# RECENT ADVANCES IN OTITIS MEDIA

Report of the Ninth Research Conference
2007

# *Contents*



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# INTRODUCTION

The Ninth Post-Symposium Research Conference was held at the Tradewinds Resort and Conference Center in St. Pete Beach, Florida, on June 7 and 8, 2007, immediately following the Symposium. The approximately 57 participants, who included otolaryngologists, pediatricians, audiologists, epidemiologists, microbiologists, immunologists, and cell and molecular biologists from the United States, Europe, Asia, and South America, were assigned to respective Panels and developed Panel Reports. In addition, another 22 individuals unable to attend the Research Conference contributed to this Research Conference Report.

The Post-Symposium Research Conferences were initiated to further capitalize upon the gathering of leading researchers in the field of otitis media that occurs for the International Symposium in Recent Advances in Otitis Media. Thus, the Research Conferences have taken place since the first International Symposium was held in 1975 in Columbus, Ohio.

The goals of the Research Conference are to 1) critically review the most recent advances and breakthroughs made during the 4 years since the previous Symposium; 2) develop consensus on definitions and classification; 3) identify new research opportunities that have arisen because of technical advances; 4) promote research on otitis media among young scientists; 5) identify critical research questions; and 6) identify short-term and long-term research goals. The Research Conference Report was designed to be as critical, selective, and succinct as possible so that the reader may use this handy research resource to follow recent advances and to understand the important questions that are challenging researchers.

All of the Research Conference Reports have been published by the *Annals of Otology, Rhinology and Laryngology* as supplements to ensure wide dissemination and distribution. Along with the Proceedings of the Otitis Media Symposium, the Research Conference Reports have been valuable resources for investigators interested in otitis media research and are especially helpful for those new to the field. Prohibitive costs of publication and difficulty of finding sponsors, forced us to resort to in-house publication. However, the report will be posted in the Otitis Media Symposium website maintained by the House Ear Institute.

Research on otitis media has been advancing at a rather rapid pace, especially in recent years, in part because of the development of new technology and methodology. We believe that the Post-Symposium Research Conferences have also made significant contributions in stimulating otitis media research in many areas, including clinical and basic research.

Generating the Research Conference Report is not an easy task. A great deal of effort is required of the Panel Chairs, Co-Chairs, and Panel Members. Because of the international composition of the Panels, there are many divergent philosophies and opinions, and we have tried to incorporate these differences in the Research Conference Report. We would like to thank the Panel Chairs and Co-Chairs for gathering information from the Panel Members and for generating these excellent reports, as well as all of the Panel Members who contributed to these reports.

On behalf of the Planning Committee of the Research Conference, we especially thank the National Institute on Deafness and Other Communication Disorders (NIDCD), the National Institutes of Health, the Deafness Research Foundation, the House Ear Institute, and the International Otitis Media Symposium Inc for their support for this Research Conference and for the partial funding of publication of the Report. We are especially grateful to Dr Bracie Watson, Program Director, Hearing Program, NIDCD, who participated in the Research Conference representing the NIDCD Extramural Program.

We also thank Lisa Astorga of the Talley Management Group and Janet Lauder at the House Ear Institute for providing logistical support for the Research Conference. We are particularly indebted to Robert Gellibolian (who coordinated the Research Conference), to Colleen Young (who assisted with correspondence as well as grant management), to Deborah Minette and Rebecca Juárez (who assisted

in the final phase of editing the Conference Report) from the Office of the Executive Vice President of Research at the House Ear Institute.

We also acknowledge the grant support of NIDCD, the National Institutes of Health and the Deafness Research Foundation, that made this Report possible.

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# SPECIAL REMARKS

BRACIE WATSON, JR, PHD

PROGRAM DIRECTOR, HEARING PROGRAM

NATIONAL INSTITUTE ON DEAFNESS AND OTHER COMMUNICATION DISORDERS

Otitis media is a complex disorder which involves both genetic and environmental factors. It has its highest incidence among infants and children and is a major health issue for children not only in this country but also for children elsewhere in the world. Most children in the United States will experience at least one episode of otitis media during their first three years of life and many will have multiple episodes. The potential long-term detrimental effects on hearing, speech, and language development in children with persistent middle ear effusion are of major concern.

Both basic and clinical research is needed to increase our understanding of the pathogenesis of the disease, discovery of new mechanisms/interactions, and the ultimate translation of this knowledge into new treatments and prevention. The presentations and discussion at the Ninth Research Symposium on Recent Advances in Otitis Media as well as the critical review and assessment of recent discoveries at the Post-Symposium Research Conference were greatly facilitated by the targeted assembly of researchers with novel ideas, excellent clinical knowledge, and researchers with expertise in multiple scientific disciplines. This forum for sharing and reviewing research data is extremely useful in advancing research in otitis media. The success of the current Symposium is in part based upon research conducted during the years covered by, and data presented at, the prior symposia. The planning committee should to be commended for their efforts in making this Symposium such a success.

The National Institute on Deafness and Other Communication Disorders is pleased to have sponsored the Post-Symposium Research Conference and look forward to future years of productive interactions with the otitis media research community.

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# PANEL REPORTS

## 1. EPIDEMIOLOGY, NATURAL HISTORY, AND RISK FACTORS

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### RECENT SIGNIFICANT CONTRIBUTIONS

Since the last Recent Advances in Otitis Media Research conference in 2003, researchers have conducted studies to reinforce and support past findings, and to offer intriguing new hypotheses. This report reviews the epidemiological literature published since the 2003 conference, and recommends short and long-term goals relevant to otitis media (OM) epidemiology. It also provides recent information about physician adherence to acute otitis media (AOM) guidelines and the effect of pneumococcal conjugate vaccine on population based rates of AOM.

### PREVALENCE AND INCIDENCE

*High Risk Populations.* Epidemiological reports of OM in high risk populations have been published over the past four years. These studies from many countries and populations continue to show a substantial OM burden with evidence of chronicity, particularly among indigenous populations. Studies from a Canadian indigenous population reported 2300 outpatient AOM visits/1,000 child-years among infants <2 years.[1]

Community-based epidemiologic studies that examined children in Bangladesh,[2] Nigeria,[3] and Australia[4] reported prevalence of chronic suppurative otitis media (CSOM), which was 12%, 2.5%, and 15% respectively. In the Australian aboriginal study 41% had OME, 33% had AOM, and tympanic membrane perforations affected 40% of children in their first 18 months of life.[4] Incidence of OM by 6 months was 63% in a cohort of American Indian children.[5]

Another study of Australian aboriginal children and adults reported a low incidence of cholesteatoma (1-3%) compared to higher rates of chronic otitis media (25-45%).[6] A report from Greenland describes a family cluster of cholesteatoma, suggesting a possible role for genetic factors and anatomical mechanisms for this middle ear disorder.[7] Australian researchers documented procedures for implementing a community participatory model for a double blind, multi-center RCT of ototopical treatments for chronic suppurative otitis media (CSOM).[8] This research model was highly successful among the participating Aboriginal communities, and shifted the balance of control to the participants and their communities, who were integral to every stage of study planning, conduct, analyses, and dissemination of findings. This model could be used to conduct research in other high risk populations.

Gunasekera[9] conducted a systematic review of population-based studies with OM incidence and prevalence data in <18 year-olds that included >250,000 children in the analyses. Inuits (81%) and Australian Aborigines (84%) had the highest prevalence of OM. Prevalence of hearing impairment ranged from <1% in Greece to 23% among Australian aborigines and was significantly more common in children with OM. Other studies reported hearing screening fail rates (>22dB HL) among 19% of 5 and 6 year old Inuit children;[10] 30% of Minnesota American Indians infants failed OAE by age 6 months.[11] One-third of aboriginal

school children had unilateral or bilateral hearing loss of 30 dB or greater.[12]

*Trends Over Time.* Studies from several countries show a general decline in OM incidence that was not reported in the last review. Kvaerner[13] conducted a study of Norwegian hospital admissions for AOM from 1999−2005 among children ≤7 years using national treatment data. Hospitalization for AOM was less frequent, and incidence of acute mastoiditis remained stable at approximately 6 per 100,000 children over time.

A study using data from the Third National Health and Nutrition Examination Survey (NHANES III) in the U.S., conducted in 1988−91 and 1991−94 among >8000 parents of preschool aged children, showed a nonsignificant 3% increase in reported rates of OM.[14] This study did show statistically significant increases in reported rates of OM onset by 12 months (5%) and recurrent OM (≥3 episodes; 6%). The greatest increase in prevalence of early and recurrent OM was for poor children and those with less educated parents. However, more recent U.S. data from the National Health Interview Survey (NHIS), which collects information on over 12,000 children annually, has shown declining rates of parent-reported recurrent OM (≥3 episodes in the past 12 months) for all ages up to 15 between 2001 and 2005.[15]

In addition, data collected annually in the U.S. National Ambulatory Medical Care Survey (NAMCS) and National Hospital Ambulatory Medical Care Survey (NHAMCS) on the number of office visits with OM as the primary diagnosis for children under the age of 18 years has shown a declining trend from a baseline rate of 344.7 per 1,000 children in 1997 to a rate of 213.5 per 1,000 children in 2004.[16] The largest decline has occurred for young children under 3 years of age in whom OM prevalence is highest. Introduction of the pneumococcal conjugate vaccine, which was licensed for routine infant administration in 2000, could partially explain the decline.[17, 18] Studies have shown a decline in OM diagnoses in vaccinated children beginning in 2002. Comparing OM rates in 1994-99 with 2002-03 among children <2 years, a significant 20% decline (246 OM visits per 1,000 children per year) was observed.[17] Among children from Tennessee and New York born in 2000−01, 29% had frequent OM by age 2, and 6% had been treated with tympanostomy tubes, which represented a decrease of 17-28% in frequent OM and 16-23% in tympanostomy tube treatment.[18] These declines may be the result of infant immunization, catch-up doses in older children, and herd immunity. Decreases in invasive pneumococcal disease have also been seen in all age groups between 1998 and 2003.[19]

In the Netherlands, a cohort study included all children <13 years between 1995−2003 in the University Medical Center Utrecht Primary Care Network research database.[20] OM incidence, antibiotic prescription and referral rates were calculated. General practitioner consultation rates for AOM and OME declined by 9% and 34%, respectively. Among 2-6 year-olds, AOM and OME incidence rates declined by 15% and 41% respectively; for 6-13 year-olds, rates declined by 40% and 48%. For those <2 years, AOM and OME incidence increased by 46% and 66%, while antibiotic prescription rates for AOM and OME increased by 45% and 25%. AOM referral rates were constant in this age group, whereas OME referral rates increased by 45%.

This research database was also used to study antibiotic prescription and referral rates for respiratory tract infections (RTIs) among children ≤5 years of age between 1998 and 2002.[21] Antibiotics were prescribed in >50% of episodes of lower RTI, sinusitis, AOM, and acute tonsillitis; 98% of RTIs were managed in primary care, and about 1% per year were referred to a pediatrician or ENT specialist, mostly for AOM. Children <2 years were significantly more likely than older children to be treated with antibiotics (RR 1.4, 95% confidence interval (CI) 1.3,1.6) or referred to a specialist (RR 2.3, 95% CI 1.8, 3.0). Speets et al.[22] analyzed the 2005 Netherlands National Network of General Practitioners (GP) database of >9000 patients to estimate OM burden in their pediatric patients. GPs diagnosed 16% of children ≤4 years with OM (85% was AOM), and they had an average of 1.2 AOM/year requiring an average of 1.3 visits/year. These rates may be an underestimate of OM burden since GPs see only about 30% of all OM episodes.

A database from 291 general practices in the U.K. with records for more than 2 million patients was used to evaluate changes in middle ear disease workload and the impact of prescribing antibiotics

on repeat surgery.[23] Middle ear disease coded as AOM or glue ear subcategories (excluding CSOM) with a first episode in 1991−2001 were included in analyses. Total AOM consultations decreased markedly over the period, while glue ear consultations increased somewhat. Among 2-10 year olds, AOM consultations decreased from 105.3 to 34.7/1000 per year, while glue ear consultations were relatively stable (15.2 to 16.7/1000 per year). Antibiotic prescribing for AOM was constant (80-84% of consultations), but rose sharply for glue ear (13 to 62%). Prescribing antibiotics slightly increased the risk of a return visit for AOM (hazard ratio [HR] = 1.09, 95% C 1.07, 1.10) while reducing the likelihood for a return visit for glue ear somewhat (HR = 0.92, 95% CI = 0.88 to 0.96).

## RISK FACTORS: AGE, GENDER, RACE, SOCIAL CLASS

*Gender and Race.* The Oslo Birth Cohort enrolled infants born in 1992-93 and collected data from children at birth and several later time periods, the latest was a questionnaire in 2001–02.[24] Among 10 year olds, 13% had at least one OM episode during the previous 12 months, and OM was somewhat more common in girls ($OR_{adj}$ 1.2, 95% CI 1.0,1.4).[25]

Racial/ethnic disparities in OM diagnosis were examined in a well controlled study of >11,000 infants followed from 1-6 months at two urban and one suburban location in the U.S.[26] After adjusting for gender, maternal age, marital status, parity, number of children in the home, breastfeeding at 6 months, daycare attendance and size, non-Hispanic white children had significantly higher OM rates. Non-Hispanic black children had significantly reduced rates of OM (OR = 0.74), as did non-Hispanic Asian children (OR = 0.77). Hispanic and non-Hispanic children had similarly increased rates of OM after multivariable adjustment. When analysis was stratified by racial/ethnic subgroup, day care attendance was not a significant risk factor among Hispanics (<10% of the sample), but it was the most significant risk factor for non-Hispanic Asian, white, and black children. In the full multivariable model, a dose-response relationship with day care size predicting OM risk was demonstrated, i.e., ORs = 1.5, 2.0, 3.0, and 3.8 as number in daycare increased from 1–3, 4–6, 7–12, ≥13 children.

## DAY CARE, EXPOSURE TO OTHER CHILDREN, RESPIRATORY INFECTIONS

Koch et al.[27] performed a prospective study of 0-2 year-old Greenland Inuit children. Risk factors such as age 6-17 months, attending a child care center, and sharing a bedroom with adults were associated with upper respiratory tract infection (URTI), which included AOM. In a systematic review of studies of children around the world, OM prevalence was associated with lack of breast feeding and parental smoking.[9] Amusa et al. reported that in Nigeria, significant risk factors for OM were exposure to wood smoke and sleeping in a room with >4 children.[3] In clinical and epidemiologic studies[28, 29] regular exposure to children was not a useful factor in distinguishing children who presented with recurrent acute OM (RAOM) from those who developed chronic otitis media with effusion (COME).

Hoffman et al. analyzed the OM data from the Early Childhood Longitudinal Study−Birth Cohort (ECLS-B), a nationally-representative longitudinal study of U.S. births in 2001, including >8000 children studied at 9 months and 2 years of age.[15] Parents reported medically diagnosed OM in 39% by 9 months and 62% by 2 years of age. Treatment for the most recent episode was antibiotics (96%), ear drops (8%), analgesic (8%) tympanostomy tubes (3%) and observation (<1%). Significantly increased risk of ROM was found for male, non-Hispanic white infants. Daycare, low birth weight, and breast feeding <9 months were also significant predictors for ROM, which in turn increased the likelihood of getting tubes.

Because quality daycare may not be available or affordable for disadvantaged families, a randomized controlled trial of 143 children between 6 months and 3.5 years was conducted in a borough of London, U.K. to determine if daycare type influenced OM incidence.[30] Mothers and children randomized to the intervention were provided high quality daycare at the center, while the controls used whatever means of child care they could arrange. Providing daycare had a borderline effect on maternal employment (RR 1.23, 95% CI 0.99 to

1.52), but did not affect household income. Children in the intervention group had more OME (RR 1.74, 95% CI 1.02, 2.96) and used more health services (RR 1.58, 95% CI 1.05, 2.38) than those in the control group, which may be due to control children being cared for in smaller settings with fewer children.

A total of 623 URTI in 612 children <3 years were evaluated for bacterial complications (e.g., AOM and sinusitis), 30% of URTIs were complicated by AOM and 8% by sinusitis.[31] The rate of AOM after URTI declined with increasing age, whereas the rate of sinusitis after URTI peaked in the second year of life. A study using data from the FINOM Cohort Study and Vaccine Trial reported that respiratory viruses can be detected in >50% AOM episodes.[32] In 19%, virus was the sole pathogen, and no pathogens were recovered in 25%. The study did not provide evidence that a specific virus type would predispose to a given OM bacterial pathogen.

