agents shown to moderate ciliary beat frequency, ET clearance function, or ET pressure-regulating function by means of standard models of OM pathogenesis.
11. Identify the progressive stages in the pathogenesis of OME in a manner similar to the recent progress made in elucidating these stages in the development of AOM. Broaden the age ranges evaluated to include infants and older children identifying the anatomical and physiological markers responsible for increased OME susceptibility and persistence in some subgroups.
12. Investigate the role of viruses, and their synergies with bacteria, in the pathogenesis of ETD and OM to identify targets or promising interventions that prevent the development of OM during viral upper respiratory tract infections.
13. Study the possible role of laryngopharyngeal reflux in the pathogenesis of OM, with special attention on the pathogenesis.
14. Examine the role the TM plays in MEP regulation, through pars flaccida accommodation or via gas exchange under normal and inflammatory conditions. Integrate these results into the computational models of MEP regulation.
15. Investigate the etiology, pathogenesis, and management of the patulous ET and its impact on ME pathophysiology.
16. Investigate neural reflex control of ET function and mucosal blood flow and their mechanisms.
17. Differentiate between the pathophysiology of MEP regulation in recurrent AOM and OME.
18. Investigate the role of neurogenic inflammation in MEP regulation and the potential role of the naso-ME cleft reflex.
19. Investigate the impact of negative MEP and gas composition on biofilm formation and, conversely, the effects of biofilms on MEM gas exchange and ET function.
20. Develop novel methods to measure surface area, mucosal blood vessel density, and distribution in the ME cleft.

Long-term goal. The long-term objective of this research remains to increase our knowledge of the physiology and pathophysiology of the ME/ET system in relation to the pathogenesis of OM. Critical to that objective is a more quantitative understanding of our current models of ME pressure regulation, extending those models to the mucosal changes that are precipitated at threshold underpressures, defining rational interventions that reestablish adequate

pressure regulation, and evaluating those interventions in the clinical population. The long-term goal of the research is to implement this paradigm and thereby define rational treatments for the prevention of OM.

Author Contributions

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Panel 3: Recent Advances in Anatomy, Pathology, and Cell Biology in Relation to Otitis Media Pathogenesis

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Abstract

Background and Objectives. The pathogenesis of otitis media (OM) involves a number of factors related to the anatomy, pathology, and cell biology of the middle ear, the mastoid, the Eustachian tube, and the nasopharynx. Although some issues of pathogenesis are fairly well established, others are only marginally indicated by current knowledge, and yet others remain undisclosed. The objective of this article is to provide a state-of-the-art review on recent scientific achievements in the pathogenesis of OM, as related to anatomy, pathology, and cell biology.

Data Sources. PubMed, Ovid Medline, and Cochrane Library.

Review Methods. Articles published on the pathogenesis of OM and the anatomy, pathology, and cell biology of the middle ear, the mastoid, the Eustachian tube, and the nasopharynx between January 2007 and June 2011 were identified. Among almost 1900 abstracts, the authors selected 130 articles for full article review and inclusion in this report.

Results. New knowledge on a number of issues emerged, including cell-specific expression and function of fluid transportation and innate immune system molecules, mucous cell metaplasia, mucin expression, bacterial adherence, and epithelial internalization, as well as the occurrence, composition, dynamics, and potential role of bacterial biofilm. In addition, the potential role of gastroesophageal reflux disease and cigarette smoke exposure has been explored further.

Conclusions and Implications for Practice. Over the past 4 years, considerable scientific progress has been made on the pathogenesis of OM, as related to issues of anatomy, pathology, and cell biology. Based on these new achievements and a sustained lack of essential knowledge, suggestions for future research are outlined.

Keywords

anatomy, pathology, cell biology, otitis media

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The pathogenesis of otitis media (OM) involves a number of factors related to the anatomy, pathology, and cell biology of the middle ear, the mastoid, the Eustachian tube (ET), and the nasopharynx. Although some issues of the pathogenesis are fairly well established, a lack of knowledge exists within many areas. The objective of this article is to provide a state-of-the-art review on recent scientific achievements in the pathogenesis of OM as related to anatomy, pathology, and cell biology. Based on these

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new achievements and a sustained lack of essential knowledge, suggestions for future research are outlined.

The review represents the report of Panel 3 at the Post-Symposium Research Conference, convened in conjunction with the 10th International Symposium on Recent Advances in Otitis Media, in New Orleans, Louisiana, June 2011. As such, the review is an extension of the report from the previous Post-Symposium Research Conference, held in St Petersburg, Florida, in 2007.[1] At the Post-Symposium Research Conference in 2007, several goals for future research that pertain to topics of the present headline were suggested.[1] These goals are listed below, and references are given to section(s) addressing the specific goal in the review (if any).

Goals for Future Research Suggested at the Post-Symposium Research Conference 2007

1. Additional studies examining the specific cell cycle of middle ear epithelial cells that have the potential to increase our knowledge of otitis media and the cell biology of the middle ear are needed.
2. Further study of the relationship and synergy between viral and bacterial middle ear pathology, particularly in animal models, is warranted (see the ''Pathogens in Biofilm Formation'' section).
3. Further extensions of experiments examining other pathogens in OM are needed (see ''Pathogens in Biofilm Formation'').
4. Additional methods, both in vivo and in vitro, are needed for studying OM, particularly otitis media with effusion (OME; see the ''Tympanic Membrane'' and ''Middle Ear'' sections).
5. Creation of cDNA libraries in animal models of infected and uninfected middle ear mucosa to provide for additional molecular analysis of changes in OM is warranted (see the ''Mucin Up-regulation in the Middle Ear'' section).
6. Creation of goblet cell lines and mucous cell lines, potentially using stem cells, to further enhance the understanding of mucin biology and pathophysiology in the middle ear would be of value (see the ''Mucous Cell Metaplasia'' section).
7. Difficulties in creation of middle ear–specific cell lines or in vitro models should be supplemented with the use of similar respiratory epithelium if possible, such as bronchial epithelium (see the ''Mucous Cell Metaplasia'' section).
8. Host intracellular responses to bacterial or inflammatory molecule endocytosis should be characterized (see the ''Tissue-Pathogen Interaction'' section).
9. Additional study is needed to determine whether mouse susceptibility gene homologues also predispose humans to OM.
10. Additional clinical and basic science research is needed with respect to pathology in OM and specific potential modulators of middle ear function including, but not limited to, gastroesophageal reflux, nasopharyngeal anatomy, and bacteriology and allergy (see all sections).
11. Additional studies are needed that examine biofilm pathogenesis in relation to middle ear disease (see reference 1 for subheadings; see the sections ''Biofilm Formation'' and ''Pathogens in Biofilm Formation'').
12. Additional research into the specific relationships between mucin and other cellular components affecting mucin regulation and OM pathogenesis is required, namely, to describe the potential for mucin gene polymorphisms and disease, to describe mucin gene regulation in patients with chronic otitis media (COM), and to fully characterize the mucin gene profile in the middle ear and describe the impact of inflammatory conditions on these genes (see the ''Mucous Cell Metaplasia'' and ''Mucin Up-regulation in the Middle Ear'' sections).
13. Correlation of middle ear epithelial inflammatory components, including cytokines and defensins, to middle ear pathogenesis with specific emphasis on bacterial and viral survival, adhesion, biofilm formation, and defense mechanisms is needed (see the sections ''Cytokines and Pathways in the Mucosal Response to Middle Ear Infection,'' ''Interactions between the Middle and Inner Ear in OM,'' ''Tissue-Pathogen Interaction,'' ''Biofilm Formation,'' ''Pathogens in Biofilm Formation,'' ''Innate Immune Response in the Middle Ear and Eustachian Tube,'' ''Adaptive Immune Response,'' and ''Neurogenic Control of Immune Response'').

Methods

For this review, the literature was searched in PubMed, Ovid Medline, and the Cochrane Library. In PubMed and Ovid Medline, the search terms *anatomy, pathology, pathogenesis*, or *cell biology* and *otitis media* or *mastoid, middle ear, Eustachian tube*, or *nasopharynx* were used, for the period January 1, 2007, to June 30, 2011, including the use of Mesh tools. Almost 1900 hits were encountered, and thus, the review includes a selection of publications on the topics, guided by the relevance in relation to the goals listed above, significance, and novelty. The vast number of retrieved publications at the initial search indicates the wide spectrum of the review headline themes and thus that the search readily includes publications also or primarily focusing on molecular biology, biochemistry, immunology, microbiology, or other subjects that are closely related to anatomy, pathology, cell biology, and/or the pathogenesis of OM. Thus, a necessary selection among the retrieved publications has been made, and some of the subjects and publications focused below may overlap partly with the main subjects of some of the other reviews/panel reports performed for the same occasion (eg, microbiology or

immunology). An example is biofilm formation, which is a given subject in reviews dealing with both microbiology and pathogenesis.

Discussion

Anatomy and Pathology

Tympanic membrane (TM). In a study by Knutsson et al,[2] the distribution of collagen fibers in the rat's TM and its suspending structures was evaluated by immunohistochemistry. The pars tensa mainly stained for collagen types II and IV, whereas the annulus fibrosus inner portion stained for type II and the outer portion for types III and IV collagen. The importance of the integrity of these collagen fibers was studied, via laser Doppler vibrometry of the motion of the stapes footplate, by a group of mechanical engineers.[3] It was reported that radial myringotomy induced fewer effects on the stapes velocity, greater than 4 KHz, than circumferentially oriented incisions, independently of the quadrant tested.

The use of optical coherence tomography to study the microstructure of the human TM in vivo was introduced by Djalilian et al.[4] Both normal and diseased TMs were observed, with characterization of the epithelial and connective tissue layers of the TM and quantification of the TM thickness in different quadrants.

The Umeå group continued the research line in the evaluation of the healing phenomena that occurs in the TM of plasminogen-deficient mice. Prestwich et al[5] found that the lack of plasminogen does not interfere with the early stages of TM healing after myringotomy, in contrast to the great influence at later stages. A mechanism of interference with the activation of inflammatory cells was postulated. Another group studied the effect of stem cell treatment on TM perforation, and although a marked thickening of the lamina propria was observed, no enhanced healing of the perforation occurred.[6] Alzbutiene et al[7] also studied TM healing in a rat model. Following ME inoculation of nontypeable *Haemophilus influenzae* (NTHi) and subsequent myringotomy, peak invasion of neutrophils, macrophages, and lymphocytes was seen on days 4, 7, and 14, respectively. Interestingly, calcium deposits indicative of myringosclerosis formation were more abundant in myringotomized TMs than in the nonmyringotomized animals.

Middle ear. Two studies reported the connections between the middle ear and the surrounding bone marrow. Whyte Orozco et al[8] observed transitory connections between the ossicle marrow and the mesenchyme filling the middle ear in 90 temporal bones from embryos and fetuses. These connections disappear earlier in the malleus and incus than in the stapes. Miura et al[9] studied 58 temporal bones of nonmalformed fetuses and infants. In most cases, the connections were established through mesenchymatous tissue. Moreover, mesenchyme was more profuse in the bones presenting inflammation in the middle ear. It was suggested that these connections may act as pathways for the induction of osteomyelitis in cases of otitis media, in particular in younger children.

The anatomy of the posterior region of the middle ear, an area of difficult access and importance in chronic ear surgery, was the focus in 2 reports. Holt[10] used 51 human cadaver temporal bones to describe the anatomy of the posterior sinus of the middle ear cleft and its relationship to the more inferiorly located sinus tympani. Thomassin et al[11] used the surgical otoscope to evaluate serially the reliefs of the posterior tympanum in 120 surgical cases of chronic otitis. Hidden areas and rates of anatomical variations were reported, emphasizing the usefulness of endoscopy.

In an anatomic study on the black rat (*Rattius rattus*), Wysocki[12] performed a systematic description and parameterization of the temporal bone structures. Such studies are of importance to those interested in this species as a research tool.

OME is characterized by an accumulation of fluid in the middle ear, and chronicity is associated with structural changes in the TM. Moon et al[13] hypothesized that degradation of macromolecules of connective tissues by host matrix metalloproteinases (MMPs) contributes to these structural changes. Middle ear effusions were collected and classified as serous, mucous, and hemorrhagic and analyzed for the presence and activity of gelatinases and caseinases (MMP activity). Gelatinase and caseinase activity was present in all effusions but was significantly higher in mucous effusions, whereas the tissue inhibitor of MMPs was highest in serous effusions. Inhibition of MMP activity alone did not prevent histological changes. The authors suggest that MMP activity of middle ear effusions, in combination with other factors such as chronicity, inflammatory mediators, other proteases, and pressure effects, causes structural damage to the TM.

The existence of eosinophilic OM has been suggested, characterized by the accumulation of eosinophils in the middle ear mucosa and in a highly viscous yellow middle ear effusion. Increased levels of immunoglobulin E (IgE) occur in the middle ear effusion, and IgE staining has been observed on mast cell surfaces but also partially in the cytoplasm of plasma cells, suggesting that IgE is produced locally in the middle ear mucosa. Eosinophilic OM is associated with a high incidence of sensory hearing loss independent of age, granulation tissue formation, nasal polyposis, and chronic rhinosinusitis.[14,15]

Ossicles. The step-by-step ontogenic development of the incudo-stapedial joint was studied by Whyte Orozco et al[16] in serial slices of 46 temporal bones from fetuses and newborns. From the 16th week of gestation to birth, this joint becomes fully developed. Farahani and Nooranipour[17] performed an accurate electron microscopic analysis of the anatomy and anthropometry in 12 human stapes on a micrometer scale.

With respect to the radiological anatomy of the middle ear ossicles, both normal and after reconstruction, rotational tomography appears to offer a more precise representation of anatomical structures.[18] Also, Sim and Puria[19] performed an original soft-tissue morphometry of the malleus-incus complex through microscale x-ray computed tomography (micro-CT) imaging. This tool is capable of imaging high-

density tissues of the middle ear and reconstructing them in 3D. In the same context, Eom et al[20] reported the virtual endoscopy findings of topographical 3D reconstructions of the middle ear and ossicles in the dog.

A number of middle ear diseases are associated with pathologic bone modeling, either formative or resorptive. Thus, the ossicles are subject to initial resorption and subsequent new bone formation during acute otitis media (AOM) although postponed and to a far lesser extent than the bone tissue structures surrounding the middle ear cavity. Potentially, this may alter the properties of ossicular chain sound conduction.[21] The bone resorption or destruction occurs by osteoclast activity, which may be facilitated through lipopolysaccharide-induced osteoclastogenesis from mononuclear precursors.[22] A number of bone-modeling molecules (eg, receptor activator of nuclear factor kappa B [NF−κB] ligand and osteoprotegerin) are expressed during OM,[23] and the significance of especially osteoprotegerin on inner ear and ossicular bone modeling has been demonstrated previously.[24,25]

Mastoid. In a study of 298 human temporal bones from a Mediterranean population, Manolis et al[26] determined the dimensions of the mastoid air cell system, according to the distances between 6 different landmarks. Gender differences were registered, both for the vertical and transverse diameters, as well as for the anterior and posterior margins of the mastoid. In another anatomical investigation, 33 temporal bones of human adults were studied with respect to the relative position of the sigmoid sinus, TM, and digastric ridge with the mastoid segment of the facial nerve. A proportional and lineal order relationship could be established with consequent advantages in describing surgical landmarks for mastoid surgery.[27]

The relationship between mastoid aeration and autophony was studied in patients with patulous ET.[28] From the combination of an audiologic and imagiologic analysis of morphology, it was concluded that patients with poorly developed mastoids are more prone to distress by symptoms of patulous ET.

ET and nasopharynx. The association between hypertrophic adenoids and OM has been known since the Danish otolaryngologist Wilhelm Meyer published his work in the 18th century. A recent African study confirmed this association, as Orji et al[29] determined the degree of nasopharyngeal obstruction in adenoidal patients and normal controls by an evaluation of the adenoidal-nasopharyngeal ratio obtained from soft-tissue radiographs. The risk of OME was more than 7 times higher among the adenoidal patients, and the diagnosis of OME correlated significantly with the degree of nasopharyngeal obstruction. Although such a correlation has been absent in previous studies in the Western world, it was supported by a study from Nepal using rigid nasal endoscopy for the grading of adenoid hypertrophy.[30] There has also been speculations on the possibility that malocclusion in children could affect the occurrence of OME in children possibly by interfering with tubal function.[31]

A Finnish group proposed a correlation between nasopharyngeal dimensions obtained by magnetic resonance imaging and the risk of OM.[32] Both the height of the nasopharynx (distance from the caudal edge of the septum to the midpoint of the sella) and the nasal base angle were smaller in children who had had attacks of AOM during the previous 12 months. A history of adenoidectomy did not have any effect on the dimensions.

A number of other studies have examined possible relationships between craniofacial anatomy and OM . Using 2-dimensional reformatted CT, multiple linear, angular, and area measurements were performed by Avci et al.[33] No differences were found regarding craniofacial variables except mastoid size, which was smaller in COM patients, as known for decades. Remarkably, Di Francesco et al[34] found significant differences in maxillary depth, upper and anterior facial height, and the angle between the anterior and medial skull base between adult OM patients and normal controls. In a study in children, Di Francesco et al[35] found a number of differences in the morphology of the face between OM patients and controls. In addition, Kim et al[36] revealed a relationship between the presence of high palatal vaults and early, recurrent AOM in young children. However, a study of patients seen in a cleft and craniofacial deformities clinic for a 2-year period did not show deformational plagiocephaly to be a significant risk factor for OM, although a trend correlating OM and the severity level of deformational plagiocephaly was observed.[37] In a novel murine model of craniofacial dysmorphologies, all animals went on to develop OM. Changes in cilia and goblet cells of the middle ear mucosa were seen, and the expression of tumor necrosis factor α (TNF-α) and toll-like receptor 2 (TLR2) was upregulated.[38] Also, *Eya4*-deficient (*Eya4–/–*) mice have severe hearing deficits, middle ear and ET dysmorphology, and develop OME. *EYA4* mutations cause sensorineural hearing loss in humans, and this gene may be critical for normal development and function of the middle ear and ET.[39]

The angle and length of the ET are more horizontal and shorter in infants than in adults. However, there is no difference between infants with and without OME. Thus, a short and horizontal ET may not be a main etiologic factor related to OME in infants and children.[40] Habesoglu et al[41] assessed the cross-sectional area of the mastoid air cells and the auditory tube angle in healthy ears and COM ears. Confirming the study above, the mastoid areas were greater in the healthy ears, suggesting that middle ear inflammation in childhood may affect mastoid size. The auditory tube angle did not correlate with mastoid size.

Although the diagnosis of a patulous ET is essentially clinical, the study by Kikuchi et al[42] demonstrated that 3D CT imaging with the patient in a sitting position may be useful to confirm the diagnosis. An absence of the occlusion zone in the cartilaginous portion medial to the isthmus of the tube was identified in these patients when compared with normal controls. Another team studied the ET cartilage

by magnetic resonance imaging, showing a wide interindividual variability of configuration.[43]

Cell Biology

Middle ear fluid homeostasis. Aquaporin molecules (AQPs) are involved in fluid transportation across cell membranes. As fluid accumulation is a hallmark of OME, the expression of aquaporins was studied in the normal middle ear and ET and during OME. Transcripts for AQP1, 4, and 5 were detected in the rat ET and middle ear. AQP4 was localized to the basolateral membranes of ciliated epithelial cells, while AQP5 was localized to the apical surface of serous gland cells, but not goblet cells, in the rat ET. AQP1 was expressed by subepithelial fibroblasts.[44] Four subtypes of AQPs were detected in the mouse ET. AQP1 was detected in fibroblasts, endothelial cells of capillary vessels, and cartilage cells. AQP3 was distinctly detected in the basal membrane of epithelial cells, whereas AQP4 was detected in the basal membrane of epithelial cells. AQP5 was expressed in the luminal side of the ET epithelial cells and also in the apical surface of the cells of the serous glands.[45] In the guinea pig, AQP1 was localized on ME capillary endothelial cells and fibroblasts in the lamina propria, as well as on flat and cubical epithelial cells. AQP4 and AQP5 were also expressed in the middle ear mucosa, and the expression of the 3 aquaporin molecules was increased in OME.[46,47] Another molecule important for fluid transportation, the Na(+)-K(+)-2Cl(-) co-transporter (NKCC), was located to the basolateral human middle ear epithelial cell membrane. Interestingly, interleukin 1β (IL-1β) treatment augmented NKCC activity and increased NKCC1 expression.[48] In addition, the treatment stimulated bumetanide-sensitive fluid transport across cell monolayers. The authors thus conclude that the inflammatory cytokine IL-1β up-regulates NKCC1 in middle ear epithelial cells.

Cytokines and pathways in the mucosal response to middle ear infection. Numerous cytokine genes related to tissue remodeling, angiogenesis, and inflammatory cell proliferation are overexpressed in the middle ear but also the inner ear during both AOM and COM (TNF-α, bone morphogenetic proteins, fibroblast growth factors, vascular endothelial growth factor, IL-1α, β, IL-2, IL-6). Thus, inner ear cytokine expression contributes to the inflammation and remodeling that occur in association with middle ear disease, which provides a potential molecular basis for the transient and permanent sensorineural hearing loss reported with OM.[49,50]

Rat OM models were used to investigate the activation of the transforming growth factor β (TGF-beta) signaling pathway in the formation of granulation tissue in response to middle ear pathogens. The TGF-β signaling pathway was highly regulated in the middle ear cleft in bacterial OM but not in ears with ET obstruction. In ears with bacterial OM, the TGF-β signaling pathway products were more abundant in *Haemophilus*-infected ears than those infected with pneumococci.[51]

TNF and TNF receptor superfamily gene expression during bacterial OM was examined in the mouse, combined with a characterization of OM in TNF-α–deficient mice. TNFs and TNF receptors were broadly regulated during OM, with TNF-α showing the highest level of up-regulation. TNF-deficient mice exhibited mucosal hyperplasia even in the absence of infection and exuberant growth of the mucosa during OM, including the formation of mucosal polyps. Mucosal recovery during OM was also delayed, in parallel with a delay in mucosal apoptosis and reduced caspase gene expression. Thus, TNF appears to be critical for the maintenance of mucosal architecture in both the normal and infected middle ear.[52] This may be related to the fact that in the absence of TNF, mice fail to up-regulate both TLRs and downstream genes and proteins, such as CCL3, resulting in defects in both inflammatory cell recruitment and macrophage function.[53] In vivo administration of rCCL3 to animals deficient in TNF fully restores the ability to control OM due to NTHi, whereas CCL3-blocking impairs the ability of wild-type mice to recover from OM. Thus, CCL3 is a potent downstream effector of TNF-mediated inflammation in vitro and in vivo.[53]

Phosphorylation of JNK isoforms in the middle ear mucosa precedes but parallels mucosal hyperplasia in a rat model of NTHi OM. Nuclear JNK phosphorylation was observed in many cells of both the mucosal epithelium and stroma by immunohistochemistry. Several JNK inhibitors inhibit middle ear mucosal growth in vitro and mucosal hyperplasia during OM in vivo. Thus, activation of JNK is a critical pathway for bacterially induced mucosal hyperplasia during otitis media, influencing tissue proliferation.[54]

By real-time polymerase chain reaction (rtPCR) and immunohistochemistry, Granath et al[55] investigated the endothelial nitric oxide synthase and inducible nitric oxide synthase (iNOS) expression in adenoids from children with OME. Also, IL-1β and TNF-α in nasopharyngeal secretions were analyzed because of their role in the iNOS-induction pathway. Children with OME exhibited lower levels of iNOS compared with controls. The corresponding proteins were found mainly in conjunction with the surface epithelium. No significant differences were seen among the cytokines tested. Results indicate that local induction of adenoidal iNOS might be of importance for preventing development of OME.

Mucous cell metaplasia. Middle ear infection triggers mucous cell metaplasia, and cytokines are well known to be involved in this process, especially proinflammatory cytokines and T-helper 2 lymphocyte subset–derived cytokines. Thus, IL-10 knockout mice do not develop mucous cell metaplasia/hyperplasia,[56] and blocking of the epidermal growth factor receptor (EGFR) and IL-13 inhibits mucous cell metaplasia.[57] These studies suggest that cytokines derived from T-helper 2 lymphocytes play a critical role in mucous cell metaplasia. It was also shown that inflammation stimulates epidermal growth factor receptor activation and IL-13 to induce both Clara and ciliated cells to transition into goblet cells through the coordinated actions of the forehead box transcription factor (FoxA2), thyroid transcription factor 1, SAM pointed domain ETS factor (SPDEF),

and γ-aminobutyric acid receptor (GABAR), in which FoxA2 is down-regulated and SPDEF and GABAR are up-regulated, with a net result of mucin MUC5AC, mucin chaperone trefoils, and chloride channel up-regulation.[58] This is in further support of the fact that mucous cell metaplasia involves increased mucin expression, production, and release.

Mucin up-regulation in the middle ear. Nineteen mucin genes have been identified, and most of these are expressed in the human middle ear mucosa, in a similar manner in both in vivo and in vitro models.[59-61] Among the mucin genes, only MUC6, MUC12, and MUC17 are not expressed in the middle ear.[60]

In terms of mucin members' being regulated in in OM, MUC2 and MUC5B seem to be predominant,[61-63] whereas MUC5AC may be up-regulated occasionally.[62,64] MUC5B expression is also induced by cigarette smoking, through NF-κB activation in mouse middle ear epithelial cells.[63] The mucin expression pattern in OM is considered unusual because MUC5B is a major mucin expressed in submucosal glands (mucous cells) but not in epithelial mucous cells. Thus, COM triggers gland formation and glandlike cell differentiation of middle ear epithelial cells in response to infection.

The newly identified MUC19 was found to be expressed in the middle ear mucosa, responding to inflammatory cytokines' challenge.[65] In addition, MUC2 mucin may be expressed in the human middle ear epithelium of patients with otitis media.[65] Further progress has been made in chinchilla models in terms of mucin gene investigation,[66] and it has also been revealed that mucin polymorphisms may play a role in otitis media.[67]

Interactions between the middle and inner ear in otitis media. Schachern et al[68] studied the middle ear, the round window membrane, and the inner ear for differences in tissue response based on the virulence of nontypeable *H influenzae* 2019 (NTHi 2019) and its 2 lipo-oligosaccharide (LOS) mutant strains, B29 (gene htrB) and DK1 (gene rfaD). The wild-type NTHi caused greater thickness and infiltration of the round window membrane and a significant increase in both inflammatory cell infiltration and bacteria presence in the scala tympani area of the inner ear. Strial edema was observed only in wild-type–inoculated animals. Thus, LOS mutants of NTHi appear to have a reduced ability to pass through the round window membrane, resulting in less inner ear inflammation and pathological changes.[68]

The same group looked at the virulence characteristics of specific pneumococcal proteins on the inner ear by histologic comparison of inflammatory cell infiltration and pathologic changes in the round window membrane and the inner ear. Most animals inoculated with pneumolysin or wild-type bacteria showed severe pathologic changes of the inner ears, whereas animals inoculated with surface protein A or surface antigen A–deficient bacteria appeared normal.[69]

Moon and colleagues[70] hypothesized that NTHi pathogen-associated molecular patterns trigger host spiral ligament fibrocytes to release inflammatory cytokines that attract effector cells and cause inner ear dysfunction. Spiral ligament fibrocytes secrete monocyte chemotactic protein 1 (MCP-1) dependent on NTHi binding TLR-2 and subsequent NF-κB activation and binding an NF-κB enhancer region of MCP-1.[70] Further studies demonstrated that MCP-1 induction contributes to inner ear inflammation via chemokine receptor 2 (CCR2)–mediated recruitment of monocytes. Inhibition of MCP-1 or CCR2 did not abrogate inner ear inflammation since targeting these molecules resulted in compensatory up-regulation of alternative genes such as MCP-2/CCL8.[71]

Tissue-pathogen interaction. The normal ET epithelium contains galactose N-acetylgalactosamine (GalNAc) residues that promote adherence of *Streptococcus pneumoniae.* GalNAc residues were mapped by lectin binding and were shown to increase significantly both in the submucosal serous glands and in the surface epithelium in a rat OM model.[72]

Intracellular bacterial infection of middle ear mucosal epithelial cells may be an important mechanism for bacterial persistence and survival during antibiotic treatment. It might thus promote the development of bacterial resistance and contribute to inflammation and mucus production in OME. Coates et al[73] examined middle ear mucosal biopsies from children with OME for bacteria using transmission electron microscopy. Gram-positive coccal bacteria were demonstrated in the epithelial cells of 4 of 11 children (36%). In the middle ear fluid, pneumolysin DNA was detected, and taken together, these findings suggest a role for persistent intracellular infection with gram-positive cocci in some cases of OME.

Clinical isolates of NTHi were cultured with human epithelial cells and monitored for NTHi internalization and penetration of host epithelial cells grown on Transwells. Three of 5 clinical isolates were found to be internalized into host cells. Azithromycin showed a marked bacteriological efficacy against cell-internalized NTHi.[74]

Biofilm formation. In a biofilm, bacterial colonies reside in a matrix, protecting them from immune response and effects of antibiotic treatment. It has lately been shown that the bacteria can communicate inside the biofilm, not only within the same species but also between different bacteria. This might have implications for the development of bacterial resistance and treatment failures in infections caused by fully sensitive bacteria. Thus, biofilms of polymicrobial nature are believed to contribute to the persistence of pathogens in the middle ear during COM. In the short interval between this publication and the most recent publication of this type following the 9th International Symposium in 2007,[1] there has been a substantial paradigm shift in the understanding and acceptance of biofilm formation as a primary determinant of OM pathogenesis in children.

Kania et al[75] demonstrated the presence of mucosal biofilm in adenoid tissue using double staining for visualization of both the bacterial matrix and the bacterial cells. A total of 54% of adenoids from children with chronic and/or recurrent OM showed evidence of mucosal biofilms. Saylam et al[76] observed biofilms on adenoid surface

removed from patients with COM with effusion (COME) to a greater extent than those not diagnosed with COME. Hoa et al[77] also demonstrated that middle ear pathogens form biofilm on the adenoid surface, by scanning electron microscopy and fluorescence in situ hybridization (FISH) with confocal microscopy. The same investigators compared the extent of biofilm infection of the mucosal surface of adenoids removed from children with OME, recurrent AOM, and obstructive sleep apnea (OSA).[78] Adenoids from patients with OME were characterized by a distinctly different percentage of biofilm mucosal surface area coverage (28%), with significantly more biofilm presence than OSA patients (0.1%) but significantly less biofilm presence than recurrent AOM patients (98%). Although previous investigations have supported a dominant role of nasopharyngeal biofilms in recurrent AOM pathogenesis, these results suggest that nasopharyngeal biofilms may play a different role in the pathogenesis of OME and that this clinical entity may be more multifactorial in nature. The results corroborated those of Zuliani et al,[79] who used scanning electron microscopy to show 94% biofilm coverage of the adenoids in recurrent AOM and 1% in OSA. The authors suggest that these dense biofilms act as a reservoir for reinfection of the tubotympanum and may play a role in viral-induced development of recurring AOM.

Homøe et al[80] analyzed smears and biopsies from Greenlanders with COME and chronic suppurative otitis media (CSOM) with microscopy and PNA-FISH. Biofilm was confirmed in 83% of CSOM smears but in none of the COME smears. Mucosal biofilm was confirmed in 80% of the biopsies from adults with CSOM.

An examination of the extracellular polymeric substance and bacterial adhesins that influence biofilm formation at the nanoscale was performed by Arce and colleagues,[81] using atomic force microscopy to visualize structural details of live NTHi at the early stages of biofilm formation. In this manner, structural and mechanical properties of NTHi biofilms were examined at the molecular level.

Pathogens in biofilm formation. De Baere et al[82] assessed the presence of middle ear pathogens in the nasopharynx, the middle ear fluid, and middle ear mucosal swabs of patients undergoing middle ear surgery. An association between *H influenzae* and middle ear biofilm was confirmed, and a potential role of *Pseudomonas aeruginosa* in middle ear inflammation and biofilm formation was indicated. Interestingly, the biofilm did not seem to cause inflammation. The association between NTHi, biofilm, and COM was further indicated by Nistico and colleagues.[83] *H influenzae* was primarily present in adenoids from COM patients (compared with those from a sleep apnea group), and the difference did not occur for *Streptococcus* and *Staphylococcus* colonization.

NTHi biofilms contain significant host components including neutrophil extracellular traps (NETs). Juneau and colleagues[84] investigated the ability of NTHi to initiate NET formation in vitro. NET formation was induced by endotoxin, and NTHi entrapped within NET structures were resistant to both extracellular killing and phagocytic killing, primarily because of oligosaccharide moieties within the LOS. Thus, NTHi-induced NET formation may provide a niche for NTHi within the middle ear and contribute to NTHi persistence. Phosphorylcholine (PCho) is added to some LOS forms in a phase-variable manner, and these PCho(+) variants predominate in vivo. Hong et al[85] found that PCho promotes NTHi infection and persistence by reducing the host inflammatory response and by promoting formation of stable biofilm communities. In another study, they investigated the composition of the NTHi biofilm matrix and the contribution of biofilms to bacterial persistence in vivo. The presence of biofilms within the chinchilla middle ear correlated significantly with increased bacterial load in middle ear effusions and tissue. Examination of thin sections revealed polymorphonuclear cells within a DNA lattice containing elastase and histones, which is consistent with the definition of neutrophil extracellular traps. Viable multicellular biofilm communities were found within the DNA lattice throughout the biofilm. Further, NTHi was resistant to both phagocytic and extracellular neutrophil killing in vitro by means of LOS moieties that promote biofilm formation. These data support the conclusion that NTHi subverts neutrophil extracellular traps to persist in vivo.[86]

Although NTHi, as referred above, is associated with COM(E) and biofilm formation, other bacteria also form biofilm communities. Thus, Hoa and colleagues[87] recently examined the ability of *S pneumoniae* to form biofilms on nasopharyngeal and middle ear mucosae in a viral-induced animal model of pneumococcus infection. Eight days following infection (viral and bacterial), biofilms were detected on 83% of nasopharynges and 67% of middle ears in experimental animals. Likewise, Reid and colleagues[88] demonstrated the formation of pneumococcal biofilms in chinchilla middle ears by scanning electron microscopy.

P aeruginosa is associated with CSOM. Thus, early and late histological features of a rat model of *P aeruginosa* OM were explored, showing mucoperiosteal inflammatory changes similar to those observed in human middle ear infection. Acute inflammatory cell infiltration was seen at 7 and 14 days, gradually decreasing to chronic inflammatory changes with fibroplasia at 60 days. Bone resorption was observed at 7 and 14 days, changing to a bony deposition at 30 and 60 days.[89] Byrd et al[90] used a chinchilla model to evaluate virulence mediators that contributes to *Pseudomonas* middle ear biofilm formation in vivo. The chinchilla model provided a means to evaluate pathogenic mediators of *Pseudomonas* biofilm formation.

An increasing role for group A *Streptococcus* (GAS) in OM has been appreciated recently. Roberts and colleagues[91] sought to test the hypothesis that GAS colonizes the middle ear in localized communities or biofilms. Using a chinchilla model, the authors observed GAS to form densely packed microcolonies in the middle ear. Biofilm formation did not occur using a strain lacking the transcriptional regulator Srv, suggesting that genetic regulatory events in response to environmental cues may contribute to biofilm formation.