In the population-based Oslo Birth Cohort study, Karevold[25] reported substantial infectious comorbidity in a subgroup of 10 year-old children. OM was associated with both tonsillopharyngitis ($OR_{adj}$ 3.1, 95% CI 2.6, 3.9) and lower respiratory infections ($OR_{adj}$ 3.5, 95% CI 2.7, 4.6), indicating a relationship between upper and lower airways infections. Shared immunological mechanisms were explored by Brandtzæg[33] who reported a common distribution of antibodies in the respiratory tract enhancing the importance of the Waldeyer ringfor the immunocompetence of lower airways as well.

Daly et al.[5] conducted a prospective study of American Indian infants in Minnesota. Infant history of URTI (OR 5.45, 2.62, 11.36) and maternal history of OM (OR 1.77, 95% CI 1.02, 3.08) were risk factors for OM onset in the first 6 months of life. Mothers' knowledge of OM before birth did not predict their avoidance of potential modifiable risk factors (smoke exposure, child care, short duration or no breastfeeding).

In a case-control study of CSOM in the Netherlands, previous tube treatment was the most important risk factor (OR, 121.4, 95% CI 38.9, 379.3).[34] Recent recurrent respiratory infections, less educated parents, and older siblings also significantly increased CSOM risk. Most predictive factor for CSOM among children with tubes was >3 OM episodes in the past year ($OR_{adj}$ 4.9, 95% CI 2.2, 11.0). Attending day care ($OR_{adj}$ 3.6, 95% CI, 1.7, 7.8) and having older siblings ($OR_{adj}$ 2.6, 95% CI, 1.2, 5.5) also significantly increased CSOM risk.

## BREAST-FEEDING, PASSIVE SMOKE EXPOSURE, POLLUTANTS

*Breast-Feeding.* Pettigrew and colleagues[35] conducted phone interviews among middle income mothers of infants at 3, 6, 9, and 12 weeks of age. A survey was completed at 6 months to ascertain illness visits; 42% were for eye or ear conditions. Less than 3 months of breast-feeding compared to ≥6 months increased the likelihood of an illness visit (RR 1.42, 95% CI 1.11, 1.81) using a generalized linear model. An interaction between breast-feeding and number of children at home was observed; breast feeding was protective only if there was no regular exposure to other children.

Using 1988-94 NHANES data from 6-24 month olds, exclusive breast-feeding for ≤4 to 6 months compared >6 months increased the risk or having ≥ 3 OM in 12 months (OR 1.95, 95% CI 1.06, 3.59).[36] Exposure to both pre- and postnatal smoking showed borderline significance (OR 1.46, 95% CI 1.01, 2.16).

*Passive Smoke Exposure.* Factors that predicted ROM and COME were studied among 210 children <2 years diagnosed with AOM and participating in a randomized clinical trial.[29] With multivariate analyses, exposure to passive smoke was protective against ROM ($OR_{adj}$= 0.5, 95% CI 0.3, 0.9), whereas male gender ($OR_{adj}$ 1.8, 95% CI, 1.0, 3.3), winter season ($OR_{adj}$ 2.4, 95% CI, 1.3, 4.4), and persistent symptoms ($OR_{adj}$ 2.3, 95% CI, 1.2, 4.1) significantly increased the risk of subsequent OM. Neither passive smoke nor breastfeeding were related to COME, but winter season, bilateral AOM, sibling history of ROM, and previous AOM were related to COME. However, the attempt to use risk scores as prognostic indicators of COME was not useful because of high false positive and negative rates.

A case-control study from Mozambique examined OM risk factors in 750 children < 6 years old.[37] Exposure to tobacco, wood and charcoal

smoke, short term breast-feeding, and overcrowding were associated with OM. Among Greek 6-12 year olds identified with unilateral or bilateral OME in a screening program (n=250), 22% had OME 16 months later.[38] Neither passive smoke nor breast-feeding were related to OME at 16 months in multivariate analyses.

Kaplan–Meier analyses were used to determine the effect of parent smoking on tube survival among 606 children with tympanostomy tubes in a prospective clinical study conducted by Praveen and Terry.[39] For those exposed to parental smoking, tubes stayed in place for 59 weeks, compared to 86 weeks for those not exposed to smoke. Post extrusion myringosclerosis was more prevalent in children with two smoking parents compared to those with no smoking parents (64% vs. 20%).

*Pollutants.* Brauer et al.[40] conducted a study of traffic-related pollutants and OM in German and Dutch birth cohorts. Estimates of environmental concentrations of pollutants (nitrogen dioxide, particulate matter with aerodynamic diameters ≤2.5 micron, and elemental carbon) were calculated by home address for about 4000 infants and related to physician OM diagnosis by age 2. Adjusted ORs (1.10–1.24) indicated small, but significant relationships between pollutants and OM, a finding with potential public health significance because of common exposure.

## GENETIC SUSCEPTIBILITY

The field of genetics in OM is evolving rapidly and significant contributions have been added to the existing body of knowledge. Substantial OM heritability has been reported in several papers, but previous studies have not been designed to explore sex differences in susceptibility genes. The substantial heritability of OM has been replicated in a study from Norway[41] including >9000 twins. This study reports that the same genes give rise to OM risk in males and females. A five-year follow up of the Pittsburgh twin and triplet cohort[42] reports the heritability of time with middle ear effusion decreased after the third year, but its cumulative effect remained significant after five years; the heritability for proportion of time with middle ear effusion in the first 5 years of life was 0.72. Previous epidemiological studies have reported a significant co-morbidity between OM and tonsillitis, and a recent twin study suggests that this disease overlap may be partly due to a shared genetic risk factor for the two diseases.[43] Co-morbidity between OM and tonsillitis was expressed as a tetrachoric correlation of 0.52 and genetic effects explained 59% of the correlation.

Heritability is a population statistic and is not useful in identifying individual risk of developing disease, but is a necessary step in the ongoing process of identifying genes responsible for OM susceptibility. Once a trait is found to be heritable, the next step is to design gene finding studies, including linkage and association studies, to identify the genetic region or specific genes influencing the particular trait or disease. Two gene finding studies of OM have been published.[44, 45] In a study by Daly et al,[44] linkage was found between COME/ROM and regions on chromosomes 10 and 19. Subsequent fine mapping of both regions strengthened evidence for linkage, with the LOD score increasing from 2.56 to 3.85 on chromosome 19, and from LOD 1.80 to 2.20 on chromosome 10.[45] FBXO11, the human homologue of a susceptibility gene for chronic forms of OM detected in a mouse model, was associated with COME/ROM in one study.[46] These findings have yet to be investigated by others for replication.

OM is a complex disease involving multiple genetic and environmental factors and does not follow strict Mendelian inheritance. Linkage studies of OM and other complex traits are limited in regard to gene finding. A large number of genes may be involved in complex diseases or traits, each contributing to a small increase in disease risk; these individual genes are difficult to detect. OM varies in severity of symptoms, age of onset, recurrence and chronicity, which results in difficulty defining an appropriate phenotype and selecting the optimal population to study. The disease can also vary in etiological mechanisms and involve a variety of biological pathways.

Another approach to gene finding is to explore functional candidate genes, i.e., those that may be important in the OM etiologic pathway. A group from Galveston studied SNPs (single nucleotide polymorphisms) for proinflammatory cytokines ($TNF\alpha^{-308}$, $IL6^{-174}$, and $IL1\beta^{+3953}$) to determine the relationship between these polymorphisms, atopy,

family history of OM, breastfeeding and OM susceptibility among otitis prone.[47] With logistic regression, both $TNF\alpha^{-308}$ and $IL6^{-174}$ were related to OM susceptibility and treatment with tympanostomy tubes. Post hoc analyses for $TNF\alpha^{-308}$ revealed that children exposed to smoke were more susceptible to OM than those not exposed to smoke. The relationship between mannan binding lectin (MBL2) haplotypes and MBL serum levels was determined in 204 Dutch children enrolled in a vaccine clinical trial.[48] For seven common MBL2 haplotypes, the level of serum MBL was determined; LXPA carriers with SNP 3130G had significantly lower levels of circulating MBL than those with SNP 3130C (0.19 vs. 070 mcg/ml, p =.026). Children with non-wild type MBL2 carriers had significantly more OM episodes per year than wild-type carriers (p = .027).

## PRENATAL AND PERINATAL FACTORS

Potential associations between well-known perinatal risk factors, such as low birth weight, preterm birth, low Apgar scores, and the risk of AOM, RAOM, or COME have been studied in many epidemiologic studies. These studies have been hampered by relatively small sample sizes, given that very low birth weight (<1500 g) or very preterm birth (<34 weeks gestational age) occur in only 1% to 3% of live births. Imprecise classification of OM status may also have contributed to attenuation of risk estimates.

Data were presented at the 9th International Symposium on Recent Advances in Otitis Media from a large nationally representative cohort (n~10,000) of births in 2001.[15] Analyses showed some relationship between very low birth weight (<1500 g) and frequent OM (ORadj 1.24, 95% CI 1.01-1.6). There was no increase in ROM risk for moderately low birth weight (1500 to 2499g) among infants through 2 years of age. Increased OR for very low birth weight remained after adjusting for gender, race/ethnicity, center-based child care, and breast feeding. In a much smaller (n=136) prospective cohort study of children treated with tubes for bilateral OME, multivariate analyses showed positive, but weak, nonsignificant associations between low birth weight and/or low gestational age or history of incubator care and OME recurrence.[49]

## OTHER RISK AND PROTECTIVE FACTORS

***Laterality.*** McCormick et al.[50] characterized risk factors for bilateral AOM and the link between specific viral and bacterial pathogens in bilateral versus unilateral disease among 566 children in a clinical study. Children with bilateral AOM were younger (p<.001), more likely to have *H. influenza* AOM (p<.0001) and severe inflammation of the tympanic membrane (p<.0001) than children with unilateral disease. These findings may explain why children with bilateral AOM are more likely to experience persistent symptoms. Other studies reviewed in this panel report showed associations between bilateral AOM and persistent middle ear effusion[29] and between age <2 years, bilateral AOM and prolonged AOM.[51]

***Atopy/Allergic Disease.*** Conflicting data have been reported about the significance of reported allergic disease, skin prick test sensitivity and OM. Bentdal and colleagues[52] demonstrated an association between reported allergic disease and both single and recurrent episodes of AOM in a population-based study of 10 year-olds. Children with negative skin prick tests and reported asthma and rhinoconjunctivitis also had increased risk of AOM. The association between allergic disease and AOM was strongest for asthma ($OR_{adj}$ = 2.7, 95% CI 1.8, 4.0). Children with ≥2 OM episodes were not more prone to allergic disease than those with one episode. However, there was an inverse relation between single episodes of AOM and a positive skin prick test to any allergen (OR 0.7, 95% CI 0.5, 1.0). Among children with asthma, only 52% were skin prick positive to one or more allergens. Skin prick negative children were at higher risk of having ≥1 AOM than skin prick positive children (OR 3.0, 95% CI 1.7, 5.4 vs. OR 1.5, 95% CI 0.8, 2.8). In the same cohort, Nafstad et al.[53] reported that OM in the first year of life was a risk factor for asthma at age ten ($OR_{adj}$ 1.3, 95% CI 1.0, 1.7), which does not support the hypothesis that early infections protect against later atopy and allergy.

Bentdal et al.[54] studied the effects of early atopy on OM status at age 10 in the Oslo Birth Cohort.

She reported that atopic eczema in early life increased the risk of AOM in children who were OM prone or had early AOM onset. Allergic rhinitis and asthma also increased the risk of OM, tonsillopharyngitis and lower respiratory infections.[52] Parental smoking at birth and female gender were weakly associated with AOM, whereas OM surgery predicted AOM.[55] In the same cohort, Bentdal[24] also investigated the relationships between early OM and OM proneness to determine whether they were predictors for infectious respiratory morbidity at age 10. OM prone children had an increased risk of AOM at age 10, a significantly increased probability of OM surgery, but no increased risk for other upper respiratory infections.

In a clinical study, Finnish children referred for COME or RAOM had similar numbers of siblings and rates of smoke exposure and day care attendance.[28] However, parental asthma or allergy to pollen, dust or animal hair was significantly less common in children with COME than those with RAOM ($OR_{adj}$ 0.49, 95% CI 0.23, 0.84), while age at diagnosis predicted COME ($OR_{adj}$ 1.58, 95% CI 1.01, 2.45). Children <2 years with a family history of asthma had significantly higher rates of parent reported and MD–diagnosed OM than children without a family history of asthma.[56] In multivariate analyses controlling for other risk factors, breastfeeding decreased the risk of AOM and tonsillitis.

Umapathy et al. distributed a questionnaire to parents of 332 primary school children to ascertain symptoms suggestive of OME, rhinitis, asthma, atopy, treatment for these conditions, and family history of atopy.[57] Symptoms of OME, rhinitis, and asthma were documented in 33%, 37% and 24% of children, respectively. There were highly significant correlations between otologic and nasal scores, particularly nasal obstruction and >6 URTI/year; and between otologic and chest scores suggestive of asthma and family history of asthma ($p < .001$).

*Other Factors.* In studies of 2000 Norwegian school children, parental report of baby-swimming was not significantly associated with OM in the first year of life ($OR_{adj}$ 1.77, 95% CI 0.96, 3.25).[58] Dampness in the home and African ancestry were risk factors for OM at age 10 in the Oslo Birth Cohort Study ($OR_{adj}$ 1.4, 95% CI 1.1, 1.6; $OR_{adj}$ 1.5, 95% CI 1.0, 2.2 respectively).[25] Birth in summer or fall predicted RAOM, but not AOM or early AOM in a cross-sectional study of Greenlandic children (RR 1.44, 95% CI 1.04, 1.99).[59]

Sulyman[60] conducted a study at a pediatric emergency unit in Nigeria over 10 months to determine the prevalence of OM among children 0-15 years with pyrexia. Among 100 children with pyrexia, AOM was diagnosed in 16 children; two-thirds were from the lower socio-economic class. AOM appears to be an important differential diagnosis in children with pyrexia in this population.

## OM AS A RISK FACTOR

In the Oslo Birth Cohort Study, a significant co-morbidity was found between otitis proneness (OM in the first year of life or 4 AOM before age 2) and diagnosis of bronchial obstruction at age two.[54]

Data presented at the 9th International Symposium on Recent Advances in Otitis Media explored the relationship between OM and body mass index. Nelson et al.[61] studied OM, tube treatment, and overweight in two cohorts of infants followed prospectively from birth to age two. Weight-for-length and BMI-for-age were calculated using data collected at well child visits. In the cohort of predominantly white children, history of tympanostomy tube treatment was significantly associated with risk of body mass index (BMI) >85th percentile at 2 years of age ($OR_{adj}$ 2.42, 95% CI 1.24, 4.71). ROM increased the likelihood of weight-for-length >95$^{th}$ percentile in an American Indian cohort (ORadj 3.32, 95% CI 1.19, 9.28). Both analyses controlled for gender, birth weight, breast feeding, maternal smoking and education.

Hoffman and colleagues[62] studied the role of tonsillectomy and OM on BMI in 6-17 year olds who participated in the U.S. National Health Examination Surveys, 1963-70. In the younger group, ages 6-11 years, history of tonsillectomy was significantly related to BMI >85th percentile for both genders ($OR_{adj}$ for males = 1.33, 95% CI 1.06, 1.69, and for females $OR_{adj}$ 1.58, 95% CI 1.16, 2.14). In the older group, histories of tonsillectomy and otorrhea among females were

related to BMI >85th percentile (OR 1.31, 95% CI 1.03, 1.65; OR 1.81, 95% CI 1.15, 2.85, respectively). A Korean study reported a significant relationship between tube treatment for OME and mean BMI and total cholesterol.[63]

## PREVALENCE OF AND KNOWLEDGE ABOUT OM RISK FACTORS

Rovers et al.[64] used population based data to demonstrate variation in OM risk factors across countries. Among women, the highest smoking rates were in Norway, Germany and France (30% - 39%), whereas male smoking was most common in Greece, Poland and Spain (40% - 49%). The lowest rate of breastfeeding initiation was in France (≈50%), and <10% were still breastfeeding in Poland and Belgium at 6 months. The country with the highest percentage of children aged 1 to 3 years in daycare was Sweden (75%), compared to Italy with the lowest rate (6%). The U.S., U.K. and Finland were in the middle range with percentages from 30% - 40%, whereas the Netherlands, Denmark, Belgium, Canada, and Norway had higher percentages (>40% to 70%).

Kerschner and colleagues[65] investigated knowledge of OM risk factors among parents/caregivers of children from 6 months to 3 years in urban and suburban pediatric practices in Wisconsin. The questionnaire focused on knowledge of potentially modifiable risk factors (e.g., smoke exposure, child care, breastfeeding, and immunization). Nearly 44% reported they had not been educated about OM risk factors by their primary care physician. Day care did not differ by location of practice; however, knowledge of day care as a risk factor was significantly greater in the suburban setting. Both groups indicated a willingness to change behavior with regard to smoke exposure and child care arrangements.

## PREVENTIVE STRATEGIES

Xylitol has been shown to be effective in preventing AOM when given 5 times daily to children in daycare, but was not effective when given only during the respiratory season.[66] Because frequent doses are inconvenient to administer, the investigators studied a 3 dose regimen in a daycare setting and found it to be ineffective in preventing AOM; 28% of those using the xylitol product and 30% of those using the control product had an AOM episode during the 3 month follow-up.[67]

Probiotics were identified in the last report as a promising strategy for preventing AOM.[68] However, a recent double-blind randomized, controlled trial (RCT) in children <6 years reported no effect of probiotics taken once daily on AOM incidence and duration.[69] No effect was demonstrated on *S. pneumoniae* and *H influenzae* nasopharyngeal colonization, but *M. catarrhalis* colonization was significantly more common among those randomized to probiotics (OR 1.79, 95% CI 1.06, 3.00).

A small feasibility study of a pacifier used to dispense xylitol and Bifidobacterium lactis Bb-12 at a slow rate reported that all subjects showed at least one salivary xylitol concentration >1%.[70] No efficacy studies are reported, but this may be a possible approach to OM prevention in infancy.

An intervention study assessed the prevalence of tympanic membrane perforations before and after the opening of swimming pools in two Aboriginal communities in Western Australia.[71] Prevalence was 32% in both communities, reduced to 13% and 8% 18 months later. This effect is probably due to hygienic improvements caused by the swimming pools. A small pilot study of Aboriginal school children who received fresh fruit each school day showed a reduction in antibiotic prescriptions for CSOM, suggesting improvements in nutrition could improve health.[72] A randomized controlled trial of pre- and postnatal lactation advice (n = 338) failed to demonstrate a significant increase in exclusive breastfeeding or reduction of AOM.[73] Only 61% had prenatal intervention, 60% had postnatal intervention and 44% had interventions at both time periods. Number of OM visits was significantly higher among control infants in the subgroup not receiving Medicaid.

## GUIDELINES FOR AOM TREATMENT

Shortly after the 2004 U.S. AOM guidelines were published, Vernacchio conducted a survey to determine family practitioner and pediatrician knowledge and adoption of the guidelines.[74] Only

39% of respondents reported using pneumatic otoscopy on a regular basis. Median percent time the "observation only" option was used was 10%, and the majority of physicians (76%) had no or little concern about AOM complications when using the observation option.

After adoption of restrictive Norwegian guidelines for antibiotic treatment of acute OM in children ≥1 year, Kvaerner used national hospitalization data to study whether reduced antibiotic use affected complication rates and incidence of severe AOM, particularly in very young children.[13] She found no evidence for increased incidence of mastoiditis between 1999 and 2005. Mean age of hospitalization for AOM decreased from 2.4 to 1.7 years and hospitalization for AOM was less frequent over time. Peak hospitalization occurred in the second and third year of life, which corresponds with incidence estimates for the disease. Severe complications such as acute mastoiditis (10.6/100,000), septicemia (1.2/100,000), meningitis (0.2/100,000), Bells palsy (0.5/100,000) were rare in children <7 years. Acute mastoiditis was diagnosed in 1% of children hospitalized for AOM at age one year, and in 2% and 4% at ages two and four respectively.

The Boston-Based Pediatric Research Group used a cluster randomization study to investigate compliance with the Centers for Disease Control and Prevention (CDC) recommendations to use specific antimicrobial agents for AOM.[75] Despite use of evidence-based approaches known to influence physician behavior, the study failed to increase adherence to CDC recommendations for AOM treatment: amoxicillin as first-line therapy for children at low risk for resistant otopathogens and high-Dose amoxicillin, high-dose amoxicillin /clavulanate, or cefuroxime for children at higher risk. CDC recommendations were based on expert consensus and evidence that antibiotic use in the previous 30 days was the main risk factor for antibiotic-resistant pathogens. The study design used the assumption that adherence to the CDC guidelines would result in reduced rates of treatment failure for the second AOM episode. Using multivariate analyses, intervention sites were slightly more likely to follow CDC recommendations than control sites for both initial and second episodes, but differences were not statistically significant.

A total of 146 Dutch general practitioners (GPs) included all patients with AOM during a 4 week period in the winter to assess clinical determinants of under- and over-prescribing of antibiotics according to national guidelines.[76] For 71% of visits, antibiotic prescribing was consistent with the guidelines, for 11% there was under prescribing (an antibiotic indication, no antibiotic prescription), and in 18% there was over prescribing (no antibiotic indication, an antibiotic prescription). Compared with patients without an antibiotic indication or prescription, patients in the over-prescribed group were more likely to be <2 years old ($OR_{adj}$ 0.34, 95% CI 0.16, 0.75), more severely ill ($OR_{adj}$ 3.30, 95% CI 2.08, 5.22) and expected an antibiotic as perceived by the GP ($OR_{adj}$ 2.11, 95% CI 1.47, 3.02).

## NATURAL HISTORY, COMPLICATIONS, AND SEQUELAE

*Natural History.* Bentdal[24] reported longitudinal data on the course of early AOM and its affect on later respiratory infections in the Oslo Birth Cohort. Only 5% of children had ≥1 AOM before age 6 months, 25% had ≥1 AOM between 6 and 12 months, 28% had ≥1 AOM by 12 months and 13% had ≥1 AOM at age 10. Early AOM was not significantly related to AOM and respiratory infections at age 10. Karevold et al.[55] conducted a study on the same population-based cohort (3754 children) to evaluate if surgery performed for airway infections in early childhood modified the risk of OM and other airway infections after the children entered school. Main outcome measures were parent reported OM and other respiratory infections at ages 2 and 10 years. Early OM was significantly associated with later surgical treatment, and children with early OM remained predisposed to OM during school age. Early middle ear surgery was also associated with OM at age 10. However, children having tonsillectomy and/or adenoidectomy did not have increased risk of tonsillopharyngitis at age 10, suggesting that surgery for OM may prevent long-term sequelae, but does not affect the underlying infectious liability in these children.

Meta-analysis of 6 randomized, controlled trials for AOM was performed to determine predictors for the 37% of children not treated with antibiotics who had fever and/or pain 3-7 days after AOM onset. Predictors included age and bilateral AOM. Prolonged AOM was twice as common among children <2 years with bilateral AOM compared to children ≥ 2 years with unilateral AOM.[51]

In an Australian study,[77] simultaneous nasal carriage of *S. pneumoniae* and/or noncapsular *H. influenzae* and risk of hand contamination were more frequent in Aboriginal children than in other children in urban child-care centers. A large prospective study examined nasopharyngeal carriage rates of *S. pneumoniae, H. influenzae* and *M. catarrhalis* in Aboriginal and non-Aboriginal children followed from birth to age two in the same region in Western Australia.[78] Aboriginal children had carriage rates twice as high as non-Aboriginal children; colonization by 2 months was almost three-fold higher in the Aboriginal group.

*Complications.* In a prospective hospital based Nigerian study[79] of 95 children, researchers reported an average duration of otorrhea of 15 months; 64% received some form of treatment. Complications included disabling hearing loss (35%), subperiosteal abscess (5%), intracranial suppuration (6%), meningitis (4%), and facial nerve palsy (2%).

*Sequelae.* Three hundred fifty-eight subjects from a birth cohort with and without OM histories and/or ventilation tube insertion were followed to adulthood to study the course of tympanic membrane pathology.[80] Otomicroscopic examinations at 8 and 18 years revealed that abnormalities (tympanosclerosis, atrophy, atelectasis, pars tensa retraction pockets, and pars flaccida retraction) were prevalent in the cohort with OM, with or without tympanostomy tubes (92% and 46% of ears respectively). Among those without an OM history, tympanic membrane abnormalities were rare. By age 18, many tympanic membrane abnormalities disappeared spontaneously, although the prevalence of tympanosclerosis remained substantial in those treated with tubes. Excluding tympanosclerosis, the course of most OM tympanic membrane pathology is favorable over time, suggesting a natural repair capacity of the tympanic membrane.

## METHODOLOGICAL AND ANALYTICAL STRATEGIES

The test-retest reliability for parent-reported upper airway surgery was estimated in the Oslo Birth Cohort at ages 4 and 10, respectively.[81] Test-retest comparison for different procedures estimated Cohen's Kappa at 0.6 for myringotomy, 0.9 for adenoidectomy, and 1.0 for tympanostomy tubes, respectively.

Reliability of retrospective questionnaire data for ROM among >4000 respondents in the Norwegian Twin Study was also studied.[82] Retrospective self-report of OM was a relatively reliable measure ($\kappa$ =0.53), and reporting inconsistency appeared to be related to less severe disease.

Recent innovations using the Bayesian statistical approach have led to improved estimators of effect size and, hence, better interpretation of findings in OM studies.[83] After an intervention, disease status may be diagnosed using the same fallible instrument. Potential misclassification based on the diagnostic test causes regression to the mean, biasing inferences about the true intervention effect. The existing likelihood approach suffers in situations where either sensitivity or specificity is near 1. In such cases, common in many diagnostic tests, confidence interval coverage can often be below nominal for the likelihood approach. Another potential drawback of the maximum likelihood estimator (MLE) method is that it requires validation data to eliminate identification problems. The proposed Bayesian approach offers improved performance in general, but substantially better performance than the MLE method in the realistic case of a highly accurate diagnostic test. This superior performance is obtained using no more information than that employed in the likelihood method. The approach is also more flexible, doing without validation data if necessary, but accommodating multiple sources of information, if available, thereby systematically eliminating identification problems. A simulation study was used to show that the Bayesian approach outperforms the MLE method, especially when the diagnostic test has high sensitivity, specificity, or both. A real data example for which the diagnostic test specificity is close to 1 (false positive

probability close to 0) was also provided in the paper.

Another statistical problem that occurs often in epidemiological research studies is how to treat missing data. In most situations, simple techniques for handling missing data (such as complete case analysis, overall mean imputation, and the missing-indicator method) produce biased results, whereas imputation techniques yield valid results without complicating the analysis once the imputations are carried out.[84] Imputation techniques are based on the idea that any subject in a study sample can be replaced by a new randomly chosen subject from the same source population. Imputation of missing data on a variable is replacing that missing by a value that is drawn from an estimate of the distribution of this variable. In single imputation, only one estimate is used. In multiple imputations various estimates are used, reflecting the uncertainty in the estimation of this distribution. Under the general conditions of so-called missing at random and missing completely at random, both single and multiple imputations result in unbiased estimates of study associations. But single imputation results in too small estimated standard errors, whereas multiple imputation results in correctly estimated standard errors and confidence intervals. Donders and colleagues[84] explain why all this is the case, and use a simple simulation study to demonstrate and explain the problems encountered. They also explain and illustrate why two frequently used methods to handle missing data, i.e., overall mean imputation and the missing-indicator method, almost always result in biased estimates.

## SHORT-TERM GOALS

1. The relationship between atopy and OM is incompletely understood, and there is a need for clarification of diagnostic criteria for allergic diseases as well as Ig E-mediated susceptibility.

**Comment:** Several studies exploring the relationships between allergy, atopy, and OM were published in the last few years and are described in the Panel report, but the association is still not well understood and more studies are needed.

2. Sharing data from existing studies should be encouraged. It will enhance collaborative research by providing the opportunity to test new hypotheses, use different analytic methods, and explore topics not envisioned by original investigators.

**Comment:** Data sharing is a useful approach to explore new hypotheses using existing datasets. Several examples of research generated by data sharing using data from individual researchers and large national studies appear in this Panel report.

3. Research to determine whether there is a diminished OM risk related to daycare and other risk factors among children >3 years of age, and if so, determine the factors and mechanisms associated with the lower risk in this age group.

**Comment:** Only a few studies have evaluated traditional OM risk factors in cohorts older than three years. Several studies of comorbidity among atopy, allergy, and OM in older children appear in this report.

4. A recent RCT reported that probiotic (lactobacillus) ingestion is related to reduced risk of respiratory disease, including OM. Studies are needed to clarify the specific effect of probiotics on OM, and double-blind RCTs are needed to verify this as a plausible, low impact treatment**.**

**Comment**: A recent double-blind RCT reported no effect of probiotics taken once daily on AOM incidence and duration. *S. pneumoniae* and *H. influenzae* nasopharygeal colonization were also unaffected, while *M. catarrhalis* colonization actually increased significantly in the probiotic group.

5. Recent studies show that OM incidence has decreased following the introduction and routine use of 7-valent pneumococcal conjugate vaccine in children, as well as the introduction of guidelines for diagnosis and treatment of AOM (both presented in this panel report). Data are needed to determine 1) whether 7-valent pneumococcal vaccine will continue to reduce OM incidence or will confer changes in the microbiology of otitis media pathogens, and 2) whether other factors are contributing to the reported decline in OM incidence.

**Comment:** Studies in several countries have reported declining OM incidence. Additional studies are needed to determine trends in OM

incidence and evolution of the most prevalent organisms and serotypes**.**

6. More research is needed into mechanisms involved in OM associated with pacifier use. This is especially important in light of recent research showing that pacifier use is related to reduced risk of sudden infant death syndrome (SIDS).[68]

**Comment:** A study presented at the 2003 Recent Advances in Otitis Media meeting reported a significant effect on OM among children using pacifiers that dispensed xylitol. A more recent study of a xylitol releasing pacifier reported on salvia concentrations in adults. The pacifier may be a useful mechanism for xylitol administration in infants.

7. Use multivariate methods that take into account complexity of the data and account for correlated outcomes. This includes general estimating equation, structured equation 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, use confidence intervals to provide information on the magnitude of risk.

**Comment:** Researchers have made much progress toward this goal. Nearly all studies in this report used multivariate analyses to control for other variables in estimating the effect in the variable of interest. In addition, most studies now report confidence intervals in place of or in addition to p values.

LONG-TERM GOALS

1. There is a need for large, well planned, prospective studies to research OM etiology and pathogenesis. These studies should include the elements needed to make scientific conclusions (e.g., Eustachian tube dysfunction, microbiological, immunological, genetic and environmental factors) as well as interactions between these factors.

**Comment:** Several studies meeting this description are included in this Panel report, although it is the rare study that includes all the variables described in the Goal. Including clinical measures in large studies is expensive, and must typically be conducted or sponsored by governmental health agencies. In the U.S., the National Children's Study planned for 2008 and other large prospective cohorts could be a valuable resource in meeting this goal.

2. Perform genetic studies, including family linkage and large scale population association studies. Assess possible endophenotypes. Large cohort studies in many countries could be used to examine genetic loci and alleles that predispose to OM. Conduct genetic studies in high-risk populations especially prone to OM (e.g., American and Canadian Indians, Alaskan and Greenland Inuits, and Australian aboriginals). Gene-gene and gene environment interactions should also be explored.

**Comment:** There has been considerable progress in exploring the genetics of OM. This panel report summarizes 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. For example, there are no genetic studies of OM in high risk populations, gene-gene/gene-environment interactions, or large scale association studies.

3. Compare data from population-based studies to determine whether actual treatment for OM in various countries is in accordance with proposed treatment guidelines. This should be monitored over time to determine what effect guidelines have on actual practice. Where possible, national level data with longitudinal tracking should be used.

**Comment**: This Panel report includes several reports assessing adherence to OM treatment guidelines, but more studies are needed to detect changes over time.