Allelic replacement of the chromosomally encoded streptococcal cysteine protease gene restored biofilm formation in vivo. The authors demonstrated that GAS naturally forms a biofilm in the middle ear, although biofilm formation was not required to establish infection.

An increased focus on the polymicrobial nature of biofilms in OM has spawned the study of multispecies biofilms in animal models of infection. Thus, Weimer and colleagues[92] demonstrated that chinchillas co-infected with *H influenzae* develop increased pneumococcal biofilms, yet this co-infection impedes the progression of pneumococcal disease. Furthermore, the polymicrobial nature of biofilms can impart a survival advantage for the microorganisms as a whole in the community. As an example, NTHi provides passive protection by means of β-lactamase production and biofilm formation for *S pneumoniae*, both in vitro and in a chinchilla model of OM.[93] These data provide insight into the recalcitrant nature of biofilms to antibiotic treatment. A recent study demonstrates that polymicrobial infection alters the course, severity, and treatability of otitis media.[94] Co-infection with *H influenzae* and *Moraxella catarrhalis* promotes increased resistance of biofilms to antibiotics and host clearance, shown to be dependent on interspecies quorum signaling. In a chinchilla model, polymicrobial infection promoted *M catarrhalis* persistence beyond the levels in animals infected with *M catarrhalis* alone. The authors demonstrated that increased *Moraxella* survival was dependent on an autoinducer 2 (AI-2) signal released by the *Haemophilus* bacteria.[94]

It has become increasingly evident that bacteria communicate via intracellular messengers and signaling events, particularly in multispecies biofilm formation. It has been demonstrated that soluble mediators, released by bacteria, modulate the composition of NTHi LOS, resulting in effects on biofilm maturation and persistence in vivo.[95] Further work has identified the ribose ABC transporter and RbsB as a quorum-regulated protein required for uptake of and response to AI-2.[96]

Xylitol has an inhibitory effect on growth of *S pneumoniae* and decreases the occurrence of AOM in children but does not appear to alter nasopharyngeal carriage of this microorganism. It does, however, lead to a decreased biofilm formation when exposed to *S pneumoniae* alone.[97] Glucose and fructose reversed this inhibitory effect, stimulating biofilm growth. An analysis of biofilm gene expression revealed that xylitol lowered capsule, autolysin, and competence gene expression in *S pneumoniae*. The authors conclude that changes in biofilm formation in response to different sugar compounds may partly explain the efficacy of xylitol to prevent AOM in previous clinical trials.

Another study examined the effect of ciprofloxacin and azithromycin on *H influenzae* biofilms.[98] Initial biofilm synthesis was inhibited by both drugs at concentrations higher than 2-fold minimal inhibitory concentration. Thus, sufficient dosage might control early biofilm formation. Ciprofloxacin was superior with respect to breakdown of biofilms.

Significant advances have been made in the field of therapeutic immunization to resolve OM biofilms. Novotny and colleagues[99] recently demonstrated a therapeutic immunization strategy, transcutaneous immunization, that resulted in significant resolution of established NTHi biofilms from the middle ear space of animals compared with controls, when targeting the surface-exposed outer membrane protein P5 adhesin and type IV pili of NTHi. These data advocate transcutaneous immunization with the adhesin-directed immunogens as an efficacious regimen for prevention and resolution of experimental NTHi-induced OM.[99]

Innate immune response in the middle ear and ET. A number of tissue responses may be considered as part of the innate immune system (eg, the secretion of mucus, the beating of cilia, and the expression of molecules with, eg, antibacterial activity). The innate immune molecules with antimicrobial activity are key components of the host innate immune defense of the middle ear.

TLRs are important mediators of the host innate immune response. TLR9 recognizes CpG DNA motifs in bacterial DNA. TLR9 genes were up-regulated during OM and in middle ear mucosal cells and infiltrating leukocytes. Leichtle and colleagues[100] demonstrated that TLR9 depletion significantly prolonged the inflammatory response induced by NTHi in the middle ear and delayed bacterial clearance. In the adenoids, TLR4 and TLR7 expression was demonstrated by Granath et al,[101] who also found that the mRNA levels for TLR7 were increased among children with a history of OME.

In normal mice, the severity of AOM decreases promptly, with a significant reduction in bacterial recovery from middle ear effusions already 48 hours after injection of NTHi. In C3H/HeJ mice, which have nonfunctional TLR4, OM is prolonged, and the bacterial counts from middle ear effusions are increased 72 hours after injection of NTHi. Transmission electron microscopy revealed that phagocytosis and phagosome maturation of polymorphonuclear cells was impaired in C3H/HeJ mice. Thus, TLR4 plays a part in the early accumulation and functional promotion of polymorphonuclear cells in the middle ear and subsequently the eradication of NTHi.[102]

β-Defensin 2 is one of the most potent molecules of the innate immune system. The molecule is inducible by exposure to inflammatory stimuli such as bacterial components or proinflammatory cytokines, although the induction mechanism has not been clearly established. It has previously been demonstrated, however, that the major NTHi-specific receptor in human middle ear epithelial cell line 1 (HMEEC-1) is TLR2 and that recognition of NTHi component(s)/ligand(s) by TLR2 activates the toll/IL-1 receptor (TIR)-MyD88-IRAK1-TRAF6-MKK3/6-p38 MAPK signal transduction pathway, ultimately leading to the induction of β-defensin 2, and that this pathway acts synergistically with IL-1α–induced β-defensin 2 induction through a MyD88-independent Raf-MEK1/2-ERK MAPK pathway. In extension of these findings, Lee et al[103] found that the induction of β-defensin 2 is highest in whole-cell lysate preparations

of NTHi, suggesting that the ligand(s) responsible for this up-regulation may be soluble macromolecule(s). The authors propose that this confers an essential evolutionary advantage to the cells in coping with infections and may serve to amplify the innate immune response through paracrine signaling.

Lysozyme is another innate immune molecule and the expression of lysozyme M and lysozyme P, as well as the effect of lysozyme depletion on pneumococcal clearance from the middle ear cavity, was investigated by Shimada et al.[104] Immunolabeling revealed that localization of lysozyme M and lysozyme P is specific to some/particular cell types of the ET. Lysozyme P of lysozyme M–/– mice was mainly expressed in the submucosal glands but not in the epithelium. Although lysozyme M–/– mice showed compensatory up-regulation of lysozyme P, lysozyme M depletion resulted in a decrease in both muramidase and antimicrobial activities. Deficiency in lysozyme M led to an increased susceptibility to middle ear infection with *S pneumoniae* 6B and resulted in severe middle ear inflammation compared with wild-type mice. The results suggest that lysozyme M plays an important role in protecting the middle ear from invading pathogens.

Schachern et al[105] studied the effect of administration of another innate immune molecule, apolactoferrin, on the middle and inner ear after experimentally induced pneumococcal OM. The thickness and the bacterial and inflammatory cell infiltration of the round window membrane were monitored, along with the infiltration of scala tympani and damage to hair cells and stria vascularis. Bacterial counts and the number of inflammatory cells in the round window membrane were significantly lower after administration of apolactoferrin.

Cationic antimicrobial peptides (AMPs), a component of the innate immune system, play a major role in the defense of mucosal surfaces against a wide spectrum of microorganisms such as viral and bacterial co-pathogens of OM. To further understand the role of AMPs in OM, McGillivary et al[106] cloned a cDNA encoding a cathelicidin homolog (cCRAMP) from upper respiratory tract mucosae of the chinchilla. In situ hybridization showed cCRAMP mRNA production in epithelium of the chinchilla ET. Recombinant cCRAMP killed the 3 main bacterial pathogens of OM, and quantitative rtPCR analysis of chinchilla middle ear epithelial cells incubated with either viral or bacterial pathogens associated with OM demonstrated distinct microbe-specific patterns of altered expression. Collectively, the data show that viruses and bacteria modulate AMP messages, which likely contributes to the disease course of OM.

Heo et al[107] found an overexpression of acidic mammalian chitinase and chitotriosidase in adenoidal histiocytes and vascular endothelial cells, suggesting that chitin-containing pathogens or a dysregulated immune response to such pathogens may be factors contributing to OME.

Aryl hydrocarbon receptor and aryl hydrocarbon receptor nuclear translocator expression was demonstrated in human adenoid tissue, in the epithelium, subepithelial layer, germinal centers, mantle zone, and interfollicular areas, suggesting a role in local immune response.[108]

NTHi response to innate immune pressure has prompted studies on bacterial response mechanisms to components of innate immunity. Sharpe et al[109] examined production of NTHi outer membrane vesicles, particularly as a result of innate immune assault. The authors suggest that vesicles may play a significant role in NTHi biofilm architecture. Further work has identified an ABC transporter system, Sap transporter, as a mechanism to bind and transport host-derived AMPs away from the inner membrane target of these biocide peptides, to intracytoplasmic degradation.[110,111] Sap-deficient NTHi strains are attenuated for infection of the chinchilla middle ear. This attenuation is primarily due to NTHi interaction with host AMPs.

Adaptive immune response. Dendritic cells (DCs) are potent antigen-presenting cells involved in the initiation and modulation of immune responses after immunization via their ability to process and present antigen to naive T cells. Novotny et al[112] examined the role of DCs in the development of protective immunity against NTHi-induced experimental OM after intranasal immunization of chinchillas with an NTHi P5-derived synthetic peptide immunogen called LB1. In vitro, chinchilla DCs readily internalized LB1, up-regulated expression of the maturation markers CD80 and major histocompatibility complex class II, and presented processed LB1 to primed CD3+ T cells, which resulted in antigen-specific T-cell proliferation. In vivo, LB1-activated DCs trafficked from the nasal cavity primarily to the nasal-associated lymphoid tissues and were detected in close proximity to CD3+ T cells within this lymphoid aggregate. The data suggest an important role for DCs in the development of protective immunity against OM.[112]

The presence of dendritic cells and lymphocyte subpopulations in the adenoids and peripheral blood were evaluated in patients with adenoid hypertrophy, compared with patients with coexisting OME. A significantly lower percentage of CD3+CD69+, CD4+CD69+, CD8+CD69+, and CD19+CD69+ cells was found in the adenoids in patients with OME, attesting to reduced T-cell activation.[113]

The percentages of apoptotic lymphocytes and CD4+, CD8+, and CD19+ cells with CD95+ antigen and Bcl-2 protein in the adenoid tissue were examined by Zelazowska-Rutkowska et al.[114] The percentages of CD4+Bcl-2+, CD8+Bcl-2+, and CD19+Bcl-2+ lymphocytes were lower in OM patients, whereas the percentages of CD4+, CD8+, and CD19+ cells with CD95+ antigen were higher. The tendency of reduced percentages of T and B lymphocytes with Bcl-2 expression (inhibits apoptosis) and elevated percentages of these cells with CD95+ expression may reflect local immunity disorders.

The expressions of the B-lymphocyte inducer of maturation program 1, a promoter of plasmacytosis; B-cell leukemia/lymphoma-6 (BCL-6), a repressor of plasmacytosis and IgA, IgG, IgD, and IgM in the adenoids of patients with OME, chronic rhinosinusitis, or adenoid hyperplasia were

evaluated by immunohistochemistry.[115] The expression of antibody to IgA in the OME was significantly lower than the score in the adenoid hyperplasia group. Yeo et al,[116] however, did not find a difference in IgA secretion between OME and non-OME patients with adenoid hypertrophy. However, the OME group showed a greater incidence of squamous metaplasia, fewer ciliated cells, and lower expression of BCL-6. Their results suggest that increased squamous metaplasia and lower BCL-6 expression in adenoids may be associated with increased susceptibility to OME. The same group found that differences exist in adenoid size, squamous metaplasia, IgA secretion, and BCL-6 expression between patients with OME alone, patients with OME and sinusitis, and controls with adenoid hypertrophy alone.[117]

Neurogenic control of immune response. Very interestingly, Maison et al[118] probed into neurogenic control of immune response and found that mice with targeted deletion of the gene for dopamine β-hydroxylase, which catalyzes the conversion of dopamine to noradrenaline (thus, these mutant mice have no measurable adrenaline or noradrenaline), are more susceptible to spontaneous middle ear infection than their control littermates, consistent with a role for sympathetics in systemic and/or local immune response.

Exposure and OM pathogenesis. The role of gastroesophageal reflux disease (GERD) in the pathogenesis of OM has been addressed by a number of groups during the recent years. Accordingly, gastroesophageal reflux induced by relaxation of the lower esophageal sphincter has been shown to reach the middle ear in a Mongolian gerbil model.[119] The histologic changes in the ET mucosa after exposure to gastric reflux were studied in the rat by Yazici et al.[120] An increase in goblet cell density as well as polymorphonuclear neutrophils and lymphocytes infiltrating the mucosa was found.[120]

Most clinical studies have measured the content of pepsinogen in middle ear effusion obtained from OM patients undergoing tympanostomy tube insertion. Pepsinogen was present in 14% to 100% of samples and 20% to 100% of patients.[121-123] A significant positive correlation was found between the level of pepsin/pepsinogen in middle ear effusions and the number of pharyngeal reflux episodes measured by pH monitoring in 17 children,[124] and an increased adenoid immunoreactivity for pepsinogen was found in OME patients undergoing adenoidectomy, although pepsinogen messenger ribonucleic acid was absent.[125] As further evidence of gastric content reaching the middle ear, bile acids were found in 32% of middle ear effusion samples and 42% of 38 children undergoing ventilation tube insertion for OME.[126]

Treatment studies have shown a middle ear pepsinogen level decrease in 7 of 10 patients after antireflux therapy treatment[127] and that treatment improves the quality of life in patients with OME and reflux.[128]

Miura et al[129] performed a systematic review of the literature for the period 1974 to September 2011 and concluded that the prevalence of GERD in children with COME/recurrent AOM may be higher than the overall prevalence for children and that the presence of pepsin/pepsinogen in the middle ear could be related to physiologic reflux but also that a cause-effect relationship between pepsin/pepsinogen in the middle ear and OM is still unclear.[129]

Cigarette smoke. In a line of immortalized human middle ear epithelial cells, application of cigarette smoke solution increases the expression of TNF-α, EGFR, and MUC5AC. The up-regulation of EGFR and MUC5AC was suppressed by the pretreatment with a EGFR tyrosine kinase inhibitor, suggesting that cigarette smoke may contribute to increased middle ear mucus production and that the MUC5A up-regulation is dependent on EGFR activation.[130] Another group showed that cigarette smoke condensate activates NF-κB in a middle ear epithelial cell line and that this activation induced TNF-α promoter activation, gene expression, and levels in cell secretions.[63]

Implication for Practice

This review has identified a number of significant new publications contributing to the knowledge on a number of issues pertaining to anatomy, pathology, and cell biology in relation to the pathogenesis of OM, for example, cell-specific expression and function of fluid transportation and innate immune system molecules, mucous cell metaplasia, mucin expression, bacterial adherence and epithelial internalization, as well as the occurrence, composition, dynamics, and potential role of bacterial biofilm. In addition, the potential role of GERD and cigarette smoke exposition has been explored further. Thus, over the past 4 years, considerable scientific progress has been made. Based on these new achievements and a sustained lack of essential knowledge, suggestions for future research are outlined randomly below.

Recommendations for Future Research

1. Develop synergies with stem cell biologists in creating models for studying the middle ear, ET, and nasopharynx with respect to identification of stem cells, cell differentiation, and turnover
2. Extend work on cDNA libraries in OM models
3. Expand models to study the effects of various agents and combinations of those on nasopharyngeal, ET, and middle ear histopathology
4. Intracellular uptake of pathogenic particles/molecules should be explored further
5. Study mucosal changes and cellular interactions in relation to biofilm formation, persistence, and eradication
6. Correlate inflammatory molecules with middle ear, ET, and nasopharyngeal histopathology, with specific emphasis on bacterial adhesion, viral infection, and colonization, as well as biofilm formation
7. Histopathological changes in the nasopharynx, ET, and middle ear mucosa caused by noninfectious factors should be further explored (eg, allergy, pollutants, GERD, smoke)

8. Explore new imaging techniques for studies of anatomy, function, and disease (eg, microendoscopy, functional magnetic resonance imaging, single-photon emission CT)
9. Further studies on finite element models should be performed to elucidate anatomy and function
10. Study locally applied, sustained-release formulations of agents of relevance with respect to tissue response

Abbreviations

AI, autoinducer; AMP, antimicrobial peptide; AOM, acute otitis media; AQP, aquaporin; Bcl, B-cell lymphoma; CCR, chemokine receptor; COM(E), chronic otitis media (with effusion); CSOM, chronic suppurative otitis media; DC, dendritic cell; EGFR, epidermal growth factor receptor; iNOS, induction nitric oxide synthase; ET, Eustachian tube; GABAR, γ-aminobutyric acid receptor; GalNAc, galactose N-acetylgalactosamine; GAS, group A streptococci; IL, interleukin; LOS: lipo-oligosaccharide; MCP, monocyte chemoattractant protein; MMP, matrix metalloproteinase; NET, neutrophil extracellular traps; NF-κB, nuclear factor-kappa beta; NKCC, Na(+)-K(+)-2Cl(-) co-transporter; NTHi, nontypeable *Haemophilus influenzae*; OM, otitis media; OME, otitis media with effusion; PCho: phosphorylcholine; SPDEF, SAM pointed domain ETS factor; TGF, transforming growth factor; TLR, toll-like receptor; TM, tympanic membrane; TNF, tumor necrosis factor; rtPCR: real-time polymerase chain reaction.

Author Contributions

Per Cayé-Thomasen, contribution to conception and design; acquisition, analysis, and interpretation of data; drafting the article and revising the article critically for important intellectual content; final approval of the version to be published. **Ann Hermansson**, acquisition, analysis, and interpretation of data; revising the article critically for important intellectual content; final approval of the version to be published. **Lauren Bakaletz**, acquisition, analysis, and interpretation of data; revising the article critically for important intellectual content; final approval of the version to be published. **Sten Hellstrøm**, acquisition, analysis, and interpretation of data; revising the article critically for important intellectual content; final approval of the version to be published. **Sho Kanzaki**, acquisition, analysis, and interpretation of data; revising the article critically for important intellectual content; final approval of the version to be published. **Joseph Kerschner**, contribution to conception and design; acquisition, analysis, and interpretation of data; revising the article critically for important intellectual content; final approval of the version to be published. **David Lim**, contribution to conception and design; acquisition, analysis, and interpretation of data; revising the article critically for important intellectual content; final approval of the version to be published. **Jizhen Lin**, acquisition, analysis, and interpretation of data; revising the article critically for important intellectual content; final approval of the version to be published. **Kevin Mason**, acquisition, analysis, and interpretation of data; revising the article critically for important intellectual content; final approval of the version to be published. **Jorge Spratley**, acquisition, analysis, and interpretation of data; revising the article critically for important intellectual content; final approval of the version to be published.

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Panel 4: Recent Advances in Otitis Media in Molecular Biology, Biochemistry, Genetics, and Animal Models

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Abstract

Background. Otitis media (OM) is the most common childhood bacterial infection and also the leading cause of conductive hearing loss in children. Currently, there is an urgent need for developing novel therapeutic agents for treating OM based on full understanding of molecular pathogenesis in the areas of molecular biology, biochemistry, genetics, and animal model studies in OM.

Objective. To provide a state-of-the-art review concerning recent advances in OM in the areas of molecular biology, biochemistry, genetics, and animal model studies and to discuss the future directions of OM studies in these areas.

Data Sources and Review Methods. A structured search of the current literature (since June 2007). The authors searched PubMed for published literature in the areas of molecular biology, biochemistry, genetics, and animal model studies in OM.

Results. Over the past 4 years, significant progress has been made in the areas of molecular biology, biochemistry, genetics, and animal model studies in OM. These studies brought new insights into our understanding of the molecular and biochemical mechanisms underlying the molecular pathogenesis of OM and helped identify novel therapeutic targets for OM.

Conclusions and Implications for Practice. Our understanding of the molecular pathogenesis of OM has been significantly advanced, particularly in the areas of inflammation, innate immunity, mucus overproduction, mucosal hyperplasia, middle ear and inner ear interaction, genetics, genome sequencing, and animal model studies. Although these studies are still in their experimental stages, they help identify new potential therapeutic targets. Future preclinical and clinical studies will help to translate these exciting experimental research findings into clinical applications.

Keywords

otitis media, molecular biology, biochemistry, inflammation, cytokine, chemokine, innate immunity, cell signaling, tissue remodeling

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The host responses to viral and bacterial pathogens during otitis media (OM) are determined, in large part, by the expression of genes during host-pathogen interaction. The identification of genomic and expressed sequences and the recent advances in the experimental methods of molecular biology, genomics, proteomics,

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biochemistry, and genetics permit the assessment of gene activity in humans and animals experiencing OM. The increasing availability of genome information on pathogens also allows assessment of OM-related bacterial and viral gene expression and activity. Finally, molecular, genetic, and biochemical tools allow the manipulation of genes and their products in both hosts and pathogens. This provides powerful methods for evaluating the functional roles of genes in this disease. Significant progress has been made in the areas of molecular, genetic, and biochemical studies in OM over the past 4 years.

Methodology/Search Strategy

We searched PubMed for published literatures. The starting date for the search was June 1, 2007, and the ending date, May 30, 2011. Search terms included *otitis media, molecular biology, biochemistry, inflammation, cytokine, chemokine, innate immunity, Toll-like receptor, host response, cell signaling, mucus, mucin, mucosal hyperplasia, tissue remodeling, cholesteatoma, genetics, chinchilla genome*, and *animal model*. The search included all published literature with the abstract available in English. No further exclusion selection criteria were applied.

Discussion

Recent Advances in the Molecular Biology and Genetics of Otitis Media

Significant advancements have been made in developing molecular, genetic, and biochemical tools that allow the manipulation of genes and their products in both hosts and pathogens. These technological advancements led to substantial progress in improving our understanding of the molecular basis of host responses in OM in a number of areas.

Innate Immunity

The innate immune system, known as the nonspecific immune system and the first line of defense, is an evolutionarily conserved mechanism that provides an early and effective response against invading microbial pathogens. Extensive studies on the role of innate immune molecules were carried out using in vitro and in vivo approaches. Genome-wide array studies using cells and knockout (KO) mice, partial characterization of complementary DNA (cDNA) library in normal and nontypeable *Haemophilus influenzae* (NTHi)–treated chinchilla middle ear (ME), and identification of SPLUNC having a role in the host defense against NTHi in the chinchilla are some of the highlights.

A number of studies have added to our understanding of innate immune defects as a risk factor for OM in children. Wiertsema and Leachl[1] noted that several such defects are associated with increased risk of OM and may help to explain the high incidence of disease in Australian Aboriginal children. Prommalikit et al[2] noted that children with defects in either innate or cognate immunity were at increased risk for pneumococcal OM. A similar conclusion regarding innate immunity was reached by Ilia et al.[3] Kim et al[4] detected several innate immune receptors in OM effusions, including Toll-like receptor 9 (TLR9), as well as NOD1 and NOD2. Lee et al[5] found that induction of host response by NTHi in human ME epithelial cells requires TLR2 signaling mediated by the TLR adaptor MyD88. Lee et al[6] identified mutations in TLR2 and TLR4 in patients with OM.

Studies in animals have added in vivo evidence regarding the importance of innate immunity in OM resistance. Song et al[7] observed the expression of TLR2 and TLR4 in the rat ME during OM. Hirano et al[8] and Leichtle et al[9] found that mutations in TLR4 resulted in prolongation of OM in the mouse. MacArthur et al[10] observed spontaneous OM in mice with a TLR4 deficit and identified *Klebsiella oxytoca* as a pathogen. Leichtle et al[11] noted that lack of TLR2 resulted in a more profound deficit in OM recovery than lack of TLR4 in mice. Moreover, they found that lack of TLR2 blocked upregulation of TLR4 by ME inoculation with NTHi. Leichtle et al investigated mice deficient in TLR9, an intracellular receptor for foreign DNA, and found prolonged OM induced by NTHi. Most TLRs signal through the adaptor molecule MyD88, with only TLR3 and TLR4 signaling through the related adaptor TRIF. Hernandez et al[12] found that mice deficient in MyD88 exhibited greatly prolonged OM induced by NTHi, whereas Leichtle et al[11] found a more modest deficit in OM recovery due to lack of TRIF. Activation of TLRs induces the expression of inflammatory cytokines, one of the most important of which is tumor necrosis factor (TNF). Leichtle et al[13] found that mice deficient in TNF showed greatly prolonged OM that was only partially rescued by exogenous TNF. Interestingly, exogenous chemokine CCL3, normally expressed at high levels during OM but not expressed in TNF-deficient mice, completely rescued normal OM recovery. Genetic defects in complement genes also reduced the ability of mice to resist and recover from OM.[14,15] In a chinchilla model, McGillivary et al[16] found that dysregulation of the innate immune effector β-defensin by respiratory syncytial virus enhanced colonization of the upper airway by NTHi. It is clear that animals with innate immune defects are excellent models of persistent/recurrent OM. Moreover, the fact that defects in so many innate immune genes prolong OM in animal models underscores the importance of this system in resistance to, and recovery from, OM.

Inflammation

Appropriate inflammation is critical for host defense. However, uncontrolled and excessive inflammation is clearly detrimental to the host. Inflammation is a hallmark of OM, and dysregulated inflammation in the middle ear contributes significantly to the development and progression of OM. Therefore, maintaining adequate levels of inflammation is critical for preventing middle ear damage. Otitis media is a multifactorial disease arising from the complex interactions among otopathogens, environmental risk factors, and host genetic factors. Thus, it is of particular

importance to understand the mechanisms by which these factors regulate immune/inflammatory responses in the middle ear, either alone or in combination.

Microbial Factors. Among many factors for OM, infection with otopathogens was found to be the most frequent cause of OM. Otitis media is a polymicrobial disease caused by bacterial and viral pathogens. Although single-pathogen infection is typical, it is common to have coinfections with more than one type of bacterial pathogens (up to 55%) as well as bacterial and viral coinfections (up to 70%).[17] Moreover, otopathogens can not only colonize together but also synergize with each other to induce host responses in multiple ways. For example, one of the most common bacterial otopathogens, *Streptococcus pneumoniae*, synergistically enhances NTHi-induced inflammation via upregulating TLR2 expression, one of the major host receptors for the NTHi pathogen.[18] The incidence of OM by *S pneumoniae* is also significantly enhanced by other otopathogens, particularly by *Moraxella catarrhalis*.[19] In addition, it has also been found that coinfection with NTHi facilitates pneumococcal biofilm formation and increases its persistence on middle ear mucosal surfaces. The enhanced biofilm persistence also correlates well with delayed emergence of opaque colony variants within the bacterial population and a decrease in the systemic infection.[20] Of particular interest are the clinical trials studying the use of spray bacteriotherapy. Nasal spray with probiotic bacteria (eg, *Streptococcus sanguinis* or *Lactobacillus rhamnosus*) showed significant therapeutic effects, including complete or significant recovery from serous OM.[21] Although the underlying mechanism for the observed effect remains to be investigated, the study suggests that the microbial physiology in the middle ear inflammation is far more complex than what we initially thought. Therefore, this complexity should be taken into consideration in designing treatments for controlling infection and inflammation in OM treatment.

Host Genetic Factors. There is also considerable evidence for a genetic predisposition for the development of OM. However, little is known about the genetic factors underlying susceptibility to OM. Recently, many studies have been focused on identifying the genetic factors underlying the regulation of immune/inflammatory responses in the middle ear, and they have identified a number of genetic factors regulating immune/inflammatory responses in the pathogenesis of OM. However, the mechanism underlying regulation of inflammation in vivo by these genetic factors is not well understood. For instance, TNF-α and TNF receptor pathways have been found to be critical for inflammation in the middle ear. Recent studies found that infliximab, the monoclonal TNF-α antibody, reduces inflammatory responses in experimental OM in rats.[22] However, deficiency of TNF resulted in prolonged inflammation following infection of the middle ear with NTHi, whereas TNF-deficient mice failed to upregulate both TLRs and the downstream genes and proteins, such as CCL3, resulting in defects in both inflammatory cell recruitment and macrophage activation. Of particular interest in this study is that in vivo administration of rCCL3 to TNF-deficient mice restores their ability to control OM caused by NTHi.[13] Moreover, genetic studies with TNF mutant mice also showed that TNF and TNF receptors are required for appropriate regulation of caspase genes and are critical for the maintenance of mucosal architecture in both normal and infected ME.[23] The role of innate immune regulators in inflammation is also demonstrated in the recent study by Leichtle et al.[11] This study showed that TRIF (Tir domain–containing adaptor inducing interferon β)–deficient mice showed reduced mucosal hyperplasia and had decreased leukocyte infiltration into the middle ear in response to NTHi infection as compared with wild-type (WT) mice. However, mucosal hyperplasia was found to be more persistent and bacterial clearance was delayed in these TRIF-deficient mice.[11] Nevertheless, due to the functional and anatomical similarities between the ears of mice and humans, the mouse OM model has been found to be an excellent model system for unraveling the complex genetic susceptibilities underlying OM.[24] Mouse genetic mutants with phenotypes comparable to OM in humans may be helpful for the identification of the precise genetic determinants underlying the increased heritability to OM. Many innate immune factors regulating inflammation have been tested in the mouse model of OM using gene-knockout approaches. Shimada et al[25] found that lysozyme M deficiency led to an increased susceptibility to middle ear infection with *S pneumoniae* and resulted in severe middle ear inflammation by studying *S pneumoniae*–induced OM in lysozyme M–deficient mice. In addition to TLR4 mutation, which was originally found to contribute to the development of OM, MyD88, TLR2, and TLR9 have also been found to be critical for regulating inflammatory responses in the middle ear.[12,26,27] MyD88, a universal TLR adaptor protein (except for TLR3), has also been found to be critical for middle ear inflammation, as evidenced by the experiment showing that MyD88 KO mice displayed prolonged ME mucosal thickening and delayed recruitment of neutrophils and macrophages in OM.[12] TLR2 KO mice were also found to produce relatively low levels of proinflammatory cytokines following pneumococcal challenge, thus hindering the clearance of bacteria from the middle ear and leading to sepsis and a high mortality rate.[27] Deficiency of TLR9 resulted in significantly prolonged inflammatory responses in the mouse model of NTHi-induced OM, indicating that DNA sensing by TLR9 may contribute to the pathogenesis and recovery of OM.[28] In addition, cylindromatosis (CYLD), a negative regulator of nuclear factor–κB (NF-κB), has been shown to play an important role in regulating NTHi-induced inflammation in the pathogenesis of cholesteatoma in human patients.[29,30] In a later study by Byun et al,[29] CYLD expression was found to be significantly low in the cholesteatoma epithelium of human patients while enhanced NF-κB activation was observed,[29] suggesting that the downregulation of CYLD may contribute not only to the inflammatory responses against otopathogens but also to the tissue remodeling

process in middle ear disease. In addition, single-gene mouse mutants with OM have identified a number of genes—namely, Eya4, Tlr4, p73, MyD88, Fas, E2f4, Plg, Fbxo11, and Evi1—as potential and biologically relevant candidates for human disease. Human polymorphisms in FBXO11, TLR4, and PAI1 genes have been identified to be significantly associated with human disease.[31,32] Rpl38 (encoding a ribosomal protein of the large subunit) mutation exhibited the OM phenotype in Tail-short (Ts) mice.[33]

MicroRNAs in OM. Recent advances in molecular biology have revealed the potential role of microRNAs (miRNAs) as an important regulator of inflammation in many pathological processes. In a recent study, Song et al[34] found that a number of miRNAs, which regulate important biological processes, including developmental process, acute inflammatory responses, and innate immune responses, were differentially regulated (either upregulated or downregulated) during lipopolysaccharide-induced acute inflammation in the human middle ear epithelial cells (HMEECs). This study suggests that miRNAs may play important roles in the regulation of inflammation in OM as it does in the other inflammatory diseases.

Sequencing Chinchilla Genome

The chinchilla OM model is a robust, reproducible, and polymicrobial model of OM. Recently, significant progress has been made in obtaining genomic information from sequencing, which will be valuable for further promoting our understanding of OM pathogenesis.[35]

Mucosal hyperplasia. It is now well accepted that middle ear infection triggers mucous cell metaplasia. Experimentally, it has been shown that bacterial infection or cytokine challenge of the middle ear mucosa results in mucous cell metaplasia/hyperplasia. Cytokines, especially the proinflammatory cytokines and T-helper 2 subset–derived cytokines, are well known to be involved in mucous cell metaplasia/hyperplasia. One such example is that interleukin (IL)–10 knockout mice developed no mucous cell metaplasia/hyperplasia.[36] These studies suggest that cytokines derived from T-helper 2 lymphocytes play a critical role in the mucous cell metaplasia. Mucous cell metaplasia involves increased mucin expression, production, and release. It is becoming clear that inflammation stimulates epidermal growth factor receptor activation and IL-13 to induce transition of both Clara and ciliated cells into goblet cells through the coordinated actions of the forkhead box transcription factor A2 (FoxA2), thyroid transcription factor 1 (TTF-1), SPDEF, and GABAαR in which FoxA2 is downregulated and SPDEF and GABAαR are upregulated, resulting in an upregulation of mucin MUC5AC, mucin chaperone trefoils, and CLCA.[37] Moreover, Tsuchiya et al[36] found that IL-10 plays a significant role in mucoid metaplasia of the middle ear. Lee et al[38] found that transforming growth factor–β (TGF-β) signaling is activated during mucosal hyperplasia. Furukawa et al[39] found that inhibition of JNK reduced mucosal hyperplasia during OM, implicating the involvement of JNK signaling. Ebmeyer et al[23] reported that TNFA deletion altered apoptosis and caspase expression during OM, suggesting that this factor is involved in the recovery of the mucosa from hyperplasia. Yaguchi et al[40] successfully generated an artificial mucosa for the regeneration of the damaged or absent ME lining.

Molecular Biology of Cholesteatoma. Interesting progress has also been made in cholesteatoma studies. One interesting finding is the identification of the transcription factor ID1 (inhibitor of differentiation 1) as an important regulator that drives the proliferation and/or immortalization of the skin keratinocytes,[41] thereby conferring more aggressive and ever-proliferating characteristics to keratinocytes in the middle ear cavity. The upregulation of ID1 in the middle ear epithelial cells is triggered by infectious agents such as *S pneumoniae*, and this upregulation persists in the middle ear cholesteatoma matrix.[41] Recent studies indicate that ID1 is involved in aggressive behaviors of the cholesteatomal matrix through regulation of NF-κB activity, a key regulator for keratinocyte proliferation and differentiation, and suppression of $p16^{INK4a}$ expression, a checkpoint protein for the cell cycle progression of keratinocytes.[42] These factors contribute significantly to the pathogenesis of cholesteatoma. In addition, ID1 has also been suggested to play a role in the pathogenesis of the cholesteatomal development[42] via a TGF-β–dependent mechanism in OM.[38]

Mucus and mucin. Although undoubtedly important in the mucosal defense against microbes, it is clear that excessive and exacerbated production of mucus in OM contributes significantly to the pathogenesis of OM by overloading the host mucociliary escalator function and resulting in conductive hearing loss. Thus, mucus production in the middle ear must be tightly regulated. Understanding the molecular mechanisms underlying mucin production and regulation is important for the development of adequate therapies to prevent mucus overproduction.

Mucous Production. In humans, mucins are encoded by 20 mucin genes (MUCs). Among these, 16 MUC genes are found to be expressed in the normal human middle ear epithelium in vivo and also in HMEECs in vitro,[43] as well as mouse middle ear epithelium (MMEE)[44] and chinchilla middle ear epithelium (CMEE).[35] Three MUC genes (MUC6, MUC12, MUC17) were not expressed and MUC21 was not evaluated in the middle ear.[43] Although most of the mucin genes are expressed in the middle ear mucosa, the major mucin genes in OM appear to be MUC2, MUC5AC, and MUC5B.[45-47] In addition, recent studies also suggest the involvement of other MUC genes, such as MUC1, MUC3, MUC4, MUC6, MUC7, MUC8, MUC9, MUC11, MUC12, and MUC19, in clinical human OM effusion, cultured middle ear epithelial cells, and animal models with middle ear inflammation.[43-50] However, their roles and the underlying regulatory mechanism in OM have yet to be fully investigated.

Regulation of Mucin Expression. Upregulation of mucin expression has been found to be induced by both microbial and host-derived factors, including OM pathogens, cytokines, and growth factors. Otitis media pathogens not only activate host positive signaling pathways but also suppress inhibitory signaling pathways to upregulate mucin expression as exemplified by *S pneumonia* pneumolysin-mediated inhibition of the TLR4-dependent JNK signaling pathway via MAPK phosphatase-1 (MKP-1) to enhance extracellular signal-regulated kinase (ERK)–mediated MUC5AC expression.[51] In addition, OM pathogens also cooperate with other pathogens or pathogenic factors to synergistically upregulate the expression of mucin. For example, NTHi and *S pneumonia*e synergistically induce MUC5AC expression.[52] Epidermal growth factor (EGF) synergistically upregulates NTHi-induced MUC5AC expression.[53] Moreover, *S pneumoniae* was found to activate 2 activator protein 1 (AP-1) sites in the promoter region of the MUC5AC gene via distinct signaling pathways, leading to differential regulation of MUC5AC gene expression.[54] All of these studies demonstrate the complexity of the tight regulation of mucin gene expression via various mechanisms.

In addition, cytokines are also found to upregulate mucin. Interleukin-10 induces mucin overproduction via mediating NTHi- and *S pneumoniae*–induced mucous cell metaplasia and hyperplasia in mice.[36] Interleukin-1β (IL-1β) regulates mucus secretion via activation of the Na-K-2Cl-cotransporter (NKCC).[55] Exposure to cigarette smoke was thought as one of the potential risk factors for OM. The molecular mechanism by which cigarette smoke regulates mucin expression in the middle ear was not well understood. Recent studies have shown that cigarette smoke induces MUC5AC expression via EGFR[56] and upregulates MUC5B expression via NF-κB pathways.[57] In addition to MUC gene expression, cigarette smoke also induces goblet cell proliferation and excess mucus secretion.[58] Moreover, host factors, such as gastric acids, were also found to upregulate MUC5B in middle ear epithelial cells.[59] Thus, it is evident that multiple factors, including both host- and non–host-derived factors contribute to the pathogenesis of OM.

Recent Advances in Biochemistry of Otitis Media

Significant progress in OM research has also been made in the area of biochemistry. These advances include the identification of biochemical markers during various stages of OM pathogenesis through the characterization of the components of chronic otitis media (COM) effusion (eg, mucins, cytokines, chemokines, bactericidal molecules, and growth factors) and the elucidation of the biochemical basis of middle ear and inner ear interaction.

Biochemical Basis of Otitis Media

Otitis media is an inflammatory response to either acute or persistent stimuli characterized by the accumulation of both cellular and chemical mediators in the middle ear cavity. It is caused by multiple factors, including bacterial or viral infection, eustachian tube dysfunction, allergy, and barotraumas. There are many cellular and biochemical events as a result of tissue injury induced by inflammation. Central factors to the formation of inflammation are the presence of inflammatory mediators, which include proteins (glycoproteins), peptides, cytokines, arachidonic acid metabolites, macrophage migration inhibitory factor, nitric oxide, and free radicals. These compounds are produced by epithelial cells, middle ear mucosa, and infiltrating inflammatory cells.

Inflammatory Cytokines in Middle Ear Effusion. Inflammatory mediators are known to diffuse into the inner ear across the round window membrane, possibly causing damage to the cells of the inner ear and leading to various types of auditory dysfunction.[60-63] The genes of numerous inflammatory cytokines are either up- or downregulated in murine inner ear cells in response to acute or chronic inflammation of the middle ear.[64]

It appears that IL-1β and TNF-α are involved in the initial phase of the inflammatory processes. Interleukin-2 is involved in T-cell proliferation and induces other cytokines, including IL-4, IL-5, IL-13, and granulocyte macrophage colony-stimulating factor (GM-CSF). These cytokines participate in the regulation of molecular and cellular processes involved in different types of chronic inflammation. The presence of cytokines in the middle ear cavity may cause disruption in the normal balance of inflammatory cytokines within the lateral wall and may hinder the recycling of ions causing hearing impairment. It has been reported that eosinophilia in middle ear effusions (MEEs), as well as in middle ear mucosa (MEM), is closely related to atopy and asthma. Upregulation of thymus and activation-regulated chemokine (TARC) in the cultured middle ear–derived fibroblasts by the Th2 cytokines (IL-4 and IL-13) was reported.[65] The production of these inflammatory mediators, along with the production of VEGF, causes an increase in the vascular permeability that results in the formation of MEE. Mucoid otitis media (MOM) middle ear effusions found in COM are characterized by the presence of mucoid glycoproteins. Both MUC5AC and MUC5B were detected in mucous middle ear effusions.[66] However, factors participating in the regulation of molecular and cellular processes leading to the chronic inflammation remain to be further investigated. The availability of MEE for laboratory analyses provides a means for identifying biochemical markers that may help characterize the types and stages of inflammation in OM.

Aquaporins. Aquaporins (AQPs) facilitate water movement within specific organs, allowing water to move along the osmotic gradient. They appear to be involved in the water balance of the middle ear. The expression of various AQPs (AQPs 1, 3, 4, 5, 7, 8, and 9) has been reported in the tissues and cell linings of tubotympanum.[67-69] The role of these AQPs in the MEE formation needs to be further studied.

Apolactoferrin. Management of OM is one of the important issues to be investigated. Studies have been performed on the inhibition of certain biochemical components involved in the pathological changes in the middle ear cavity. The effect of administration of apolactoferrin, the iron-free form of lactoferrin, on the middle and inner ears after experimentally induced pneumococcal OM was also studied.[70] Bacterial counts of MEE and the number of inflammatory cells in the round window membrane (RWM) were significantly lower in the apolactoferrin-treated group compared with the control group. Since antibiotic-resistant bacteria have become a problem, the significant reduction of bacteria in the middle and inner ear, as well as reduced damage to the RWM compared with the controls, is noteworthy. Further studies, using a topical application of exogenous apolactoferrin alone or in combination with other antimicrobial and/or anti-inflammatory agents for the treatment of acute OM (AOM), would be helpful in evaluating their therapeutic potential.

Middle Ear and Inner Ear Interaction

It has been well known that patients with COM develop sensorineural hearing loss, suggesting an interaction between the middle and inner ear.

Human Studies. Paparella[71] suggested that certain sudden deafness associated with vestibular symptoms can be caused by middle and inner ear interactions. It was postulated that the 2 most common causes of idiopathic sudden deafness were viral endolymphatic labyrinthitis and middle ear/inner ear interaction, commonly caused by infections or barometric trauma to the middle ear, generated by activities such as nose blowing or scuba diving. These events can damage the inner ear via the RWM, resulting in impairment to inner ear elements, including sensory cells.

Yoshida et al[72] reported sensorineural hearing loss with COM, specifically examining the role infection and aging played in older patients. Bone conduction (BC) hearing thresholds of 180 preoperative patients (207 ears) with COM and 226 normal individuals (289 ears) were measured by audiometry, and the percentage of ears with BC thresholds being higher than normal range was evaluated in the COM group. In the COM group, the size of the perforation on the eardrum (n=196) and the cross-sectional area of the mastoid air cells based on the axial computed tomography (CT) image (n=103) were also measured and correlated with the results of the BC threshold. When compared with the control group, the percentage of ears with higher than normal BC thresholds tended to increase with age, ranging from 4.5% in the 20s to 34.1% in the 60s. The increase in the BC thresholds did not correlate with the size of eardrum perforation but instead was closely associated with the size of the mastoid air cells. The authors suggested that all measures for an early cure, including surgery, should be considered as rapidly as possible for patients with COM.

Joglekar et al[61] described cochlear pathology in human temporal bones with OM. They used 614 temporal bones with OM and selected 47 with chronic and 35 with purulent OM following strict exclusion of subjects with a history of acoustic trauma, head trauma, ototoxic drugs, and other diseases affecting the cochlear labyrinth. Temporal bones with labyrinthine inflammatory changes were further evaluated for loss of hair cells and other histopathologic changes compared with age-matched controls. In all, 19% of temporal bones with chronic and 9% with purulent OM showed labyrinthine inflammatory changes. In chronic OM, inflammatory changes were as follows: 56% localized purulent, 22% localized serous, 11% generalized seropurulent, and 11% generalized serous. Inflammatory changes in temporal bones with purulent OM included 67% localized purulent and 33% generalized seropurulent. Pathological findings included serofibrinous precipitates and inflammatory cells in scala tympani of basal turn and cochlear aqueduct, significant loss of outer and inner hair cells, and significant decrease in the area of stria vascularis in the basal turn of the cochlea, as compared with controls. They concluded that middle and inner ear interactions in OM can lead to cochlear pathology. More severe pathological changes observed in the basal turn of the cochlea are consistent with prevalence of sensorineural hearing loss at higher frequencies in patients with OM.

Animal Studies and Molecular Biological Basis. The existence and characterization of the blood labyrinthine barrier (BLB) have been previously reported. The integrity of this barrier is essential for auditory function. The inflammatory cytokines (IL-1β and TNF-α) present on the round window membrane caused an increase in the permeability of the BLB and resulted in auditory dysfunction. It is quite conceivable that an elevation of cytokines in the middle ear cavity due to OM can cause hearing impairment.

MacArthur et al[73] investigated the control of COM and sensorineural hearing loss in C3H/HeJ mice using glucocorticoids vs mineralocorticoids. They used 7 to 17 mice per treatment group. Auditory brain stem response (ABR) thresholds were performed at baseline, 2 weeks, and 4 weeks. Histopathologic test results were evaluated on all mice ears at the end of the study. Analysis of variance (ANOVA) of ABR threshold change showed significant treatment effects ($P < .05$) by both steroid types at all time intervals and ABR frequencies except 4 weeks/8 kHz. Histologic assessment showed prednisolone-treated mice (62%) had a higher rate of clearance of middle and inner ear inflammation than did control mice (4%). They concluded that steroid treatments can improve the physiology of chronic middle and inner ear disease seen with COM.

Cytokines in the middle ear cavity may cause disruption in the normal balance of inflammatory cytokines within the lateral wall and may hinder the recycling of ions, causing hearing impairment. Recurrent AOM leads to sensorineural hearing loss by unknown mechanisms. It is widely accepted that inflammatory cytokines diffuse across the round window membrane to exert cytotoxic effects. Ghaheri et al[74] investigated cochlear cytokine gene expression in murine AOM. BALB/c mice underwent transtympanic

injection of heat-killed *H influenzae* to create an acute inflammatory response. These mice were compared with a control group, in addition to a group of uninjected mice, and found to have otomicroscopic changes consistent with persistent or COM. The cochleas of these mice were obtained, their RNA harvested, and cytokine gene expression analyzed using prefabricated cDNA arrays. Four groups of mice (control, 3-day postinjection, 7-day postinjection, and mice with COM) with 5 mice in each group were analyzed. Numerous classes of genes were found to be upregulated or downregulated by more than 2-fold. Some genes differed from control mice by more than 10-fold. These genes included numerous fibroblast growth factors, interleukins, tumor necrosis factors, and colony-stimulating factors. They concluded that the genes of numerous inflammatory cytokines are either up- or downregulated in murine inner ear cells in response to either acute or chronic inflammation of the middle ear. Their study provides a novel site of production of cytokines that may be responsible for the damage seen in sensorineural hearing loss.

Previous gene expression array studies have shown that cytokine genes might be upregulated in the cochleas of mice with acute and chronic OM. This finding implies that the inner ear could manifest a direct inflammatory response to OM that may cause sensorineural damage. Therefore, to better understand inner ear cytokine gene expression during OM, quantitative real-time polymerase chain reaction and immunohistochemistry were used in mouse models to evaluate middle and inner ear inflammatory and remodeling cytokines. MacArthur et al[64] reported altered expression of middle and inner ear cytokines in mouse OM. They induced AOM in BALB/c mice by a transtympanic injection of *S pneumoniae* in one ear while the other ear was used as a control. C3H/HeJ mice were screened for unilateral COM, with the noninfected ear serving as a control. Both acute and chronic OM caused both the middle ear and inner tissues in these 2 mouse models to overexpress numerous cytokine genes related to tissue remodeling (TNF-α, bone morphogenetic proteins, fibroblast growth factors) and angiogenesis (VEGF), as well as inflammatory cell proliferation (IL-1β, IL-2, IL-6). Immunohistochemistry confirmed that both the middle ear and inner ear tissues expressed these cytokines. They concluded that cochlear tissues do express cytokine mRNA that contributes to the inflammation and remodeling that occur in association with middle ear disease, thereby providing a potential molecular basis for the transient and permanent sensorineural hearing loss often reported with acute and chronic OM.

Recent Advances in Animal Models of Otitis Media

Animal models of OM are important research tools, since they allow access to the entire course of the disease and are subject to experimental manipulation. Because of their importance, there has been continuous and significant work done to develop additional and improved animal models for this condition. Those advances include (1) animal model of spontaneous OM using MyD88 and TLR2 KO mice; (2) animal model of induced OM with increased severity and duration using TLR2, 4, 9, Trif, dynactin subunit 4 (DYA4) KO mice and ribosomal protein L38 (RPL38) mutant mice; (3) animal model of polymicrobial infection, including virus and bacteria; and (4) animal model of viral infection.

Mouse Genetics and Animal Models

Genetic Resources. Recently, novel OM genes in chemical (ENU) mutagenesis programs linked to phenotypic screens for deafness and vestibular signs were discovered. The gene targeting and the international efforts, including the European Mouse Disease Clinic (EUMODIC), Knockout Mouse Project (KOMP), and North American Conditional *Mouse* Mutagenesis (NORCOM) programs, to produce KO of all genes in the mouse genome are gaining momentum. In addition, embryonic stem cells are a publicly available resource for the scientific community. The International Mouse Phenotyping Consortium (IMPC) is funded by the National Institutes of Health (NIH), MRC Wellcome Trust, and Genome Canada. The 3 consortia—UC Davis–Toronto, Regeneron-Jax, and Baylor–Sanger Wellcome Trust–MRC Harwell—are funded to share the work. Phase 1 (2011-2016) aims to turn 5000 targeted embryonic stem cells into mutant mice and also perform the baseline phenotyping (includes ABRs). Studies at Sanger Wellcome Trust indicate that this effort will lead to the identification of novel sensorineural and OM mutants. Phase 2 (2016-2021) of the program will aim to deliver an additional 15,000 mutants.

Translation of Genetic Insights. The findings from mouse studies can be applied in a myriad of ways to humans through genetic studies. Candidate genes for human disease association studies (eg, polymorphisms in F-box protein 11 are associated with recurrent or chronic OM replicated in 2 separate populations in the United States and Australia) have been identified. Identification of genetic pathways and mechanisms; the interest in identifying the gene underlying *Jeff*, a dominant mouse mutant displaying chronic OM; and a TGF-β signaling mutant highlighted the possible role of TGF-β–induced factor, another TGF-β signaling pathway member (TGF-β–induced factor KO used for the study had an OM phenotype). Possibility of testing the candidate genes identified in *genome-wide association studies* (GWAS) by making mouse models (eg, fat mass obesity gene) is a great opportunity. New regulatory mechanisms were also identified (eg, identification of Evi1 as a negative regulator of NF-κB).[75]

Enhanced Animal Models. In addition to KO alleles, EUCOMM alleles will produce a β-*galactosidase* (LacZ) reporter mouse by crossing to a ubiquitous Cre recombinase line and a conditional floxed allele (the sandwiching of a DNA sequence between 2 lox P sites) by breeding with an Flp recombinase transgene mouse. Availability of various Cre recombinase driver lines will provide opportunities for site-specific or temporal gene deletion. Hypomorphic and

hypermorphic alleles can be generated by screening of the mutagen ENU archive and regenerating mice by in vitro fertilization from sperm. Acute OM challenge models and enhanced models for challenge studies with human pathogens (eg, C3H/HeJ TLR4-deficient background enhances susceptibility to gram-negative bacteria) will provide a platform for studying pathogen-specific patterns. Transgenic mice with humanized receptors (eg, rhinovirus) and chronic OM models (*Junbo* and *Jeff*, 2 novel deaf mutant mice) that developed chronic lifelong disease are new additions to the growing list of animal models.

Translational Opportunities

When designing and developing new treatments, testing the efficacy of drugs (eg, moderation of acute inflammatory response using EGFR kinase inhibitor in the NTHi-challenged model and moderation of hearing loss in the chronic model using VEGFR kinase inhibitor in the *Junbo* mutant mouse) is critical. New models for vaccine research (eg, proposed plan to establish a chronic NTHi infection in *Junbo* mouse model) and testing novel drug delivery systems will be crucial in shaping the translational approaches.

Implications for Clinical Practice

Otitis media is the most common childhood bacterial infection and also the leading cause of conductive hearing loss in children. Despite an obvious need for prophylactic measures, development of highly effective vaccines for OM still remains a great challenge. Moreover, inappropriate antibiotic treatment of OM has increased antibiotic resistance substantially. Currently, there are no effective therapeutic agents available for treating OM due to the poor understanding of the molecular, cellular, and biochemical basis of the pathogenesis of OM. Therefore, development of novel therapeutic strategies is urgently needed for treating OM based on fully investigating the molecular mechanism and identifying the key molecular therapeutic targets. To this end, the following short- and long-term goals in the areas of molecular biology, genetics, biochemistry, and animal model studies need to be pursued. Successfully achieving these goals would ultimately lead to the development of novel therapeutics for treating OM.

Molecular Biology and Genetics of Otitis Media

Short-Term Goals.

1. Further study of host gene expression in OM, including differences in the responses to various pathogens
2. Further study of bioinformatics analysis of gene networks activated in the ME during OM, using genomics and proteomics approaches
3. Further study of pathogen gene expression during OM, including viruses
4. Further study of the interaction of host and pathogen gene expression using both mouse and pathogen mutants as well as genomics such as gene arrays and deep sequencing
5. Identify the gene targets and functional consequences of various cell signaling networks in ME cells
6. Develop transfection, transduction, small interfering RNA (siRNA), and other technologies for in vivo up- and downregulation of genes as well as gene therapies in the ME
7. Use transgenic and mutant bacterial models to understand pathogenesis, virulence, and biofilm formation
8. Study regulatory sequences that target gene expression to the ME and epigenetic modification and its influence on OM pathogenesis
9. Further identify additional mutations that cause OM in human and animals
10. Further identify polymorphisms contributing to susceptibility to OM

Long-Term Goals.

1. Understand how the complex cell signaling pathways and gene regulatory networks are induced to produce various outcomes in OM
2. Translate molecular findings on cell signaling and gene regulation during OM into improvements in patient care by targeting key regulators of pathogenesis and recovery

Biochemistry of Otitis Media

Short-Term Goals.

1. Explore the link between pathogens and pathogen combinations and the production of key molecules such as cytokines, chemokines, growth factors, and bactericidal molecules
2. More precisely define mucus components and their modifications in various types of ME effusions and nasopharyngeal secretions
3. Explore combinatorial strategies for modifying the various phenotypes of ME cells
4. Explore new methods for high-throughput screening in ME cells to identify compounds that have therapeutic potentials

Long-Term Goals.

1. Perform more detailed proteomic analysis of ME, eustachian tube, and nasopharynx during OM to explore posttranslational processing of gene products
2. Define the biochemistry of ME mucin degradation to aid in the development of treatments for mucoid OM
3. Identify diagnostic and prognostic markers of different stages and varieties of OM using various

more advanced biochemical methods, including proteomic and glycobiology methods
4. Develop new biochemistry-based therapeutic strategies

Animal Models of Otitis Media

Short-Term Goals.

1. Further standardize phenotype determination in mouse models of OM
2. Develop better animal models of chronic OM, including sequelae such as cholesteatoma
3. Develop better models of mucoid and serous OM (OME)
4. Develop models of conditional gene expression in the ME, including both site specificity and inducibility
5. Study polymicrobial effects in ME, ET, and nasopharynx in vivo
6. Identify the susceptibility of inbred mouse strains to induced OM
7. Generate mouse models of candidate OM disease genes

Long-Term Goals.

1. Improve all of our existing animal models of OM and continue to develop new models
2. Use diversity of animal models to study OM and to ensure that differences between any one species and humans do not bias our data

Author Contributions

Jian-Dong Li, literature search and review, preparation and discussion of the manuscript, correction and refinement; **Ann Hermansson**, literature search and review, preparation and discussion of the manuscript; **Allen F. Ryan**, literature search and review, preparation and discussion of the manuscript; **Lauren O. Bakaletz**, literature search and review, preparation and discussion of the manuscript; **Steve D. Brown**, literature search and review, preparation and discussion of the manuscript; **Michael T. Cheeseman**, literature search and review, preparation and discussion of the manuscript; **Steven K. Juhn**, literature search and review, preparation and discussion of the manuscript; **Timothy T. K. Jung**, literature search and review, preparation and discussion of the manuscript; **David J. Lim**, literature search and review, preparation and discussion of the manuscript; **Jae Hyang Lim**, literature search and review, preparation and discussion of the manuscript; **Jizhen Lin**, literature search and review, preparation and discussion of the manuscript; **Sung-Kyun Moon**, literature search and review, preparation and discussion of the manuscript; **J. Christopher Post**, literature search and review, preparation and discussion of the manuscript.

Appendix

Abbreviations

Auditory brain stem response	ABR
Acute otitis media	AOM
Activator protein 1	AP-1
Aquaporins	AQPs
Blood labyrinthine barrier	BLB
Chronic otitis media	COM
Cylindromatosis	CYLD
Dynactin subunit 4	DYA4
Epidermal growth factor	EGF
Extracellular signal-regulated kinases	ERK
Ecotropic viral integration site 1	Evi1
European Mouse Disease Clinic	EUMODIC
Forkhead box transcription factor A2	FoxA2
Granulocyte macrophage colony-stimulating factor	GM-CSF
Genome-wide association studies	GWAS
Human middle ear epithelial cell	HMEEC
Inhibitor of differentiation 1	ID1
Interleukin	IL
c-Jun N-terminal kinases/stress-activated protein kinase	JNK
Knockout mouse project	KOMP
Mitogen-activated protein kinase	MAPK
Middle ear effusion	MEE
Middle ear mucosa	MEM
MicroRNA	miRNA
MAPK phosphatase-1	MKP-1
Mucoid otitis media	MOM
Myeloid differentiation primary response gene 88	MyD88
Nuclear factor–κB	NF-κB
North American Conditional Mouse Mutagenesis	NORCOM
Nontypeable *Haemophilus influenzae*	NTHi
Round window membrane	RWM
Ribosomal protein L38	RPL38
Short palate lung and nasal epithelial clone	SPLUNC
Streptococcus pneumoniae	*S pneumoniae*
Thymus and activation-regulated chemokine	TARC
Transforming growth factor–β	TGF-β
Toll-like receptor	TLR
Tumor necrosis factor–α	TNF-α
Tir domain–containing adaptor inducing interferon β	TRIF
Thyroid transcription factor 1	TTF-1
Vascular endothelial growth factor	VEGF

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Panel 5: Microbiology and Immunology Panel

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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.

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

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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

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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

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

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, antimeningococcal 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.

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.

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Panel 6: Vaccines

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

Abstract

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

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

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

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

Keywords

otitis media, vaccines, vaccine antigens, otitis pathogens, candidate antigens

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

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its complications have a significant economic cost for society.

Methods

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

Results

Effectiveness of Pneumococcal Conjugate Vaccine on Incidence of AOM

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

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

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

Progress in the Identification of Vaccine Candidates

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

Pneumococcal Surface Protein A. Pneumococcal surface protein A (PspA) has been a promising vaccine candidate. Recent studies demonstrate sequence heterogeneity among pneumococcal (SP) isolates, and considerable work has gone into identifying PspA epitopes critical for eliciting broadly protective antibodies.[14-16] Previous research focused primarily on the N-terminal alpha helical region of PspA; however, the proline-rich region is antigenically conserved and present in all PspA and the majority of PspC molecules. Recombinant fragments of the proline-rich region elicited cross-clade protective immunity to invasive infection.[17]

Research has also focused on adjuvants for PspA such as whole-cell pertussis,[18] delivery systems such as outer membrane vesicles from *Salmonella*,[19,20] DNA vectors,[21] and mucosal delivery systems that use *Lactobacillus*[22,23] or *Lactococcus*.[24] PspA conjugates have been prepared with polysaccharide capsule 23F, and immunization with such polysaccharide-protein conjugates enhances survival from invasive pneumococcal disease in animals beyond that achieved with either component alone.[25]

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

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

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

Pneumolysin. Pneumolysin (Ply) is pore-forming toxin that is critical for virulence, immunogenic, and conserved across *S pneumoniae* serotypes. Native pneumolysin is not surface expressed and is associated with toxicity. Oloo and colleagues[36] used a structure-based approach to design nontoxic forms of pneumolysin that retained immunogenic and protective epitopes.

Additional Protein Targets. Pneumococcal serine-rich repeat protein (PsrP) is a serine-rich repeat protein that functions as an adhesion and promotes the formation of *S pneumoniae* aggregates.[37,38] Passive immunization with antiserum against recombinant PsrP resulted in lower *S pneumoniae* titers in the lungs and blood but did not affect colonization.[37] Heat shock protein caseinolytic protease (Clp) delivered through the mucosal route increased survival in a murine sepsis model and decreased bacterial counts in a pneumonia model.[39] Pneumococcal choline binding protein A (PcpA) is regulated in a manganese-dependent fashion.[40] Immunization with recombinant PcpA provides protection in pneumonia and sepsis models but does not affect nasopharyngeal colonization. Sortase A (SrtA) plays a key role in pathogenesis and is highly conserved at the DNA level.[41] SrtA intraperitoneal immunization protects against intraperitoneal challenge but not lung infection or colonization. Bacterial counts in the lung were reduced following intranasal immunization. Polyamine transport operon (potD) is membrane associated and antigenically conserved among pneumococci.[42] Recombinant PotD was used for mucosal immunization of mice. Significantly lower counts of bacteria were observed in nasal washes and brain tissues but not in the lungs and olfactory bulbs. Pneumococcal protective protein A (PppA) expressed on *Lactococcus lactis* generates mucosal and systemic immunity and may provide protection against multiple *S pneumoniae* serotypes.[43]

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

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

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

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

Haemophilus influenzae

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

P6. Although regarded as a highly conserved antigen, a recent study of 151 NTHi respiratory isolates reported variant P6 proteins in 9% of strains.[54] Another study from the same group used molecular modeling, site-directed mutagenesis, and nuclear magnetic resonance (NMR) spectroscopy to characterize the P6 protein and concluded that P6 was not a transmembrane protein at all and was unlikely to be exposed on the bacterial surface or accessible to host antibody.[55] Noda and coworkers[56] attempted to identify P6 peptides that might be incorporated into a universal vaccine and identified promiscuous T-cell epitopes that could, in theory, provide broad-based protection against NTHi disease. Using P6 as a model vaccine antigen, Kodama and coworkers reported studies investigating a variety of different immunization protocols intended to induce a protective mucosal antibody response in a murine model of NTHi nasopharyngeal colonization. They found that administration of CpG oligodeoxynucleotides,[57] fms-like tyrosine kinase receptor-3 ligands,[58] α-galactosylceramide,[56] or plasmid DNA encoding P6 given with immunostimulatory complexes[59] was each capable of inducing an enhanced mucosal antibody response in the nasopharynx and more rapid clearance of NTHi from the nasopharynx. Sabirov and coworkers[60] reported a series of studies characterizing the antibody response of children with NTHi otitis media directed against P6. They reported that breastfed infants experienced a lower incidence of AOM and had higher levels of P6-specific serum antibodies, and these antibody levels correlated with the level of serum bactericidal activity. In a subsequent study of otitis-prone children and non–otitis-prone controls, the authors found that otitis-prone children had lower levels of anti-P6 antibodies in their acute phase sera and demonstrated a much decreased antibody response to P6 (and OMP 26 and protein D) in their convalescent sera, providing a possible explanation for the increased risk of infection in otitis-prone children.[61]

Pilin and OMP P5. Novotny and coworkers[62] continued their studies of OMP P5 and the type IV pilin protein of NTHi as potential vaccine candidates. This group constructed a chimeric peptide vaccine consisting of a P5-derived B-cell epitope fused to the N-terminus of recombinant soluble pilin and reported protection against infection in a passive immunization model. In subsequent work, this group reported that transcutaneous immunization with an OMP P5 peptide, a pilin-derived peptide, and the P5-pilin fusion peptide just described were each capable of providing protection in the chinchilla otitis media model and also enhanced resolution of preformed biofilms.[63]

Protein D. The prior Vaccine Report included the results of the POET study, which employed an 11-valent pneumococcal polysaccharide vaccine with a protein D carrier and demonstrated protection against both vaccine-type pneumococcal otitis and disease due to NTHi.[64,65] These clinical studies failed to demonstrate a clear correlation between serum antibody levels in protein D–immunized children and the observed protection seen against NTHi disease. To better understand the potential mechanisms of protection, investigators constructed a protein D knockout mutant. They demonstrated that the mutant exhibited reduced adherence, diminished amounts of ChoP in NTHi biofilms, and diminished survival in vivo, and antibody against protein D had similar effects on the phenotype of the bacteria.[66] A functional assay measuring inhibition of protein D phosphodiesterase activity in serum samples of children immunized with the protein D conjugate vaccine was described, and a modest, but imperfect, correlation was noted between serum protein D antibody levels and enzymatic inhibition.[67] A follow-up clinical study of the protective ability of the protein D conjugate vaccine against NTHi nasopharyngeal carriage in a population of young children demonstrated marginal protection.[65]

HMW1/HMW2 and Hia. Winter and coworkers[68] continued studies of the NTHi high molecular weight proteins and their potential as vaccine candidates. One study reported that the Hia autotransporter proteins of NTHi are targets of opsonophagocytic antibodies and that shared epitopes recognized by such antibodies are present on the Hia proteins of unrelated NTHi strains. Another study from that same group

reported the construction of recombinant adenovirus vaccines that expressed the HMW1/HMW2 or Hia proteins of prototype NTHi strains and demonstrated the immunogenicity of the constructs given by either the parenteral or intranasal route in the chinchilla model.[69]

Lipooligosaccharide. Hong and coworkers[70] continued their investigations of NTHi lipooligosaccharide as a potential vaccine candidate. They described a mucosal vaccination approach with a detoxified lipooligosaccharide-tetanus conjugate vaccine. They reported that intranasal immunization of chinchillas with the conjugate vaccine was associated with decreased nasopharyngeal colonization and decreased otitis media development in an NP challenge model and with earlier clearance of bacteria and lesser disease severity in an intrabullar challenge model.

Moraxella catarrhalis

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

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

A genome mining approach, which has been successful in several bacterial species, has been applied to *M catarrhalis* and has resulted in the identification of several new promising vaccine antigens.[77-79] Ruckdeschel et al[77] examined genome sequences from *M catarrhalis* to identify open reading frames that encode 348 putative surface-expressed proteins. Three *Moraxella* surface proteins, *msp22, msp75*, and *msp78*, were shown to be conserved among a collection of clinical isolates; are transcribed during in vitro growth; and are expressed during carriage in the respiratory tract of patients with chronic obstructive pulmonary disease (COPD). These genes exhibited homology to cytochrome c, class II, succinic semialdehyde dehydrogenase, and an outer membrane nitrite reductase from *Neisseria*, respectively. A subset of patients with COPD demonstrated systemic and mucosal antibody responses to each protein after acquisition and clearance of *M catarrhalis*. Thus, these proteins appear to be expressed in the respiratory tracts and immunogenic.[77] A follow-up study demonstrated that antisera to recombinant Msp22 and Msp75 recognizes the native protein in heterologous strains. Both subcutaneous and intranasal immunization with recombinant proteins resulted in enhanced clearance of *M catarrhalis* in the murine pulmonary clearance model.[78] Oligopeptide permease A (OppA) was also identified in the report by Ruckdeschel et al.[77] Despite the predicted periplasmic location of OppA based on homology analyses, OppA was shown to express epitopes on the surface of *M catarrhalis* by flow cytometry and whole-cell enzyme-linked immunosorbent assay (ELISA). OppA is highly conserved among *M catarrhalis* isolates from subjects with otitis media or COPD. Intranasal immunization with recombinant OppA results in enhanced clearance in the mouse pulmonary clearance model.[79]

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

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

Viral Vaccines

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

Marchisio et al[82] reported the use of a newer formulation of an inactivated, injectable influenza vaccine that had the neuramindase and hemagglutinin antigens of the donor viruses integrated into the lipid membrane of the virosome (Inflexal V; Berna Biotech, Milan, Italy). The vaccine was tested in Italian children aged 1 to 5 years with a history of recurrent AOM and previously unvaccinated against influenza. Half of the children (n = 90) received the vaccine, whereas the other half (n = 90) received no vaccine. The number of children experiencing at least 1 AOM episode was significantly smaller in the vaccinated group, as was the mean number of AOM episodes, the mean number of AOM episodes without perforation, and the mean number of antibiotic courses. The mean duration of bilateral otitis

Table 1. Potential Vaccine Antigens of *Moraxella catarrhalis*

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

[a]Indicates that immunization with antigen induces enhance clearance in the mouse pulmonary clearance model.
[b]References from 2007 to present.

media with effusion was also significantly shorter. The vaccine had significantly greater efficacy in preventing AOM in the absence of a history of recurrent perforation. Based on this study, it is not clear if this new virosome formulation of the vaccine is better than the standard injectable vaccine for protection against AOM.

Bracco Neto et al[83] investigated the efficacy and safety of 1 vs 2 doses of live attenuated influenza vaccine (LAIV) in 3200 influenza vaccine–naive Brazilian children aged 6 to <36 months over a 2-year period. The 2-dose regimen in year 1 was significantly effective against all febrile episodes of AOM (34% efficacy compared with placebo) and all episodes of influenza-associated AOM caused by strains antigenically similar to those in the vaccine (74% efficacy). The 1-dose regimen was also similarly effective. A similar trend was observed in the second year of study. However, this study does not allow us to compare the efficacy of live, intranasal vaccine with that of killed vaccine for protection against AOM. Nonetheless, this study from a country with developing economy produced results similar to the previous studies in the developed nations of North America and Europe.