4. Guidelines to reduce antibiotic use in OM treatment have been proposed. Rates of mastoiditis and other intratemporal and intracranial complications should be tracked to determine the population effect of these guidelines. Prospective monitoring will determine the effect of guidelines on actual clinical practice.

**Comment:** Studies tracking changes in hospital admissions for AOM and OM complications in Norway are included in this report.

5. Intervention studies designed to determine the impact of risk factor reduction on OM incidence and prevalence will enhance knowledge about OM prevention strategies.

**Comment:** Randomized studies were conducted over the last 4 years on lactation advice to increase breastfeeding and to assess the effect of daycare setting.

6. As more large population based studies are conducted, coordinated standardization of methods and definitions would facilitate further meta-analyses. This should better quantify the magnitude of the effect of common risk factors for OM.

**Comment:** This Panel Report includes a study that used meta-analysis of randomized treatment trials for AOM to determine predictors for fever and/or pain persisting 3-7 days after AOM onset.

7. OM is a challenge in many populations around the world. To increase our understanding and efforts toward reducing OM in these populations, studies of genetic and immunological factors are needed, along with research on the effect of pneumococcal vaccines. Risk factor reduction studies would also shed light on other potentially effective approaches to reducing the OM burden.

8. Develop valid and accurate clinical prediction rules. Additional predictive factors need to be identified so models will be useful tools. The goal of an accurate prediction rule is to help clinicians distinguish children with a poor natural course (those that probably benefit most from an intervention) from those with a favorable natural course. Predictors are applied to subgroups of patients or population groups in order to guide management decisions for individuals.

9. 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.

**Comment:** Studies on the role of perinatal factors on early OM onset are included in this panel report**.**

Abbreviations used: OM, otitis media; OME, otitis media with effusion; COME, chronic otitis media with effusion; CSOM, chronic suppurative otitis media; COM, chronic otitis media; AOM, acute otitis media; ROM, recurrent otitis media; RAOM, recurrent acute otitis media; URTI, upper respiratory tract infection; RR, relative risk; OR, odds ratio; ORadj, adjusted odds ratio; CI, confidence interval; BMI, body mass index; HR; hazard ratio; IG, immunoglobulin, MLE; maximum likelihood ratio; RCT, randomized controlled trial; GP, general practitioner; SIDS; sudden infant death syndrome; ENT, ear, nose and throat specialist, SNP, single nucleotide polymorphism; CDC, Centers for Disease Control and Prevention; NHANES; National Health and Nutritional Examination Survey; National Health Interview Survey; NHAMCS; National Hospital Ambulatory Care Survey.

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# 2. EUSTACHIAN TUBE, MIDDLE EAR, AND MASTOID: ANATOMY, PHYSIOLOGY, PATHOPHYSIOLOGY, AND PATHOGENESIS

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## PANEL OBJECTIVES

1. Summarize important new contributions to our understanding of the anatomy, physiology, and pathophysiology of the middle ear (ME) system relevant to otitis media (OM) and certain related diseases and disorders, published since the Eighth International Symposium on Recent Advances in Otitis Media in June 2003.

2. Determine whether the short- and long-term research goals identified at the previous meeting of this Panel have been met.

3. Identify deficiencies in our understanding of the anatomy, physiology, and pathophysiology of the ME System related to OM.

4. Define and prioritize research goals that address deficiencies in our knowledge for investigation during the next 4 years.

5. Identify the short- and long-term goals for the future and methods by which they can be accomplished.

The following are publications related to the Eustachian tube (ET), middle ear and mastoid gas-cell system reported in the literature since the last research meeting in 2003.[2] They include the anatomy, physiology, pathophysiology, and tests of function of the tube, as well as the role of the ET in the pathogenesis of middle-ear disorders and diseases. Also, these aspects related to the middle ear and mastoid gas-cell system are reviewed. But, there has been no attempt to include every publication reported during this time. This review is meant to be a state of knowledge since the last research conference up until the deliberations at this meeting.

## EUSTACHIAN TUBE

Certain aspects of the ET were addressed in a recently published book by Bluestone (*Eustachian tube: structure, function and role in otitis media*), which viewed the ET as part of a system in which the nasal cavities, nasopharynx and palate are at the tube's anterior end and the middle ear and mastoid gas cells are at its posterior end.[3] Also, a summary of research on otitis media, including the ET, middle ear and mastoid, conducted at the Children's Hospital of Pittsburgh and University of Pittsburgh since 1969 has been published.[4]

*Anatomy.* Human babies are born with immature structures and functions which includes the ET and its immature immunity. This fact contributes to a high incidence of otitis media in human during the first year of life.[5] Abe assessed the origin and insertion of the human tensor veli palatini muscle from an anatomic standpoint and confirmed that the dilator tubae of the tensor muscle acts to dilate the tube.[6] They concluded the hamulus of the pterygoid acts strictly as a pulley for the tensor tendon and that its function could be maintained by preserving or reconstructing the maxillary placing during a push-back procedure for cleft palate repair, even if a hamulectomy is performed, it should not affect the function of the tensor. Studies of human temporal bone specimens from fetus to adults have shown that Ostmann's fat pad could vary in its location, which hypothetically could enhance or deter ET function.[7] Orita reported that incomplete development of connective tissue in the region lateral to the ET

could be related to dysfunction of the tube.[8] In addition, Suzuki examined the ratio of the tensor veli palatini muscle insertion to the length of the cartilaginous ET in human temporal bone specimens and found the ratio increased from infancy to adulthood, but then decreased with age.[9] They postulated that this change may have some effect on pressure-regulation function in the elderly. Renko and colleagues used MRI imaging to investigate the nasopharyngeal airway in healthy children and compared the findings with their history information.[10] They found that the dimension from caudal edge of septum to the midpoint of sella, which reflects the height of the nasopharynx, was on average 2.2mm smaller in children with history of OM, and that the nasal base angle was on average 2.1 degrees smaller in children with OM in the prior year.

*Physiology.* At the Symposium that preceded this Panel, two reports considered the physiology of the human ET: one was related to the impact of human evolution and the other compared the ET function between species. Bluestone presented the hypotheses that humans are the only species afflicted with otitis media, in particular with its extraordinary rate in infants and young children, in other species, such non-human primates, otitis media associated hearing loss would more likely lead to death due to their natural predators.[11] Differences in comparative anatomy and physiology were proposed as the cause of relatively poor ET function in humans. Indeed, Swarts and Ghadiali, using the forced-response test, described interspecies differences in function which and showed that the human functioned relatively poor compared with the monkey.[12] In an ET compliance model of the monkey, Ghadiali and co-workers described the viscoelastic properties of the ET using pressure-flow hysteresis loops and a computer model. Later, the same group, developed a computational technique, which quantified the structure-function relationships of ET and, which the investigators believed, could be helpful in targeting treatment.[13] Compliance of the ET was also assessed with a new functional 3-D model digital image system with the emphasis on the role of the medial pterygoid muscle. It concluded that this muscle may influence the opening of the ET, and this could be related to the patulous ET disorder and to patients with cleft palate.[14] Brattmo fitted tympanostomy tubes in healthy volunteers in which the external canal was plugged with a pressure transducer.[15] They found negative pressure in the ME and difficulty in equilibrating pressure differences through the ET. For the first time in humans, surfactant proteins have been detected in the Eustachian tube as well as in the organ of Corti and kidney.[16] Mandell and colleagues (2003) reported that there is an effect in ambient oxygen concentration on the bioelectric properties of the middle-ear mucosa in experiments on the gerbil, which suggested a role for calcium-activated chloride channels in the middle ear.[17] Franz and Anderson, (2007) demonstrated that sympathetic axons were sparse, but CGRP-immunoreactive, nociceptive axons formed a dense subepethelial plexus beneath the ET epithelium, and that capsaicin alone did not affect ET function but capsaicin applied with an alpha adrenoceptor agonist impaired ET function.

Picciotti and colleagues using a novel electromyography technique for TVP muscle, studied electrophysiological parameters in patients affected by chronic OM and in normal subjects.[18] No differences were found between the affected by OM and the normal subjects. However, significant differences were found in the OM group between normal and affected sides. Didyk investigated the role of atmospheric pressure fluctuations (APF) in developing psychophysiological and autonomic reactions. Their results suggest that humans react to slight APF at the subcortical level.[19]

*Pathophysiology.* Thirty-eight older children, adolescents and adults who had tympanostomy tubes placed for chronic otitis media with effusion were found to have ET dysfunction, primarily paradoxical constriction of the tube upon swallowing, as opposed to normal dilation.[20] In studies in the monkey model, Ghadiali and co-workers injected botulinum into the tensor veli palatini muscle then assessed compliance using a new test of this function, continuous pressure—flow rate, and reported the lack of tubal dilation, increase in tubal compliance and abnormal forced-response ET function, but the tube did not abnormally constrict when the animal swallowed.[21] Investigators from Iran reported that children with a deep dental overbite were 10.6 times more likely to have ET

dysfunction than those without this dental abnormality.[22]

Using Bluestone's 9-step inflation-deflation ET test in patients with or without ventilated intranasal packs showed no difference in ET function; the authors noted that this is the first ET function test reported for packs, as previous studies only used tympanometry.[23]

There were many reports concerning the patulous ET, especially from Japan. A patulous ET was reported as a sequela following a Le Fort 1 osteotomy in a cleft palate patient.[24] Also, in a review article on ET dysfunction, Grimmer and Poe reported a patulous ET is associated with a defect in the longitudinal scaphoid in the antero-lateral wall of the cartilaginous portion of the ET which results in a closing failure of the tube; they identified this with endoscopy and reported that there is now CT scan evidence of this defect.[25] In an earlier study, Yoshida and co-workers also used high resolution CT scans, with multiplaner reconstruction technique, to identify a patulous ET.[26] An earlier study from this group compared the outcomes of sitting versus recumbent positions during the imaging.[27] In an interesting report from Japan, investigators found that 8.8% of 147 patients undergoing hemodialysis had a patulous ET and considered this as the dysfunction associated with patients who report ear symptoms during this procedure.[28] In an earlier report from the same group, Kawase and co-workers using an artificial middle ear studied autophony associated with a patulous ET.[29] Also from Japan, an interesting risk factor for the occurrence of a patulous ET was a previous episode of otitis media, which was attributed to a pathologic sequela of infection in the ET.[30] Again from Japan, Kikuchi and colleagues reported that three-dimensional CT scans were able to diagnose (100% specificity) the patulous ET in patients in the sitting position.[31] An unusual cause of a patulous ET was identified on the CT scan of a patient with oculoauriculovertebral spectrum in which the osseous portion, as opposed to the usual cartilaginous portion, was grossly enlarged, which indicates that imaging in these patients with symptomatic patulous ET could be diagnostic of an unusual cause.[32] And still another interesting report from Japan described a patient who had a patulous ET as the presenting symptoms related to an underlying intracranial hypotension syndrome which resolved after an epidural blood patch.[33] The development of the patulous ET was attributed to changes in venous blood distribution which led to collapse of the dural sac of the cervical spine which presumably caused decreased size of the pterygoid plexus around the ET.

Yoshida and colleagues examined the bony portion of stenotic ETs, as defined by high opening pressures using multiplanar CT.[34] They found that the bony framework was smaller and the soft tissue ratio was greater in patients with stenotic tube than in normals.

Models of the ET and middle ear are being developed to better understand the physiology and pathophysiology of the system. Kanick and Doyle developed a mathematical model to estimate the probability of otic barotrauma based on ET function and concluded that individuals with obstructed ET would be less likely to experience this problem than those who have difficulty in dilating the tube during swallowing.[35] This is consistent with the report that children with middle-ear effusion usually do not have symptoms during air flight, whereas those who have recurrent otitis media, but who have no effusion at the time of flight, frequently do have complaints referable to the ear. Using a physical model of the middle-ear cleft, Cinamon determined that when the ET is blocked, the smaller the mastoid gas system, the greater the negative middle-ear pressure change.[36] Also, with the aid of this model, these investigators concluded that under physiologic conditions, the amount of gas flow through the ET is more than sufficient to equalize middle-ear negative pressure that might occur when the tube is very narrow or only open for a very short time, but flow will not occur if the ET is completely occluded.[37] In a unique study using the Visible Woman Project, a virtual reality model of ET dilator function was developed and used to assess methods of cleft palate repair.[38]

Similar to flying, alternobaric vertigo (i.e., unequal ET equilibration function between the two middle ears during rapid atmospheric pressure changes) can occur in scuba divers which was studied by investigators from Turkey who identified a past history of otitis media, ET dysfunction (as determined by Bluestone's 9-step test and Toynbee test), and difficulty clearing ears during diving were risk factors.[39] In a later study from Germany, investigators tested 63 sport scuba divers and

reported that 27% had alternobaric vertigo, more common in females than males, but this disorder was not life-threatening.[40] A case was reported in the neuroradiology literature describing severe middle-ear barotrauma in a scuba diver that resulted in rupture of the tegmen tympani with blood and gas in the epidural space of the middle cranial fossa.[41] Also, a report from Denmark described 4 cases of cabin attendants who developed a perilymphatic fistula during air flights after which the authors advised against those who have poor ET, especially during a period of an upper respiratory tract infection to fly.[42]

In a case report in the radiology literature, the authors described a habitual sniffer for chronic ET dysfunction who developed vertigo associated with calvarial (occipitoparietal) and upper cervical hyperpneumatization due to the Valsalva maneuvers.[43]

Investigators from Turkey demonstrated, using a radionuclide in patients without any head and neck pathology, that nasopharyngeal secretions do not reflux into the middle ear during the recovery phase from a general anesthesia.[44]

From Italy, employing audiometry and tympanometry, orthodontists studied children with maxillary constriction during rapid maxillary expansion and reported that there was improvement in conductive hearing loss, which was attributed to changes in palatal-paratubal musculature.[45] Even though this was an uncontrolled study without valid evidence of ET function and presence of middle-ear effusion, it provides possible insight into the pathogenesis of otitis media in some children.

The pathophysiology of the ET associated with cleft palate in children continues to be an area of interest. From Pittsburgh, Gungor and associates reported at the Symposium that preceded this Panel meeting that ET dysfunction can be assessed using not only manometric tests, but also that MRI of the tube is a potential method to evaluate structure of the tube.[46]

*Pathology.* In a recently reported temporal bone study of a term fetus with bilateral cleft palate from Germany, the levator palatini muscle was found to have an abnormal course, which was postulated to possibly related to the high incidence of otitis media in these children.[47] In a brief report of an apparently unique case of a 35-year old male with relapsing polychondritis had a symptomatic patulous ET associated with an anacoustic ear as well as vertigo and tinnitus.[48] From Korea, investigators exposed the ET of rats to relatively short-term cigarette smoke and found histologic changes in the tubal mucosa, e.g., squamous metaplasia.[49] Also from Korea, Baek and colleagues described only the fourth reported case of a melanoma arising from the mucosa of the ET.[50] In a rare case report from Taiwan, using CT scans and MRI, a nasopharyngeal carcinoma was identified as spreading along the ET into the middle ear and mastoid, which caused a middle-ear effusion.[51] From China, surgeons reported that the pathologic changes in the ET following radiotherapy for carcinoma of the nasopharynx is not always obstructive, but may result in a patulous ET and concluded that examinations (e.g., tubal endoscopy) of the tube should be performed to determine the type and severity of the ET pathology for appropriate treatment.[52] A case of a mature teratoma of the ET was reported from Thailand.[53]

*Pathogenesis.* In a study in children who had recurrence otitis media following extrusion of tympanostomy tubes, Straetemans and colleagues determined that the combination of ET dysfunction and low IgA or low IgG2 levels, and decrease in mannose-binding protein was associated with recurrence.[54] From Japan, investigators identified 20 patients with eosinophilic otitis media who had an associated patulous ET, diagnosed by sonotubometry, and who also had bronchial asthma; they posited the eosinophilic-laden secretions entered (refluxed) into the middle ear.[55] Investigators from Pittsburgh demonstrated on rat otitis media model that while gastroesophageal reflux do induce Eustachian tube dysfunction, this response is insufficient to cause otitis media with effusion or prolong an episode of acute otitis media.[56] From Serbia, surgeons assessed mucociliary transport through the middle ear and ET in patients with chronic suppurative otitis media with the saccharine test and found that transport was dramatically decreased, compared to control patients who had traumatic perforations of the tympanic membrane.[57] In a study of 7047 Japanese Air Force trainees tested in a hypobaric chamber, 6.1% had ear pain and of these airmen there was an association between the presence of allergic rhinitis and

otalgia, especially in the spring of the year, which suggested ET dysfunction.[58] From Pittsburgh, exposure of middle ear epithelial cells to physiologically relevant negative pressure was shown to trigger the release of key inflammatory mediators, which may be analogous to the middle ear underpressure in Eustachian tube dysfunction.[59]

*Tests of Function.* In addition to the new tests of function described above (see Physiology), especially to assess compliance of the ET[14, 60] there have been other new, or modifications of old, ET function tests described. Radiopaque contrast material was placed at the nasopharyngeal orifice of the ET, which refluxed into the middle ear during swallowing and yawning as demonstrated on CT scans; this finding was similar tests in the past that used standard radiographs or fluoroscopy.[61] Also, 3-dimensional CT scans were reported to be diagnostic in identifying the patulous ET.[31] Another diagnostic test to identify a patulous tube used the acoustic transfer function of the ET using audiometric measurements, which also was purported to assess severity of the disorder.[62] Employing Bluestone's 9-step ET inflation-deflation function test, Adali and Uzun evaluated the effect of dry versus wet swallows on the outcome and concluded the dry swallows provided more accurate assessments of function when only one test is performed.[63] Using a 2.5-mm flexible endoscope, seven normal adults had a successful examination of the structure and function of the ET and in some, topical anesthesia was needed.[64] From China, surgeons, using a rhinopharyngoscope, compared the nasopharyngeal orifice of the ET in patients with otitis media with effusion to a control group, and reported the shapes in the otitis media group were abnormal, and variable in shape, compared to the control group.[65] Ghadiali investigated the physical properties that may influence ET opening phenomena, including tissue mechanical properties (viscoelasticity), micro-scale adhesion properties within mucosa.[66] The standard force-response test was modified to generate oscillatory pressure-wave forms in the ET. Analysis of the FRT results of adults using a mathematical model provided important insights into the mechanisms responsible for ET dysfunction.