Although influenza and pneumococcal vaccines independently show protection against AOM, it may be postulated that the combined effect of both vaccines may be more robust. However, this assumption was proven not to be correct. Jansen et al[84] evaluated the effects of influenza vaccination with or without heptavalent pneumococcal conjugate vaccination on respiratory tract infections (RTIs) in 579 Dutch children aged 18 to 72 with a previous history of physician-diagnosed respiratory infection. The children were assigned to 2 doses of parenteral inactivated trivalent subunit influenza plus heptavalent pneumococcal conjugate vaccination (TIV+PCV7), influenza plus placebo vaccination (TIV+plac), or control hepatitis B virus vaccination plus placebo (HBV+plac). During influenza seasons, febrile RTIs were reduced by 24% in the combined TIV+PCV7 group and by 13% in the TIV-alone group compared with the control group. However, episodes of AOM were reduced by 57% in the TIV+PCV7 group and by 71% in the TIV-alone group. The reasons for the reduced efficacy for the combined vaccines as compared with TIV alone are not clear.

Measles is well known to cause suppurative otitis media as a complication. Although measles has been well controlled through the use of a live, injectable vaccine for the past several decades, its impact on AOM and other long-term otologic complications has not been published. Recently, Arnold et al[85] studied the influence of measles vaccination on the incidence of otosclerosis in Germany. The pathologic process of otosclerosis is characterized by an inflammatory lytic phase followed by an abnormal bone remodeling at very specific sites of predilection. There is a clear genetic predisposition, with about half of all cases occurring in families with more than one affected member. N, H, and F measles proteins as well as measles virus RNA have been demonstrated in osteoblasts, chondroblasts, and macrophages of the inflammatory phase of the disease. In the absence of official data, the investigators reconstructed the rate of vaccination coverage between 1974 and 2004 using information from the Robert Koch Institute and from the literature. Between 1993 and 2004, the incidence of hospital treatments for otosclerosis decreased to a significantly greater extent in the vaccinated patients than in the

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

Discussion

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

Prevention of AOM would have its highest value in children at risk for recurrent otitis media; children at risk for development of CSOM, including indigenous peoples; and children at risk for comorbidities associated with recurrent AOM such as persistent otitis media with effusion, conductive hearing loss, and language delay. The challenge is that studies to date suggest that pneumococcal conjugate vaccine is not effective when initially administered to children who are already suffering from recurrent otitis media,[86] suggesting that prevention of early episodes is critical or that otitis-prone children may have suboptimal immune response to vaccines. Dagan and colleagues (personal communication, 2011) recently reported that early disease is more often due to vaccine serotypes of *S pneumoniae*. They hypothesize that nonvaccine serotypes, NTHi, and *M catarrhalis* often are ''opportunistic'' invaders that follow damage to the middle ear after early, recurrent otitis occurs. Clinical trials of PCV in California and Finland reported efficacy for children beginning after the primary series of immunizations at 7 months of age.[87,88] Poehling et al[89] reported a decline in invasive pneumococcal disease in the first 90 days of life, prior to expected protection from the direct effect of immunization, presumably as a result of indirect protection. Prevention of early episodes is potentially one strategy for reducing the burden of middle ear disease in childhood. Both the induction of immunity early in life as well as the impact of early disease on subsequent risk should be a focus of research activity for both industry and government (National Institutes of Health [NIH]). Indeed, maternal immunization strategy needs to be investigated with respect to the benefit to the infant.

Despite progress in identifying and characterizing promising candidate antigens from *S pneumoniae*, NTHi, and *M catarrhalis*, moving them from animal studies to human studies is a critical step that has limited progress in the prevention of AOM for a number of reasons. Among them, feasibility and enthusiasm for a vaccine that targets primarily AOM pathogens is a significant hurdle for the pharmaceutical industry, which may consider prevention of AOM to be of limited marketplace potential. The pneumococcal conjugate vaccines have been developed primarily to address invasive pneumococcal disease, usually defined as invasive disease and pneumonia. PHiD-CV, a pneumococcal conjugate vaccine that incorporates protein D as a carrier as well as to elicit protection against disease due to NTHi, was developed to extend prevention to include middle ear pathogens beyond the pneumococcus. Further work defining the value of AOM prevention and where such a vaccine strategy fits into the priorities for child health would provide guidance to industry as to whether such vaccines are likely to be viewed as cost-effective and can be fiscally responsible investments. If the model is to build on a pneumococcal polysaccharide conjugate vaccine, then encouraging industry-academic collaborations will be a critical piece in developing a future vaccine combination that will include AOM pathogens among the targets for prevention.

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

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

evaluating identical antigens in 2 or more models, and limited appreciation of the burden of middle ear disease on child health and specifically its global impact must be solved if we are to hasten the pace of progress.

Selected Future Research Objectives

1. Induction of early immune protective immune responses
2. Evaluation of maternal immunization for prevention of AOM
3. Development of correlates of protection for acute bacterial otitis media
4. Definition of the role of potential vaccine antigens in the pathogenesis of AOM
5. Evaluation of the candidate antigen in human trials

Implications for Practice

The recurrent nature of acute otitis media continues to be burdensome to children and families, especially those who suffer from frequent recurrences and in disadvantaged populations where disease progresses to chronic suppurative otitis media with associated impacts on hearing loss and educational potential. PCV7 has reduced the burden of vaccine-serotype disease as well as shifted the pneumococcal serotypes carried in the nasopharynx toward those with lower disease-causing potential. Antibiotic resistance remains a challenge to successful therapy with ceftriaxone-resistant pneumococci present in the community and increasing emergence of β-lactamase–negative, amoxicillin-resistant NTHi identified globally. The next-generation PCV13 has been introduced, and early data suggest efficacy against invasive pneumococcal disease and carriage of SP19A, the multidrug resistance isolate that has been associated with both treatment failure in AOM[90] and the increasing number of cases of pneumococcal mastoiditis.[91] Promising data on an 11-valent pneumococcal polysaccharide conjugate vaccine with protein D as a carrier was published in 2006,[65] but additional confirmation of efficacy against NTHi otitis media with the licensed formulation, PHiD-CV (a 10-valent conjugate), is pending data from the COMPAS trial in South America.

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

Author Contributions

Stephen I. Pelton, coordination of postsymposium meeting, substantial contributions to writing and editing; **Melinda M. Pettigrew**, coordination of postsymposium meeting, substantial contributions to writing and editing; **Stephen J. Barenkamp**, participation in postsymposium meeting and substantial contributions to writing; **Fabrice Godfroid**, participation in postsymposium meeting and substantial contributions to writing; **Carlos G. Grijalva**, participation in postsymposium meeting and substantial contributions to writing; **Amanda Leach**, participation in postsymposium meeting and substantial contributions to writing; **Janak Patel**, participation in postsymposium meeting and substantial contributions to writing; **Timothy F. Murphy**, participation in postsymposium meeting and substantial contributions to writing; **Sanja Selak**, participation in post symposium meeting and substantial contributions to writing; **Lauren O. Bakaletz**, contributions to conception and design of postmeeting conference and revision and approval of manuscript.

Disclosures

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Panel 7: Treatment and Comparative Effectiveness Research

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Abstract

Background and Objectives. Otitis media (OM) is one of the most common reasons for antibiotic treatment in children. Controversies regarding antibiotic treatment for OM have accumulated in the past decade, and there seem to be more dilemmas than certainties. The objectives of this article are to provide the state-of-the art review on achievements in treatment of all different stages of OM, including acute otitis media (AOM), otitis media with effusion (OME), and chronic suppurative otitis media, and to outline the future research areas.

Data Sources. PubMed, Ovid Medline, the Cochrane Database, and Clinical Evidence (BMJ Publishing).

Review Methods. All types of articles related to OM treatment published in English between January 2007 and June 2011 were identified. A total of 286 articles related to OM treatment were reviewed by the panel members; 114 relevant quality articles were identified and summarized.

Results. New evidence emerged on beneficial results of antibiotic treatment, compared with observation of AOM in young children who were diagnosed based on stringent criteria. In OME, the main results were related to a nonsignificant benefit of adenoidectomy versus tympanostomy tube placement alone in the treatment of chronic OME in younger children. Other modalities of OM treatment were studied and described herein.

Conclusions and Implications for Practice. Significant progress has been made in advancing the knowledge on the treatment of OM. Areas of potential future research have been identified and outlined.

Keywords

otitis media, treatment, antibiotics

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Controversies regarding treatment for otitis media (OM) have accumulated for nearly a decade, and there seem to be more dilemmas than certainties.[1,2] Diagnosis is often a challenge in everyday practice of pediatricians, who have to cope with difficulties in performing and interpreting otoscopy.[3,4] Conflicting diagnoses between pediatricians and otolaryngologists have been reported, suggesting differences in diagnostic instruments and skills.[5] Despite the dilemmas, and direct and indirect consequences, the costs and the negative impact on the quality of life of the patients, their family, and the community are significant. Optimizing treatment for all the different stages of OM is desirable.

The Post-symposium Research Conference was sponsored by the National Institute on Deafness and Other Communication Disorders and was held in New Orleans, Louisiana, on June 9 and 10, 2011, immediately following

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Abbreviations: A, adenoidectomy; AOM, acute otitis media; CI, confidence intervals; CSOM, chronic suppurative otitis media; M, miringotomy; MEE, middle-ear effusion; MEF, middle-ear fluid; OM, otitis media; OME, otitis media with effusion; OR, odds ratio; PCV, pneumococcal conjugate vaccine; RCT, randomized controlled trial; T, tympanostomy tube; TTO, tympanostomy-tube, otorrhea.

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the 10th International Symposium on Recent Advances in Otitis Media. Taking into account the goals of the Research Conference, The Panel on Treatment convened (1) to critically review the most recent advances and breakthroughs made during the 4 years since the previous symposium on questions related to treatment of OM and (2) to identify new research opportunities and critical research questions.

Methods

A multidisciplinary panel of clinician experts in OM was identified by the Scientific Committee of the Research Conference. Specifically, the panel included experts in the fields of general pediatrics, otorhinolaryngology, microbiology, pharmacology, and infectious diseases. The Panel was formed in December 2010, and many of the consultations involved in the report development and draft processes took place interactively by email or telephone contact. The panel members first agreed on the objectives of the report, the essential clinical questions, and the appropriate inclusion and exclusion criteria for the studies to be included. They also identified the information sources and biomedical databases that would be consulted and the search terms that would be used in constructing the search strategy. Once the specific clinical questions were developed, literature searches were performed for each question. The literature search included PubMed, Ovid Medline, the Cochrane Database (Wiley Publishing), and Clinical Evidence (BMJ Publishing), supplemented by additional articles identified by panel members after review of each document. Evidence-based guidelines, systematic reviews of randomized controlled trials (RCTs), and RCTs were described in detail. The results of observational study designs were described briefly. We did not include publications that had been cited in the previous review.[6] The date range for the literature search was January 2007, with the most recent search being June 30, 2011.

Search terms included *otitis media* and/or *acute otitis media* and/or *otitis media with effusion* and/or *chronic suppurative otitis media* either in combination with, or separate to, the following terms: *treatment, practice guidelines, discharge, otorrhea, prevention, tympanostomy tubes, adenoidectomy*, and *surgery*. The only restriction on search was English language. There was no restriction on the type of articles (included original articles, reviews, meta-analyses, etc).

The initial literature search identified 286 potential articles, which was reduced to 240 after excluding narrative (nonsystematic) review articles and letters to the editor. The exclusion decision was made by group consensus. A final data set of 114 articles was obtained after excluding small case series (5 or fewer participants), research performed using animal models, articles dealing only with surgical technique, tutorial or dissemination articles, and research for which the condition of interest was poorly defined.

Panel members reviewed assigned areas, wrote initial drafts summarizing the areas, and shared the drafts with members of the subpanels (acute otitis media [AOM]; otitis media with effusion [OME]; chronic suppurative otitis media [CSOM]; recurrent AOM). A draft of the full document was circulated to all panel members before convening at the 10th Symposium in New Orleans, where the panel met, reviewed the draft, discussed the literature, and developed research goals for the next 4 years. A revised draft of the report was circulated to the panel for further comments and approval following the meeting at the Symposium.

Discussion

Key findings are summarized based on the following major categories: AOM, recurrent AOM (rAOM), OME, tympanostomy tube othorrea (TTO), and CSOM.

Acute Otitis Media

Clinical practice guidelines on the management of AOM have been issued from various countries around the globe since 2000. Currently, there are nearly 20 published national or regional AOM guidelines.[7] One common feature of the guidelines has been the recommendation of an initial observation option without antibiotic treatment in selected patients, mainly for those older than 2 years. The other common feature has been the recommendation for amoxicillin as the first-line antibiotic, while specifying alternative antibiotic drugs. Recent studies had focused on various aspects of the AOM guideline recommendations, including trials comparing antibiotic versus placebo and/or observation. There have been very few new prospective trials comparing alternate antibiotics to the usually recommended antibiotic, amoxicillin.

Antibiotic versus Placebo: Randomized, Double-Blind Trials in Young Children. As more and more published studies have shown resolution of AOM without antibiotic treatment in a good proportion of children, especially in those older than 2 years, the benefit and cost-effectiveness of antibiotics in improving AOM clinical outcome has become questionable. Nevertheless, most clinical practice guidelines still recommend immediate antibiotic for AOM in children younger than 2 years because there had been no definitive study performed in a large number of young children. Two trials from the United States and Finland were the first to compare the effect of antibiotic versus placebo for AOM in young children in a randomized, double-blind, placebo-controlled setting. The significance of these 2 studies described below also lies in the definitive AOM diagnostic criteria and careful study design.

The criteria for AOM diagnosis in the study performed in Pittsburgh by Hoberman et al[8] included acute symptoms with specific AOM severity of symptom score (AOM-SOS, 0-14) of at least 3, middle-ear effusion (MEE), and moderate or marked bulging of the tympanic membrane or slight bulging accompanied by either otalgia or marked erythema of the membrane. To have maximal antimicrobial coverage, high-dose amoxicillin-clavulanate (90 mg/kg per day) was chosen as the active treatment. Altogether, 291 patients

aged 6 to 23 months were included: 144 in the antibiotic group and 147 in the placebo group.

The primary outcome measures were the time to resolution of symptoms and the symptom burden over time. The initial resolution of symptoms (ie, the first recording of an AOM-SOS score of 0 or 1) was 35%, 61%, and 80% among children who received amoxicillin-clavulanate and 28%, 54%, and 74% among children who received placebo by days 2, 4, and 7, respectively ($P = .14$ for the overall comparison). For sustained resolution of symptoms (ie, the time to the second of 2 successive recordings of an AOM-SOS score of 0 or 1), the corresponding values were 20%, 41%, and 67% with amoxicillin-clavulanate as compared with 14%, 36%, and 53% with placebo ($P = .04$ for the overall comparison). The symptom burden (ie, mean AOM-SOS scores) over the first 7 days was lower for the children treated with amoxicillin-clavulanate than for those who received placebo ($P = .02$).

Treatment failure by the day 4 to 5 visit was defined as no improvement in symptoms, worsening of otoscopic examination, or both, and treatment failure at the day 10 to 12 visit was defined as lack of resolution of symptoms and of otoscopic signs (excluding MEE). Treatment failure occurred by day 4 to 5 in 4% of the antimicrobial treatment group and 23% in placebo group (number needed to treat [NNT] = 6, $P < .001$) and at day 10 to 12 in 16% versus 51% (NNT = 3, $P < .001$). The most common adverse events were expectedly diarrhea (25% vs 15%; $P = .05$) and diaper dermatitis (51% vs 35%; $P = .008$). One placebo recipient developed mastoiditis.

Tähtinen et al[9] studied 319 children aged 6 to 35 months; 161 in the antibiotic group (amoxicillin-clavulanate, 40 mg/kg per day) and 158 in the placebo group. The definition of AOM was the presence of MEE, distinct erythema over a bulging or yellow tympanic membrane (TM), and acute symptoms. Compliance was measured using daily patient diaries and number of capsules remaining at the end of the study. Primary outcome was time to treatment failure defined as a composite of 6 independent components: no improvement in overall condition by day 3, worsening of the child's condition at any time, no improvement in otoscopic signs by day 8, perforation of the TM, development of severe infection (eg, pneumonia, mastoiditis), and any other reason for stopping the study drug/placebo. Groups were comparable on multiple characteristics: 85% of patients in both groups used analgesic medicines. Of the patients, 135 of the 161 (84%) of the treatment group and 124 of 158 placebo patients (78%) were <24 months old.

Treatment failure occurred in 18.6% of the treatment group and 44.9% of the placebo group (NNT = 4, $P < .001$). Rescue treatment was needed in 6.8% of the treatment group and 33.5% of placebo patients (NNT = 4, $P < .001$). Contralateral AOM developed in 8.2% and 18.6% of treatment and placebo groups, respectively ($P = .007$). There was no significant difference in use of analgesic or antipyretic medicine. Parents of daycare attendees on placebo missed more days of work ($P = .005$). Adverse events, primarily diarrhea and/or rash, occurred in 52.8% of the treatment group and 36.1% of the placebo group ($P = .003$). Overall condition as evaluated by the parents and otoscopic appearance of the TM showed a benefit of antibiotics over placebo at the end of the treatment visit ($P < .001$).

These 2 trials have clearly demonstrated a short-term benefit of antimicrobial treatment (amoxicillin-clavulanate) over placebo for clinical resolution as defined in the trials. It is worthy to note that about half of the patients who received placebo did not have treatment failure and two-thirds did not need rescue treatment, indicating that even in young children 6 to 35 months of age with carefully diagnosed AOM may do well without antibiotics.

Antibiotic versus Initial Observation. There have been continued reports on the positive outcome of initial observation without antibiotic treatment for AOM. In the pediatric emergency department setting, observation therapy reduces antibiotic use without compromising satisfaction with the visit. In a prospective randomized trial, Chao et al[10] compared 2 approaches of observation therapy for AOM in the emergency department: observation therapy with and without a prescription. All 232 children (aged 2-12 years) were provided with pain relief medication. A total of 81% of the observation group reported no use of antibiotics, compared with 53% in the prescription group. The authors concluded that adherence to delayed antibiotic therapy was better for those not offered a prescription. In another prospective case series with telephone follow-up of an observation option in the emergency department, parents of children aged ≥2 years with AOM were given wait-and-see antibiotic prescriptions.[11] A total of 105 of 144 (73%) patients enrolled in the study recovered without requiring antibiotics. Vomiting or diarrhea was noted in 28% of the antibiotic group and 6% of the group without. No complications such as mastoiditis or meningitis were noted among the study participants. Parents were supportive of the observation option.

A study from Serbia evaluated the necessity of antibiotic treatment in children with AOM (6 months to 6 years).[12] Children with less severe disease (nonbulging TM erythema and fever ≤38.5°C) were treated with watchful waiting (n = 237); children with purulent AOM, middle-ear fluid (MEF), and/or bulging of the TM and fever >38.5°C were treated with immediate antibiotics (n = 77). Resolution of symptoms occurred in 81% of children in the less severe group, many of whom may not have fit the stringent criteria of AOM. Of 123 children treated with antibiotics at any time during the course, 63% had recovery; the remaining experienced 1 or more relapses. The investigators supported the wait-and-see approach in children with AOM without serious signs and symptoms.

In a meta-analysis of 7 RCTs on antibiotics versus placebo or watchful waiting for AOM, clinical success was more likely with antibiotics than the comparators in placebo-controlled trials, watchful waiting trials, and all trials combined.[13] Persistence of symptoms 2 to 4 days after treatment initiation was less likely with antibiotics. The

pooled risk ratio for placebo versus immediate antibiotics was 1.10 (confidence interval [CI], 1.05-1.18) and for watchful waiting trials was 1.18 (CI, 1.07-1.32). Overall, diarrhea was more likely in patients treated with antibiotics than in the comparator treatments. The authors concluded that the clinical significance of the results needed careful interpretation since the benefits of antibiotics over placebo in achieving a favorable clinical course did not appear to be large.

Spiro and Arnold[14] reviewed current guidelines and contemporary management of AOM, including symptomatic care, rationale and literature for using the wait-and-see approach, and indications of immediate therapy with antibiotics. The authors concluded that there is marginal benefit from antibiotics for most children with AOM and proposed an algorithm for AOM management. Indications for use of an immediate antibiotic for AOM included (1) age <6 months, (2) ill-appearing, (3) suspicion of another bacterial illness, (4) rAOM, (5) compromised immunity, (6) patient treated with an antibiotic within 7 days of presentation, (7) perforation of TM, (8) uncertain access to medical care, (9) hearing impairment, and (10) craniofacial anomalies. In older children without the foregoing risk factors, the authors recommended appropriate pain management and a wait-and-see antibiotic prescription; they noted that two-thirds of families receiving a wait-and-see prescription will not fill it. Although the cited data were not entirely supportive of the following conclusion, the authors stated that the wait-and-see approach is safe, empowers families, and will reduce the development of antibiotic resistance and the cost and side effects of antibiotics.

Antibiotic Treatment: Clinical Trials/Retrospective Studies/Meta-analyses and Reviews. A multicenter study was performed in the United States and Costa Rica: 1650 infants and young children (6 months to <5 years) with recurrent or persistent AOM were enrolled in an evaluator-blinded, noninferiority, randomized comparative study of levofloxacin (10 mg/kg twice daily) versus amoxicillin/clavulanate (14:1; amoxicillin 45 mg/kg twice daily).[15] A total of 1305 (79%) were clinically evaluable; clinical cure rates at 2 to 5 days after completing therapy were 72.4% in levofloxacin-treated and 69.9% in amoxicillin/clavulanate-treated children. Levofloxacin was not inferior to amoxicillin/clavulanate for the treatment of recurrent and/or persistent AOM.

In a small prospective, comparative, open randomized trial from Turkey, 104 children with AOM (6 months to 10 years of age) were evaluated. Short-course antimicrobial therapies: single intramuscular (IM) ceftriaxone (50 mg/kg) and 5 days of azithromycin (10 mg/kg on day 1, then 5 mg/kg daily on days 2-5) were compared with the traditional 10-day course of amoxicillin-clavulanate (90/6.4 mg/kg per day in 2 doses).[16] Clinical success was achieved in 85.3% in the ceftriaxone group, 87.1% in the azithromycin group, and 87.2% in the amoxicillin-clavulanate group. The rate of persistence of MEF at day 30 did not differ between the 3 groups. The children in this study were relatively older (mean age, 3.8 ± 2.3 years).

A double-blind RCT compared the clinical effectiveness of single-dose azithromycin with 7 days of amoxicillin (50 mg/kg per day in 2 doses) for AOM in 306 Aboriginal children (6 months to 6 years of age) in rural and remote communities.[17] Single-dose azithromycin did not reduce or increase the risk of clinical failure (50%) compared with amoxicillin (54%). Azithromycin significantly reduced ($P < .001$) the proportion of children with nasal carriage of *Streptococcus pneumoniae* and nontypeable *Haemophilus influenzae*. Nasal carriage of *S pneumoniae* with intermediate or full resistance to penicillin was lower (not significant) in the azithromycin group (10% vs 16%), but this group had significantly increased carriage of azithromycin-resistant *S pneumoniae* ($P = .001$). Carriage of β-lactamase–producing nontypeable *H influenzae* was 5% in both groups. In this high-risk population, the treatment failure rate was high in either group; poor compliance may have played a role in the higher than expected failure rate in the amoxicillin group.

Investigators in Sweden performed a nonblinded, prospective randomized trial of 268 children with AOM (2 to 13 years of age), seen by 72 general practitioners, to compare the effect of oral penicillin V or placebo.[18] Parents completed a diary recording the child's symptoms. The cumulative number of recoveries by day was similar in the 2 groups. The median recovery time was 4 days in each group. Children in the penicillin group had less initial pain compared with children in the placebo group, but pain was not different between the groups after 2 days of treatment. Children randomized to no antibiotic used more pain medication. Subsequent clinic visits for perforation, ear pain, and hearing disturbance were higher in the nonantibiotic group, but treatment failures and perforations were not different. The authors concluded that the benefits of antibiotic treatment in children with AOM are limited and supported watchful waiting as an option in the treatment of uncomplicated AOM in children aged 2 to 16 years.

In a retrospective study of 50 patients, Brook and Gober[19] compared the effects on the nasopharyngeal flora in children with AOM treated with low-dose (45 mg/kg per day) or high-dose (90 mg/kg per day) amoxicillin. The number of penicillin-susceptible isolates was equally reduced after both therapies. *Staphylococcus aureus* recovery was increased in the high-dose group, along with depletion of microbial flora such as α-hemolytic streptococci, Peptostreptococcus, and Prevotella species. Another retrospective review was performed on 111,335 AOM visits for children (aged 2 months to 12 years) between 1996 and 2004 in a large-group practice, using computerized data.[20] The incidence of AOM decreased from 385.1 visits/1000 enrollees in 1996 to 188.8 visits in 2004. The proportion of cases treated with high-dose amoxicillin increased from 1.7% to 41.9%. Both treatment failure and relapse rates decreased from 1996 to 2004 (from 3.9% to 2.6% and from 9.2% to 8.9%, respectively). The odds of treatment failure or relapse did not differ between AOM episodes treated

with high-dose and low-dose amoxicillin. AOM, treatment failure, and relapse became less common and high-dose amoxicillin use increased, but high-dose amoxicillin did not reduce the risk of individual infections, resulting in adverse outcomes. The authors related their findings to change in treatment thresholds for AOM among the practicing physicians (most probably in response to pressure to decrease the antibiotic prescriptions) rather than as a result of the introduction of the pneumococcal vaccine.

Pichichero and Reed[21] aimed to define the biologic variations in amoxicillin pharmacokinetic/pharmacodynamic (PK/PD) parameters for AOM treatment in children and assess whether these variations could explain why the commonly employed amoxicillin PK/PD model is imperfect in predicting outcome for every patient in this clinical setting. A literature search was conducted to identify studies that evaluated amoxicillin intestinal absorption, serum concentrations, and/or MEF concentrations. Results showed that the intestinal bioavailability of amoxicillin depends on passive diffusion and a saturable pump mechanism that produces variable serum concentrations of the drug. Substantial differences from patient to patient in serum (5- to 30-fold) and MEF (up to 20-fold) concentrations of amoxicillin occur following oral administration, and 15% to 35% of children have no detectable amoxicillin in MEF. These findings suggest that variability in PK/PD parameters may affect amoxicillin concentrations in serum and MEF, possibly explaining some AOM treatment failures. Dagan et al[22] determined the association between early bacteriologic failure and clinical failure in AOM by analyzing 907 AOM episodes in children (aged 3-35 months). Clinical failure occurred in 7.3% of patients with bacterial eradication and 32.8% of patients with bacteriologic failures. The overall unadjusted relative risk for clinical failure was 4.41 (confidence interval [CI], 3.19-6.11). After adjustment for any differences in age, sex, ethnic origin, previous otitis history, and previous antibiotic treatment, the rate was 6.52 (CI, 4.26-9.99). Across clinical studies with 8 antibiotic drug regimens for AOM, the rate of clinical failure correlated with bacteriologic failure (P = .003). The authors concluded that in young children with culture-positive AOM, failure to eradicate the pathogen from MEF within the first few days of treatment leads to a significant risk for clinical failure.

Two meta-analyses were published related to antibiotic use in AOM. Courter et al[23] performed a meta-analysis of clinical trials comparing amoxicillin or amoxicillin-clavulanate with macrolide antibiotics, azithromycin or clarithromycin. Ten RCTs, single- or double-blinded, were included (n = 2766 children 6 months to 15 years old). The primary outcome was clinical failure measured between days 10 and 16 after starting antibiotic therapy. The use of macrolides was associated with an increased risk of clinical failure (relative risk [RR], 1.31; CI, 1.07 to 1.60), but the adverse reaction rate was significantly lower. The results support the current American Academy of Pediatrics (AAP) AOM recommendation that macrolides be reserved only for patients who cannot receive first-line antibiotics. Thanaviratananich et al[24] aimed to compare the effectiveness of 1 or 2 daily doses with 3 or 4 daily doses of amoxicillin, with or without clavulanate, for the treatment of AOM. The authors could not draw a firm conclusion as there was insufficient evidence addressing this question.

The intermediate- and long-term effects of AOM treatment with no antibiotic have not yet been carefully studied. Koopman et al[25] attempted to assess the effect of antibiotic therapy in preventing the development of MEE and to determine predictors of the development of asymptomatic MEE by performing a meta-analysis of 5 randomized controlled trials (1328 children 6 months to 12 years). The overall relative risk of antibiotic therapy in preventing the development of asymptomatic MEE after 1 month was 0.9 (CI, 0.8-1.0). Independent predictors of the development of asymptomatic MEE were age younger than 2 years and rAOM.

Bezáková et al[26] reported the rate of recurrence obtained from parental survey 3.5 years after a double-blind trial of 240 Dutch children (aged 6 months to 2 years); children in the original study were randomized to receive low-dose amoxicillin for 10 days or placebo. One hundred sixty-eight (70%) parents returned the questionnaire addressing the history of rAOM; referral for specialist care; and ear, nose, and throat surgery in the period 6 months to 3 years after the trial. rAOM occurred in 63% and 43% of children randomized to amoxicillin and placebo (RR, 1.5; CI, 1.1-2.0). The referral rate and surgery rate were not significantly different between groups: 31% and 21% in the amoxicillin group and 30% and 30% in the placebo group, respectively. Results of these 2 studies are not conclusive for the benefit of antibiotic treatment on duration of MEE and recurrences.

Acute Otitis Media: Clinical Practice Guidelines and Related Studies. A summary of the Italian guideline (Italian Society of Pediatric Otolaryngology and Italian Society of Pediatrics) was recently published.[7] This guideline was written to update prior guidelines and address unique characteristics in Italy. The guideline deals with AOM in otherwise healthy children, 2 months to 12 years of age, and also addresses issues on AOM diagnosis, complications, and prevention. For AOM treatment, systemic analgesics are recommended for earache. Immediate antibiotic treatment is recommended for certain AOM cases in children younger than 6 months of age. In children 6 to 24 months of age, immediate antibiotic is recommended except in cases of unilateral AOM with mild symptoms for which watchful waiting is recommended. For children older than 2 years, immediate antibiotic treatment is recommended only for children with bilateral AOM and severe symptoms; watchful waiting is recommended for the remaining. Amoxicillin (50 mg/kg per day) is recommended as the first-choice drug, with cefaclor as the alternate drug in cases with mild symptoms and low-risk factors. In children with severe symptoms or at high risk for resistant bacteria, amoxicillin-clavulanate (80-90 mg/kg per day) is recommended as the first-choice drug, with cefpodoxime proxetil or cefuroxime axetil as alternatives. IM

or intravenous ceftriaxone is recommended for treatment failure, while the use of quinolones is discouraged. The recommended duration of antibiotic therapy is 10 days; it can be reduced to 5 days in children older than 2 years. The use of other treatments such as systemic or topical decongestants, steroids, or antihistamines is not recommended. The panel also stated that the immediate antibiotic therapy does not prevent the development of OME, does not reduce the persistence of MEE, and does not reduce the risk for recurrent episodes.

In 2004, the Israel Medical Association issued a guideline recommending delaying for 24 to 48 hours antibiotic therapy for nonsevere AOM in children older than 6 months, using analgesics for symptomatic relief instead. Grossman et al[27] assessed the effect of this guideline on systemic antibiotic and topical analgesic use in children aged 6 months and 5 years. Between 2004 and 2007, the rate of antibiotic treatment for first documented AOM treatment rates decreased from 61% to 54% in children aged 6 months to 1 year, 63% to 54% in children aged 1 to 2 years, and 56% to 47% in children aged 2 to 5 years ($P < .001$). Proportions of cases treated exclusively with topical therapy increased from 5% to 9%, 4% to 8%, and 8% to 14% for the respective categories ($P < .001$). In Israel, implementation of the delayed antibiotic treatment approach was associated with a significant reduction in use of antibiotics associated with first documented AOM in children.

The National Institute for Health and Clinical Excellence (NICE) guideline issued in the United Kingdom recommends treatment of AOM in older children immediately with antibiotics only if they have ear discharge. In an observational cohort study, Smith et al[28] determined the clinical significance and outcome of ear discharge in children with AOM, in UK general practice. They prospectively followed a cohort of consecutive children aged 6 months to 10 years with AOM. Of 256 children, 38 (15%) had ear discharge, and in 22 (58%) cases, a bacterial pathogen was identified. Children with ear discharge were more likely to be treated with antibiotics irrespective of age (odds ratio [OR], 15; CI, 3-66); they had more severe systemic illness and an increased likelihood of adverse outcome. The investigators concluded that their data supported the NICE guideline to treat these children with antibiotics.

A clinical practice guideline was developed for local use in a medical center in Canada for IM ceftriaxone use in children with AOM who had not responded to high-dose amoxicillin or amoxicillin-clavulanate.[29] The guideline recommendations were similar to that of the 2004 AAP/American Academy of Family Physicians (AAFP) AOM guideline. A retrospective review was performed on 127 charts of emergency department patients treated in 18-month periods before and after initiation of the guideline. Indications for prescription of ceftriaxone were adequate in 16.7% of the preguideline and 22.4% of the postguideline groups ($P = .4$). Adequate dosing in the postguideline group was significantly better ($P < .001$). Implementation of the guideline did not improve indications for ceftriaxone use. The authors cited guideline fatigue and parent and physician preferences to avoid 3 painful injections, although 1 injection was often seen as preferable to 10 days of oral antibiotics. The findings are consistent with other studies of compliance with AOM guidelines (51%-64% adherence).

In the past 4 years, many studies have addressed various effects of the 2004 AAP/AAFP AOM guideline. To study primary care physicians' trend in management of AOM after the guideline's recommendations, Vernacchio et al[30] in 2006 resurveyed primary care physicians who were members of a national practice-based pediatric network. A similar survey had been performed in the same group in 2004, 6 months after publication of the guideline. Of 477 physicians, 62.7% completed the survey. The guideline was accepted as reasonable by 83.3% of respondents (compared with 88.0% in 2004); however, it was used only 15% of the time. The biggest obstacle was the perceived parental reluctance to withhold antibiotics, and the cost of follow-up of children who did not improve. The other important finding was relative nonadherence to antibiotic recommendations, especially a reluctance to use IM ceftriaxone after failure with high-dose amoxicillin-clavulanate. Most primary care physicians accept the concept of an observation option for AOM but use it only occasionally. Antibiotics prescribed for AOM differ markedly from the guideline's recommendations, and the difference has increased since 2004.

A retrospective chart review on adherence to the 2004 AAP/AAFP AOM guideline was published by 2 pharmacologists.[31] Of 200 cases of AOM, the ''observation option'' would have been appropriate in 62.5% but was used in only 11.5% of patients. Having a certain diagnosis was a significant predictor for not using the observation ($P < .005$). High-dose amoxicillin was used in 15.5% of cases; 58.5% received low-dose amoxicillin. The authors estimated that about 50% of antibiotic prescriptions for AOM were inappropriate.

Meropol et al[32] used complex decision analysis methodology to evaluate the 2004 AAP/AAFP guideline. The objective was to compare strategies for diagnosing and treating AOM: (1) a commonly used, 2-criteria strategy (acute symptoms and signs of inflammation), (2) the guidelines' 3-criteria algorithm (acute symptoms, presence of MEE, and signs of inflammation), and (3) initial observation without antibiotics. The model used 3 age groups: 2 to <6 months, 6 to 24 months, and 2 to 12 years. Some assumptions used in the analyses (such as observation without antibiotic for children 2 to 6 months and the 2 criteria for AOM diagnosis) are not part of the AAP/AAFP guideline. Model probabilities were derived from previously published studies, and 26 assumptions were made. The model predicted that the guideline strategy reduces antibiotic use but increases sick days and cost. For children <24 months, sick days are increased by 13% and cost increases to $178 to $283 per antibiotic prescription avoided. For the >24 month group, sick days are increased by only 4% and costs are not increased. The authors conclude that there is age inconsistency in the guidelines in that there is a lower threshold for treatment in the older than in younger children. The overall implication of this study is unclear. In

another publication by the same author,[33] cost-utility analysis was performed. The analysis suggested that following the AAP/AAFP guideline to avoid one prescription of antibiotic, parents would have to trade 0.3 to 4 quality-adjusted life-days. Although this might be a desirable tradeoff from a societal perspective, the author argued that it might not be as desirable from the parental perspective.

Coco et al[34] analyzed data from the National Ambulatory Medical Care Survey, 2002-2006 (N = 1114), which occurred in US physicians' offices. Children (aged 6 months to 12 years) who were diagnosed with AOM were included. The time comparisons were the 30-month periods before and after the guideline. The proportion of AOM cases not managed with antibiotics pre- and postguideline were not significantly different (11% pre, 16% post; $P = .103$). The proportion of visits for which amoxicillin was prescribed increased (40% pre, 49% post; $P = .039$). The prescribing of amoxicillin-clavulanate decreased (23% pre, 16% post; $P = .043$). Cefdinir prescribing increased (7% pre, 14% post; $P < .004$), while macrolide prescribing did not change (14% pre, 13% post; $P = .82$). The rate of analgesic prescribing increased from 14% to 24% ($P = .038$). Conclusions are that for the 30 months following publication of the guideline, the only recommendations that were significantly different were the management of pain and the use of amoxicillin as the first-line antibiotic. Contrary to the guideline, the prescribing of amoxicillin-clavulanate has decreased, whereas the prescribing of cefdinir has increased.

The AAP/AAFP guideline recommends using high-dose amoxicillin, 80 to 90 mg/kg per day. With rising childhood obesity, a child weighing more than 19 kg will exceed the standard adult dose of 1500 mg/d. Christian-Kopp et al[35] reviewed charts of 359 qualifying patients. Children weighing <20 kg received an average dose of 74.2 mg/kg per day; those weighing ≥20 kg received an average dose of 40.4 mg/kg per day ($P < .00$). The maximum daily dose given was 2400 mg/d. Overall, 20.1% of the patients 2 to 18 years old exceeded the 97th percentile for weight. Primary care physicians prescribed a significantly lower than recommended dose of amoxicillin in older children and those in the higher weight category. They also performed a web-based survey of 14 members of the AOM guidelines subcommittee; 9 of 14 responded to the survey. All affirmed scientific discussion of the 80 to 90 mg/kg per day dose and indicated that maximum dosing for obese patients was not discussed during guideline formulation. The opinion among subcommittee members regarding maximum dose specification of amoxicillin varied.

Acute Otitis Media: Diagnosis and General Treatment. There has been a strong emphasis on the need for a stringent definition of AOM used in treatment studies. In the 2 recent studies on AOM treatment, bulging of the TM was included in the AOM definition in one study,[8] and 92% of enrolled cases in the other study had full or bulging TM.[9] While it is important for treatment studies to enroll children with well-developed AOM (eg, with bulging TM) to ensure certainty of the diagnosis and to assess treatment efficacy from a uniform pool of cases, it is also important to understand that the process of ear infection likely develops soon after the infectious pathogens enter the middle ear and before the middle ear is filled with pus. Kalu et al[36] reported a spectrum of AOM signs as the disease developed in young children followed from the occurrence of common cold. Early and mild AOM may improve without treatment or progress to later require antibiotic. Based on our current understanding, the 3 cardinal criteria for AOM including acute symptoms, signs of TM inflammation, and presence of MEF provide a comprehensive definition of AOM. A bulging TM is becoming more commonly required in the AOM treatment definition.

A randomized, double-blind, placebo-controlled trial from Australia compared topical aqueous 2% lignocaine eardrops with a placebo (saline) for relief of pain related to AOM.[37] Children 3 to 17 years (n = 63) were included; oral analgesia was available to all. Three drops of either lignocaine or placebo were instilled after which the child was laid with that ear upward for 5 minutes. Significant reduction by 50% in pain score was achieved within 10 and 30 minutes in the lignocaine group (52% and 90%, respectively) compared with the placebo group (25% and 63%). This study suggests the added benefit of concurrent use of topical analgesic eardrops in addition to oral analgesia in providing rapid relief of ear pain attributed to AOM.

In a randomized, double-blind, placebo-controlled trial from Finland, Hatakka et al[38] determined whether probiotic use for 24 weeks would reduce the occurrence or duration of AOM or the nasopharyngeal carriage of otitis pathogens in otitis-prone children (10 months to 6 years). Of the 135 children in the probiotic group, 72% had at least 1 episode of AOM, while 65% of the 134 placebo recipients had at least 1 episode of AOM during the 6-month follow-up. rAOM affected 18% and 17% of cases in the 2 treatment groups, respectively. The median duration of AOM episodes was 5.6 days in the probiotic group compared with 6.0 in the placebo group. Probiotics used in this study did not prevent the occurrence of AOM, reduce the duration of AOM, or reduce nasopharyngeal carriage of otitis pathogens in otitis-prone children.

The effects of adjunctive treatment such as the use of decongestants, antihistamines, or steroids, in addition to antibiotic, to improve clinical resolution of AOM have not recently been studied in prospective randomized trials. A Cochrane review published in 2003 on decongestants and antihistamines for AOM was updated in 2008,[39] but there were no new studies to be added. The 2011 review was withdrawn because there were no data to update.[40] In a retrospective review in children aged 1 to 13 years treated in a tertiary referral center for AOM, without tympanic membrane perforation, Eyibilen et al[41] analyzed the effects of nasal and oral decongestants and antihistamines as adjunctive drugs to antimicrobial treatment of AOM. MEE was resolved by day 5 to 7, most rapidly in children not receiving decongestants and/or antihistamines. By day 25 to 30,

MEE was resolved in 73%, 81%, 74%, and 71% in children receiving no adjunctive drug, topical decongestants, oral decongestants, and decongestants and antihistamines, respectively. In virtually all children, MEE was resolved by day 90, the end of follow-up. This study is in line with results from a previously published prospective, randomized, double-blind study[42] showing the negative effect of antihistamine on resolution of MEE and transient positive effect of steroids in the resolution of AOM. The use of decongestants and/or antihistamines as adjunctive drugs for AOM treatment is discouraged.

In a private practice group in the United States, a physician has performed tympanocentesis as a mode of treatment for AOM. The procedure was recommended to all AOM patients younger than 3 years of age and received a high acceptance rate.[43] A retrospective review was performed to compare treatment failure, recurrence, and antibiotic prescription rates in different AOM treatment modalities. AOM treatment failure rates (recurrence of symptoms within 10 days) and recurrence of symptoms 11 to 30 days after the initial visit were reviewed in 3 treatment groups: immediate antibiotic (n = 233), tympanocentesis and observation (n = 154), and tympanocentesis with immediate antibiotic (n = 46). There was no significant difference in rates of treatment failure and recurrence between groups; however, treatment failure was higher among AOM episodes caused by *S pneumoniae* (OR, 2.5; CI, 1.1-5.9). The authors suggested that alternative AOM therapy such as tympanocentesis can help reduce antibiotic use. Another different treatment approach was reported from Bratislava, Slovakia.[44] Between January 2005 and December 2006, 76 children (aged 4 months to 14 years) hospitalized for severe AOM and were retrospectively reviewed. The most frequent pathogen was *S pneumoniae* (n = 37), resistant to routine antibiotics in 70% of the cases. Initial treatment was intravenous antibiotics, most commonly with second- or third-generation cephalosporins. Mastoiditis occurred in 7 cases, and 4 had subperiosteal abscess. Mastoidectomy or antrotomy was performed in 6 cases, and tubes were inserted in 43% of cases.

Clinical/otologic scores before and during treatment of AOM were analyzed in Israel.[45] It would be useful for the clinician to have some ways to differentiate AOM cases associated with bacterial or a nonbacterial etiology. Satran et al[45] reviewed data from 1003 children with AOM who underwent tympanocentesis and MEF culture at enrollment and follow-up. They compared culture results with initial clinical/otologic scores. A score was calculated based on temperature, irritability, ear tugging, redness, and bulging, each graded from 0 to 3. Possible scores ranged from 0 to 15. Upon enrollment, children with positive cultures had scores that were 0.48 points higher than those whose cultures were negative. No differences were observed in scores from children who were culture positive for *S pneumoniae* versus *H influenzae* or mixed infection. A marked improvement in symptom scores was noted at day 4 to 6 in all patients regardless of their culture status at day 1 (6.5-point difference). At the second visit, improvement in scores was greater in patients who demonstrated bacterial eradication than in those for whom a bacterial pathogen was isolated. An accurate prediction of the bacterial etiology could not be made based on the clinical appearance of the disease.

Two studies were published on the effectiveness of a short course of antibiotics for AOM in children. The Cochrane update compared a short course of less than 7 days to a long course ($\geq$7days).[46] The review included 49 trials containing 12,045 participants aged 1 month to 18 years, no previous antibiotic therapy, and randomization to treatment with $<$7 days or $\geq$7days of antibiotics. Risk of treatment failure, relapse, or recurrence within 1 month was higher (21%) with short-course antibiotic than with a longer course (18%). There were no significant differences between IM ceftriaxone versus $>$7 days of short-acting antibiotic or between short-course azithromycin and $>$7 days of short-acting antibiotic. Gulani et al[47] reviewed 35 trials that compared the efficacy of a short course of antibiotics ($<$4 days) with a longer course ($>$4 days) for AOM treatment in children (age 4 weeks to 18 years). Overall, there was no increased risk with shorter course for treatment failure, bacteriologic failure, persistent MEF, relapse, or recurrence until 1 month, and there was no increase in MEE at 10 to 14 days or 1 to 3 months. However, when evaluating only oral antibiotic, use of a short course was associated with increased risk of treatment failure. Limitations of these reviews include differences in pharmacologic properties of the studied drugs, variations in diagnostic and outcome criteria, lack of information on both bacteriologic and clinical outcomes, limited enrollment of high-risk groups, and the statistical possibility of finding false-positive results due to the multiple analyses.

A cost-effectiveness analysis of treatment options for AOM was performed by Coco.[48] The model compared the cost and utility of 4 management strategies: watchful waiting, delayed prescription, routine treatment with 5 days of amoxicillin, and routine treatment with 7 to 10 days of amoxicillin. Multiple assumptions were made based on review of prior literature. Results indicated a tradeoff between 7 to 10 days of amoxicillin, which was the most effective but second most costly, and delayed prescription, which was least costly but less effective. Watchful waiting and 5 days of amoxicillin were neither more effective nor less costly. While the difference in effectiveness was small, the cost difference was significant, with 7 to 10 days costing $22.90 more per episode than delayed prescription. For the 13.6 million cases of AOM in the United States, this amounted to $311 million.

Coker et al[4] performed a systematic review on AOM diagnosis, treatment, and the association of heptavalent pneumococcal conjugate vaccine (PCV7) use with AOM microbiology; this is part of the Agency for Healthcare Research and Quality (AHRQ) evidence report. Of 8945 citations screened, 135 were included. Meta-analysis was performed for comparisons with 3 or more trials. Conclusions were (1) AOM is a clinical diagnosis. Red and immobile TM or bulging of the TM predicts AOM, but

accuracy and precision of a clinical diagnosis has not been determined. (2) Since the release of PCV7, the prevalence of *H influenzae* has increased and *S pneumoniae* has decreased. However, this may be changing because of the emergence of nonvaccine serotypes of *S pneumoniae.* (3) Initial amoxicillin treatment has a modest benefit compared with placebo or delayed antibiotics but also may be associated with more diarrhea or rash. (4) Most antibiotics used to treat uncomplicated AOM have similar rates of clinical success. In most cases, there is no evidence for use of higher cost antibiotics.

In a review of the literature on diagnosis and treatment of AOM, Powers[49] pointed out that despite the frequency with which the disease is diagnosed and treated, the present evidence on which diagnosis and management is based is not conclusive. The authors proposed that bias and confounding are common problems associated with AOM clinical trials. While the main goal of antimicrobial therapy is to improve how patients feel, function, or survive, the AOM literature has often focused on outcomes describing the effect of antibiotic on the organism. Difficulties with diagnosis are highlighted by the observation that although bulging of the TM is considered to be the most helpful in evaluating TM position, pediatric trainees agreed with a pediatric otolaryngologist on bulging with a Kappa coefficient of only 0.16. Acute onset of less than 48 hours of ear pain in association with a bulging, cloudy or distinctly red, immobile TM may provide the highest likelihood of selecting patients with bacterial AOM. However, even under these conditions, as many as 25% of patients may not have bacterial disease. The author recommends that all clinical AOM antibiotic trials should use a tympanocentesis to verify the diagnosis. Care should be used when interpreting the results of meta-analyses, since meta-analysis does not control for the effects of bias and confounding on measured outcomes. ''The more precise answer obtained by a meta-analysis may be more precisely wrong, and this possibility is of most concern when the trials included in the meta-analyses have known biases and confounders.'' The author concludes that noninferiority trials do not provide evidence that a new drug is more effective than placebo in AOM and that future trials should be placebo controlled. Future clinical trials would benefit from the use of novel endpoints, such as time to resolution of symptoms using validated patient-reported outcome instruments. This would provide more accurate assessments of outcomes and might yield valuable information regarding duration of therapy.

Two reports have addressed parental experiences, opinions, and knowledge on management of AOM. Tahtinen et al[50] compared data from parents in Finland and the Netherlands. Questionnaires were sent to daycare centers and distributed to parents of children aged <4 years. Of 1151 participants, 83% in Finland and 49% in the Netherlands had at least 1 episode of AOM. Antibiotics were used more frequently in Finland (99%) than in the Netherlands (78%). More Finnish parents reported a belief that antibiotics are necessary for AOM. Use of analgesics for AOM was similar (80% vs 86%). One-third of the parents had discussed resistance with their doctor; 88% of parents in Finland and 65% in the Netherlands were concerned that bacteria could become resistant to antibiotics. According to parental experiences, antimicrobial resistance had caused more problems in Finland than in the Netherlands (20% vs 2%). The authors concluded that treatment practices and parental expectations interact with each other. Therefore, to change AOM treatment practices, modification is needed for both guidelines and parental expectations. In another study, Holland et al[51] conducted a systematic review of web sites to determine if parents of children with AOM are likely to find updated and correct information on AOM management. Search terms were *ear infection* and *ear ache*. Of 400 search results, 105 sites contained information about AOM and were included in the study. Only 31% of sites explained the watch and wait option; 41% recommended finishing the full course of antibiotic. Only 13% included both recommendations. Sites with the appropriate recommendations were more likely to have an update documented, to have been written or reviewed by a physician, to cite the source of the recommendation, to have a nonprofit domain name, to have a ''fairly difficult'' reading ease score, and to have been updated within the past year. Health care providers should provide updated information on the guidelines and should refer patients to reliable sites. Physicians should be aware that their patients might visit the office with expectations based on outdated information found on the web. Organizations making recommendations should consider how to disseminate new information through the web.

Recurrent Acute Otitis Media

rAOM is common in otitis-prone children (defined as those children with ≥3 AOM episodes occurring in the previous 6 months or ≥4 episodes in 1 year preceding the current AOM episode). Clinical rAOM is defined as the reappearance of AOM after completion of treatment of an initial episode of AOM accompanied by clinical cure. True bacteriologically rAOM requires the presence of an organism identical to that isolated during the original AOM episode.[52,53]

Medical Treatment. The AAP/AAFP guideline does not provide standard recommendations for the treatment of a child suffering from rAOM episodes.[54] In a recent blinded, noninferiority, prospective, comparative multicenter study, 1305 children aged 6 months to 5 years with rAOM or persistent AOM were randomized to receive levofloxacin or low-dose amoxicillin/clavulanate.[15] Clinical cure rates were similar (72.4% and 69.9%) in the levofloxacin- and amoxicillin-clavulanate–treated children, without difference between the younger (≤24 months) and older (>24 months) patients and without difference in the incidence of adverse events between the 2 antibiotics. This study suggests the potential of fluoroquinolones as an effective therapy in children with rAOM or persistent AOM. However, these compounds are not approved for use in children.

Convincing evidence was demonstrated for the relationship between the efficacy of antimicrobial treatment in eradication of the causative bacteria, clinical responses, and the rates of early clinical recurrences of AOM. Asher et al[55] reported on the outcome of 673 culture-positive patients with AOM enrolled in double-tympanocentesis studies and treated with various antibiotics. Of these, 28% still had culture-positive MEFs on days 4 to 6 of treatment. Patients with clinical improvement/cure on days 11 to 14 after initiation of therapy, despite showing persistence of bacterial pathogens in the MEF culture performed on days 4 to 6, had more episodes of rAOM (35% occurring 3-4 weeks following the initial episodes) compared with those with culture-negative examination on days 4 to 6 (24%, P = .007). Of the 53 culture-positive (on day 4-6) patients with clinical improvement/cure at the end-of-therapy visit, 41 (77%) underwent tympanocentesis when AOM recurred and 29 (71%) of them were culture positive. Pulsed field gel electrophoresis identity between the pathogens isolated at recurrence and those persisting on days 4 to 6 were found in 66% of the patients compared with only 36% of the evaluable patients with recurrence of AOM and culture negative on day 4 to 6 (P = .005). These results demonstrate that AOM recurrences were caused, in most cases, by pathogens initially present in the MEF and not eradicated by antibiotics during treatment.

Nonvaccine Prevention. The use of xylitol, a 5-carbon polyol (sugar alcohol) produced from birch trees and a variety of berries, was systematically analyzed. The review included 4 RCTs and concluded that xylitol showed benefit as prophylaxis for AOM with few side effects when administered via chewing gum or syrup at 10 g/d given 5 times daily.[56] The treatment duration needed, cost, and expected long-term effects are yet to be established.

Marchisio et al[57] analyzed, in a prospective, blind, RCT, the effectiveness of a propolis and zinc solution in preventing AOM in 122 children aged 1 to 5 years with rAOM. AOM was diagnosed during the 3-month follow-up period in significantly less patients given propolis and zinc suspension compared with the group of children in which only elimination of environmental risk factors was achieved (50.8% vs 70.5%, P = .04). The mean number of AOM episodes per child/month was 0.23 $\pm$ 0.26 in the treatment group compared with controls (0.34 $\pm$ 0.29, P = .03). However, no effect could be shown on the effect of propolis and zinc solution on respiratory infections other than AOM.

The Italian AOM guidelines panel on AOM summarized up-to-date evidence accumulated in the literature regarding the prevention of AOM and rAOM. The guideline recommended specific preventive goals to be achieved: breast-feeding for at least 3 months, attendance at daycare centers only where appropriate hygiene measures are practiced, reducing the use of pacifiers to minimum, and avoidance of passive smoking.[7]

Adenoidectomy. Van den Aardweg et al[58] summarized RCTs comparing adenoidectomy, with or without tubes, versus nonsurgical management or tubes only in children with OM. The primary outcome studied was the proportion of time with OME. Secondary outcomes were mean number of episodes, mean number of days per episode and per year, and proportion of children with AOM or OME, as well as mean hearing level. While a significant benefit of adenoidectomy was demonstrated, in terms of resolution of the MEE in children with OME, the authors did not find a significant benefit of adenoidectomy on AOM. The trials were too heterogeneous to pool in a meta-analysis, especially because rAOM often overlapped with recurrent OME, and in some studies the 2 different entities were not separated. The authors' conclusion was that routine surgery for rAOM is not warranted.

Tympanostomy Tubes. A systematic review was published by McDonald et al[59] investigating whether tubes insertion reduces the frequency of episodes of rAOM and the proportion of children with symptoms of ear diseases. Of 5 RCTs, only 2 (including 148 children) were deemed to fulfill the inclusion criteria. The combined results of the latter suggested that more children treated with tubes are rendered symptom free in the 6 months following surgery compared with those who received other treatments or no treatment. One of the 2 studies involving 95 children showed that tubes reduce the number of AOM episodes in the first 6 months after surgery by an average of 1.5 episodes per child. A significant increase in the proportion of children with no episodes of AOM in the tubes group, compared with no treatment or antibiotic treatment, was also showed. The author concluded that tubes have a significant role in maintaining a disease-free state in the first 6 months after insertion but advised clinicians to consider the possible adverse effects of tube insertion before surgery is undertaken.

The conclusions of McDonald's review were questioned by Lous et al[60] because of disagreement with the selection and exclusion of the studies. Lous et al used information from all 5 RCTs, accounting for 519 children. Between 2 and 5 children have to be treated with tubes to prevent 1 child from having AOM in the next 6 months, and overall, tubes prevent 1 new episode of AOM in 6 months compared with no treatment. Moreover, considering the possible adverse effects of tube placement, the authors stated that the long-term effect of treatment with amoxicillin seems to be better than treatment with ventilation tubes but that long-term antibiotic treatment is problematic because of the risk of development of resistant bacteria.

Keyhani et al,[61] in a retrospective study, analyzed clinical data for children living in the New York metropolitan area and undergoing tubes insertion in 2002. In a sample of 682 children for whom data for the preceding year were abstracted, the mean age was 3.8 years, 57% were male, and 74% had private insurance. More than 25% of children had received tubes previously. The stated reason for surgery was OME in 60.4% of children, rAOM in 20.7%, and Eustachian tube dysfunction in 10.6%. Children with rAOM

averaged 3.1 ± 0.2 episodes (median, 3.0) in the previous year; those with OME averaged effusions that were 29 ± 1.7 days long (median, 16 days) at surgery. Twenty-five percent of children had bilateral effusions of >42 days' duration at surgery.

The same group[62] compared the use of tubes insertion for children with OM in the same population living in the New York metropolitan area with the recommendations of 2 sets of expert guidelines. Overall, 48% of the cases with alleged rAOM undergoing tube placement were not concordant with the explicit criteria, mainly because of low frequency of infection; 48% were uncertain, and only 4% were appropriate. The study panel believed that the benefit of delaying surgery until after a failure of antibiotic prophylaxis for rAOM outweighed concerns about the development of antimicrobial resistance. Even reanalyzing the data excluding a trial of antibiotic prophylaxis, the proportion of appropriate cases only increased from 7% to 22%.

In a formal decision analysis, Higgins et al[63] compared utility estimates between tubes and short-course antibiotics in children with rAOM. The appropriateness of considering tubes over a course of antibiotic varies by age at first episode of AOM and number of prior episodes of AOM at presentation. In children in whom the first AOM episode occurred after 12 months of age, tubes were recommended over a course of antibiotics when they presented with 7 episodes in a 24-month time span, 5 episodes in a 12-month time span, and 3 episodes in a 6-month time span. In contrast, the model recommended tubes in children with the first AOM episode before age 6 months when they presented with 3 episodes in 24 months or 2 episodes in either a 12-month or 6-month time span.

Otitis Media with Effusion

Medical/Nonsurgical Treatment. Earlier reviews have shown no long-term efficacy of antibiotics as a treatment for OME. Leach et al[64] reported the results of a double-blind study in a high-risk population in northern Australia in which 103 Aboriginal infants who had been examined since birth were randomized at the time OME was first detected to receive either amoxicillin (50 mg/kg per day twice daily) or placebo for 24 weeks or until bilateral aerated middle ears were noted at 2 consecutive monthly examinations (''success''). Five of 52 infants in the amoxicillin group and none of 51 infants in the placebo group achieved success at the end of therapy (risk difference [RD], 9.6% [95% CI, 1.6-17.6]). Amoxicillin also significantly reduced the proportion of children with perforation (27% to 12%). During therapy, the proportion of examinations with penicillin nonsusceptible (minimum inhibitory concentration >0.1 μg/mL) pneumococci was not significantly different between the amoxicillin group (34%) and the placebo group (40%). Beta-lactamase–positive noncapsular *H influenzae* was uncommon during therapy but more frequent in the amoxicillin group (10%) than in the placebo group (5%).

In their systematic review, Griffin et al[65] found no benefit of antihistamines and/or decongestants for any of the interventions or outcomes studied. However, treated study subjects experienced 11% more side effects than untreated subjects did. No new randomized studies on antihistamines or decongestants have been published since that review.

The effect of autoinflation with either a Politzer balloon or Otovent on OME is described in a Cochrane review by Perera et al.[66] They concluded that all of the studies were small, of limited treatment duration, and had short follow-up; however, because of the low cost and absence of adverse effects, it is reasonable to consider autoinflation while awaiting natural resolution of OME. Since that review, no other RCTs have been published.

In a Cochrane review, Simpson et al[67] found, on the basis of 9 RCTs with oral steroid (doses equivalent to 1-2 mg/kg per day of prednisone for 7-14 days) and 3 studies with topical intranasal steroids, that oral steroids, particularly in combination with an antibiotic, speed the resolution of OME in the short term, but there was no long-term evidence from trials to show lasting benefit or improved hearing in treated children. Unlike previous editions of this review, they did not find evidence of any short-term improvement with topical intranasal steroid with or without antibiotics. Included in this review is a well-designed multicenter RCT of mometasone furoate 50 μg or placebo once a day in each nostril for 3 months in 217 children aged 4 to 11 years.[68] Subjects had at least 1 recorded episode of OM or related ear problem in the previous 12 months and bilateral OME confirmed by otoscopy and tympanometry at entry. The authors found no improvement with use of the intranasal steroid at 1, 3, or 9 months and concluded that intranasal steroids are not likely to be an effective treatment for OME.

Skovbjerg et al[69] published a double-blind study of probiotics in 60 children with long-standing OME (median, 6 months) who were scheduled for tubes insertion. The children were randomized to 3 nasal spray treatment groups with *Streptococcus sanguinis, Lactobacillius rhamnosus*, or placebo for 10 days before surgery. Complete or significant clinical recovery occurred in 7 of 19 patients treated with *S sanguinis* compared with 1 of 17 patients in the placebo group ($P < .05$). In the *L rhamnosus* treatment group, 3 of 18 patients were cured or improved.

Schoem et al[70] studied a leukotriene inhibitor in a small study of 38 children between 2 and 6 years of age. Nineteen were randomized to receive 4 mg of montelukast (Singulair) 4 mg orally once daily, and 19 received placebo. Early in the study, it became apparent that the montelukast regimen was not having any effect. An interim analysis was performed after 38 patients. OME clearance was seen in only 3 montelukast patients and 4 controls ($P > .9$). Based on this early trend, the study was terminated by the funding sponsor.

McCoul et al[71] published a study on children with OM and gastroesophageal reflux disease. In an observational study of 37 children (mean age, 19.5 months), they found

standard antireflux treatment (2 consecutive 12-week periods) had a beneficial effect on quality of life (OM-6), hearing, and clinical examination.

Prevention. Le et al[72] studied the use of combined pneumococcal conjugate and polysaccharide vaccination in 383 children 1 to 7 years of age, with a history of $\geq$2 episodes of AOM in the preceding year, approximately 50% of whom had OME at entry. The control children received hepatitis A+B vaccine. They concluded that ''the combined pneumococcal conjugate and the polysaccharide vaccination had no beneficial effect on OME in children aged 1 year or older with a history of recurrent OM. Therefore, these vaccines are not indicated in the prevention of OME in these children.''

In 2010, a Cochrane review on zinc supplements for preventing OM was published.[73] On the basis of 10 trials, all in children younger than 5 years, they concluded ''evidence on whether zinc supplementation can reduce the incidence of OM in healthy children under the age of five years living in low- and middle-income countries is mixed.'' They also acknowledged that in one small trial in children with severe malnutrition, those given zinc supplementation had fewer episode of OM.

Surgical Treatment.

Tympanostomy tubes. Popova et al[74] randomized 78 children 3 to 7 years of age with bilateral MEE for at least 3 months to either A+M&T or A+M and followed them monthly for 1 year. Recurrence of OME was documented in 10% of the A+M&T group and in 14% of the A+M group. By 1 year, 7 tubes were noted to be occluded, and 1 child in the A+M group and 1 child in the A+M&T group underwent another surgical procedure for tube insertion. They found no difference in hearing testing at 6 and 12 months postoperatively. They concluded that insertion of tubes provided no additional benefit in regard to hearing loss when adenoidectomy was performed as the first-line treatment for chronic OME.

Rosenfeld et al[75] reported the efficacy of tube insertion in terms of caregiver responses to a survey. Parents of 168 children who had previously undergone tube insertion without concurrent adenoidectomy or tonsillectomy for AOM or OME were interviewed at a median of 2.0 years after tube insertion. At-risk children were considered as those having 1 or more predefined risk factors for developmental delay. Fifty-nine percent of caregivers reported that their expectations regarding impact of the tubes were met, and another 38% reported they were exceeded. Caregivers of at-risk children reported greater changes after tubes than did caregivers of children not at risk for issues with speech and language and for learning or school performance.

Browning et al[76] published a Cochrane Review that included 10 trials (1728 participants), with unpublished data from the MRC TARGET-trial concluding that, in children with OME, the effect of grommets on hearing is small and short term, decreasing after 6 to 9 months as natural resolution of OME leads to improved hearing. There are no conclusions on the effect of grommets in children with known speech, language, or developmental delay (as there have been no studies in these populations).

Knutsson and von Unge[77] retrospectively reviewed the medical records of 348 patients (640 tubes) who underwent tympanostomy tube insertion with short-term single-flanged fluoroplastic tubes for OME (75.4%), rAOM (20.2%), or retraction pathology (4.4%). By 12 months, 36.4% and by 24 months, 71.0% of tubes were extruded. Reasons for removal were prolonged retention (14.1%; mean time, 38.9 months) and local infection with suppuration not cured by local or systemic antibiotics (4.5%). Of ears without a previous tube, 77.9% did not need a second tube, while 15.9% did have further procedures for tube insertion during the 5-year follow-up. Persistent perforations after tube extrusion were noted in 4.5% ears; when including only those without previous tubes, perforations were found in 3.6%.

Yaman et al[78] reported their complication rates in a retrospective review of 162 ears of 87 children who underwent insertion of Shepard tubes between 2003 and 2008 in Turkey. All children were reexamined between January and May 2009, with the follow-up period being between 6 and 66 months (median, 23.3 $\pm$ 14.9). They found 8.1% of children had otorrhea, 46% had myringosclerosis, 9.2% had persistent (>3 months) perforation, 29% had atrophy, and 2% had medial displacement of a tube.

Hong et al[79] compared phosphorylcholine-coated fluoroplastic tubes to standard (uncoated fluoroplastic) tubes in children undergoing tube insertion for rAOM (74%) or chronic OME (26%). Children served as their own controls, as they were randomly assigned to receive one type of tube in one ear and the other type in the other ear. Of the 219 children who were followed (up to 24 months), the authors noted no difference between the tubes in the incidence of postoperative otorrhea (9%-19% at the various visits), tube obstruction (9% overall), or extrusion (mean survival time 11.4 months).

With regard to new tubes, Sherman et al[80] reported their work in developing a tube with the desirable characteristic of being able to be dissolved on demand rather than require a further surgical procedure to remove it. The authors tested a calcium alginate tube in vitro, comparing it to a commercial silicone tube. The alginate tube had a greater compressive strength than the silicon tube, and although exposure to such liquids as chlorinated pool water, soapy water, salt water, blood, vinegar, ear mucus, Ciprodex, and ofloxacin reduced the compressive strength of both tubes significantly, the alginate tube had greater compressive strength. Also, the alginate tube had less propensity for occlusion compared with commercial stainless-steel Reuter bobbin tubes; the rate of occlusion was reduced even further when coated with human serum albumen.

Adenoidectomy. Casselbrant et al[81] randomly assigned 98 children (24-47 months of age) with chronic MEE to M&T, A+M&T, or A+M and followed subjects monthly and with any signs or symptoms of ear disease for up to 36 months. Adenoidectomy was performed using electrocautery or

curette or both. In these young children, A+M&T provided no advantage over M&T alone with regard to mean percentage of time with MEE during the 36-month follow-up (21% vs 19%, respectively). However, the mean percentage of time with MEE in the A+M alone was significantly higher than in both the M&T and A+M&T groups (31% in the A+M group).

Van den Aardweg et al[58] published a Cochrane Review that included one new study since the previous Treatment Panel report.[81] The conclusions of the review are that there is a significant benefit of adenoidectomy in the resolution of MEF in children with OME, but the benefit to hearing is small and the effects on changes in the tympanic membrane are unknown. The authors concluded that the risks of the surgical procedure should be balanced with the potential benefits in making a decision for or against this surgery.

Mattila et al[82] looked at the development of asthma and atopy after randomization to adenoidectomy or no adenoidectomy in 166 children aged 12 to 48 months with recurrent or persistent OM; approximately 30% of subjects were entered with persistent OME. After 3 years, they found that adenoidectomy did not appear to promote exercise-induced bronchoconstriction or bronchial inflammation, nor were children undergoing adenoidectomy more likely to be atopic on prick skin testing than those not undergoing adenoidectomy.

The same group[83] looked at the effect of adenoidectomy on the nasopharyngeal carriage of pathogens associated with OM in children aged 12 to 48 months with either rAOM or chronic OME. Two hundred seventeen children were randomly assigned to undergo tubes with either adenoidectomy or no adenoidectomy and were followed with nasopharyngeal cultures yearly for 3 years; study children had not received any pneumococcal vaccine. No baseline cultures were reported, but at the 1-year follow-up, carriage of *S pneumoniae* occurred in 50% more children in the adenoidectomy group compared with those who did not undergo adenoidectomy (RR, 1.47; 95% CI, 1.04-2.07), but carriage was less markedly increased during the second- and third-year follow-ups (RR, 1.22 and 1.19, respectively). There were no differences observed in carriage of *H influenzae* and *Moraxella catarrhalis* during any of the years. When pneumococcal serotypes were looked at, the serotype 19F was statistically increased.

Esposito et al[84] looked at the incidence of bacteremia during and after adenoidectomy for middle-ear disease. Of 33 children undergoing the surgical procedure because of persistent OME, 10 (30.3%) children had positive nasopharyngeal swabs and none developed a positive blood culture. Of 15 children undergoing adenoidectomy for rAOM, 7 (46.7%) had positive nasopharyngeal cultures, of which 4 developed a positive blood culture at 30 seconds after beginning surgery (1 with *S aureus*, 3 with *H influenzae*); 3 children also had positive blood cultures at 20 minutes after surgery.

Worley et al[85] prospectively compared, in a nonrandomized study, adenoidectomy done by laser to that using curettage. One hundred children aged 8 to 48 months underwent adenoidectomy with tube placement for chronic OME of at least 12 weeks' duration and symptoms of adenoid hypertrophy: 50 patients underwent adenoidectomy with the laser, while 50 underwent traditional curettage adenoidectomy. All received ofloxacin otic solution 0.3% 2 times daily for 7 days postoperatively and were followed up for 4 months. The authors reported that the laser adenoidectomy time was shorter than that for curettage (4.6 vs 7.7 minutes) and there were fewer subjects with postoperative otorrhea by 4 months in the laser group (12% vs 42%). Long-term follow-up after the tubes are extruded has not yet available.