In monkeys, Alper and colleagues (2003) assessed the accuracy of tympanometry in correctly measuring middle-ear underpressures and concluded it was an unbiased, accurate and non-invasive method and that tympanometry could be used in other species, as well.[67] In a report from Denmark, the rapid rate of pressure change in the newer tympanometers did not decrease the accuracy of measuring the middle-ear pressure.[68]

Investigators in Germany used sonotubometry with a 8 kHz signal in healthy adults, but reported that it was not reliable.[69] But, in another updated sonotubometry test in healthy adults (and a later publication in healthy children) was found to have high reproducibility, but was not tested in patients with middle-ear disease.[70, 71] One test thought to be an effective test of middle-ear ventilation through the ET was scintigraphy using technetium-99m, but a study in the rabbit model failed to demonstrate effectiveness.[72] Investigators from Japan used several tests of ET function, including an updated sonotubometry, which they termed tubo-tympanum-aero-dynamography, which was useful to diagnose various ET disorders, especially patulous ET.[73]

Investigators from Japan reported that audiometry with nasally presented noise was successful in diagnosing a patulous Eustachian tube.[74] In a report from the Netherlands, a study of Eustachian tube function testing (forced-response, pressure equilibration, protective function) was not predicative of recurrence of otitis media with effusion in children.[75]

Using the tests of ET function (forced-response, pressure equilibration, sniff), van Heerbeek and colleagues showed that children, who had tympanostomy tubes in place for otitis media, had different function test results between ears, thus the authors recommend against using split-level design studies.[76]

From India, investigators used slow motion video endoscopy to assess the function of the ET in patients with and without middle-ear disease which was reported to be helpful in the diagnosis of ET dysfunction.[77]

*Management.* There have been studies addressing the potential treatments for ET dysfunction. The efficacy of direct application of dexamethasone into the middle ear through a tympanostomy tube in 11 patients with ET dysfunction appeared to be promising in the short-term but long-term, randomized, placebo-controlled trials are yet to be

reported for this promising therapeutic option.[78] In a study of 40 patients who had intra-nasal surgery for nasal obstruction and reported "ear fullness" preoperatively showed improvement of their symptoms and ET function postoperatively, but no improvement in tympanometric findings.[79] In one study in the monkey, Ghadiali and colleagues performed experiments with surfactant and concluded this therapy might only be beneficial in rigid and inelastic ETs.[80]

Five-fluorouracil (5FU) ointment was reported to be effective for prolonging the opening time of the myringotomy, thus effective for the treatment of OME.[81]

In an effort to determine the efficacy of a gold tube wire (tube conductor), which was previously recommended for chronic ET dysfunction, investigators in Germany found it to be ineffective.[82] From a study of rats, investigators from Japan showed that the ET possesses the immunologic characteristics of a mucosal effector site which responds to P6 outer-membrane protein of *Haemophilus influenzae*, lending support to the development of a vaccine against this bacterium.[83]

Surfactant therapy for otitis media continues to be a subject of interest. Indeed, in a randomized, double-blind, placebo-controlled study of exogenous surfactant administered into the middle ear of rats, the surfactant decreased closing forces of the ET, but had no significant effect on mucociliary clearance.[84] Indeed, intranasal aerosolized surfactant, alone or in conjunction with steroids, was employed in gerbils and mice reduced the passive ET opening pressure in normal animals and duration of middle-ear effusion in animals with experimental effusions.[85] In an earlier experiment using guinea pigs, artificial surfactant was as effective as natural surfactant in facilitating ET opening function.[86]

In an effort to alleviate the symptoms of a patulous ET in 10 patients (15ears), investigators in Japan ligated the pharyngeal end of the ET with the aid of an endoscope, and reported this method to be effective in nine ears.[87] In another report from Japan, surgeons using a modification of the previously reported transcranial method to plug the middle-ear end of the ET in patients with a patulous tube (see: Bluestone CD, et al. Management of the patulous Eustachian tube. *Laryngoscope* 1981; 91:149-152.), was successful in relieving symptoms in 60% of 37 patients.[88] In a similar surgical treatment, a patient with intractable tinnitus secondary to palatomyoclonus was corrected by blocking the protympanic portion of the ET with bone cement.[89] Approaching the patulous ET from the nasopharyngeal end, surgeons inserted an autologous fat plug prior to cauterizing the proximal end of the tube, which was successful in 2 patients.[90] Also approaching the ET from the nasopharyngeal end, Orlandi and Shelton, using an endoscope, cauterized the lumen and sutured the proximal end with success in 3 cases of cerebrospinal fluid otorhinorrhea and one patient with a patulous ET.[91] Surgeons in Japan found that patients who had cholesteatoma and were habitual sniffers due to closing failure of the ET had relief of their habit following surgery when there was an expanded air-bone gap in the lower frequencies of hearing after surgery.[92]

In a review of autoinflation of the ET and middle ear for hearing loss due to otitis media with effusion, the Cochrane Database concluded that despite the small size of the published studies, limited follow-up and treatment duration, auto-inflation seems a reasonable option while awaiting natural resolution of the effusion; further research was recommended, especially related to the duration of treatment and long-term impact of this management on developmental outcomes.[93] But, at the Symposium that preceded this Panel discussion, Alper and colleagues presented the results of a randomized clinical trial of air inflation with the Otovent inflator, with and without corticosteroid therapy, compared with placebo, for otitis media with effusion, in which they concluded the treatment is very limited in its practical applicability to children, not acceptable to most parents, and holds little or no promise of efficacy.[94] Interestingly and related to the previous report, Mudry reviewed the contributions made by Adam Politzer in developing inflation techniques to inflate the middle ear through the ET which dates back to 1863.[95]

In a review of three randomized clinical trials of decongestant therapy to prevent otic barotrauma during flying, Mirza and Richardson found in one study of oral pseudoephedrine, adults had reduced symptoms, but the same medication and route in children did not prevent this complication. The third trial showed that oxymetazoline nasal spray taken 30 minutes before the flight did not prevent ear pain.[96]

In Denmark, investigators assessed the gland tissue changes in the ET of a rat model of acute otitis media, and reported that goblet cells increased in density for up to six months after a pneumococcal acute otitis media,[97] and a second similar experiment showed this sequela after the infection irrespective of the bacterial etiology except for *Moraxella catarrhalis* which lasted only a few weeks.[98] This finding is consistent with clinical observations that this bacterium is not as virulent as the other common otic bacteria. In a follow-up study, Andersen and co-workers demonstrated that penicillin treatment of rats reduced the gland tissue changes in the mucosa of the ET during a pneumococcal acute otitis media; they posited that penicillin could reduce ET dysfunction by this process.[99]

Even though nasopharyngeal carcinoma is relatively uncommon in the West, it is quite common in Asia and thus is a therapeutic challenge. Indeed, investigators in China reported success in dilating the ET that was obstructed following radiotherapy using a Swan-Gans thermodilution catheter.[100] In a later report, investigators reported that patients who had had nasopharyngeal radiotherapy were possible candidates for tympanostomy tube placement to prevent otic barotrauma if they were to receive hyperbaric oxygen therapy.[101] Investigators found that slow compression during hyperbaric oxygen therapy was helpful in prevention of otic barotrauma.[102] Evidently slow compression prevents the ET from locking or near locking, which is consistent with the mechanics of the ET.

The use of lasers to ablate a portion of the nasopharygeal end of the ET has been reported in 56 adults with chronic otitis media, with a reported 68.51% improvement, but the study was not a randomized, control clinical trial.[103] More recently but still without a control, these surgeons reported 13 adults had this procedure performed with success in some, and that those who failed had allergic rhinitis or laryngopharyngeal reflux.[104] In a later report, these investigators used the microdebrider to excise a portion of the proximal end of the ET in 20 patients which had a 70% rate of improvement in symptoms.

## MIDDLE EAR

Even though there has been very little in the literature related to the anatomy of the middle ear during the last four years, there has been significant advances in our state of knowledge of its physiology, pathophysiology and role in middle-ear diseases and disorders related to ET function.

*Anatomy.* In a potentially very useful method to study the middle-ear anatomy, investigators from Taiwan, using 3-dimensional reconstructions and modeling of the middle-ear biomechanics was performed using high-resolution CT scans and finite analysis after which the investigators concluded that this method is quick, practical, low-cost, and most importantly noninvasive as compared with histologic sectioning.[1] Anatomists in Hungary studied 70 macerated temporal bones stereomicroscopically to understand the protympanum of the middle ear and found the main structures of the medial wall of the protypanum are the carotid canal with the internal carotid artery and concluded that the presence of the artery is necessary for development of the canal, and if the artery takes an aberrant pathway, or is absent, there is no sign of the carotid canal.[105] In study of human cadaver crania, Todd attempted to answer the question as to why the manubrium of the malleus appears to be pointing downward and posterior, but could only conclude that its orientation is widely variable.[106] Hypothetically, the fore-shortening position of the malleus could be explained by the presence of commonly encountered, subclinical middle-ear underpressures, but this explanation does not explain the persistence of this deformity in the presence of a functioning tympanostomy tube. Palva and Ramsay dissected 145 temporal bones and described, in detail, the state of the soft tissue structures of the epitympanum, especially Prussak's space, which is relevant for surgery of this anatomic area.[107]

*Physiology and Pathophysiology.* From France, investigators using the experimental rat and a mathematical model studied the role of nitrogen in transmucosal gas exchange rates in the middle ear,

and concluded that gas absorption of the rat middle ear during steady-state conditions is governed mainly by diffusive nitrogen exchange between the middle-ear gas and its mucosal blood circulation.[108] There was an earlier similar report from this team related to the latest publication.[109] Herman demonstrated in vitro and in vivo fluid absorption through epithelium sodium channel during otitis media.[110] Variation of fluid volume after LPS inoculation compared to control side was monitored by a special capillary tube. Compared to the control side, the fluid absorption rate in the LPS-treated middle ear was dramatically affected, but recovered over the 3 days, suggesting that function of sodium channel is well preserved and is a major factor in elimination of fluid development. Ar et al. (2007) utilized a rat experimental model to study trans-mucosal gas exchanges by measuring volume changes in the middle ear. Middle ear gas exchange was recorded after resolution of effusion caused by LPS inoculation.[111] The middle ear gas volume decreased significantly faster with time in inflamed ears compared to the normal control. Mucosal thickness was significantly greater in the inflamed ears. These changes are consistent with increased mucosal blood flow. Also from the same group and reported at the Symposium that preceded this Panel's deliberations, Kania and colleagues, employing similar methods and animal model as described above, as well as a mathematical model, concluded that transmucosal gas absorption in the middle ear during steady state conditions is governed mainly by diffusive $N_2$ exchange between the middle ear and its mucosal blood circulation.[112] From the same group in another report in the Symposium, Ar compared the mucosal blood flow in normal and inflamed ears, concluding that the increased gas exchange, despite the doubling of the mucosal thickness may be explained by doubling the mucosal blood flow.[113]

Another report from Pittsburgh also addressing middle-ear gas exchange of nitrogen, Doyle and colleagues, measured the nitrous oxide time constant for middle ear transmucosal gas exchange in monkeys and using a mathematical model concluded that there is an asymmetric rate of nitrous oxide and by extension, nitrogen exchange for the middle ear.[114] In an earlier report from the same laboratory, Doyle and Banks, using monkeys, showed that breathing gas mixtures containing nitrous oxide causes predictable and quantifiable increases in middle-ear pressure.[115] Middle-ear gas loss was assessed when its mucosa was inflamed in the rat, and from a mathematical model investigators in Israel concluded the model predicts that despite almost doubling mucosal thickness in the middle ear the increased gas loss may be explained by increased blood flow rate.[111] Yuksel demonstrated that nasal inflammation induced by bradykinin and prostoglandin D2 challenge increased the inert gas exchange rate, indicating that persistent nasal inflammation would increase the demand on the Eustachian tube for the gas supply, and may result in otitis media with effusion when this demand is not met.[116] Investigators from Israel also used a mathematical model to determine that diffusive gas transfer in relation to blood gas content is the leading mechanism to alterations in middle-ear pressure and volume in patients with tympanostomy tubes in place.[117] The mechanism and rate of middle-ear fluid absorption was assessed in guinea pigs by investigators in Israel who reported that absorption is related to osmotic gradients within the middle ear.[118] High resolution measurements of middle-ear gas volume changes enabled investigators in the Netherlands to estimate the mucosal $CO_2$ conductance in the rabbit.[119]

Periodic assessments of middle-ear function using tympanometry over a five-year period in older adults, 28-92 years of age, were essentially stable.[120] Gaihede and co-workers (2005) reported that viscosity and amount of middle-ear effusion can influence the tympanogram by increasing the peak pressure difference and that errors of more than 100 daPa can be anticipated.[121]

*Pathogenesis.* In a study from Japan, Noda and colleagues reported at the Symposium that preceded this Panel's discussions, sonotubometry did not reflect the precise condition of the cholesteatoma, but was helpful in assessing the prognosis for hearing levels and recurrence of the disease.[122] An article was published that addressed the unresolved question as to why Australian aborigines have such a high rate of chronic suppurative otitis media, but a low incidence of cholesteatoma. Surgeons from Australia observed the aborigines had "crowding" of the posterior attic, due the incudostapedial assembly, which compromised drainage that hypothetically could result in non-cholesteatomatous disease,

whereas these structures supported the tympanic membrane preventing cholesteatoma.[123]

The possible role of gastrointestinal reflux in the pathogenesis of otitis media continues to be investigated. From Egypt, investigators performed dual 24-hour pH probe testing for reflux in 31 children with otitis media with effusion, of which 71% were considered having the disorder. Also, the middle-ear effusions were tested using ELISA for pepsin/pepsinogen concentrations and they reported that there was a significant positive correlation between the presence of reflux and middle-ear levels, after which they concluded that children with otitis media should be suspect for reflux.[124] A study of adults with otitis media with effusion in Japan also revealed high pepsinogen levels in the middle-ear effusions which was common in those patients who also had symptoms of gastroesophageal reflux by questionnaire, but not by pH probe testing.[125] In an earlier report, *Helicobacter pylori* was detected in middle-ear effusions in children.[126] The problem with these reports is that we remain uncertain if these contents from the stomach are just refluxing through the ET and entering the middle ear, or are these findings are indicative of a true pathogenesis of middle-ear disease. Indeed, stomach contents were not detected in post-tympanostomy tube otorrhea by Antonelli and colleagues.[127]

*Management.* To date, attempts to employ preoperative ET function testing in predicting the outcome of tympanoplasty have been a subject that has not generated any specific recommendations. But, Takahashi and colleagues (2007) report that preoperative ET function tests (inflation-deflation and passive opening via the middle ear) were predictive of outcome; ET mechanical obstruction was associated with failure, and poor inflation-deflation testing and decreased mastoid aeration were also correlated with less favorable outcomes.[128] In a related study, surgeons assessed the predictive value of tympanometric volume in children prior to tympanoplasty and reported that the larger the volume the better the success rate of the procedure.[129] No doubt the volume size is related to the size of the mastoid gas-cell system.