Haapkyla et al[86] looked at trends in surgery for AOM and OME in Norway and Finland over the years 1999 to 2005. Examining national databases for surgery rates in children between 0 and 7 years of age, they found the rate of adenoidectomy always higher in Finland than Norway but a decrease in adenoidectomies in both countries. Tube insertions increased by 52% in Finland but remained stable in Norway. The increase in tube insertions might be explained by the decrease in adenoidectomies, with the need for some type of treatment for ongoing disease.

Tympanostomy Tube Otorrhea

Postoperative otorrhea (discharge) is the most common complication of tube insertion, with a reported incidence ranging from 10% to 50%. Many otolaryngologists treat with topical antibiotics/steroid combinations, but general practitioners, mainly through fears of ototoxicity, are unlikely to prescribe topical antibiotics and choose systemic broad-spectrum antibiotics.

The Cochrane review in 2006[87] found only 1 relevant study: oral amoxicillin-clavulanate was compared with placebo in 79 patients with duration of otorrhea as outcome. The odds of having a discharge persisting 8 days after starting treatment was 0.19 (95% CI, 0.07-0.49), and the number needed to treat to achieve that benefit was 3. No significant benefit was shown in the 2 studies investigating steroids (oral prednisolone with oral amoxicillin-clavulanate and topical dexamethasone with topical ciprofloxacin ear drops) or in the 1 study comparing an antibiotic-steroid combination (Otosporin) drops versus spray (Otomize).

In 2008, Granath et al[88] published an RCT on 50 children with TTO randomized to either treatment with topical ear drops (hydrocortisone + oxytetracycline + polymyxin B; n = 24) or with topical ear drops + amoxicillin with or without clavulanic acid (n= 26). They found no difference in days with otorrhea, and 88% in both groups were cured within 7 days regardless of treatment.

Chronic Suppurative Otitis Media

CSOM is defined as the presence of a tympanic membrane perforation plus the presence of discharge for a minimum of 2 to 6 weeks. The onset of disease is usually early childhood. It occurs either following persistent AOM with perforation or persistent middle ear infection in the presence of an established perforation. In developed countries, it usually

occurs as a complication of the insertion of tubes and in developing countries as a complication of AOM with perforation.

Medical/Nonsurgical Interventions. The most commonly described nonsurgical treatments for CSOM are ear cleaning, antiseptics, antibiotics, and steroids. Boonacker et al[89] reported the economic evaluation and Van der Veen[90] reported the microbiological outcomes of the study by Van der Veen et al.[91] The study assessed the impact of topical antibiotics plus 6 to 12 weeks of cotrimoxazole (compared with topical antibiotics plus oral placebo) on persistent discharge in an RCT of 101 Dutch children with CSOM. After 6 weeks of treatment, 28% of children still had ear discharge in the oral antibiotic group compared with 53% in the group receiving topical antibiotics alone. There was no difference at the 12-month follow up (25% vs 20%). In subsequent publications, the authors found that the additional costs associated with the oral antibiotics were modest (US $100-$200) in the short term (6-12 weeks). The mean costs were greater at 12 months (US $500). After 6 weeks of treatment, 32 (91%) children in the cotrimoxazole group carried cotrimoxazole-resistant *Enterobacteriaceae* versus 10 (21%) in the placebo group (RD, 70; 95% CI, 55, 85). The integron prevalence was 26 (79%) in the cotrimoxazole group and 10 (22%) in the placebo group (RD, 57; 95% CI, 39, 75). After 1 year, the susceptibility levels had returned to baseline values.

Leach et al[92] assessed the impact of topical ciprofloxacin drops (compared with topical framycetin-gramicidin-dexamethasone drops) in an RCT of 97 Australian Indigenous children with CSOM who had previously failed treatment with topical framycetin-gramicidn-dexamethasone drops. After 6 to 8 weeks of treatment, 70% of children still had ear discharge, and there was no difference between the treatment groups (RD, –2%; 95% CI, –20 to 16). This lack of clinical difference was observed despite topical ciprofloxacin reducing *Pseudomonas* in the ear discharge. Wright et al[93] assessed the impact of a therapeutic bacteriophage preparation (Biophage-PA) targeting antibiotic-resistant *Pseudomonas aeruginosa* (compared with placebo) in an RCT of 24 British adults with CSOM. This phase 1 study was stopped early to allow a larger trial to proceed. Overall, there was a 50% reduction in visual analogue scores by clinician and patient in the bacteriophage group compared with a 20% reduction in the placebo group. This difference was associated with an even larger difference in bacterial counts.

Observational studies over the same period addressed topical antibiotics, treatment of CSOM associated with methicillin-resistant *S aureus* (MRSA) detection, and quality-of-life assessment. Haynes et al[94] provided an update on the risks of otoxicity associated with topical ear treatments in the presence of a perforated tympanic membrane. Wall et al[95] systematically reviewed 47 studies of ciprofloxacin (0.3%) plus dexamethasone (0.1%) drops used in the treatment of both OM and otitis externa, concluding that this topical treatment was safe and effective. In their evidence summary, Woodfield and Dugdale[96] described the evidence for the choice of antibiotics in the treatment of CSOM. Park et al[97] described the recent increase in community-acquired MRSA in ear discharge detected on bacterial culture in Korea. Choi et al[98] described the potential impact of increasing MRSA in the same country. In their available retrospective data, cleaning and irrigation with topical antiseptics was equivalent to intravenous teicoplanin or vancomycin.

Surgical Interventions. Surgical interventions of CSOM usually focus on the removal of infection from the middle ear space and mastoid cavities and repair of the tympanic membrane. These interventions are usually associated with reductions in conductive hearing loss. Bhat et al[99] assessed the impact of type 1 tympanoplasty plus cortical mastoidectomy (compared with type 1 tympanoplasty alone) in an RCT of 68 Indian adults with CSOM. At 3 and 6 months after surgery, there was no difference in clinical outcomes (tympanic membrane closure and hearing level) between the 2 groups. At 6 months, both groups had 6 participants with residual perforation and 2 participants with perforation and discharge. Cabra and Monux[100] assessed the impact of cartilage palisade tympanoplasty (CPT; compared with temporalis muscle fascia graft tympanoplasty [FT]) in an RCT of 123 Spanish adults. Morphological success (clinical assessment of perforation, atelectasis, atrophy, lateralization, otorrhea, and blunting) was present in 82% of the CPT group compared with 64% of the FT group (RR, 1.28; 95% CI, 1.02, 1.6).

Ramakrishnan et al[101] assessed the impact of tympanoplasty with mastoidectomy (compared with tympanoplasty without mastoidectomy) in an RCT of 62 Indian adults with uncomplicated mucosal chronic OM. After 3 months, there was no difference in graft uptake (94% vs 97%) or residual air-bone gap (13 dB vs 13 dB). Raj et al[102] assessed the impact of using acellular dermis in type 1 tympanoplasties (compared with the usual temporalis fascia graft) in an RCT of 42 Indian adults with inactive disease. The use of acellular dermis was associated with a shorter operating time and less pain. Other outcomes (including graft success rate and hearing level) were similar.

Observational studies addressed myringoplasty and tympanoplasty techniques and outcomes, ossiculoplasty or ossicular replacement, single-stage surgery, preoperative assessment by audiometry or high-resolution computed tomography or virtual otoscopy, cochlear implantation in people affected by CSOM, and use of bone-anchored hearing aids (where discharge makes behind-the-ear hearing aids unsuitable). Yung et al[103] described outcomes following pediatric myringoplasty in 51 British children. More than 80% had successful perforation closure, with no difference between younger (4-8 years old) and older children (9-13 years old). Seidman[104] described a novel minimally invasive technique for anterior tympanic membrane repair. He reported the outcomes following transcanal repair using an anterior tympanoplasty technique. Overall, 40 of 45 had closure of their perforations and avoided the need for a large postauricular incision. Webb and Chang[105] reported on the

outcomes following tympanoplasty without mastoidectomy in 150 patients with either CSOM or dry perforation of the tympanic membrane. There were no important differences in outcomes between the 2 disease categories. Ebenezer and Rupa[106] reported on 150 patients older than 5 years undergoing tympanomastoid surgery for tubotympanic CSOM. They found incus necrosis in 16%. This was best predicted by the presence of middle ear granulations and moderate to severe hearing loss (40-70 dB). Knowledge of these risk factors can assist with preparation and the consent process. Homoe et al[107] described a program of 1-stage bilateral CSOM/COM ear surgery in 17 Greenlandic children and young adults. At 3 weeks and 2 years postsurgery, 50% to 60% of tympanic membrane perforations were intact. Hellingham and Dunnebier[108] reviewed the available published literature on cochlear implantation in people with AOM or CSOM. The authors found no good evidence to prevent implantation in those with middle ear disease so as long as precautions (usually by careful staging) are taken to protect the electrode. Watson et al[109] conducted a small exploratory study looking at the costs of treatment following surgery for bone-anchored hearing aids in British adults with CSOM. The costs associated with management of CSOM decreased after surgery. Vlastos et al[110] described the changes in a chronic OM score (COM-5) before and after surgery in 45 Greek children. Successful tympanoplasty surgery was associated with improved quality of life as measured by the COM-5, global ear-related quality of life rating, and the change reported by the caregiver.

Implications for Practice

Remarkable accomplishments regarding the treatment of AOM have been reached. The 2 high-quality RCTs published in 2011 have demonstrated short-term benefit of immediate antimicrobial treatment (amoxicillin-clavulanate) over placebo on clinical resolution. However, about half of the patients who received placebo did not have treatment failure and two-thirds did not need rescue treatment, indicating that even in young children aged 6 and 35 months with carefully diagnosed AOM may do well without antibiotics.

Specific diagnosis has become a key factor in AOM treatment[111]; we are moving toward AOM with bulging TM as the AOM treatment definition. This would provide greater uniformity in treatment studies and support more precise AOM diagnosis in general practice. There is also a consensus that ''AOM is a treatable disease for all infants with a definite diagnosis of AOM.''[111]

Optimizing antibiotic treatment and diagnosing AOM with stringent criteria are important to reduce direct and indirect costs and to avoid the emergence of antibiotic resistance. It is noteworthy that no trials on new antibiotics have been published. As a result, we have to make the best use of the available antibiotics.

Clinical practice guidelines on diagnosis and treatment of AOM advise against an indiscriminate use of antibiotics (which can result in adverse effects and increased antibiotic resistance). Miscellaneous studies continue to support observation for selected cases, and watchful waiting is now an agreed treatment option. The identification of the children who benefit most from immediate antibiotic treatment or can be managed by watchful waiting is still debatable. Current guidelines do not appear to have been fully embraced by clinicians and parents.

For OME, the main 2007-2011 results were related to no significant benefit of adenoidectomy compared with tube insertion alone in the treatment of chronic OME in children 2 to 4 years of age. On the contrary, there was new evidence that tubes can maintain a disease-free state for 6 months in children with rAOM.

CSOM remains a neglected and difficult disease. There was no new study on prevention and only a few studies on medical treatment with controversial results. The publication of several randomized controlled trials assessing surgical interventions is an important development and suggests that better evidence on the impact of surgery will be available in the future.

In conclusion, while research progress has been made in the field of OM treatment, further research is still required. The panel has identified a series of short- and long-term research goals below.

Short-term Research Goals (1 to 3 Years)

- Differentiate young children who would benefit from antibiotics from those who would not.
- Study the effect of antibiotics versus no antibiotic on duration of MEE and recurrences.
- Improve education and tools to promote accuracy in diagnosis of AOM and OME.
- Establish safety and efficacy of topical analgesic drops in children with AOM, especially in young children who have been excluded from existing studies.
- Evaluate the impact of clinical practice guidelines, including part of the observation option guidelines that may lead to clinicians' deviation in practice. Document, analyze, and revise the specific parts that lead to noncompliance.
- Establish optimal strategies for managing AOM and OME in the types of children typically excluded from RCTs, especially those with baseline health or developmental disorders or conditions placing them at risk for developmental sequelae.
- Develop alternative delivery systems of antimicrobial agents directly to the middle ear, especially with an intact tympanic membrane.
- Conduct prospective studies to define the natural history and spontaneous resolution of TTO, to assess the efficacy of nonantimicrobial strategies for TTO and to define the risk-versus-benefit profile of topical antibiotics for TTO.
- Evaluate the efficacy of surgery versus medical management versus both for treating CSOM, including alternative medical strategies.

- Include intermediate and long-term outcome measures in OM treatment studies of medication versus placebo, therapy versus no therapy, and surgery versus no surgery.
- Establish the role of gastroesophageal reflux management and anti-allergy therapy in managing the child with middle ear disease.
- Evaluate the efficacy of immunomodulation of the nasopharyngeal mucosal immune system, such as by vaccination, probiotic and prebiotic agents, and commensal bacteria such as alpha hemolytic streptococcus, in reducing the duration of MEE after AOM.

Long-term Research Goals (>3 Years)

- Achieve a uniform definition of disease state/severity and a uniform way to assess outcome.
- Better understanding of individual host factors, moving toward personalized medicine.
- Encourage international collaboration in designing and conducting clinical trials in OM through practice-based networks.
- Monitor shifts in bacteriological epidemiology caused by vaccination and determine their implications on antimicrobial use.
- Acquire additional information on selective pressure for bacterial resistance caused by antimicrobial therapy for OM.
- Determine the rate of biofilm formation in experimental models of AOM treated with immediate, delayed, or no antibiotic.
- Detect surrogates that may allow reduction in the numbers of participants in clinical trials, so as to promptly differentiate ''good'' versus ''bad'' antimicrobials for AOM.
- Better understanding of complications/sequelae of surgical procedures for chronic OM.
- Identify uniform criteria for children with OM who are likely to benefit most from surgery and should be referred to surgeon.
- Conduct large, observational studies to define more precisely the stay-time (functional duration) of short-, medium-, and long-term tubes and to document the impact of stay-times on OM recurrence. Define the harm versus benefit ratio of tubes with different functional durations.
- Conduct randomized trials to determine the impact of tympanostomy tube stay-time on developmental outcomes in severely affected children with OME, including the tradeoff with adenoidectomy as a known extender of the benefit from short-term tubes.
- Define watchful waiting and surveillance strategies for children who are not immediate candidates for surgery in OME.
- Conduct well-designed RCTs with adequate statistical power to assess the efficacy of novel treatment modalities (complementary and alternative therapies) for AOM and OME.
- Conduct well-designed RCTs with adequate statistical power to assess the efficacy of Eustachian tube autoinflation for OME.
- Establish the impact of tubes on developmental sequelae in children with special needs (Down syndrome, cleft palate, cerebral palsy, developmental delays).

Author Contributions

Paola Marchisio, panel chair, conception, acquisition of data, interpretation of data, drafting and revising, final approval; **Tasnee Chonmaitree**, panel co-chair, conception, acquisition of data, interpretation of data, drafting and revising, final approval; **Eugene Leibovitz**, conception, acquisition of data, interpretation of data, drafting and revising, final approval; **Allan Lieberthal**, conception, acquisition of data, interpretation of data, drafting and revising, final approval; **Jorgen Lous**, conception, acquisition of data, interpretation of data, drafting and revising, final approval; **Ellen Mandel**, conception, acquisition of data, interpretation of data, drafting and revising, final approval; **David McCormick**, conception, acquisition of data, interpretation of data, drafting and revising, final approval; **Peter Morris**, conception, acquisition of data, interpretation of data, drafting and revising, final approval; **Aino Ruohola**, conception, acquisition of data, interpretation of data, drafting and revising, final approval.

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Panel 8: Complications and Sequelae

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Abstract

Background and Objectives. Although serious complications of otitis media (OM) such as brain abscess are rare, sequelae of OM such as tympanic membrane perforation and atelectatic tympanic membrane are quite common. Inner ear sequelae can cause hearing loss and speech and language problems. The objectives of this article are to provide a state-of-the-art review on recent articles on complications and sequelae of OM in different anatomic locations, from the tympanic membrane to intracranial sites, as well as hearing loss and speech and language development.

Data Sources. Primarily PubMed supplemented by Ovid MEDLINE and the Cochrane Database.

Review Methods. All types of articles related to OM complications and sequelae published in English between January 2007 and June 2011 were identified. A total of 127 relevant quality articles are summarized and included in this report.

Results. Key findings are summarized based on the following major anatomic locations and categories: tympanic membrane; cholesteatoma; ossicular problems; mucosal sequelae; inner ear sequelae; speech and language development; extracranial areas, including mastoiditis and facial nerve paralysis; intracranial complications; and future research goals. New information and insights were gained to prevent complications and sequelae.

Conclusion and Implications for Practice. Over the past 4 years, progress has been made in advancing the knowledge on the complications and sequelae of OM, which can be used to prevent and treat them effectively. Areas of potential future research have been identified and outlined.

Keywords

otitis media, tympanic membrane perforation, atelectasis of TM, cholesteatoma, hearing loss, speech and language, facial nerve paralysis, mastoiditis, cranial complications

Although serious complications of otitis media (OM) such as brain abscess are rare, sequelae of OM such as tympanic membrane perforation and atelectasis of the tympanic membrane are quite common. Inner ear sequelae can cause hearing loss and speech and language problems. The objectives of this article are to provide a state-of-the art review on recent publications on complications and sequelae of OM in different anatomic locations, from the tympanic membrane (TM) to intracranial sites, as well as hearing loss and speech and language development.

Published reports from the past 5 years were included for sequelae or complications ensuing from OM in childhood to adulthood. The specific panel objectives include following: (1) to summarize important new contributions to our understanding of the complications and sequelae of OM, published since the Ninth International Symposium on Recent Advances in Otitis Media in 2007; (2) to determine whether

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the short- and long-term research goals identified at the previous meeting of this panel have been met; (3) to identify deficiencies in our understanding of the complications and sequelae of OM in specific sites related to the TM (including cholesteatoma), middle ear, inner ear, and intratemporal and intracranial sites, as well as the impact of OM on sound transmission in the middle ear (including TM and ossicles) on hearing and speech and language; (4) to define and prioritize research goals that address deficiencies in our knowledge for investigation during the next 4 years; and (5) to identify the short- and long-term goals for the future and methods by which they can be accomplished.

Methods

Articles came primarily from PubMed supplemented by Ovid MEDLINE and the Cochrane Database.

All types of articles related to OM complications and sequelae published in English between January 2007 and June 2011 were identified. A total of 127 relevant quality articles are summarized and included in this report. All articles in English with a keyword of *otitis media*, complications, or sequelae were evaluated and included. Articles considered new and significant were included.

Discussion

Key findings are summarized based on the following major anatomic locations and categories: TM; cholesteatoma; ossicular problems; mucosal sequelae; inner ear sequelae; speech and language development; extracranial areas, including mastoiditis and facial nerve paralysis; intracranial complications; and future research goals.

Tympanic Membrane

Perforation. Von Unge et al[1] evaluated a model for studies on sequelae after acute otitis media (AOM) in the Mongolian gerbil. The middle ears of 16 Mongolian gerbils were inoculated with type 6a *Streptococcus pneumoniae*. Half of the animals were treated with antibiotics on days 4 to 6, when otoscopy was performed as well. After 1, 2, 3, or 4 weeks, the animals were sacrificed and their TMs were examined. On days 4 and 6, AOM was produced in approximately 80% of the animals, and perforations prevailed in approximately 30% at the study end points. Clinical signs of AOM and edema of the TM had already started to reduce after 1 week and often resolved within 2 weeks. The mechanical stiffness of TM remained relatively unharmed in the nonperforated ears. The antibiotic treatment seemed to reduce the duration of edema but not the perforation rate.

Santa Maria et al[2] reported keratinocyte growth factor (KGF) 1, fibroblast growth factor (FGF) 2, and FGF10 in the healing TM following perforation in rats. The TMs of rats were perforated and sacrificed at time points over a 14-day period. KGF1, FGF2, and FGF10 play important roles in TM healing following perforation. KGF1 and FGF2 appeared to be involved in the proliferation and migration of keratinocytes. The role of KGF1 appeared to be exclusively connected to an increased proliferation and migration at the perforation site. The continued expression of FGF2, beyond perforation closure, indicated also another role of this molecule. The effect of FGF10 on keratinocytes in wound healing appeared to emanate from the connective tissue layer.

In a retrospective study, Iacovidou et al[3] investigated 12 cases of spontaneous perforation of the TM in the first 10 days of life. Cultures of the middle ear exudate grew *Pseudomonas aeruginosa* in 10, *Serratia marcescens* in 1, and *Staphylococcus aureus* in 1. Cultures of nasopharyngeal secretions grew *P aeruginosa* in 9, *S marcescens* in 1, *S aureus* in 1, and *Streptococcus viridans* in 1. Middle ear vs nasopharyngeal secretion cultures grew the same organism in 11 neonates. A 10-day course of parenteral antibiotics was administered (ampicillin-ceftazidime for all neonates except for the 1 neonate with the *S aureus* otitis who received netilmicin-cloxacillin). All neonates had an uneventful course and were discharged home in good clinical condition. The authors suggest that neonates with TM perforation should receive antibiotics parenterally, as the most common pathogen is *P aeruginosa*.

Tympanic membrane perforation causes a sound conduction disturbance, and the size of this conduction disturbance is proportional to the perforation area.

In an article from Japan, Matsuda et al[4] conducted a quantitative evaluation of TM perforations using image analysis equipment. A significant correlation was found between the degree of sound conduction disturbance and the perforation area. That correlation was greater at low frequencies following a traumatic perforation. The conductive disturbance associated with chronic otitis media was significantly greater at low frequencies. Circular perforations caused only minor conduction disturbance. Perforations in the anteroinferior quadrant were associated with greater conduction disturbance.

Tympanostomy tube. Yaman et al[5] investigated Shepard grommet tympanostomy tube complications in children with chronic otitis media with effusion (OME). The medical records of 162 ears of 87 children were reviewed retrospectively. Otorrhea occurred in 9 ears (5.6%). Granulation tissue was seen in 2 ears (1.2%). Complications after tympanostomy tube extrusion included myringosclerosis (34.6%), persistent perforation (5.6%), atrophy (23.5%), retraction (16.7%), and medial displacement of tubes (1.2%). The authors concluded that myringosclerosis, tympanic membrane atrophy, and otorrhea are the most frequently appearing complications, but they are generally insignificant and cosmetic. Consequently, in the majority of these complications, there is no need for any management.

Sherman et al[6] proposed dissolvable tympanostomy tubes (TTs) developed from calcium alginate to reduce complications and the need for removal if TTs fail to extrude. Alginate TTs had a greater compressive strength than commercial silicone tubes. The TTs composed of 0.5M CaCl were stronger than high-molarity CaCl concentrations. Uncoated alginate TTs showed a 20% reduction in occlusion propensity. The authors proposed alginate TTs to be a

good alternative to commercial tubes based on their high mechanical strength and low occlusion propensity. Furthermore, alginate TTs have the potential to be dissolved in vivo if retained.

Daudia et al[7] retrospectively investigated long-term middle ear ventilation with 57 subannular tubes in 45 patients. They proposed that subannular ventilation tubes provide an effective option for management of intractable middle ear effusion and eustachian tube dysfunction.

Popova et al[8] compared myringotomy and tympanostomy tubes in combination with adenoidectomy in seventy-eight 3- to 7-year-old children with OME. They studied 2 surgical combinations: adenoidectomy with myringotomy and tympanostomy (A + T) and adenoidectomy with myringotomy only (A + M). Seventy-two percent of the patients in the A + T group and 75% of those in the A + M group were free of AOM episodes. None of the patients with A + M had episodes with otorrhea, which contrasted with the 40% occurrence rate in the A + T group. During the follow-up period, the authors documented a 10% recurrence rate of OME in the A + T group and a 14% recurrence rate in the A + M group. They suggest that A + T provides no additional benefit to A + M in hearing loss or AOM episode occurrences.

Barañano et al[9] retrospectively evaluated the management of TTs in pediatric cochlear implant (CI) recipients. Sixty-two patients received a TT before CI, and 68 ears received simultaneously CIs and TTs. The TTs were removed and allowed to extrude before CI (59%) or were kept in place until CI (41%). Forty ears (51%) required more than 1 set of TTs. Ten ears (22%) in which the TTs were removed before CI required a separate TT after CI compared with 6 ears (19%) in which the TTs remained in place until CI. The TTs that were present during CI were either removed with myringoplasty (31%) or retained after surgery (69%). Tympanostomy tubes do not appear to adversely affect the final outcomes of pediatric CI recipients and can be managed similarly to TTs in otitis media–prone children.

Myringosclerosis and atrophy. Cankaya et al[10] studied the effects of topical mitomycin and trimetazidine on the development of myringosclerosis (MS), which was induced in rabbits by paracentesis (myringotomy) with or without tympanostomy tube placement. Mitomycin, having an anti-inflammatory effect, and trimetazidine, inhibiting the oxygen-free radicals, were applied topically to the tympanic membrane, and MS was graded histopathologically after Masson's trichrome staining. In TMs subjected to paracentesis or paracentesis plus tube application or those that received trimetazidine, MS was significantly more extensive than in unoperated animals and in mitomycin-treated animals. The authors concluded that only topical use of mitomycin had alleviating effects on MS development.

Park et al[11] studied the effect of topical sodium thiosulfate (STS) in experimentally induced MS. Thirty Wistar albino rats were bilaterally myringotomized. The number of MS ears was significantly reduced in the STS group, and in tympanometric measurements, significantly reduced magnitudes of maximum admittance were observed in control and saline groups. The TM of the STS group appeared thinner than that of the control group ($P < .05$) and with a reduced calcium deposition. The authors showed that STS has a preventive role in the development of MS in the experimental animal model.

Song et al[12] investigated the effect of caffeic acid phenethyl ester (CAPE) on the prevention of experimentally induced MS. Thirty-five Sprague-Dawley rats were divided into 3 groups; group 1 received no treatment, group 2 received intraperitoneally administered saline, and group 3 received intraperitoneally administered CAPE. The TMs were examined on the 15th day after treatment. Group 1 showed extensive MS, and group 2 showed a similar occurrence of MS. Group 3 had reduced MS. The lamina propria of the pars tensa was thicker and more sclerotic in groups 1 and 2. The authors concluded that systemic CAPE was effective in the prevention of sclerotic lesions in myringotomized rat TMs.

Acute OM. Dai and Gan[13] developed an OME model in guinea pigs, in which middle ear transfer functions could be measured. Evidence of OME was assessed by otoscopy, tympanometry, histology, and measurement of the volume of fluid in the middle ear. Vibrations of the umbo and round window membrane were measured with a laser Doppler vibrometer at a frequency range of 200 to 40 kHz in 3 groups 3, 7, and 14 days after injection of lipopolysaccharide (LPS). Changes in displacement of the umbo and round window membrane in response to an 80-dB sound pressure level (SPL) in the ear canal were measured across the frequency range. Displacement of both the umbo and round window membrane was reduced at all time points following LPS injections. Furthermore, the change of the displacement transmission ratio (DTR) from the TM to the round window occurred mainly in chronic OME ears. This study provides useful data for analyzing the change of middle ear transfer function in OME ears.

Basic science of TM/gas exchange. Knutsson et al[14] located progenitor/stem cells in the human TM. Human TMs obtained at translabyrinthine surgery were investigated using immunohistochemistry and immunofluorescence to detect the progenitor/stem cell markers α_6-integrin, β_1-integrin, and cytokeratin 19 (CK19). α_6-Integrin was detected in the basal layer of the keratinizing epithelium in the umbo, in the annular region, and along the malleus but not in the intermediate portion of the pars tensa. β_1-Integrin and CK19 were found in the same locations, not only in the basal layer but also in the suprabasal layers of the keratinizing epithelium. The authors concluded that possible progenitor cells are found in the umbo, in the annular region, and along the malleus.

Redmond et al[15] compared phenotypic and genotypic profiles of human TM (hTM) derived from hTM tissue explants with epithelial and mesenchymal reference cells. Epithelium-specific ets-1 (ESE-1), E-cadherin, KGF1/FGF7, KGF2/FGF10, fibroblast growth factor receptor (FGFR) 1,

variants of FGFR2, fibroblast surface protein (FSP), and vimentin proteins were used to assess the phenotypes of all cultured cells. The tissues were stained with ESE-1 and E-cadherin. Immunofluorescent (IF) cell staining of hTM epithelial cells (hTMk) demonstrated coexpression of both epithelial- and mesenchymal-specific proteins. Flow cytometry (FCM) analysis further demonstrated coexpression of these epithelial and mesenchymal-specific proteins, indicating that the subcultured hTMk cells possess a transitional phenotype. Gene transcript analysis of hTMk cells by reverse transcriptase polymerase chain reaction (RT-PCR) revealed a downregulation of ESE-1, E-cadherin, FGFR2, and variant 1 and variant 2 (FGFR2v1 and FGFR2v2) between low and high passages, as well as upregulation of KGF1, KGF2, and FGFR1. The authors concluded that the results indicate a gradual shift in cell phenotype of hTMk-derived cells from epithelial to mesenchymal.

Yuksel et al[16] investigated the CO_2 gas exchange across the human TM. The study used an ear canal (EC) probe (ECP) constructed from a custom-fitted acrylic body, a glass capillary tube enclosing an oil meniscus to maintain ambient ECP + EC pressure, and a silica glass microtube linked to a mass spectrometer (MS) for measuring gas composition that was hermetically sealed within the ear canal of the test ear. The fractional CO_2 pressure measured in the ECP + EC for each sample was regressed on time and the slope of the function multiplied by the ECP + EC volume and divided by the estimated trans-TM CO_2 gradient at the start of the experiment to yield trans-TM CO_2 conductance. In comparison, the difference between sclerotic and normal TMs did not reach statistical significance. The study showed that the effect of TM pathology on CO_2 conductance is limited.

Toros et al[17] prospectively investigated gastric pepsinogen in middle ear fluid of children with glue ear. Middle ear effusion (MEE) and blood samples were obtained from 42 children. Total pepsinogen, albumin concentrations of effusions, and serum samples were measured with enzyme-linked immunosorbent assay (ELISA). The authors concluded that the occurrence of pepsinogen is a simple and reliable method for assessment of reflux in children. Pepsinogen was present in all MEEs, with levels higher than those of serum values.

Shim et al[18] reported that measurement of the largest cross-sectional areas of the aerated eustachian tube on coronal images of temporal bone computed tomography (CT) might be useful for predicting the postoperative condition of the tympanic cavity.

Cholesteatoma

Congenital or acquired. Persaud et al[19] reviewed the etiopathogenic theories of congenital and acquired cholesteatoma. Congenital cholesteatoma is explained by the persistence of fetal epidermoid formation. Acquired cholesteatoma may develop by various mechanisms: immigration, basal hyperplasia, retraction pocket, and/or trauma (iatrogenic or noniatrogenic). Chronic inflammation seems to play a fundamental role in multiple etiopathogenic mechanisms of acquired cholesteatoma. The authors recommend that early treatment of inflammatory conditions might reduce the sequelae by preventing the development of hyperplastic papillary protrusions.

Welkoborsky et al[20] reported a study designed to evaluate the cell-to-cell and epithelium-stroma interaction of acquired cholesteatoma. Surgical specimens of 54 patients who underwent primary surgery for an acquired cholesteatoma of the middle ear were examined by histopathology and DNA–image cytometry (DNA-ICM). Immunohistochemical investigations included expression of proliferation markers (proliferation cell nuclear antigen and MIB-1) along with cell surface markers reflecting the cell-to-cell interaction (ie, $\alpha_1\beta_6$-integrin, E-cadherin, I-CAM = CD54) and the epithelial-to-stroma interaction (ie, α-v and β_3-integrin chains, V-CAM = CD106, CD44v6, and fibronectin). Cell surface markers and cell adhesion molecules were equally expressed in both groups except $\alpha_1\beta_6$-integrin and fibronectin, which were significantly overexpressed in pediatric cholesteatomas.

The authors concluded that pediatric and adult cholesteatomas do not show differences at a cellular level. The observed clinically more aggressive behavior of pediatric cholesteatoma is likely due to other secondary factors such as more intense inflammation, disturbed middle ear ventilation, or the diminished calcium salt content of pediatric bone.

Basic science of cholesteatoma. Kwon et al[21] analyzed gene expression profiles in cholesteatoma using an oligonucleotide microarray. In all, 1327 upregulated or 767 downregulated genes that were over 3 times more prominent in cholesteatoma than in skin were identified by 5 samples of microarray data. Among these up- or downregulated genes in cholesteatoma, 291 genes were identified in 3 of 5 samples as upregulated expression more than 3-fold in density, and 191 genes were downregulated more than 3-fold in density. Reverse transcriptase PCR of 21 selected genes revealed that those expression levels were higher in cholesteatoma than in skin. The authors suggested that microarray analysis may be a useful tool to identify some candidate genes related to the pathogenesis of cholesteatoma.

Hamajima et al[22] investigated the role of the inhibitor of DNA binding (Id1) in the hyperproliferation of keratinocytes. Keratinocytes were transfected with Id1, and the responses of keratinocytes to Id1 were studied by using cellular and molecular biologic methods. Id1 significantly increased the promoter activity of nuclear factor (NF)–κB, which, in turn, upregulated the expression of cyclin D1 and keratin 10 in keratinocytes. Specific NF-κB inhibitors (pyrrolidine dithiocarbamate, PDTC) or a dominant-negative inhibitor (IκBαM) abrogated the Id1-induced cell proliferation and keratin 10 production, whereas p65, a subunit of the NF-κB heterodimer and an enhancer of NF-κB activity, strengthened the Id1-induced cell proliferation and keratin 10 production. The authors identified that Id1 contributed to the hyperproliferation of keratinocytes via enhancement of

cell cycle progression, removal of cell cycle inhibition, and a simultaneous increase in keratin production.

d'Alessandro et al[23] have shown paracrine loops of keratinocyte stimulation in cholesteatoma tissue by immunofluorescence, transmission electron microscopy, and molecular study. The expression of KGF, K1, and filaggrin in the samples was evaluated by quantitative RT-PCR. The authors suggest that KGF upmodulation is a consequence of fibroblast stimulation by inflammatory cells and that this paracrine loop could be responsible not only for the hyperproliferation of keratinocytes in cholesteatoma tissue but also for the deregulation of epidermal differentiation.

Franz et al[24] investigated the role of human papillomavirus (HPV) in the development of cholesteatomas. They found that only 1 of 29 biopsies showed a positive signal at the nested PCR level. The low prevalence of detected HPV DNA in cholesteatomas suggests that HPV infections are unlikely to be a causative factor.

Kuczkowski et al[25] investigated the expression of tumor necrosis factor (TNF)–α, interleukin (IL)–1α, IL-6, and IL-10 in chronic otitis media (COM) with bone destruction. They described an increased expression of TNF-α, IL-1α, and IL-6 in cholesteatoma tissue compared with that of granulation tissue. The authors found a strong positive correlation between these cytokine levels and the degree of bone destruction in cholesteatomas. The degree of correlation coefficient, calculated with Spearman's rank correlation coefficient with $P < .05$ between cytokine levels to the degree of bone destruction, was 0.72 for TNF-α, 0.61 for IL-1α, and 0.76 for IL-6.

Clinical and surgical review. Tatlipinar et al[26] reported the correlation between observations by high-definition CT in COM and the presence of cholesteatoma at surgery. In the CT scans, cholesteatoma was present in 46% showing abnormal soft tissue densities and signs of bone erosion, 52.9% showing abnormal soft tissue without osseous erosion, 66.6% showing soft tissue density with ossicular chain erosion, 77.7% showing an external ear bony canal defect, and 83.3% showing a bone-eroding soft tissue mass involving the epitympanum.

Ayache et al[27] conducted a study with the aim of determining the contribution of otoendoscopy in the surgical management of cholesteatoma. They concluded that the use of otoendoscopy significantly reduced the frequency of open tympanoplasty and recourse to posterior tympanotomy, as well as offered excellent access to numerous lesions by the transmeatal approach. However, they did not reveal a reduction in the frequency of residual cholesteatomas at revision surgery.

Magliulo et al[28] evaluated 298 adults and 38 children with cholesteatoma who underwent surgery and reported the frequency of facial nerve dehiscence to be 27.1%. Dehiscence was present in 42.3% of the patients who underwent revision surgery. The most common site of dehiscence, 92.3%, was the tympanic segment.

Haginomori et al[29] demonstrated that 21% of the 85 ears operated on had residual cholesteatomas. Six cholesteatomas were located in the epitympanum (33%), 3 in the sinus tympani (17%), 3 in the antrum (17%), 2 on the stapes (11%), 2 on the tympanic membrane (11%), 1 on the tympanic portion of the facial canal (6%), and 1 just under the skin of the external auditory canal (6%). By retrospective videotape analysis, it was observed that the main cause of residual cholesteatomas in the epitympanum and sinus tympani was incomplete removal of the matrix under an indirect surgical view because of insufficient drilling.

In a review of 1531 surgeries (1183 patients), Kaylie et al[30] revealed that smokers had more cholesteatomas and required more canal wall-down surgeries than nonsmokers. Furthermore, they required more revision surgeries and had overall worse hearing outcome than nonsmokers.

Zhang et al[31] investigated identification of Id1 in acquired middle ear cholesteatoma. Two hundred sixty-four ears with COM that had undergone ear surgery were included. Fourteen middle ear cholesteatoma specimens were collected for immunohistochemical analysis of abnormal proliferation of keratinocytes. The inhibitor of the DNA-binding (Id1) gene, which is involved in controlling cell cycle progression, was abundantly expressed in cholesteatoma epithelium. In vitro studies indicate that Id1 regulated the expression of NF-κB, cyclin D1, proliferating cell nuclear antigen, and cell cycle progression of keratinocytes. The authors concluded that chronic inflammation in the ossicular chain area (OCA) is closely related to the formation of cholesteatoma. The Id1/NF-κB/cyclin D1/proliferating cell nuclear antigen signaling pathway is involved in the abnormal proliferation of keratinocytes in acquired cholesteatoma.

Ossicular Problems

Gluth et al[32] reviewed the results of malleostapedotomy for incus replacement in 7 individuals who had undergone malleostapedotomy in the setting of quiescent COM and a mobile stapes. They investigated surgical results and hearing outcomes by preoperative and postoperative pure-tone audiometry. Improvement in the air-bone gap was noted in 6 of 7, with an average closure of 17 dB. In 5 of 7, the air-bone gap was closed to 20 dB or less, and in 3 of 7, the air-bone gap was closed to 10 dB or less. The authors suggested that malleostapedotomy is a potentially safe and effective alternative to placement of a total ossicular replacement prosthesis (TORP).

Hamilton[33] investigated systematic preservation of the ossicular chain in laser-assisted cholesteatoma surgery. In this study, ears were categorized into 2 groups based on the state of the ossicular chain at the end of surgery. Patients with a continuous ossicular chain were allocated to group A. Patients with a disrupted chain and an intact stapes superstructure onto which an ossiculoplasty had been performed were placed in group B. Ultimately, it was concluded that the fiber-guided laser allows the cholesteatoma surgeon to preserve the ossicular chain in a systematic manner that is both safe and of benefit to the patient.

Mucosal Sequelae

Ebmeyer et al[34] reported that TNF-α deletion alters apoptosis as well as caspase 3 and 4 expression during otitis

media. They evaluated middle ear (ME) expression of genes encoding the TNF and TNF receptor superfamilies during bacterial OM in the mouse, characterized OM in TNFA-deficient mice, and assessed apoptosis during OM in normal vs TNF-deficient MEs. They concluded TNF and TNF receptor superfamilies mediate both inflammation and apoptosis during OM.

Schachern et al[35] designed a study to evaluate the effect of apolactoferrin administration on the middle and inner ears after experimentally induced pneumococcal otitis media. The middle ear cavities of chinchillas were inoculated bilaterally with type 2 wild-type *S pneumoniae*. Twenty-four hours later, the ears of 5 of the animals were injected with phosphate-buffered saline (PBS) and the other 5 with human apolactoferrin. The animals were killed 24 hours after the last injection. Bacterial plate counts of middle ear effusions and the number of inflammatory cells in the round window membrane were significantly lower in the apolactoferrin group compared with the group treated with PBS.

Prulière-Escabasse et al[36] analyzed otologic features in patients with primary ciliary dyskinesia (PCD) aged 0 to 18 years and evaluated the correlation between ultrastructural defects and severity of otologic features. Fifty-eight patients with PCD were evaluated in the following 4 age intervals: group 1, preschool (n = 47); group 2, school (n = 50); group 3, teenagers (n = 34); and group 4, young adults (n = 10). Ultrastructural defects occurred in the outer dynein arm (n = 33), the inner dynein arm (n = 13), and the central complex (n = 11). One patient had typical Kartagener syndrome with typical PCD features but normal ciliary ultrastructure. Recurrent AOM decreased from group 1 (68%) to group 4 (0%). Otitis media with effusion was more severe in groups 1 through 3 than in group 4. Otorrhea decreased in group 4. Half of the patients with tympanostomy tubes eventually had TM perforation. Hearing loss was moderate in groups 1 through 3 and mild in group 4. Continuous antibiotic therapy could be slightly reduced only in group 4. Despite continuous antibiotic therapy, the middle ear condition in PCD remained severe throughout childhood, with improvement only after age 18 years. Armstrong grommet placement did not improve the middle ear condition. Central complex defect is a marker of severity.

To study treatment patterns and complications of OM, a cross-sectional survey was conducted across 9 countries over 3 continents, in France, Germany, Spain, Poland, Argentina, Mexico, South Korea, Thailand, and Saudi Arabia, by Arguedas et al.[37] Face-to-face interviews were conducted with 1800 physicians. Respondents estimated an average annual caseload of 375 children younger than 5 years with OM, 54% with an initial episode and 38% with recurrent OM (ROM). Specialist referrals were needed for an estimated 15% of children with OM with complications. There was high awareness of *S pneumoniae* and *Haemophilus influenzae* as causative bacterial pathogens: 77% and 74%, respectively. Empirical treatment with antibiotics was the most common first-line treatment (81%). The burden of disease is substantial enough that many physicians would consider vaccination to prevent OM (score 5.1). The authors reported that OM remains a significant burden for clinical practice, despite awareness of shortcomings, and antimicrobial therapy remains the most frequent treatment for OM.

Urschel[38] reported a brief insight into primary immunodeficiencies in cases of otitis media. All patients suffer from recurrent, prolonged, and/or unusual infections leading to local sequelae, failure to thrive, developmental delays, and systemic infections with severe courses. A stepwise diagnostic approach is proposed to facilitate early and accurate diagnosis, as well as effective and timely therapy to improve the patient's outcome.

Yamamoto-Fukuda et al[39] analyzed the expression of keratinocyte growth factor and its receptor in noncholesteatomatous COM (NC-COM) and cholesteatomatous COM (C-COM). The subepithelial tissue from 18 patients with NC-COM and 70 patients with C-COM was processed for immunohistochemistry for KGF and KGFR. Keratinocyte growth factor was positive in 28% of NC-COM specimens and 88% of C-COM specimens. Thirty-seven (60%) C-COM specimens were positive for KGFR, whereas 0% of the NC-COM specimens were positive. The Ki-67 labeling index (LI) was significantly smaller in NC-COM than in C-COM. B-cell LI was almost similar in the 2 groups. T-cell LI was significantly higher in C-COM than in NC-COM. T-cell LI in NC-COM was higher in KGF-positive tissues. The authors concluded that coexpression of KGF and KGFR explains the pathologic difference between C-COM and NC-COM and that KGF may play an important role in cholesteatoma.

Using noninvasive optical interferometry for the assessment of biofilm growth in the middle ear, Nguyen et al[40] identified the presence of a biofilm with 86% sensitivity and 90% specificity with a novel classification algorithm for acquired low coherence interferometry data. *Streptococcus pneumoniae* is the most common pathogen associated with otitis media.

Han et al[41] reported a role for Toll-like receptor 2 (TLR2) in the immune response to *S pneumoniae* infection in mouse otitis media. Nineteen of 37 $TLR2^{-/-}$ mice had bacteremia and died within 3 days after the challenge, compared with only 4 of 32 wild-type (WT) mice that died. Of those that survived, more severe hearing loss in the $TLR2^{-/-}$ mice was indicated by an elevation in auditory-evoked brainstem response thresholds at 3 or 7 days after inoculation. The histological pathology was characterized by effusion and tissue damage in the middle ear. At both 3 and 7 days postchallenge, the $TLR2^{-/-}$ mice had higher blood bacterial titers. By 3 days postchallenge, the mRNA accumulation levels of NF-κB, TNF-α, IL-1β, MIP1α, Muc5ac, and Muc5b were significantly lower in the ears of $TLR2^{-/-}$ mice. The authors concluded that $TLR2^{-/-}$ mice may produce relatively low levels of proinflammatory cytokines following pneumococcal challenge, thus hindering the clearance of bacteria from the middle ear and leading to sepsis and a high mortality rate. They

showed evidence that TLR2 is important in the molecular pathogenesis and host response to otitis media.

In a study by Matsuda et al,[42] data about the treatment of middle ear cholesterol granuloma in 16 patients undergoing surgical treatment were reviewed. Patients with swollen TMs had significantly poorer outcomes. Patients with retracted TMs and those undergoing ossicular chain reconstruction had significantly better outcomes. The patients' overall hearing success rate at approximately 2 weeks postoperatively was 75%. However, by 6 months postoperatively, the overall hearing success rate had declined to 62.5%. Patients with poor hearing 2 weeks postoperatively did not acquire better hearing.

Jang et al[43] reported a recent case of advanced congenital cholesteatoma (stage IV) associated with blue eardrum that was treated using preoperative tympanostomy tube insertion and pointed out that tympanostomy tubes were helpful in preventing recurrence of the cholesteatoma after surgery.

Masaany et al[44] presented a rare case of familial hypercholesterolemia (an autosomal dominant disorder, which manifests with high levels of serum cholesterol and low-density lipoprotein cholesterol) with bilateral aggressive primary middle ear cholesterol granuloma.

Inner Ear Sequelae

Vestibular disturbances. Gianoli et al[45] presented a study to determine the incidence of caloric and rotational chair testing (RCT) abnormalities in a group of patients with chronic suppurative otitis media (CSOM) and to correlate caloric test results with RCT. Nineteen (76%) demonstrated either unilateral or bilateral canal weakness (CW). Eighteen (72%) demonstrated abnormalities on ROT. Eleven (44%) had complaints of vertigo/dizziness, although 2 of these patients had both normal caloric testing and ROT. Unilateral or bilateral CW was 80% accurate in predicting an RCT abnormality, whereas the symptom of vertigo/dizziness was only 48% accurate in predicting an RCT abnormality. The authors concluded that the incidence of CW among CSOM patients in this study was high and correlated well with abnormalities on RCT. The RCT results correlated better with CW than symptoms of dizziness/vertigo.

Cohen et al,[46] using a case-control study, investigated the effect of bilateral myringotomy and tube insertion (BMT) on balance in children 4 to 7 years of age. Children with documented OME who were scheduled for BMT underwent RCT and computerized dynamic posturography (CDP) preoperatively and at 1, 3, and 6 months postoperatively. Children without any significant history of otitis media were tested as controls at the same intervals. Seventy-two cases and 56 controls were enrolled. No difference was seen between groups on RCT or sensory organizing test (SOT) scores. Higher sway velocity during CDP was observed in the OME group both preoperatively as well as 1 month postoperatively, but there was no difference 3 months postoperatively. At 6 months, the BMT group had a significant decrease in sway velocity. The authors concluded that the study supports previous findings that OME has a measurable impact on balance function and that BMT is associated with improvement in balance function. Therefore, balance in addition to hearing and speech should be considered when considering BMT as a treatment for OME.

Hearing and Auditory Sequelae

Martines et al[47] reported audiological findings on OME with or without atopy in primary school children. Three hundred ten children were screened by skin tests and divided into atopics (G1) and nonatopics (G2). The overall prevalence rate of OME was 12.9%, with 42.85% in G1 and 6.30% in G2; OME was bilateral (70%). A type B tympanogram was evidenced (70.59%), with a significant difference between G1 and G2. The analysis of mean air conduction pure tone (31.97 dB for G1 and 29.8 dB for G2) and of tympanometric measurements showed a significant difference between G1 and G2. The authors concluded that the higher prevalence of OME in atopic children and the statistically significant differences in audiometric and tympanometric measurements among atopic and nonatopic subjects with OME suggest the important role of allergy in the genesis and recurrence of OME.

Prieve et al[48] investigated changes in transient-evoked otoacoustic emission (TEOAE) levels with negative tympanometric peak pressure (TPP) in infants and toddlers. Mean TEOAE level was lower when TPP was negative, but noise levels did not change between the 2 conditions. There were no significant differences between TEOAEs collected on days when TPP was normal and when TPP was negative. Mean data indicated that when tympanograms had negative TPP, TEOAE level was lower by approximately 4 dB across all frequency bands. However, this affected the pass rate in only 5% to 6% of cases. The authors suggested that it is possible to measure TEOAEs in children with negative TPP. If the emission-to-noise ratio is used to identify hearing loss in mid- to high-frequency bands, the majority of children will still have TEOAEs that meet clinical criteria, thus providing the clinician with important information about cochlear status.

Pereira et al[49] have documented that children who failed the newborn screening showing conductive hearing loss had more episodes of OM during the first year of life than those who did not fail, with a statistically significant difference.

In prospective study by Boudewyns et al,[50] 55.3% of referred infants after automated auditory brainstem response (ABR) screening presented with OME. In this study, a spontaneous resolution was documented in 15 of 64 OME patients who were under follow-up, whereas hearing of the remaining patients was normalized after tympanocentesis or placement of ventilation tubes. The authors reported that normal hearing could be ascertained in all children at a median age of 4.8 months.

Lok et al[51] reported that after implementation of neonatal screening, there was a distinct increase in the number of children aged 6 to 11 months treated with tubes by 25%.

Lehmann et al[52] followed up 100 Aboriginal and 100 non-Aboriginal children from birth to age 2 years and

demonstrated that moderate to severe hearing loss was present in 32% of 47 Aboriginal children and 7% of 120 non-Aboriginal children aged 12 months or older. Furthermore, they found that Aboriginal children who failed TEOAE at age 1 to 2 months were 2.6 times more likely to develop OM subsequently than those who passed. They suggested measurement of TEOAEs at age 1 to 2 months to identify children at risk of developing OM in a routine health service setting in view of the frequently silent nature of OM in Aborigines.

In a study by Hunter et al,[53] hearing screening in Native American infants and toddlers was reported. Infants were prospectively assessed with pneumatic otoscopy, distortion product otoacoustic emissions (DPOAEs), and tympanometry. In the newborn period, 23.5% of infants failed hearing screening in at least 1 ear. Hearing screening failures increased to 29.9% from 2 to 5 months of age. Only 1 of 366 infants was identified with sensorineural hearing loss, and thus essentially all of the hearing screening failures reflected either a middle ear origin or other temporary problems. The authors suggested that behavioral assessment is needed after 6 months of age, when high rates of OME persist in this population.

Rosenfeld et al[54] analyzed tympanostomy tube outcomes in children at risk and not at risk for developmental delays. Fifty-five percent children had at least 1 condition placing them at risk for developmental delays. After tube insertion, 89% caregivers stated that their child's life was ''much better.'' Speech and language was ''much better'' for 55%, more often in at-risk children. Learning or school performance was ''much better'' after tubes for 55%, more often in at-risk children. Improved hearing was reported by 84% with no relationship to at-risk status. Caregivers reported favorable outcomes regardless of their child's at-risk status, but children at risk for delays had better reported outcomes for speech, language, learning, and school performance.

Daudia et al[55] reported long-term middle ear ventilation with myringotomy and tympanostomy tube (MT). They retrospectively studied 45 patients with COM and hearing loss, associated with adhesive otitis media in 7, TM retraction in 17, and TM perforation in 3. The mean improvement in air-bone gap was 14 dB. Complications included blockage (16%), perforation after extrusion (9%), granulation (5%), and infection (4%). The authors concluded that MT provides an effective option for management of intractable MEE and eustachian tube dysfunction.

Spielmann et al[56] reported a follow-up after BMT. Twenty percent of their patients required further MT insertion within the study periods. Children with abnormal clinical findings or a mean hearing threshold greater than 20 dB were significantly more likely to require further intervention. The authors recommended 1 postoperative review with audiometry 3 months after surgery.

Revai et al[57] investigated tympanometric findings in young children while they had upper respiratory tract infections (URIs) with and without AOM. The peak day for an abnormal tympanogram was day 2 of the URI. An abnormal tympanogram tended to be type B in children aged 6 to 23 months and type C in children aged 24 to 47 months. One-third of children older than 24 months had a type C tympanogram during the first week of URI. The authors concluded that eustachian tube dysfunction and middle ear abnormalities during URI are more severe in children younger than 2 years.

Al-Kandari et al[58] reported the hearing evaluation of schoolchildren in Kuwait. Although 120 children were found to have normal ear conditions, 39 children had abnormal results. In 21 children with ear wax, 3 had a normal hearing level, whereas 18 had mild conductive hearing loss in the 250-to 500-Hz frequency range. In 16 children with SOM, 3 had a normal hearing level, 9 had mild conductive hearing loss in the 250- to 500-Hz frequency range, and 4 had moderate conductive hearing loss in the 250- to 2000-Hz frequency range. The 2 children with sensorineural hearing loss (SNHL) had moderate hearing loss in the 4000- to 8000-Hz frequency range.

Browning et al[59] searched the Cochrane ENT Disorders Group Trials Register and other electronic databases to assess the effectiveness of grommet insertion compared with myringotomy or nonsurgical treatment in children with OME. They selected randomized controlled trials evaluating the effect of grommets (ventilating tubes [VTs]). Outcomes studied included hearing level, duration of MEE, language and speech development, cognitive development, behavior, and adverse effects. The authors included 10 trials (1728 participants). The VTs were mainly beneficial in the first 6 months. Only 1 high-quality trial that randomized children (n = 211) reported results at 3 months; the mean hearing level was 12 dB better in those treated with VTs. Meta-analyses of 3 high-quality trials (n = 523) showed a benefit of 4 dB at 6 to 9 months. At 12 and 18 months follow-up, no differences in mean hearing levels were found. Data from 3 trials that randomized ears (n = 230) showed similar effects to the trials that randomized children. At 4 to 6 months, mean hearing level was 10 dB better in the VT, and at 7 to 12 months and 18 to 24 months, it was 6 dB and 5 dB better, respectively. No effect was found on language or speech development or for behavior, cognitive, or quality-of-life outcomes.

Shahnaz et al[60] investigated multifrequency tympanometry in neonatal intensive care unit (NICU) and well babies. The NICU babies (n = 33), healthy 3-week-old babies (n = 16), and neonates on a high-priority hearing registry (HPHR) (n = 42) were tested. Thirty-two ears of 16 healthy white adults (compared with well babies) and 47 ears of 26 healthy white adults (compared with NICU babies) were also included in this study. Tympanograms at 226 Hz are typically multipeaked in ears that pass or are referred for TEOAE, limiting the specificity and sensitivity of this measure for differentiating normal and abnormal middle ear conditions. Tympanograms obtained at 1 kHz are potentially more sensitive and specific to presumably abnormal and normal middle ear conditions. Tympanometry at 1 kHz is also a good predictor of the presence or absence of TEOAE.

Chianese et al[61] showed spectral gradient acoustic reflectometry (SGAR) compared with tympanometry in diagnosing OME among 786 healthy children. The SGAR results were available for 3096 otoscopic examinations in 647 children. Tympanometric results were available for 2854 otoscopic examinations in 597 children. Using the recommended SGAR pass or fail cutoff, 53% of the ears in which effusion was present would have been considered effusion free. Only 10% of the ears without effusion would have been considered to have effusion. The area under the receiver operating characteristic curve was 0.78 for SGAR and 0.83 for tympanometry. The authors concluded that SGAR is slightly less discerning than tympanometry in predicting OME in children younger than 2 years.

Speech and Language Development

Speech perception and production. Bluestone and Swarts[62] speculated about human evolutionary history and the consequences for the pathogenesis of OM. They presented the possible consequences of 2 human adaptations that may have resulted in ubiquitous OM: the interaction of bipedalism and increased brain size and the loss of facial prognathism resulting from speech or cooking. Immature eustachian tube structure and function, in conjunction with an immature immune system, helps to explain the high incidence of OM in the first year of life. The morphology of the palate changed with the adaptations that produced facial flattening, with concomitant effects on eustachian tube function. These changes resulted in relatively poor human physiologic tubal function in comparison to the nonhuman primate.

Zumach et al[63] investigated OM and speech-in-noise recognition in school-aged children. Fifty-five children with a prospective 3-monthly documented middle ear status and hearing loss between birth and 24 months completed a ''speech-in-noise'' (SPiN) test at age 7 years. Both hearing loss and the accumulation of uni- and bilateral OM incidents in early life were significantly correlated to the performance on the SPiN test at school age. Only the language production score at age 7 years was also significantly related to the score on the SPiN test. The authors' study deals with the risk of OM in early life and its accompanied hearing loss on auditory processing, specifically speech perception in noise, up to school age.

Eapen et al[64] studied the development of frequency weighting for speech in children with a history of OME. The frequency bands selected were 798 to 1212 Hz (low band), 1575 to 2425 Hz (mid band), and 3000 to 5000 Hz (high band). The children in the OME group achieved results 85% to 90% correct at a lower signal-to-noise ratio than controls in the adaptive testing, where all 3 speech bands were present. Fixed block testing indicated that children with OME history gave more weight to speech frequencies in the region of 2000 Hz. The results show that the development of frequency weighting in the perception of speech can be affected by a history of OME.

Serbetcioglu et al[65] analyzed the association between hearing loss due to bilateral OME and Denver-II test results in preschool children. Sixteen children with bilateral otitis media were compared with the same number of age-matched children with normal hearing (controls). Language and verbal cognitive abilities were not affected significantly as a result of the presence of hearing loss because of OME. Using the Denver-II test to evaluate language development and other developmental screening parameters, the authors found no significant difference. They concluded no association between hearing loss due to OME and speech and language parameters in preschool children.

Kalu and Hall[66] studied clinician adherence to treatment guidelines for OME related to the documentation of presence, laterality, resolution, persistence, and surveillance for hearing loss or speech delay. Retrospective chart review of 363 children with OME was performed. The authors found a high level of documentation practices at the initial diagnosis of OME (laterality 95%) but poor documentation of follow-up factors (duration 14.9%). Documentation was not found to improve after release of the 2004 American Academy of Pediatrics (AAP) guidelines. The survey found physician knowledge lacking in terms of the decibel hearing level stratification of management and antibiotic use, although it was better for the use of pneumatic otoscopy, adenoidectomy, and myringotomy as accepted treatments.

Language. Johnson et al[67] examined early OME and language at age 7 years. It was hypothesized, on the basis of a literature review, that (1) a low but positive relation between early OME and language measures in general will be observed at age 7 years, and (2) major effects will be demonstrated for measures of articulation and phonological sensitivity. The following measures of language status were used: the Test of Auditory Analysis Skill (TAAS), the Goldman-Fristoe Articulation Test, Sounds in Words and Sounds in Sentences (GFAT), and the Clinical Evaluation of Language Fundamentals–Revised (CELF-R). The sample included 179 children who were heterogeneous for socioeconomic status (SES) and ethnicity. There were no significant correlations for MEE and language measures. These negative results were sustained when multiple regression was used with controls for SES and quality of the home environment. The authors concluded that early MEE may not pose a threat to language development in the early school years.

Cognition and academics. Hall et al[68] investigated developmental changes in word recognition threshold in children with different middle ear status. The aims were to (1) provide word recognition thresholds (WRTs) at 31, 43, and 61 months of age; (2) investigate developmental changes over time; (3) investigate the relationship between OME and WRT; and (4) investigate the relationship between WRT and hearing thresholds. Around 1000 children were tested longitudinally as part of the Avon Longitudinal Study of Parents and Children (ALSPAC) study, using an adaptive measure of word recognition in quiet. Mean WRTs were 28, 23, and 23 dB (A) at 31, 43, and 61 months, respectively. Normal auditory development is associated with a mean improvement in WRT of 5 dB between ages 31 and

61 months. There was a mean increase in WRT of +5 dB and +15 dB when OME was present in 1 and 2 ears, respectively. Thus, both unilateral and bilateral OME results in a detrimental effect on hearing ability for speech. In addition, early and ''persistent'' OME is associated with greater disability. However, by 61 months, previous OME status was not significant.

Williams and Jacobs[69] identified the impact of otitis media on cognitive and educational outcomes. Children who have early onset OM (younger than 12 months) are at high risk of developing long-term speech and language problems. For biological or environmental reasons, some populations have a pattern of early onset, higher prevalence, and episodes of longer duration; this pattern leads to a higher risk of long-term speech and language problems. These factors suggested that Australian indigenous children may be at higher risk of cognitive and educational sequelae than nonindigenous children.

Maruthy et al[70] determined the effect of early onset OM on brainstem and cortical auditory processing. Thirty children with OM were divided into 3 groups based on their age. The mean central conduction time was significantly increased and the mean amplitude of waves I and III of ABRs was significantly reduced in children with early onset OM. The latency of all late latency response (LLR) waves was significantly less in children with early onset OM. Significant differences in mean values of either ABR or LLR were observed only in 3-year-old children. There was a significant but negative association between central conduction time and latency of LLRs. Otitis media in the first year of life leads to negative effects on brainstem signal processing even if it has occurred only for a short duration (maximum of 3 months). The authors suggested that auditory cortical structures probably show compensatory changes through central gain to offset the prolonged central conduction time.

Behavior. Gouma et al[71] investigated behavioral trends in young children with conductive hearing loss as a case-control study. One hundred eighty-three children aged between 6 and 8 years participated in the study and were divided in 2 groups. The study group consisted of 117 children with a positive record of unilateral or bilateral acute otitis media at age 4 or 5 years. All patients of the study group were referred to the otolaryngology department by their attending pediatrician and had conductive hearing loss as proved by pure-tone audiometry. The rest of the children (n = 66) comprised the control group and were recruited from the orthopedics department, where they were hospitalized for minor injuries. One hundred seventeen patients with episodes of OME history at age 4 to 5 years were compared for depression according to the Achenbach system of evaluation, by application of the Child Behavior Checklist (CBCL). Patients with OME had more anxiety/related disorders and attention disorders. The psychological effect of OME in children aged 6 to 8 years is evident, with anxiety and depression disorders being especially prominent among these patients.

Parent and professional opinions about the sequelae of OME. Two studies were published that queried different groups of individuals regarding the impact of OM on development.

Sonnenschein and Cascella[72] asked 53 pediatricians in midsize New England cities in single and group practices and hospitals their opinions about the relationship between OM (both acute and OME) and children's hearing and speech-language development. Results of their survey indicate that although these pediatricians thought that OM occurring in the first 2 years of life could affect speech-language development, they were less inclined to agree that OM would have an effect in general. Moreover, they felt that parents and child care environments could lessen the impact of OM on speech-language sequelae.

The second article, by Higson and Haggard,[73] examined the differences between parents, teachers, and otolaryngologists in rating the importance of symptoms and the developmental impact of OME. Among their findings, they report that the 3 groups differed on all 4 areas of impact (ie, hearing, language-education, behavior, and balance). Both parents and teachers rated OME as having an impact on behavior. They also reported that teachers weigh language and education symptoms much more heavily than do parents and otolaryngologists and that parents give them decreasing importance as children age. The conclusions reached in both of these articles reflect findings in the research literature that indicate that by school age, there is little impact of OME on speech and language development.

Future Research

In the previous postsymposium meeting in 2007, a set of recommendations were provided that called for future studies to use a variety of research designs with varied populations; to design research that is hypothesis driven; to include reliable and valid measures of OME, hearing, and development; to include mediating and moderating measures that may affect the relationship between OME and developmental outcomes; and to provide adequate information in research reports, including power for nonsignificant findings, so that research synthesis may be completed. Several of these goals were met. Most of the studies were designed to examine specific hypotheses and used outcome measures to examine these effects. Populations included both middle-class and less advantaged groups, but in only 1 study was a clinical sample included. Many of the investigators examined hearing at the time of the developmental assessment to ensure that when outcomes were measured, their samples had hearing in the normal range.

However, hearing during the time when children experienced OME was included as an independent variable in only 2 studies—Hooper et al[74] and Paradise.[75] Aside from SES, only Feagans et al[76] and Hooper and colleagues considered other environmental variables to account for development.

Our recommendations for future studies are to more consistently apply the suggestions made previously. We encourage investigators to use more specific measures that may be

more sensitive to the effects of OME effects as have been used in retrospective studies such as rhyme detection, working memory, and verbal attention. It is possible that the effects of OME are more subtle and only apparent in acoustical analyses, although the importance of those effects may not be clear.

We again strongly recommend that hearing be measured concurrently with OME and be included as the mediating variable that may explain the relationship between OME and developmental outcomes. Given the host of other variables that affect later development, it is critical that investigators include possible confounding factors that may mediate or moderate the relationship between OME and later development. These are variables such as the quality of mother-child interaction, quality of child care experiences, and factors such as sex and the child's cognitive level.

Similarly, it will be important for investigators to include a variety of populations. As we indicated, it is important to include children at the most risk for OME, such children with Down syndrome or cleft palate who have considerable speech, language, and/or learning difficulties. In addition, understudied populations (children who are of Hispanic origin and Native Americans) should also be included in research studies. Finally, researchers must be diligent in providing detailed information about their participants and analytic procedures so that meta-analyses can be performed.

Extracranial Complications

Mastoiditis. Benito and Gorricho[77] reviewed the cases of mastoiditis over 10 years (1996-2005) at the Niño Jesús University Children Hospital in Madrid. Of the 215 children (aged 0.6-17 years) with mastoiditis, 67% were younger than 3 years and 69% were males. The number of cases doubled in 1999, with the same percentage of admissions in the pediatric service, and tripled in 2005 compared with 1996. Surgical treatment has increased from 4% to 33% in the past years and grew to 70% in 2005. Most cases (80%) had received prehospital antibiotic therapy, but individual pathogens and current complications of periostitis or subperiosteal abscess formation were equally distributed between the 2 groups. The authors detected *S pneumoniae* in 29% and a significantly high rate of *S aureus* (16%). Fifty-four percent of cases had negative cultures. There was a progressive increase in the incidence of acute mastoiditis and an increase in surgical treatments. Most cases of acute mastoiditis had responded well to medical management alone. The authors concluded that tympanocentesis for middle ear culture may become more valuable and more frequently used in cases of antibiotic treatment failures, and surgical therapy may be necessary more often in the future.

Kvaerner et al[78] studied the variation and characteristics of acute mastoiditis in 399 children aged 0 to 16 years in Norway. The study was based on a registry with complete data on hospitalization for acute mastoiditis and cortical mastoidectomy in Norway during 1999-2005. The incidence of acute mastoiditis in children younger than 2 years ranged from 13.5 to 16.8 per 100,000 during the study period. Corresponding numbers for children aged 2 to 16 years were 4.3 to 7.1 per 100,000 children. No increased incidence was found during the study period. Age-specific incidence revealed a peak during the second and third year of life, and acute mastoiditis was most common in boys. Cortical mastoidectomy was equally common in the young and older age groups; 22% received surgery. Despite the introduction of restrictive Norwegian guidelines for antibiotic treatment of acute otitis media in children 1 year and older, the data did not give evidence for an increase in acute mastoiditis.

Palma et al[79] in Italy investigated the clinical features and outcomes of acute mastoiditis in children during 1994-2005. Fifty-five cases fulfilled the inclusion criteria. Twenty-six patients were treated only with antibiotic therapy, tympanocentesis alone was performed in 11 cases, and a ventilation tube was positioned in 5 cases. Mastoidectomy was performed in 13 patients. The group who underwent mastoidectomy had a median hospital stay of 15 days (range, 5-54 days). In this group, the following complications were found: meningitis (n = 1), meningo-encephalitis (n = 1), lateral and sigmoid sinus thrombosis (n = 1), and facial palsy (n = 1). Their experience could not confirm a real increase of the incidence, but they noted periodic variations during the time of observation. They concluded that it is important that careful attention is paid to the clinical assessment of children who are 2 years or younger, as they seem to be more exposed to the risk of clinical complications; therefore, it is highly recommended that the otologist and the pediatrician collaborate closely.

Roddy et al[80] compared the etiology of mastoiditis in the pre–pneumococcal conjugate vaccine (PCV) era (1995-2000) and post-PCV era (2001 to April 2005) to guide empiric antimicrobial therapy in the pediatric emergency department. Retrospective chart review was done on all patients admitted with a diagnosis of mastoiditis from January 1995 to April 2005. Of the 122 charts reviewed, 68 were pre-PCV and 54 post-PCV. Etiological agents were determined by culture results in 60 patients. The most common bacterial isolates were *S pneumoniae* (n = 24), *P aeruginosa* (n = 12), *S aureus* (n = 12), *Streptococcus pyogenes* (n = 8), and *H influenzae* (n = 2). Acute mastoiditis was diagnosed in 93 patients, and chronic mastoiditis (defined as $\geq$3 weeks of symptoms) was diagnosed in 29 patients. *Streptococcus pneumoniae* was more likely to be implicated in acute vs chronic mastoiditis (odds ratio, 9.2; 95% confidence interval, 1.2-52.2; $P = 0.01$). *Pseudomonas aeruginosa* was more frequently implicated in chronic vs acute mastoiditis. There was no difference in the proportion of pediatric mastoiditis cases caused by *S pneumoniae* in the pre-PCV vs post-PCV eras.

Geva et al[81] studied 144 consecutive children hospitalized for acute mastoiditis between 1991 and 2002. All children were treated with parenteral antibiotics (conservative management). Myringotomy was performed in 35% of episodes at the discretion of the otolaryngologist on call. The

children who underwent myringotomy were significantly younger (22.4 vs 28.8 months; $P = .028$) and had more complications (n = 17 vs n = 8; $P < .001$). Complications overall occurred in 16% of episodes. Performing myringotomy had no significant effect on the duration of hospital stay. Children pretreated with antibiotics underwent significantly less myringotomies. There were no significant differences between children who underwent myringotomy and those who did not with regard to white blood cell (WBC) count or erythrocyte sedimentation rate (ESR). These findings suggest that myringotomy may not be required in all cases of acute mastoiditis. Parenteral antibiotics are sufficient in most cases. Criteria for myringotomy may include a younger age. Conservative management resulted in good outcomes in this series.