## MASTOID GAS-CELL SYSTEM

There are several lingering controversies regarding the mastoid gas-cell system. One is the role of the size of the mastoid system related to middle-ear disease. A recent study of 41 adult crania, without apparent clinical otitis media, were examined for the degree of attic (pars flaccida) retraction related to the degree of aeration of the mastoid system, found no relationship.[130]

Another controversial issue is the role of the mastoid gas-cell system, and its size, related to the pressure regulation function of the middle-ear cleft. Doyle and colleagues (2003) measured the rate of nitrous oxide exchange across the middle ear mucosa in monkeys before and after blocking the mastoid antrum, which essentially sealed off the mastoid gas-cell system from the middle ear, and reported that the experiment did indeed decrease the middle-ear volume but did not affect the time constant for transmucosal nitrous oxide exchange.[131] Also, in an attempt to resolve this question, Doyle (2007) developed a mathematical model of the system and fit an equation to published human pressure-regulation data and concluded that the larger the volume of the gas-cell system the longer will be the time required to develop sufficient middle-ear underpressures to cause otitis media by the ex vacuo theory or prolonged periods of ET dysfunction; the mastoid is a rate-limiter of middle-ear pressure.[132] This model implies the mastoid gas-cell system is a "gas sink." In an earlier study using a model of the middle-ear cleft, Cinamon and Sade (2003), using mastoids of various sizes concluded that the volume of the mastoid "dilutes" pressure changes, which may be related to middle-ear atelectasis and retraction of the tympanic membrane.[133] In a study in air-dried temporal bones of monkeys, Felding and co-investigators (2003) assessed the air-phase gas exchange between the tympanic cavity and the mastoid gas-cell system, which they found to be rapid.[134] Magnuson (2003) posits that one of the functions of the mastoid gas cell-system is to protect the sensitive vestibular system from stimulation by external temperature changes, and also postulated the system acts as pressure regulator.[135] In a study of

submariners, Toklu and associates (2005) assessed the susceptibility for otic barotrauma related to mastoid pneumatization and reported that mastoid aeration had no relationship.[136]

Still another persistent controversial issue is the role of early otitis media on mastoid gas cell-system growth: one school of thought posits no correlation (i.e., nature) and the other that early ET dysfunction and otitis media inhibits mastoid aeration (i.e., nurture). From Israel, Sade and colleagues (2006) compared mastoid radiographs of two groups of children, 2-11 years of age, in which one group with a history of otitis media and the other group without such a history, and concluded otitis media in infancy inhibits growth of the mastoid system could not be accepted.[137] But, there was no documentation (other than a subjective history) of the presence or absence of otitis media in infancy. Indeed, a 5-year prospective study from Finland did conclude that otitis media in infancy (<17 months of age) was associated with a small mastoid gas-cell system from which the investigators recommended early tympanostomy tube placement with repeat insertions if necessary.[138] One variant that could be improved in these studies of mastoid volume would be to use newer technology, such as proposed by investigators in Turkey who used high resolution CT scans in a 3-dimensional multiplaner volume rendering technique.[139] Also, in a later report from Korea investigators used and now advocate three-dimensional reconstructions of the mastoid using CT scans.[140] In another method, but in human temporal bone specimens, Oishi and colleagues (2003) used a computer-aided surface area measurement of temporal bone pneumatization from histologic sections.[141]

*Management.* For establishing a method for regenerating mastoid air cells after mastoidectomy in chronic otitis media, Kanemaru and colleagues, after in vitro experiments, applied a collagen-coated honeycomb-structure hydroxy apatite containing calcium phosphate in three patients with cholesteatoma, and they found that recovery of mastoid aeration and restructuring of mastoid air cells were observed in two patients.[142-144]

## PROGRESS ON GOALS OF THE 2003 PANEL REPORT

There has not been any report on the role of progenitor cells in the ET and ME epithelium. However this goal may not be a high priority. Similarly there were no reports on the lymphatic system of the ET-ME-mastoid and its role in the pathogenesis of ME disease. Further, no studies of temporal bone specimens from patients in special populations with a known high incidence of OM, such as the Aborigines of Australia and certain Native North Americans have been performed. The Panel still feels that this goal is important, but because of the limitations of temporal bone studies, an increased emphasis on evaluating temporal bones with imaging modalities is warranted. The Panel suggests the inclusion of subjects with cleft palate and other maxillofacial anomalies that predispose subjects to ear disease. There have been a couple of publications identifying the essential structural properties that govern ET function with the goal of elucidating which components could be targeted for treatment. There has been an interest on developing experimental and computational tools to evaluate the relative importance of the various ET structure-function relationships, however more work needs to be done.

There have been a number of publications and progress on determining the relative contributions of ET gas transfer and ME mucosa in the maintenance of ME gas composition and pressure under normal and inflammatory conditions, however more work needs to be done on the actual measurement of gas transfer and the ratio and relative importance of each in the disease state. Some studies were done on the physiology of ion and fluid transport in ET-ME epithelium in normal and diseased states, but this subject remains unexplored. The Panel feels that determining the response of ME mucosal cell cultures to under pressures and changes in gas composition needs to be expanded by applying the concept of "mechanotransduction" in this research.

Assessing ET function in special populations known to have a high incidence of OM, and comparing these findings with function in

individuals without OM, in order to ascertain the underlying pathophysiology in these special populations is still considered important. Evaluation of agents shown to moderate ciliary beat frequency, ET clearance function or ET pressure-regulating function by means of standard models of OM pathogenesis has not been done and panel encourages this research. Due to the ethical and technical limitations of studying ciliary clearance, in humans, novel methods need to be developed.

The Panel feels that designing studies on the ME secretory conditions of mucopolysaccharidoses and management of these conditions before and after blood, marrow, and stem cell transplantation does not fall under its purview. Identifying 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 needs to be expanded to infants and young children. Investigating certain anatomical and physiological markers making some subgroups more susceptible to OME is thought to be important. The Panel suggests continuing to investigate the role of viruses in the pathogenesis of OM and identify targets or promising interventions designed to prevent development of OM during a viral upper respiratory tract infection. The Panel feels that studying the role of OM in the pathogenesis of meningitis in patients with and without inner ear malformations, and with and without cochlear implants is also not relevant to its mandate and suggests they be assessed by the panel on sequalae. The Panel still feels that more studies are needed to evaluate possible role of gastroesophageal reflux in the pathogenesis of OM. Conducting definitive, targeted randomized clinical trials was also found to be not within the scope of this panel.

## FUTURE GOALS

1. Investigate the normal anatomy of the ET-ME-mastoid lymphatic system and determine the system's possible role in the pathogenesis of ME disease.

2. Study histologic temporal bone specimens from patients in special populations that have a known high incidence of OM, such as the Aborigines of Australia and certain Native North Americans. Assess ET function in special populations known to have a high incidence of OM, and compare these findings with function in individuals without OM, in order to understand the pathophysiology of ear disease in these special populations.

3. Identify the essential structural properties that govern ET function with the goal of elucidating which components could be targeted for treatment.

4. Develop experimental and computational tools to evaluate the relative importance of the various ET structure-function relationships.

5. Determine the relative contributions of ET gas transfer and ME mucosa in the maintenance of ME gas composition and pressure under normal and inflammatory conditions with special attention to the actual measurement of gas transfer and the ratio and relative importance of each in various conditions of disease in each disease condition.

6. Study the physiology of ion and fluid transport in ET-ME epithelium in normal and diseased states.

7. Determine the response of ME mucosal cell cultures to underpressures and changes in 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 into OM.

8. 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.

9. 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, expanding in infants and young children and investigating certain anatomical and physiological markers making some subgroups more susceptible to OME.

10. Investigate the role of viruses in the pathogenesis of OM and identify targets or promising interventions designed to prevent development of OM during a viral upper respiratory tract infection.

11. Study the possible role of gastroesophageal reflux in the pathogenesis of OM, with special attention on the pathogenesis.

12. Investigate trans TM gas exchange, with respect to its exchange or buffer or role in pressure regulation. And impact of TM inflammation or sequalae (tympanosclerosis, or atelectasis) on this function.

13. Investigate the pathogenesis of patulous ET, and its impact on ME pathophysiology.

14. Investigate neural reflex control of ETF and mucosal blood flow, and the mechanisms.

15. Investigate the role of Tensor tympani muscle and pars flaccida on MEP regulation and its mechanisms. Include these in the computational models of MEP regulation.

16. Differentiate between pathophysiology of MEP regulation system in recurrent AOM and OME.

17. Investigate the role of neurogenic inflammation in MEP regulation, potential role of naso-ME cleft reflex.

18. Investigate the impact of negative MEP and gas composition on biofilm formation. Impact of biofilms on MEM gas exchange and ETF.

19. Develop novel methods to measure surface area, mucosal blood vessel density and distribution in the ME cleft.

The long-term objective of this research remains a better understanding of the physiology and pathophysiology of the ME system in relation to the pathogenesis of OM. To accomplish that objective, it is important that we refine our current models of ME pressure regulation, include as extensions to those models the mucosal changes that are precipitated at threshold underpressures, define rational interventions that reestablish adequate pressure regulation, and evaluate those interventions in the clinical population. The long-term goal of the research is to implement this paradigm and thereby define rational treatments for OM.

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# 3. PATHOGENSIS: ANATOMY AND PATHOLOGY, AND CELL BIOLOGY

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A number of goals pertaining to the topics represented by this Panel were articulated at the Eighth Post-Symposium Research Conference in Ft. Lauderdale, FL on June 7th and 8th 2003. Those goals are outlined below. Where there has been progress in meeting these specific goals the studies pertaining to those efforts are cited. Each of these studies is further discussed in the body of this manuscript. Additional short and long-goals for the next period of study are outlined at the conclusion of this report.

1. Understand the role of progenitor cells. No studies pertaining to this.

2. Develop a model of respiratory syncitial virus infection in the chinchilla.[16]

3. Continue to investigate the role of viruses in the pathogenesis of OM and identify targets of promising interventions designed to prevent development of OM during a viral upper respiratory tract infection.[16-22]

4. Produce studies investigating the impact of cochlear implants in association with meningitis of otitic origin and the association of OM, meningitis and hearing loss.[23-28]

5. There is a need for further identification of mouse models to use in OM pathogenesis studies.

6. There is a need to determine whether mouse OM susceptibility gene homologues also predispose humans to OM. Deferred to Panel 4.

7. There is a need for the development of reagents for use in chinchilla pathogenesis studies.[29, 30]

8. There is a need for the development of a mouse cell line for *in vitro* cell culture experiments.[31]

9. Use cell lines to determine the signaling pathways activated by OM pathogens and viruses.

## ANATOMY AND PATHOLOGY

A number of studies have been published examining specific anatomic aspects of the ear with respect to pathogenesis.

*Tympanic Membrane and Middle Ear Anatomy.* In the rat model, Hellstom demonstrated that portions of the pars tensa lack vascularity but during periods of inflammation there is new vessel formation to meet the demand for increased blood flow.[1] The early inflammatory changes in the tympanic membrane were also explored in 2 rat models. The course of inflammation showed a bimodal pattern with an early deposition of a filamentous material with a band pattern, typical of fibrin.[2] Examination of vascular changes in the middle ear space during inflammation demonstrated that the distance from blood vessel to mucosa is reduced in all five regions of the middle ear cleft during inflammation. This distance is statistically the shortest in the postero-superior compartment of the middle ear cleft.[3] Related to these studies on vascularity and infection, Chae demonstrated that expression of vascular endothelial growth factors 1 and 2 (VEGFR-1 and -2) were up-regulated between 1-hour and 3-days after middle ear (ME) endotoxin instillation in the rat, with peak expression occurring at 12-hours on day-1. No

expression of VEGFR-3 was detected. These results suggest that these receptors may play different roles in the production of effusion in otitis media with effusion (OME).[4] Further studies associated with the vascular system in the ear showed that plasminogen may play an essential role in protecting against the spontaneous development of chronic otitis media. These findings by Eriksson suggest the possibility of using plasminogen for clinical therapy of certain types of otitis media.[5]

*Cellular Anatomy.* Examination of cellular anatomy within the middle ear cleft specifically focused on mast cells. The studies showed that the number of mast cells is increased in all areas of ME cleft in chronic inflammation, indicating that mast cells have a function in otitis media (OM) pathophysiology.[6] Additionally, wild-type mice, mast cell-deficient mice, and mast cell-deficient mice whose mast cell populations were restored by transplantation of bone marrow-derived mast cells, were challenged using models of bacterial and allergic middle ear inflammation. Results indicated that mast cells account for a substantial proportion of the innate immune response to bacteria in the middle ear.[7]

*Temporal Bone, Eustachian Tube and Adenoid Anatomy.* To delineate a possible role of protective function in OM by Ostmann's fatty tissue (OFT), human temporal bones were studied. In infants and younger children, a relative deficiency in OFT was found near the narrowest portion of ET compared with older patients, which was postulated as a potential cause of insufficient restoration of the ET lumen and increased risk of developing OM.[8]

In the work by Mey, a single episode of OM introduced early in life was sufficient to reduce the final volume of the bulla in rats. This finding may mimic the effect of OM contracted in early childhood on the development of the mastoid air cells in humans and has implications in the pathogenesis of OM in otitis-prone children.[9]

A number of studies focused on goblet cell anatomy and cell biology with respect to OM pathogenesis. ET goblet cell density was found to be increased during and up to 6 months after AOM, which may contribute to the deteriorated ET function found after acute otitis media (AOM).[10] The volume of the paratubal glands also increase during and up to at least 3 months after AOM, primarily because of hypertrophy of the mucous gland components. This may compromise tubal ventilatory and drainage function.[11] Also, the ET goblet cell density is increased up to at least six months after AOM regardless of bacterial species, except when employing MC, by which the density was increased for a few weeks only. NTHi induces the highest increase of both goblet cell density and mucous gland volume.[12] Finally, penicillin reduces the increase of ET goblet cell density during and after AOM, whereas the increase of paratubal gland volume remains unaffected.[13] A study examining adenoid tissue produced additional information with respect to adenoid anatomy and its potential to exert pathogenic changes contributing to OM. In this study, adenoid tissue not only exerted an obstructive influence on the ET lumen when enlarged, but also impedes mucociliary drainage of the ME by the way of extended non-ciliated metaplastic epithelium and fibrosis of connective tissue in OM.[14]

Despite its common use as a model in which to study the pathogenesis and prevention of viral and bacterial OM, there has been very little in the literature to describe the anatomy of the nasal cavity of the chinchilla host. To provide the background information needed in support of intranasal (IN) challenge regimens as well as prior to developing vaccine candidates optimized for IN delivery, Jurcisek published a detailed study of the anatomy of the chinchilla nasal cavity in which, among other observations, they established that the chinchilla has a simple ‘double scroll’ anatomy, similar to that of other rodents, as opposed to the complex turbinate structure of rabbits. Moreover, the abundant presence of nasal NALT-like tissue suggested that this well-established model would be highly suitable to developing an IN delivery system for the assay of OM vaccine candidates.[15]