Ho et al[82] determined the relationship between prior antibiotic use and the development of acute mastoiditis (AM) in children. They identified 129 patients with AM who were admitted to their center between 1996 and 2000. A total of only 67 patients (52%) had undergone any antimicrobial treatment prior to hospital admission. In 1996, 7 of 11 (64%) of patients with AM had received antibiotics for AOM prior to admission, but this number had steadily decreased to 4 of 15 (27%) by 2005. The yearly number of cases of AM treated in their institution remained stable over this period. A subperiosteal abscess was identified in 45 patients (35%). Nineteen patients with a subperiosteal abscess (42%) and 48 patients without a subperiosteal abscess (57%) had undergone prehospitalization antimicrobial therapy for suppurative AOM. There was no significant difference in antibiotic use between the numbers of patients with or without a subperiosteal abscess. Use of antibiotics to treat suppurative AOM in children might not influence the subsequent development of AM.

Ongkasuwan et al[83] reviewed the medical charts (including the number of pneumococcal conjugate vaccine doses) between January 1995 and June 2007 and studied the impact of pneumococcal conjugate vaccine on pneumococcal mastoiditis in children at Texas Children's Hospital. Isolates were serotyped with the capsular swelling method. Forty-one pneumococcal mastoiditis cases were identified, and 19A (n = 19) was the most common serotype. Before the introduction of pneumococcal conjugate vaccine (from 1995-1999), 0 of 12 cases were 19A. Between April 2000 and October 2006, 15 cases of pneumococcal mastoiditis occurred, and 5 were 19A. Fourteen cases of pneumococcal mastoiditis occurred between November 2006 and June 2007, all of which were 19A. Mastoiditis caused by 19A isolates was more likely to present with subperiosteal abscess and was more likely to need intraoperative mastoidectomy than was mastoiditis caused by non-19A isolates. At Texas Children's Hospital, 19A has become the predominant serotype causing pneumococcal mastoiditis, partly related to the emergence of multidrug-resistant clonal complex 271 strains.

Stähelin-Massik et al[84] performed a prospective, observational study in children younger than 16 years presenting to their institution during the 2-year period beginning in April 2000. The children were examined and their condition treated in accordance with a standardized protocol elaborated by the pediatric, otolaryngology, and radiology departments. Thirty-eight patients were hospitalized (22 with acute mastoiditis, 7 with subacute mastoiditis, and 9 with chronic mastoiditis). There were 30 complications present in 21 patients (55%). *Streptococcus pyogenes* was the most common pathogen (7/24 cases), followed by *S pneumoniae* (4/24 cases). Mastoid surgery was performed in 29 patients. Histology of mastoid tissue revealed predominantly acute inflammation in 2 cases, mixed acute/chronic inflammation in 19 cases, and predominantly chronic inflammation in 7 cases. Radiologic data were evaluated retrospectively. Histological evidence suggests that subacute/chronic infection underlies not only subacute and chronic mastoiditis but most cases of acute mastoiditis as well. The authors concluded that spiral, volume-based, high-resolution computed tomography of the temporal bone is effective in ruling out coalescence.

van den Aardweg et al[85] made a comprehensive literature search of studies on the diagnosis of acute mastoiditis in children published between January 1980 and September 2007. The study type and setting, diagnostic criteria for acute mastoiditis, disease-specific history, presenting otologic and systemic signs and symptoms, diagnostic procedures, and final diagnosis were identified. The initial search resulted in 1057 articles. The inclusion criteria were met in 65; 44 were retrospective case series, and 21 were case reports. These studies included 2109 children with a median age of 32 months (range, 0 months to 18 years). Only 26 of 65 articles reported the criteria upon which the diagnosis of acute mastoiditis in children was based. The criteria most frequently used were the clinical signs of postauricular swelling, erythema, tenderness, and protrusion of the auricle. The most frequently used imaging modality was CT scanning (reported in 39 of 65 studies, performed in 68% of patients). The most frequently used laboratory test was WBC count (100% of patients in 45 of 65 studies). In 63 studies, the result of culturing from the otomastoid was reported: *S pneumoniae* was the most frequently isolated bacterium. The authors concluded that there is a lack of consensus regarding the criteria and strategies for diagnosing acute mastoiditis in the pediatric population. It is crucial that such criteria are established and consensus is achieved so that prognostic and controlled studies can be initiated to identify risk factors and establish the most effective management of this condition in children.

Bilavsky et al[86] collected data from computerized files on all children who were hospitalized at a tertiary center for acute mastoiditis over a 5-year period. Findings were compared between those with simple mastoiditis vs cases with intra- or extracranial complications. Of the 308 children with acute mastoiditis, 55 (18%) had complicated disease. This group was characterized by a significantly higher maximal fever at presentation and higher absolute neutrophil count and C-reactive protein level than the children with

simple disease. There was no statistically significant between-group difference in age, history of otitis media, prior antibiotic treatment, days of illness before presentation, absolute leukocyte count, and platelet count. No difference was detected between the groups with regard to penicillin and ceftriaxone susceptibility of the *S pneumoniae* isolates. High absolute neutrophil count and high C-reactive protein level may serve as clinical and laboratory markers of complicated mastoiditis. Children with these findings warrant close follow-up and, perhaps, earlier surgical intervention.

Finnbogadóttir et al[87] evaluated the incidence of mastoiditis in Iceland at the University Hospital, especially in children, and the possible correlation with antibiotic usage. Patients with mastoiditis during 1984-2002 were identified, and information on antibiotic usage in children during 1989-2002 was obtained. Eighty-four patients were diagnosed with mastoiditis during 1984-2002, 52 (62%) of whom were younger than 18 years. Twenty-six (50%) children were younger than 3 years. During 1999-2002, 28 children were diagnosed with mastoiditis, of whom 15 (54%) were diagnosed with otitis media within a week prior to admission, and 11 (73%) were treated with antibiotics. During 1989-2002, a correlation was detected between decreased antibiotic usage in children and increased incidence of mastoiditis. Following changes in guidelines for antibiotic prescriptions for otitis media in Iceland during the 1990s, antibiotic usage decreased, but the incidence of mastoiditis increased. It is uncertain if this is a causal relationship. It is important to treat otitis media correctly while being alert for complications, especially in young children.

Flohr and Schultz[88] provided anthropologic evidence about how common acute mastoiditis was in earlier historical and prehistoric times. In this study, osseous changes because of mastoiditis were diagnosed in 83% of the temporal bones. Males were more often affected than females. But interestingly, it was reported that older individuals had mastoiditis more often than younger individuals. The authors concluded that the high frequency of mastoiditis observed, particularly in the adults, was most likely due to an accumulation of osseous changes during individual lifetimes, supporting the hypothesis that mastoiditis was a serious health problem in preantibiotic times.

Hence, as stated by Flohr et al,[89] for evaluation of mastoid cells, discrimination of primary or secondary hypocellularity seems to be important. The authors stated that mastoid hypocellularity can be caused by poor development of air cells during infancy and early childhood (primary hypocellularity) or by obliteration of air cells with bone during later life (secondary hypocellularity). The first is characterized by a poorly defined boundary between the pneumatized portion and the nonpneumatized portion and a trabecular thickening in the spongy bone of the latter. The second shows a well-defined boundary between the pneumatized portion and the nonpneumatized portion and normal spongy bone architecture in the latter. The key feature for the diagnosis of secondary hypocellularity is the recognition of the walls of former air cells. The authors point out that their observations closely match the histopathological findings by Wittmaack (Wittmaack K: *Über die normale und die pathologische Pneumatisation des Schläfenbeins*. Jena, Germany: Gustav Fischer; 1918), who developed the concept of the normal pneumatization process of the temporal bone and the pathogenesis of aberrant pneumatization.

Mallur et al[90] determined the clinical characteristics and treatment outcomes of an unusual cluster of intracranial complications seen in AM. They performed a retrospective review of pediatric patients treated for AM in a tertiary care hospital from March 2006 to March 2007. Eleven children, 6 months to 10 years of age (mean age, 3.8 years), were treated for AM confirmed by computed tomography, which identified asymptomatic intracranial complications in 8 of the 11 patients: these were sigmoid sinus thrombosis (4 patients), epidural abscess (4), perisigmoid abscess or bony erosion (2), and tegmen mastoideum dehiscence (1). All patients required operative intervention with tympanomastoidectomy, although only 2 patients required neurosurgical intervention, consisting of evacuation of epidural abscess and sigmoid sinus thrombosis, respectively. Although uncommon, intracranial complications of AM may present without clinical signs or symptoms. Computed tomography of the temporal bone with contrast is essential for identifying asymptomatic complications. Mastoidectomy remains the mainstay of surgical treatment.

Pang et al[91] studied 79 episodes of AM, managed in 76 patients in a pediatric population, in a review of 11 years of experience in management. Prehospital treatment was commenced by the family practitioner or district hospital doctor in 53 of 79 patients. In 33 episodes, a previous history of acute otitis media was noted (42%). Complications were found in 30 episodes (38%), and 36 episodes (46%) required surgical treatment. The authors suggested that children with acute mastoiditis should be managed in centers where timely and complete medical and surgical treatment is available.

Thompson et al[92] conducted a retrospective cohort study by using the UK General Practice Research Database. Children aged 3 months to 15 years between 1990 and 2006 were included. Risk of mastoiditis within 3 months after otitis media diagnosis and the protective effect of antibiotics were determined. There were 2,622,348 children within the General Practice Research Database; 854 had mastoiditis, only one-third of whom (36%) had antecedent otitis media. Mastoiditis incidence remained stable between 1990 and 2006 (approximately 1.2 per 10,000 child-years). Risk of mastoiditis, after otitis media, was 1.8 per 10,000 episodes (139 of 792,623) after antibiotics compared with 3.8 per 10,000 (149 of 389,649) without antibiotics and increased with age. Antibiotics halved the risk of mastoiditis. General practitioners would need to treat 4831 otitis media episodes with antibiotics to prevent 1 child from developing mastoiditis. If antibiotics were no longer prescribed for otitis media, an extra 255 cases of childhood mastoiditis would occur, but there would be 738,775 fewer antibiotic

prescriptions per year in the United Kingdom. The high number of episodes of otitis media needing treatment to prevent 1 case of acute mastoiditis precludes the treatment of otitis media as a strategy for preventing mastoiditis. Although mastoiditis is a serious disease, most children make an uncomplicated recovery after mastoidectomy or intravenous antibiotics. Treating these additional otitis media episodes could pose a larger public health problem in terms of antibiotic resistance.

Thorne et al[93] studied 87 children (age <18 years) with acute mastoiditis treated at their institution over 2000-2007. Acute mastoiditis was defined by evidence of inflammation in the middle ear space and signs of mastoid inflammation (postauricular swelling, redness, or tenderness) or radiographic evidence of destruction of mastoid air cells, sigmoid sinus thrombosis, or abscess formation. Patients with underlying cholesteatoma were excluded. The frequency of cases of acute mastoiditis was positively correlated with calendar time, both for all cases of acute mastoiditis (Spearman rank correlation, $r = 0.73$; $P = .04$) and for cases of mastoid subperiosteal abscess ($r = 0.96$; $P < .001$). The authors observed an increase in the frequency of cases of acute mastoiditis with subperiosteal abscess seen at their institution over the study period, controlling for case volume. These findings suggest an increase in incidence, although further population-based studies are required to definitively evaluate this possibility.

Abdel-Aziz and El-Hoshy[94] studied 19 children aged 9 months to 11 years. Medical management alone was performed in 5 cases (26%), 7 cases (37%) needed tympanostomy, and 7 cases (37%) had cortical mastoidectomy with tympanostomy. The authors concluded that conservative management is an effective treatment of noncomplicated acute mastoiditis, but tympanostomy should be considered if there is no response within 48 hours. Cortical mastoidectomy should be used with medical management in complicated cases.

Croche Santander et al[95] investigated acute mastoiditis among 145 patients, of whom 54% received preadmission oral antibiotics, mainly β-lactamase. The most frequently presenting clinical findings were fever (78%), ear displacement (74%), otalgia (72%), and postauricular swelling (70%). Microbiological cultures were performed in 53 cases; *S pneumoniae* was the most isolated microorganism. Computed tomography scans were performed in 57% of cases. All patients received parenteral antibiotic treatment with a median duration of treatment of 5 days. Surgery was performed on 33%. Of the patients, 13% had extracranial and 8% had intracranial complications. A significant increase in intracranial complications was detected in the second half of the study period.

In Greenland, Homøe et al[96] reported that the incidence of acute mastoiditis was comparable to the incidence elsewhere, although AOM occurred more frequently among small children in the Greenlandic population. Median age was 14 months (range, 5-105 months), and 8 were female (72%). Seven of 10 were exclusively treated with antibiotics, and 3 underwent additional ear surgery. Bacteriological examination was performed in 5 of 10. One 8-month-old girl presented with a temporary facial nerve paralysis and was treated with intravenous antibiotics. In one 8-year-old girl with signs of meningitis, an acute CT scan showed a cerebellar abscess and a thrombosis in the lateral sigmoid sinus vein. An extensive cholesteatoma was found and eradicated during surgery. Six weeks later, the patient returned home with a maximal conductive hearing loss as the only complication. All patients recovered from the disease.

Lin et al[97] reviewed other studies of acute mastoiditis and presented an overview of the anatomical and pathophysiological considerations in acute mastoiditis. They suggested that with thorough clinical evaluations, early diagnosis, and close follow-up, a large proportion of children with severe acute otitis media or early stage mastoiditis can be managed in the primary care setting without immediate surgical specialty involvement.

Navazo-Eguía et al[98] retrospectively reviewed 61 acute mastoiditis cases diagnosed in children younger than 14 years between 1996 and 2008. Prehospital antibacterial agent therapy had been administered in 56% of the cases. Culture of middle ear effusions revealed *S pneumoniae* in 40%, *H influenzae* in 2%, *S aureus* in 12.5%, *P aeruginosa* in 8%, and sterile in 37.5%. Of the pneumococcal isolates, 26% were resistant to penicillin or third-generation cephalosporins. Of the patients, 93% responded well to antibacterial therapy alone or with tympanostomy. There were complications in 12%. Mastoid surgery was performed in 4 patients.

Quesnel et al[99] recently conducted a retrospective study of acute mastoiditis in 188 children. The most frequently isolated germs were *S pneumoniae* (51%), *S pyogenes* (11.5%), *Anaerobes* (6.5%), and coagulase-negative *Staphylococcus* (6.5%). All the patients were hospitalized and received intravenous antibiotics, and 36% (n = 68) underwent surgery. Several surgical procedures were necessary in 4 cases (2%). Acute mastoiditis recurrences requiring a second hospitalization were observed in 8 patients (4%). The only complication was lateral sinus thrombosis (n = 6; 3%). Surgical failures, requiring more than 1 surgical procedure, were more frequent in cases that had (1) *Anaerobes* or Gram-negative bacteria in microbiological samples and (2) surgical drainage without mastoidectomy. Recurrences were more frequent in AM due to *S pneumoniae*. The authors point out that if surgery is indicated, it must encompass a mastoidectomy; broad-spectrum intravenous antibiotic treatment must cover the most commonly involved germs and secondarily be adapted to the results of microbiological samples. If the infection is not controlled after 48 hours of intravenous antibiotic therapy, a mastoidectomy has to be performed.

Rodriguez et al[100] reported treatment of acute mastoiditis in children with CI. Among 248 children, 5 patients developed acute mastoiditis (2%), 3 of them with subperiosteal abscess (1%). The mean age of implantation was 2 years

and 4 months, and the complication presented between 1 and 33 months postimplantation. Four patients had episodes of serous otitis preimplantation. The mean age of AM patients was 3 years and 4 months. The CI type was nucleus in all cases. Conservative management is suggested for AM and subperiosteal abscess in children with CI. Surgical treatment should be avoided to prevent CI contamination. The first option is intravenous antibiotics and simple puncture of the abscess. If surgical drainage is needed, the authors recommended radiological study to locate the CI electrodes.

Stenfeldt and Hermansson[101] studied the occurrence, treatment policy, and clinical course of mastoiditis before and after the new treatment recommendations for acute otitis media were introduced in Sweden. Included in the study were all patients who were admitted to 2 ear, nose, and throat (ENT) departments in southern Sweden for acute mastoiditis from 1996 to 2005. A total of 42 cases of mastoiditis were identified: 23 during the first period of 1996-2000 and 19 during 2001-2005. Mastoidectomy was performed in 14 patients during the first period and in 8 during the second period. As many as 39% of patients with mastoiditis received antibiotics before hospital care but had no improvement. There was no indication that the number of patients with acute mastoiditis increased after the new treatment recommendation for AOM. There was no increase in the occurrence of mastoidectomy. Severe complications of mastoiditis were rare. It is important to follow up the consequences when treatment recommendations of AOM have been changed.

Tamir et al[102] reviewed the medical files of pediatric patients who had AM from 2005 to 2007. Fifty patients were identified. The sex distribution was equal, and the ages ranged from 4 months to 12 years. Of the 46 patients who were admitted to the institution, only 2 underwent CT scanning on admission, and 4 other patients had CT performed during hospitalization. The majority of patients (92%) with AM did not have a CT scan performed and were treated conservatively with no complications. In most pediatric patients, CT does not seem to be indispensable in the diagnosis of AM. Conservative therapy and close follow-up seem to suffice for most.

Facial paralysis. Thorne et al[103] presented a case of delayed facial paresis after tympanomastoidectomy for chronic otitis media in a pediatric patient with a clinical course consistent with viral reactivation.

Shiva and Balasubramanian[104] reported a case of COM and facial paralysis as a presenting feature of Wegener's granulomatosis. They reported a 40-year-old woman who presented with complaints of ear discharge, deep-seated ear pain, and loss of hearing in her right ear and suggested that early diagnosis demands heightened suspicion.

In a Spanish study by Santa Cruz Ruiz et al,[105] facial paralysis of infectious origin in patients receiving CI was reported. The authors showed 3 patients who underwent CI surgery at their department and who presented peripheral facial paralysis secondary to AOM. Treatment consists of parenteral antibiotic and corticosteroid treatment. Prognosis is favorable, with a total recovery of facial function in 1 or more months.

Wang et al[106] conducted a study on facial palsy in children with regard to emergency department management and outcome. Among 85 patients, 60% of the patients were male, and 65.9% were admitted to the hospital. Bell's palsy (50.6%) was the most common etiology followed by infectious (22.4%), traumatic (16.5%), congenital (7.1%), and neoplastic etiologies (3.5%). Patients with Bell's palsy had shorter recovery times (P = .049), and traumatic cases required a longer time for recovery (P = .016). Acute otitis media–related pediatric facial nerve paralysis (FNP) had shorter recovery times than non–AOM-related cases (P = .005) in the infectious group. Patients given steroid therapy did not have a shorter recovery time (P = .237) or a better recovery (P = .269). There was no difference in the recovery rate of pediatric patients with Bell's palsy who were hospitalized or not hospitalized (P = .952). The authors concluded that Bell's palsy, infection, and trauma are the most common etiologies of pediatric FNP; recovery times are shorter in pediatric patients with Bell's palsy and AOM-related FNP, whereas recovery takes longer in traumatic cases; steroid therapy does not seem beneficial for pediatric FNP; and hospitalization is not indicated for pediatric patients with Bell's palsy.

Yonamine et al[107] studied 40 patients with coexisting acute otitis media, from a total of 2758 cases of facial paralysis seen in the department for facial nerve disorders. The authors reported that the paralysis was of sudden onset in 95% of the cases. Recovery was of 85% for grade I (House-Brackmann) and 15% for grade II (House-Brackmann). Treatment was clinical, with antibiotics and steroids yielding good results. In those patients with electrical testing indicating bad prognosis, facial nerve decompression turned their prognosis into a favorable one.

Scardapane et al[108] reported a 4-year-old child who was admitted for facial nerve palsy and abducens nerve palsy subsequent to a 2-week persistent pain in the right ear. The child was eventually diagnosed with Gradenigo's syndrome with lateral venous sinus thrombosis. Symptoms completely resolved with conservative management.

Ozbek et al[109] reported a study on the management of facial nerve paralysis in noncholesteatomatous chronic otitis media. Among 13 patients, 6 had dehiscence of the fallopian canal, whereas the bony canal was intact in the remaining patients. Decompression of the facial nerve was not performed in 5 of 7 ears with an intact fallopian canal. Four ears underwent total decompression from the geniculate ganglion to the stylomastoid foramen, whereas the remaining 4 ears underwent partial nerve decompression. However, statistical analysis did not show any difference in recovery between the patients with surgical decompression and those without decompression (P = .171). All patients not receiving decompression had successful outcomes (80% classified as grade I and 20% as grade II). The authors concluded that all patients not receiving decompression had successful outcomes; intravenous antibiotic treatment in

conjunction with steroid therapy is the mainstay management of facial paralysis due to chronic otitis media without cholesteatoma, and it is therefore not necessary to decompress the facial nerve in cases of facial paralysis in noncholesteatomatous chronic otitis media.

Intracranial Complications

Isaacson et al[110] investigated pediatric otogenic intracranial abscesses. An inpatient database was queried for the following diagnostic codes from 2000 to 2008: petrositis (383.2), acute mastoiditis (383), labyrinthitis (386.3), facial paralysis (Bell's palsy) (351.0), facial nerve disorder unspecified (351.9), other facial nerve disorders (351.8), subperiosteal abscess (383.01), Gradenigo's syndrome (383.02), meningitis (320), extradural or subdural abscess (324.9), intracranial abscess (324.0), thrombosis of intracranial venous sinus (325), and otic hydrocephalus (348.2). Forty patients were identified with an otogenic intracranial complication. Thirty patients had evidence of an intraparenchymal, epidural, subdural, or petrous apex suppurative complication of otitis media. Eighty percent of patients had a canal wall-up mastoidectomy, 10% patients had a craniotomy without a mastoidectomy, and 10% patients were managed with intravenous antibiotics with or without pressure equalization tubes. The authors showed that in selected cases, patients with intracranial abscesses can be managed with intravenous antibiotics without mastoidectomy. The use of canal wall-up mastoidectomy is an acceptable alternative to radical mastoidectomy when surgical intervention is necessary.

Lin et al[111] investigated the prevalence of chronic otitis media and its complication rates in teenagers and adult patients. Complications secondary to COM were identified in a total of 115 patients. The incidence of COM in adults decreased per year ($R^2 = 0.845$; $P < .001$) from 1998 to 2007. The mean age of patients with COM increased from 44.67 to 49.43 years (linear regression; $R^2 = 0.896$; $P < .001$). The prevalence of COM decreased considerably. However, the annual extracranial complication rate ($R^2 = 0.109$; $P = .352$) and intracranial rate ($R^2 = 0.382$; $P = .057$) have not decreased during the past 10 years. The authors concluded that the complication rate for COM has remained steady in the past 10 years, regardless of the overall reduction in the prevalence of COM with the use of antibiotics. A high index of suspicion and imaging studies for early identification are recommended. Pneumococcal vaccination is recommended, particularly for elderly patients who are treated conservatively because of relatively poor general health conditions.

Mustafa et al[112] studied complications of COM with cholesteatoma during a 10-year period in Kosovo. Among 91 patients (60.4% men and 39.6% women), 1 or 2 complications were recorded. Extracranial (EC) complications were observed in 57.1%, and intracranial (IC) complications were seen in 31.9%. Eleven percent had multiple complications. For the IC cases, meningitis (19.7%) and perisinus abscess (15.3%) were the most common complications. The most often isolated pathogen from ear swabs was *Proteus mirabilis* in 33.3% of cases. The most frequent radiological diagnostic procedures were mastoid tip X-rays, which were performed in 77% of the patients, and computed tomography in 24%; magnetic resonance imaging was not performed on any of the patients during the study period. In this series, 3.3% died as a result of complications, whereas the remaining 96.7% survived. Complications of COM with cholesteatoma can represent life-threatening conditions, and close cooperation between otosurgeons, neurosurgeons, and infectious disease specialists is mandatory.

Wanna et al[113] also reported management of intracranial complications as a result of otogenic infections. Ten cases reviewed had intracranial complications. Five patients had brain abscesses, 1 patient had a subdural empyema, and 4 patients had lateral sinus thrombosis. The authors indicated that broad-spectrum intravenous antibiotics for 6 weeks are usually sufficient treatment. Management of the intracranial disease takes precedence, but direct drainage of the abscess may not be necessary if a patient's symptoms, neurologic status, and radiographic findings progress favorably.

In Papua New Guinea, Dubey et al[114] showed intracranial spread of chronic middle ear suppuration. In 32 patients, otitic meningitis, the commonest intracranial complication, was seen in 43.7%; lateral sinus thrombosis in 31.2%; cerebellar abscess in 18.7%; epidural abscess in 21.8%; perisinus abscess in 15.6%; cerebral abscess and interhemispheric abscess in 6.2%; and subdural abscess, otitic hydrocephalus, and otogenic cavernous sinus thrombosis in 3.1%. The authors emphasized that infected thrombus in the dural venous sinus should be removed to prevent dissemination of septic emboli.

Ibrahim et al[115] reported the incidence of meningitis secondary to suppurative OM in adults. Eighty-seven cases of meningitis from 1997 to 2002 were analyzed retrospectively. Acute and chronic suppurative OM accounted for 13 and 3 cases, respectively. The overall mortality rate was 5.7%. The authors showed that the incidence of otogenic meningitis was 0.42 per 100,000 per year.

Damergis et al[116] reported a case of otogenic pneumococcal meningitis with pneumocephalus. A 33-year-old man with Crohn's disease and azathioprine use presented to their emergency department with progressive headache while taking antibiotics for OM. Initial computed tomography scan of the brain revealed pneumocephaly, and cerebrospinal fluid analysis and culture diagnosed pneumococcal meningitis. The authors stated that meningitis has been a rare complication of *S pneumoniae* infections since the advent of antibiotics; however, it may become more frequent with increasing antibiotic resistance and a growing population of immunocompromised patients. They also stated that pneumocephalus in the setting of meningitis and otitis media should raise the suspicion for mastoiditis (even without overt clinical findings), and early consultation with an otolaryngologist is warranted.

Lesnakova et al[117] investigated how many cases of bacterial meningitis in their national survey were associated with sinusitis or OM. Among 372 cases of bacterial

meningitis within their nationwide 17-year survey, 201 were community-acquired meningitis (CBM), and in 20%, OM or acute/chronic sinusitis was reported 1 to 5 weeks before onset of CBM. Diabetes mellitus (20% vs 7.5%; $P = .01$), alcohol abuse (35% vs 15.4%; $P = .003$), and trauma (30% vs 14.9%; $P = .02$) were significantly associated with CBM after ENT infections. Concerning etiology, CBM after sinusitis/otitis was not significantly associated with pneumococcal etiology (50% vs 33.8%; NS) and significantly associated with other (*Listeria monocytogenes, Streptococcus agalactiae*) bacterial agents (9.9% vs 25%; $P = .008$). However, those significant differences for new ENT-related CBM had no impact on mortality (12.4% vs 5%; NS), failure after initial antibiotics (10% vs 9.5%; NS), and neurologic sequelae (12.5% vs 15.4%; NS).

Slovik et al[118] investigated the role of surgery in the management of otogenic meningitis. Two patients had an emergency mastoidectomy, and 1 patient underwent surgery 1 month postrecovery due to the suspicion of bone erosion on a CT scan. In 2 cases, a canal wall-up procedure was performed, and 1 patient underwent revision of a radical mastoidectomy. In all cases, no pus or granulations were seen in the mastoid. Two patients fully recovered, and 1 patient died.

In a retrospective study by Bales et al,[119] the authors reviewed medical charts of 13 patients diagnosed with otogenic lateral sinus thrombosis. The diagnosis was made by using CT and magnetic resonance imaging (MRI)/venography. Treatments included MT placement, simple mastoidectomy, intravenous antibiotics, and anticoagulation. Posthospitalization follow-up data revealed no significant long-term complications. The authors emphasized that neurologic, rather than otologic, symptoms may dominate in children. In particular, signs and symptoms of sixth or seventh nerve impairment and raised intracranial pressure may be important clues to the diagnosis.

Christensen et al[120] reported a review of 7 cases of lateral sinus thrombosis and proposed a management algorithm. All patients underwent MRI with venography (MRV) for diagnosis. All patients were admitted to the hospital and treated with antibiotics. Five of 7 were treated with simple mastoidectomy and concurrent middle ear ventilation tubes, and 2 of 7 received only medical treatment. Two patients had long-term sequelae: one had persistent mild lateral gaze diplopia, and another had unilateral moderate to severe high-frequency SNHL loss. Six of 7 patients had follow-up imaging. Four of 6 patients showed recanalization of the lateral sinus on repeat imaging. The authors concluded that lateral sinus thrombosis is an uncommon cranial complication of OM, and the advent of noninvasive diagnosis and effective broad-spectrum antibiotics has drastically decreased the mortality and altered the diagnostic and treatment paradigm.

Bravo et al[121] reported mastoiditis complicated with Gradenigo's syndrome and a hypertrophic pachymeningitis with consequent communicating hydrocephalus. Documented by the development of clinical findings, magnetic resonance imaging, cerebrospinal fluid changes, histopathology findings, otosurgical intervention, and finally the insertion of a ventriculo-peritoneal shunt, the case illustrates a gradual development of pachymeningitis with consequent hydrocephalus and intracranial hypertension.

Isildak et al[122] presented a case with sigmoid sinus thrombosis of the sinus due to compression following surgical injury. The patient presented to the authors' care with otitic hydrocephalus, increased intracranial pressure, papilledema, oculomotor and abducens nerve palsy, and severe right-side visual loss as prominent features. The authors stated that the frequency of sigmoid sinus hypoplasia reported in the literature is about 17%, and considering the possibility of postoperative intracranial hypertension, sigmoid sinus and jugular bulb surgeries were contraindicated in a case with contralateral sigmoid sinus hypoplasia.

de Oliveira Penido et al[123] reported on the presentation, treatment, and clinical course of 8 patients with otogenic lateral sinus thrombosis. Fever, headache, and cranial nerve paralysis were the main clinical manifestations associated with coexisting mastoiditis, meningitis, and cerebellar and epidural abscess. All patients underwent mastoidectomy and were given broad-spectrum antibiotics for 2 months. Four patients were anticoagulated, and all patients experienced complete clinical recovery without sequelae.

Alaani et al[124] retrospectively proposed a transtemporal approach to otogenic brain abscesses. Five children had acute middle ear disease and had an extended cortical mastoidectomy approach to both intracranial pathology and ear disease. One adult patient had a petrous apex cholesteatoma and underwent petrosectomy, followed by transtemporal abscess drainage. All patients were treated by mastoidectomy and needle aspiration to drain the abscesses. The authors concluded that this approach has a low complication rate and avoids the need for a craniotomy or subsequent operations.

Morwani and Jayashankar[125] reported the management of otogenic intracranial abscess with a single-stage, transmastoid approach. Among 73 patients, 12 were lost to follow-up and were excluded from the study. Adults were more commonly affected by otogenic intracranial abscess than children, with a male preponderance. Otogenic intracranial abscess was associated with both cholesteatomatous (41%) and noncholesteatomatous ears (59%). All cases were treated with transmastoid drainage of the intracranial abscess and canal wall-up or wall-down tympanomastoidectomy, depending on the ear pathology. Of the patients, 3% had postoperative cerebrospinal fluid leakage, 3% had meningitis, and 5% of patients had recurrent abscess; 3% of the patients died but were included in the study. Three patients had residual abscess, which improved with additional management. The authors suggested that single-stage transmastoid drainage of the intracranial abscess and concurrent treatment of the otogenic pathology is an effective treatment for otogenic intracranial abscess.

Erdogan and Cansever[126] reported a review on pyogenic brain abscess. Surgical treatment options showed no significant difference with respect to mortality levels, but lower

morbidity rates were achieved with stereotactically guided aspiration. Decompression with stereotactically guided aspiration, antibiotic therapy based on results of pus culture, and repeated aspirations if indicated from results of periodic CT follow-up scans seem to be the most appropriate treatment modality for brain abscesses. Immunosuppression and comorbidities, initial neurological status, and intraventricular rupture were significant factors influencing the outcomes of patients.

Yilmaz et al[127] reported a case report on cerebellar abscess and meningitis caused by *Shewanella putrefaciens* and *Klebsiella pneumoniae* associated with COM in a river trap fisherman. *Shewanella putrefaciens* is a facultatively anaerobic, nonmotile, Gram-negative, nonfermentative bacterium, and it is a rare cause of brain abscesses and meningitis.

Implications for Practice

Over the past 4 years, progress has been made in advancing the knowledge on the complications and sequelae of OM. This knowledge can be used to prevent and treat complications and sequelae of OM more effectively. Areas of potential future research have been identified and outlined.

Goals for Future Research

Ultimate research goals are to identify ways to prevent complications and sequelae of otitis media and develop new management strategies. The sequelae related to AOM, OME, and treatment should be differentiated and studied separately. The panel considered all areas of complications and sequelae and came up with following list.

1. It is important to define early signs and symptoms of threatening complications such as mastoiditis, meningitis, epidural abscess, and sinus thrombosis.
2. It will be necessary to develop prospective monitoring on OM complications in the new era of treatment guidelines.
3. Tympanic membrane atrophy, retraction pockets, and adhesive OM have to be studied further clinically and in the new animal models.
4. The mechanism of tympanic membrane perforation and healing needs to be further clarified.
5. The pathogenesis of myringosclerosis and tympanosclerosis needs to be clarified. Treatment methods to prevent these conditions need to be studied.
6. The pathogenesis and new treatment strategies of suppurative OM with otorrhea need to be investigated.
7. Basic science and clinical research should focus on the etiopathogenesis and behavior of cholesteatoma. Novel management methods to eradicate cholesteatoma and prevent recurrence should be developed. Refined imaging techniques need to be further explored to prevent second-look surgery.
8. The mechanism of ossicular erosion and fixation associated with COM with and without cholesteatoma needs to be investigated.
9. The etiopathogenesis of vestibular disturbances associated with OM should be clarified.
10. The transient and long-term effects of inflammatory mediators of OM in the ME and the inner ear need to be studied further.
11. Studies on the demographics, severity, and prevention of complications of OM in developing countries need to be developed.
12. Consensus needs to be developed for the best minimally invasive method of treatment for acute complications of OM such as mastoiditis, facial palsy, or intracranial involvement regarding mastoidectomy, myringotomy, and antibiotics.

Author Contributions

Timothy T. K. Jung, literature search and review, preparation and discussion of the manuscript, correction and finalization; **Cuneyt M. Alper**, literature search and review, preparation and discussion of the manuscript; **Sten O. Hellstrom**, literature search and review, preparation and discussion of the manuscript; **Lisa L. Hunter**, literature search and review, preparation and discussion of the manuscript; **Margaretha L. Casselbrant**, literature search and review, preparation and discussion of the manuscript; **Anita Groth**, literature search and review, preparation and discussion of the manuscript; **Yusuf K. Kemaloglu**, literature search and review, preparation and discussion of the manuscript; **Sang Gyoon Kim**, literature search and review, preparation and discussion of the manuscript; **David Lim**, literature search and review, preparation and discussion of the manuscript; **Susan Nittrouer**, literature search and review, preparation and discussion of the manuscript; **Kee Hyun Park**, literature search and review, preparation and discussion of the manuscript; **Diane Sabo**, literature search and review, preparation and discussion of the manuscript; **Jorge Spratley**, literature search and review, preparation and discussion of the manuscript.

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