*Viral Interactions and Pathogenesis.* The association of viral infections and acute otitis media has been previously demonstrated. Significant important progress was achieved in the development of a chinchilla model of respiratory syncytial virus (RSV) infection given the primary role that has been demonstrated with respect to RSV infection as a precursor for AOM.[16] Patel presented additional information from a clinical

study demonstrating the high association of RSV in both middle ear and nasopharyngeal secretions in patients with AOM.[17] However, other studies examining viral prevalence in children with OM identified a predominance of rhinovirus. In one study, nasopharyngeal and middle ear fluid specimens for each acute otitis media event were examined for common respiratory viruses; adenoviruses, influenza viruses A and B, parainfluenza viruses 1, 2, and 3, RSV, enteroviruses, parechoviruses, and rhinoviruses (RV). Picornaviruses (rhinoviruses, enteroviruses, and parechoviruses) were determined by reverse transcription polymerase chain reaction (PCR) while antigen detection was used for the other viruses. A virus was present in either nasopharyngeal or middle ear specimen in 54% of events in the first cohort and in 67% of events in the second. RV formed the most common virus group detected (41-32%), followed by enteroviruses (25%, sought in the second cohort only) and RSV (10%). All the other viruses represented jointly 8-10% of the events. It was concluded that a specific virus infection was diagnosed in two thirds of all acute otitis media events in young children. Picornavirus RNA was detected in association with more than a half of all acute otitis media events. The use of PCR-based methods for the other respiratory viruses might have increased further the overall virus detection rate in AOM.[18] Additionally, Chantzi demonstrated that RV is the predominant virus in the middle ear cavity of asymptomatic OME children, especially those with a chronic OME, recurrent OME, or children with a family history of allergy. These observations lead to the conclusion for the authors that combinations of allergies and RV infections are predisposing factors to middle ear disease.[19] Finally, with respect to viral typing and OM, two studies commented on the herpes virus and its association with AOM and OME.[20, 21]

Additional study further demonstrated the importance of viral upper respiratory tract infection (URI) in the pathogenesis of AOM. Winther studied the relationship between colds and OM in 18 children aged 1 to 8 years followed from October 1 through April 30 using parent-completed daily diaries focused on cold/flu signs and weekly examinations using pneumatic otoscopy for diagnosis of the presence/absence of otitis media. The median duration of the OM episodes was approximately 2 weeks but this was longer for OM episodes where earache was reported. The results confirmed past observation relating new OM episodes to a concurrent URI but show these episodes to usually be of short duration.[22] Additionally Revai demonstrated that URI causation of OM was dependent upon age.[23]

*OM Pathogenesis and the Inner Ear.* A study specifically examining the presence of a cochlear implant and associated meningitis associated with AOM demonstrated an increased risk of pneumococcal meningitis regardless of the route of bacterial infection and suggested that early detection and treatment of pneumococcal infection in AOM may be required, as cochlear implantation may lead to a reduction of infectious threshold for meningitis.[24]

In considering OM pathogens in the development of meningitis it was demonstrated that sequelae are very common in pneumococcal meningitis. Poor outcome was associated with pupillary abnormality and a leukocyte count $<6{,}000/mm^3$ on admission. Leukocytosis was protective against poor outcome.[25] In another study looking at meningitis and hearing loss, the incidence of hearing loss was greater in patients with *Streptococcus pneumoniae* meningitis than in patients with *Neisseria meningitides* meningitis (35.9% and 23.9%, respectively). Length of hospitalization, development of seizures, elevated cerebrospinal fluid protein, and decreased cerebrospinal fluid glucose were significant predictors for hearing loss in children with bacterial meningitis. These factors were not found to be as strong a predictor for hearing loss in patients with *Neisseria meningitides* meningitis. Stability of hearing was demonstrated with limited follow-up audiometry.[26]

The association between AOM and inner ear pathogenic changes was characterized by Ghaheri. Recurrent AOM causes sensorineural hearing loss by unknown mechanisms. It is widely accepted that inflammatory cytokines diffuse across the round window membrane to exert cytotoxic effects. This study addressed whether inner ear cells are capable of expressing genes for inflammatory cytokines and found that the genes of numerous inflammatory cytokines are either up- or down-regulated by

murine inner ear cells in response to either acute or chronic inflammation of the middle ear. This demonstrated a novel site of production of cytokines that might be responsible for the damage seen in sensorineural hearing loss.[27]

Additional studies examining the inner ear in association with AOM demonstrated significant vestibular signs[28] and that the spiral ligament fibrocytes (SLFs) may be involved in the innate immune response of the inner ear by producing chemoattractants for recruiting inflammatory cells such as neutrophils and monocytes.[29]

## CELL BIOLOGY AND PATHOLOGY

*Cell Biology in Association with Pathogenesis - Cell Culture Models.* Significant progress has been made in identifying mouse models for the study of OM. This section is covered by Panel 4 in this conference report.

There has been little development in the creation of biologic reagents for OM studies in the chinchilla. Kerschner has produced two publications in this regard specifically looking at mucin gene sequences in the chinchilla.[30]

There has also been significant progress in the development of a mouse cell line to use in controlled *in vitro* studies of OM pathogenesis. Tsuchiya produced a temperature-sensitive mouse immortalized middle ear cell culture model and characterized this model.[31]

Although identified as a stated goal in the previous research conference, the use of middle ear epithelial cell lines to study pathogenesis and specifically viral pathogenesis has been problematic due to poor cell culture survival. However, Das presented results of respiratory epithelial response to RSV in a nasal cell culture which can serve as a reasonable surrogate for middle ear epithelium and this demonstrated significant up-regulation of inflammatory cytokines.[32]

Pathogenesis related to bacterial pathogens on a cellular level was investigated by Moon examining protein array analysis and showing that in response to NTHi or *S. pneumoniae*, rat spiral ligament fibrocytes released monocyte chemotactic protein 1, macrophage inflammatory protein 3α, TNF-α, and cytokine-induced neutrophil chemoattractant 2 and 3. Treatment with IL-1α, on the other hand, resulted in release of MCP-1 but not the other molecules.[33] The same group demonstrated that IL-1α is secreted by middle ear epithelial cells upon exposure to NTHi components and that it can synergistically act with certain of these molecules to up-regulate, innate antimicrobial molecule, β-defensin 2 via the p38 MAP kinase pathway.[34]

*Mucins.* Pathogenesis in OM is strongly correlated with mucins. Under normal physiologic conditions, membrane-bound mucins remain on the cell surface which plays a role in shielding pathogens (bacteria and viruses) from contact with toll-like receptors and thus prevents them from invasion. Soluble mucins, together with trefoil factors (mucin chaperones), form a mucus gel or blanket which discharges invading bacteria and viruses from the middle ear and Eustachian tube. In pathophysiologic conditions, mucins are overproduced and accumulated in the middle ear cavity, which makes effusion resolution difficult due to the gel property of mucins. It has been recognized clinically that mucin overproduction makes OME prolonged and treatment complicated. The rationale is that with prolonged inflammation mucin producing cells become more developed and subsequently result in mucin overproduction which is chronic and refractory to treatment. In addition, mucins are responsible for the viscosity of middle ear fluid and associated with hearing loss in chronic ear disease. Significant work has been accomplished in increasing the understanding of the function of mucins in the cellular biology of the middle ear and the pathogenesis of OM.

In a study by Solzbacher, it was demonstrated that the ability of mucins to inhibit NTHi attachment to mucosa was related to sialic acid concentration.[35] Lin demonstrated that the MUC5B and MUC4 were major mucins in mouse OM that formed distinct treelike polymers (mucus strands). The MUC5B and MUC4 mRNAs in the middle ear mucosa with mouse OM were up regulated 5-fold and 6-fold, compared with the controls.[36] Martin studied the addition of NO in LPS-induced chinchilla OM. NO was found to increase the mucin concentration in middle ear fluid and increased mucosal thickness and inflammation in

middle ear mucosa. NO was postulated as a contributor to the pathogenesis of mucoid OM.[37]

*Mucin Regulation and Infectious Agents.* An increase of mucin production is a host innate defense response to microbes and pathogens. Both gram-positive and –negative bacteria are capable of inducing mucin production in the host airway mucosa. These bacteria utilize different toll-like receptors and intracellular signaling pathways to activate numerous host mucosal epithelial cells functions including mucin production. The degree and manner of pathogen activation of toll-like receptors and their downstream signaling molecules have influence on mucin expression levels and profiles. Pneumococcus has been demonstrated to be potent in triggering mucin production and mucous cell metaplasia. Accordingly, children who frequently suffer pneumococcal infections in the middle ear tend to have otitis media with mucoid effusion. Ha recently demonstrated that pneumolysin regulated MUC5AC via TLR4-dependent activation of Erk in human epithelial cells in vitro and in mice in vivo.[38] Chen et al. revealed that nontypeable haemophilus enfluenzae regulated the expression of MUC5AC via TLR2-dependent p38 MAPK in human airway epithelial cells.[39]

*Mucins and Cytokines in Middle Ear.* There are 20 human mucin genes that encode protein backbone of mucins and 16 mucin genes that are detected in the airway. In the middle ear, major soluble or secreted mucins are MUC5B, MUC5AC, and perhaps MUC2.[40, 41] Mucin expression in the diseased human middle ear mucosa is highly related to infiltration of inflammatory cells[36] which produce abundant cytokines.[42] Kerschner has developed a number of articles in the chinchilla model demonstrating that mucin production responds to inflammatory cytokines such as tumor necrosis factor-α and interleukin-1β (IL-1β).[43-45] Elucidating the effect of specific cytokines on the regulation of mucin secretion is important in understanding the pathophysiology of otitis media and the development of novel therapeutic strategies. In chinchilla middle ear epithelial cell cultures, secretion of mucins is also triggered by interleukin-6 (IL-6).[46] One increasingly well-recognized pathway in the pathogenesis of OM is the progression from middle ear infection to inflammatory cell infiltration to cytokine production to mucous cell metaplasia.

*Mucins and Mucous Cell Metaplasia.* Mucous cell metaplasia in otitis media is a driving force for persistent mucoid or mucous-serous effusion and otitis media transition from acute to chronic. A major product of mucous cells is mucus that contains abundant soluble mucins[36] and trefoil factors. Trefoil factors are mucin chaperones. Soluble mucins are tightly packed into mucous granules that stain positive with Alcian blue-periodic acid Schiffs (AB-PAS). They are secreted onto the cell surface under the direction of trefoil factors.

*Triggers for Mucous Cell Metaplasia.* It is well accepted that middle ear infection triggers mucous cell metaplasia. Experimentally, many investigators have shown that bacterial infection or cytokine challenge of middle ear mucosa results in mucous cell metaplasia.[36] Clinically, chronic otitis media with mucoid effusion is frequently preceded by upper respiratory tract infection. However, ETO alone induces accumulation of serous fluid in the middle ear cavity but rarely induces mucous cell metaplasia in pathogen-free rats,[1, 12] whereas mucous cell metaplasia may be induced in non pathogen-free rats.[47, 48] This difference may be best explained by priming of the middle ear mucosa by pathogens prior to ETO. It has been shown that pneumococcus-induced middle ear infection in rats results in higher goblet cell numbers compared to *H. influenzae*-induced middle ear infection. Multiple challenges with pneumococcus in the middle ear induced more goblet cells than a single challenge in the middle ear of pneumococcus,[47] suggesting that repeated middle ear pneumococcal infections or a long-term exposure to pneumococcal cell envelope are important determinants for the development of mucous cell metaplasia in OM. In these experiments, it was noted that bacterial remnants may remain in the middle ear cavity despite the absence of viable bacteria, similar to the observation in humans where bacterial cell envelope components were present in the middle ear cavity, although bacterial cultures were sterile.[49, 50]

*Mucin Signaling Pathways.* Mucin signaling pathways have been significantly elucidated in recent years. In an important study it was demonstrated by Ha that pneumolysin is required for up-regulation of MUC5AC mucin via TLR4-dependent activation of ERK in human epithelial cells in vitro and in mice in vivo. Interestingly, a "second wave" of ERK activation appears to be important in mediating MUC5AC induction. Moreover, IkappaB kinase (IKK) alpha and IKKbeta are distinctly involved in MUC5AC induction via an ERK1-dependent, but IkappaBalpha-p65- and p100-p52-independent, mechanism, thereby revealing novel roles for IKKs in mediating up-regulation of MUC5AC mucin by S. pneumoniae.[46] Other focus areas of cell signaling, mucin production and OM pathogenesis are reviewed in Panel 4, however specific areas which have highlighted mucin have emphasized TLR2-TAK1-dependent p38 MAPK-AP1 and NF-kappaB.[51, 52]

*Effusion Biochemistry.* Middle ear effusions (MEE) are closely related to ME mucin production, along with other inflammatory components, and result in morbidity associated with OM. Nonaka demonstrated the importance of IL-5 in OME patients with a history of asthma.[53] Kariya investigated macrophage migration inhibitory factor in MEE and suggested an interaction with endotoxin promoting fluid collection in the ME.[54] Antonelli studied proteases in MEE and determined that ilomastat and recombinant alpha 1-antitrypsin (rAAT) are potent inhibitors of proteases in MEEs across a wide range of OM in humans.[55] Jang further elucidated specific matrix metalloproteinases (MMP) in MEE and reported that MMP-9 and MMP-2 are mediators of inflammation in the OME; in addition, MMP-9 was linked to the pathophysiology of OME in patients with allergy.[56] Russo studied OME in children and concluded that the inflammatory response leading to OME involves multiple cytokines and that IL-6 is an important cytokine in the pathogenesis of OME.[57] Kim et al. studied the effect of IL-1β on the fluid homeostasis of human middle ear epithelial cells. In their study the pro-inflammtory cytokine IL-1β increased the expression of Na-K-2Cl cotransporter 1 protein at the basolateral site of the cells and subsequently increase the transport of bumetanide-sensitive fluid across the human middle ear epithleial cells.[58]

*Biofilms and OM Pathology.* Biofilms are complex organization of bacteria, anchored to a surface, surrounded by exopolysaccharide matrix secreted by the bacteria. Bacteria in the biofilm environment have a low metabolic rate that renders them resistant to antimicrobial treatment and resistant to standard culture techniques. The exopolysaccharide matrix provides protection from phagocytosis and other host defense mechanisms due to a lack of accessibility by immunoglobulin and complement. Bacteria in a biofilm environment rely on a complex intracellular communication system that provides for organized growth characteristics; “quorum sensing.” These biofilm bacteria have altered genetic expression and ability to rapidly share genetic information. This concept of bacterial pathogenesis is distinct from the planktonic concept of bacterial pathogenesis which has been the primary model used to understand otitis media.

The possibility that chronic otitis media exists as a biofilm disease has been emerging as a concept in the past several years. Significant work has been done demonstrating that most children with chronic OM do indeed have biofilms that develop in their middle ear space.[59] Hall-Stoodley and colleagues compared patients undergoing tympanostomy tube placement with control patients without a history of otitis media. In patients with a history of chronic otitis media virtually all had evidence of biofilm formation by two separate measures and none of the patients in the control group had evidence of biofilm formation.

Identifying biofilm formation in clinical specimens suggests opportunities to investigate the potential impact of considering OM as a biofilm disease. Implications in patients with recurrent acute OM include examining evidence that these recurrent acute infections represent recrudescence of biofilm infection rather than infection with a new pathogen. Studies examining the ability of otitis-prone children to clear nasopharyngeal pathogens have been done and provide some insight into these possibilities. Yokota demonstrated that otitis-prone children are more likely to be affected by the same strain of bacterium even after antibiotic treatment and that antibiotics reduce but do not eradicate

nasopharyngeal pathogens in this patient population.[60] Consideration of biofilms in the pathogenesis model of OM also has implications in the study of immunologic defenses in children. Harimaya discussed lower antibody production and decreased cytokine release in adenoid tissue of otitis-prone children along with reduced nasopharyngeal clearance in these patients. It is possible that these factors also result in decreased ability to clear biofilms leading to chronic disease.[61]

These clinical studies demonstrating the importance of biofilms in the pathogenesis of chronic OM have led to further investigations as to the cellular biology involved in biofilm formation in the ME. Leroy showed that chinchilla infection of the ME with non-typeable Haemophilus influenzae (NTHi) demonstrated existence of morphologically distinct NTHi subpopulations with varying levels of gene expression. The biofilm-type organisms significantly differed from those in planktonic form. Additionally, this approach revealed that the actual burden of bacteria in experimental otitis media is significantly greater than previously reported and that such findings may have direct implications for antibiotic treatment and vaccine development against NTHi.[62] Slinger used NTHi and measured biofilm and planktonic minimum inhibitory concentrations (MICs) for 8 antibiotics, and multiple combination testing was performed with 66 groupings of 1, 2, or 3 antibiotics. They found that biofilm cultures were more resistant to antibiotics than planktonic ones. Antibiotic combinations containing rifampin and ciprofloxacin were most effective against biofilms. Biofilm testing revealed differences in effectiveness among antibiotics not apparent from conventional susceptibility testing.[63] Hong demonstrated that mutant bacteria induced 2 to 8-fold more pro-inflammatory cytokine production in the chinchilla model and, therefore, induced more severe immune response compared to normal bacteria. Based on these data, the authors conclude that phosphorylcholine helps NTHi to facilitate infection, establish longer infection and form stable biofilms.[64]

*Resistance Mechanisms and Bacterial Survival.* It has become evident that the diversity of mechanisms bacteria have evolved to resist killing by antimicrobial peptides (APs) suggests an important role for this component of the innate host response. In an effort to investigate AP resistance mechanisms in NTHi, Mason described the function of the *sap* (sensitivity to antimicrobial peptides) operon transporter in resistance to AP lethality.[65] Their results indicated that the products of the *sap* operon are important for resisting the activity of APs and may regulate, in part, the balance between normal carriage and disease caused by NTHi. The authors extended their investigation to show mechanistically that the Sap transporter confers to NTHi the ability to detect the presence of APs, upregulate expression of the *sap* genes in response to AP exposure, and mediate potassium-uptake that was dependent upon SapD. This mechanism of Sap system-mediated resistance to APs and Sap-dependent restoration of potassium homeostasis was critical for NTHi survival *in vivo.*[66]

Nontypeable *H. influenzae* is by definition known to be a "heme-loving" organism, thereby residence within the human airway, which is a highly iron restricted environment, requires that this heterogeneous microbe have an efficient means by which to acquire iron from its host. Since the last symposium, Morton and colleagues have extended their studies of the hemoglobin/hemoglobin-haptoglobin binding proteins expressed by NTHI by using a chinchilla model to investigate the ability of an *hgp* mutant to infect the middle ear. Compared to the wild type strain, the *hgp* mutant exhibited a significant delay in onset of detectable OM as well as a shortened disease course, during which the bacterial load present in the tympanum was significantly less than that due to the parental isolate. The authors concluded that the hemoglobin/hemoglobin-haptoglobin binding proteins of NTHI are required for bacterial proliferation during experimental OM.[67]

In addition to a requisite need for iron, NTHI that reside for a long-term in the human airway must be able to withstand oxidative stress due to the production of multiple reactive oxygen species by both other co-pathogens as well as by its human host. In an attempt to better understand how NTHI mediates resistance to reactive oxygen species, Harrison used an NTHI strain 86-028NP specific DNA microarray to identify bacterial genes that were upregulated when this microbe was exposed to oxidative stress due to hydrogen peroxide. The data demonstrated that careful balancing of intracellular

iron was critical as NTHI attempted to minimize the effects of oxidative stress during colonization and disease.[68]

OM is not only a multifactorial disease, but also a polymicrobial one as well in which viral infection predisposes to bacterial superinfection. The mechanisms by which viruses facilitate opportunistic infection of the middle ear by normal flora that colonize the pediatric nasopharynx are replete and this area of investigation has received a good deal of attention since the last symposium. One mechanism by which viruses augment bacterial infection is to induce upregulation of expression of receptors to which the bacteria adhere. Avadhanula and her colleagues showed that RSV, human parainfluenza virus 3 and influenza virus could influence the ability of both nontypeable *H. influenzae* and *S. pneumoniae* to adhere to both primary and immortalized cell lines. An important finding from their work was that different cell types respond in a distinct manner to infection with specific viruses and this variation needs to be taken into consideration when studying complex polymicrobial diseases, such as OM.[69]

*Innate Immunity.* Lee reported that lysozyme and the β-defensins can inhibit the growth of clinical isolates of otitis media pathogens, NTHi, *S. pneumoniae* 3 and 6B and *M. catarrhalis* and cause ultrastructural damage to these pathogens. Also, they demonstrated that lysozyme and β-defensin-2 can act synergistically against *S. pneumoniae*. These findings are consistent with the concept that secreted antimicrobial peptides and other components of innate immunity constitute the first line of defense protecting host mucosal surfaces, including the tubotympanal mucosa, against pathogens.[70] McGillivary et al.[71] studied the bactericidal effect of cCRAMP, a cathelicidin homolog of chinchilla, against the three major bacterial OM pathogens.[71] Interestingly, not all the OM pathogenic viruses and/or bacteria are cCRAMP inducers. Bacterial pathogens, NTHi and S. pneumoniae, revealed the inductive activity of cCRAMP in chinchilla middle ear cell line but not any of OM related viruses that include RSV, adenovirus, and influenza A.[71]

## OM PATHOGENESIS AND CLINICAL CONDITIONS

*Gastroesophageal Reflux Disease (GERD).* Helicobacter pylori and pepsin have been demonstrated to be present in the middle ear.[72, 73] However, studies aimed at elucidating a linkage of gastroesophageal reflux disease to OM pathogenesis have been relatively lacking. Agirdir demonstrated that H. pylori was more prevalent in patients with OME than in patients with no middle ear effusions at the time of myringotomy.[74] Keles and colleagues reported a significantly higher incidence of GERD in OME patients compared with controls and specifically highlighted measurements of pharyngeal reflux as well as esophageal reflux.[75] This study also demonstrated that most of these patients did not have any clinical symptoms suggestive of GERD. In a gerbil study, Sudhoff demonstrated an animal model linking GERD to OME using tracing of gastric reflux into the middle ear.[76]

*Allergy.* Allergy has been linked to OM pathogenesis in a variable fashion with some studies suggesting a strong correlation between OM and allergy and others demonstrating little association. Investigations conducted since the last symposium continued this pattern. Yeo prospectively analyzed the prevalence of allergic rhinitis (AR) in 123 children with OME (28.4%) and 141 control subjects (24.1%) finding no significant differences.[77] No differences in total eosinophil count and serum and MEE IgE concentration were found between groups. Abnormalities in eustachian tube function were the same in patients with AR and controls. Coulson studied children with chronic OME and normal children, by measuring total IgE and IgE RASTS to dust mite and mixed grass pollens. No statistical differences were found between the groups and this study did not support the role of allergy in the pathogenesis of OME.[78] Lazo-Saenz assessed eustachian tube function in patients with allergic rhinitis and compared them with a control group. Allergic rhinitis patients were found to have a higher risk of eustachian tube dysfunction, particularly during childhood.[79]

## FUTURE GOALS

1. Additional studies examining the specific cell cycle of middle ear epithelial cells has the potential to increase our knowledge of otitis media and the cell biology of the middle ear.

2. Further study of the relationship and synergy between viral and bacterial ME pathology, particularly in animal models, is warranted.

3. Further extensions of experiments examining other pathogens in OM are needed.

4. Additional models, both in vivo and in vitro, are needed for studying OM, particularly OME.

5. Creation of cDNA libraries in animal models of infected and unifected middle ear mucosa to provide for additional molecular analysis of changes in OM is warranted.

6. Creation of goblet cell lines and mucous cell lines, potentially using stem cells, to further enhance the understanding mucin biology and pathophysiology in the middle ear would be of value.

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.

8. Host intracellular responses to bacterial or inflammatory molecule endocytosis should be characterized.

9. Additional study determining whether mouse OM susceptibility gene homologues also predispose humans to OM.

10. Additional clinical and basic science research 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.

11. Additional studies examining biofilm pathogenesis in relation to middle ear disease including:

- Treatment and outcome assessment of biofilm eradication in middle ear disease.
- Additional bacterial ecological studies linking nasopharyngeal and middle ear pathogens and biofilm correlation between nasopharyngeal and middle ear tissues.
- Early onset in life of OM increases the likelihood of chronic disease – is this related to and increased likelihood of biofilm formation in this patient population.
- What is the impact of biofilm formation, especially early in life, on vaccine design, administration and scheduling.
- What is the impact of viruses on biofilm bacteria in the middle ear:
  - Interactions
  - Activation of acute infection
- What is the impact of biofilm formation on antibiotic usage:
  - Watchful waiting
  - Tympanostomy tube placement

12. Additional research into the specific relationships between mucin and other cellular components impacting mucin regulation and OM pathogenesis:

- Describe the potential for mucin gene polymorphisms and disease.
- Describe mucin gene regulation in patients with chronic OM.
- Fully characterize the mucin gene profile in the middle and describe the impact of inflammatory conditions on these genes.

13. Correlate 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.

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# 4. MOLECULAR BIOLOGY, BIOCHEMISTRY, GENETICS AND, ANIMAL MODELS

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## A. RECENT ADVANCES IN THE MOLECULAR BIOLOGY OF OTITIS MEDIA

The responses of both hosts and pathogens during otitis media (OM) are determined in large part by the expression of genes. The identification of genomic and expressed sequences, and the methods of molecular biology, permits 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 activity. Finally, molecular tools allow the manipulation of genes in both hosts and pathogens. This provides powerful methods for evaluating the functional roles of genes in this disease. Many significant molecular advances have occurred over the past four years.

*Molecular Biology of Host Responses in OM.* OM induces a variety of responses in the middle ear (ME), including inflammation, leukocyte recruitment, fluid generation, mucosal hyperplasia, mucin production and both innate and cognate immune responses. Each of these requires the coordinated activation of suites of genes. Moreover, all of these processes are reversed during recovery from OM, involving down-regulation of genes and activation of additional sets of genes that are related to this recovery.

*Mucosal Hyperplasia and Tissue Remodeling*. The ME responds to OM with profound changes in its mucosal lining. The normal ME mucosa has an extremely simple structure, consisting of a monolayer of largely squamous epithelium over a thin and sparsely vascularized stroma, with no resident lymphoid tissue and very few leukocytes. However, unlike most other mucosae, when inflamed the ME mucosa can proliferate rapidly, increasing in thickness from a few tens of μm to several hundred μm in a few days. Hyperplasia includes transformation into a pseudostratified, columnar epithelium with ciliated cells and with the ability to secrete mucus and other substances into the ME lumen. Neovascularization and expansion of the subepithelial stroma, fibrosis, osteoneogenesis and recruitment of leukocytes into the ME mucosa and lumen can also occur.[65, 46] These changes appear to occur regardless of the initiating etiology of OM, and thus form part of a common pathway for the condition. Thus a potential key to the development of new therapies for OM may lie in understanding the behavior of the ME mucosa.

ME mucosal hyperplasia is almost certainly controlled in part by growth factors (GFs), through their interaction with specific transmembrane receptors. The role of GFs in the regulation of cellular growth and differentiation in other tissues is undisputed. GFs produce their effects on cells by interacting with specific transmembrane receptors. Receptor activation can result in the activation of many intracellular signaling cascades. Of particular importance for cell proliferation are the mitogen-activated protein kinase (MAPK) pathways. Palacios et al.[64] demonstrated that intracellular signaling via p38 MAPK influences the hyperplastic response of the ME mucosa during bacterial OM. Furthermore, Furukawa et al.[20] observed that activation of JNK is a critical pathway for bacterially induced mucosal hyperplasia. They found that JNK activation in the ME paralleled tissue proliferation, and that inhibition of JNK reduced mucosal hyperplasia. Understanding the signal transduction pathways that mediate ME mucosal hyperplasia could lead to

the development of new therapeutic interventions for this disease and its sequelae.

It is well accepted that ME infection triggers mucous cell metaplasia. Experimentally, many investigators have shown that bacterial infection or cytokine challenge of ME mucosa results in mucous cell metaplasia. In response to infection, mucous cells increase in the mucosa and many transcription factors (TFs) are involved in this process. Little is known about the TFs that control ME mucosal hyperplasia. However, one of the important TFs that controls mucous cell differentiation process is Atoh1 (formerly known as Math1 in mouse and Hath1 in human), which is involved in the formation and maintenance of mucous cells in mucosal epithelia. Early deletion of this gene results in absence of mucous cells in the intestinal epithelium.[88] However, Atoh1 also appears to function as a tumor suppressor in mature tissue, and its loss is associated with several cancers. This raises the question of whether Atoh1 plays a role in ME mucosal hyperplasia or mucin regulation.

Kim and Jung[45] reported that Clara cell secretory protein, an anti-inflammatory and immunomodulatory protein known to be secreted by non-ciliated respiratory epithelial cells in the lung and to originate from cells that may become goblet cells,[29] was produced in the ME during acute OM in the rat. Expression peaked at 12 hours after endotoxin instillation into the ME, and then decreased after day 3. This suggests that goblet cell metaplasia in the ME mucosa may be initiated by the induction of Clara cells.

Not all OM recovers without permanent sequelae. Osteoneogenesis can result from severe OM. Melhus and Ryan[57] reported that amoxicillin treatment enhances expression of both osteocalcin (a bone formation marker) and IL-10 (a suppressor of bone resorption). This could contribute to increased bone formation during OM.

*Production of Mucus and Mucin Gene Expression.* Mucins, the major product of mucous cells, are highly related to ME physiology and pathology. Under physiologic conditions, membrane-bound mucins on the cell surface play a role in shielding pathogens (bacteria and viruses) from contact with Toll-like receptors (TLRs) and thus prevent them from invasion. Soluble mucins, together with trefoil factors (mucin chaperones), form a mucus gel or blanket which aid in the discharge of bacteria and viruses from the ME and Eustachian tube. Under disease conditions, mucins can be overproduced and accumulated in the ME cavity, which makes effusion resolution difficult due to the gel property of mucins. Thus production of mucins may lead to OM that is chronic and refractory to treatment. Mucin genes can be upregulated by risk factors for OM, such as smoking.[68]

Soluble mucins expressed in mucous cells of the Eustachian tube epithelium include MUC5B and MUC5AC under normal conditions[16] whereas mucin expressed in mucous cells of the ME epithelium under chronic OM conditions include MUC5B[16] and Muc2.[85] MUC5AC, MUC5B, MUC2 and MUC6 are known to be clustered at a locus on chromosome 11p15.5 in humans, bearing similar properties and functions (soluble mucins). While these mucins are prominent, however, Kerschner[44] found that of 18 mucin genes tested, 15 are expressed in the ME mucosa.

An increase of mucin production is a host innate defense response to microbes and pathogens. Bacteria, gram-positive and -negative, are capable of inducing mucin production in the host airway mucosa. The difference is which TLRs they bind and which signaling pathways they activate during the interaction between pathogens and host mucosal epithelial cells. The degree and manner of pathogen activating TLRs and their downstream signaling molecules have influence on mucin expression levels and profiles. Gram-positive bacteria such as pneumococcus tend to be potent in triggering mucin production and mucous cell metaplasia. Accordingly, children who frequently suffer pneucoccal infections in the ME tend to have OM with mucoid effusion. Ha et al.[23] recently demonstrated that pneumolysin regulated MUC5AC via TLR4-dependent activation of Erk in human epithelial cells in vitro and in mice in vivo. Chen et al.[11] revealed that nontypeable *Haemophilus influenzae* (NTHi) regulated the expression of MUC5AC via TLR2-dependent p38 MAPK in human airway epithelial cells. Shen et al.[76] reported synergistic activation of MUC5A by NTHi and *S. pneumoniae*.

Mucin expression in the diseased human ME mucosa is highly related to infiltration of inflammatory cells which produce abundant cytokines. In animal models, mucin production responds to inflammatory cytokines such as interleukin-1beta (IL-1beta) and IL-6. Elucidating the effect of specific cytokines on the regulation of mucin secretion is vital to understanding the pathophysiology of OM and the development of novel therapeutic strategies. In chinchilla ME epithelial cell cultures, secretion of mucins is triggered by IL-6. One well-recognized pathway is ME infection→inflammatory cell infiltration→cytokine production→mucous cell metaplasia. A number of cytokines generated from inflammatory cells upregulate the mucin gene expression and induce mucous cell metaplasia. They are either proinflammatory cytokines or lymphocyte (especially T helper 1 subset)-derived cytokines.[79]

*Role of Mast Cells in OM.* Ebmeyer et al.[15] evaluated acute OM in mice genetically deficient in mast cells. They found that NTHi-induced OM was reduced in severity in these animals, implicating the mast cell in innate immune defense of the ME against bacteria. When bone marrow-derived mast cells were used to reconstitute mast-cell-deficient animals, the wild-type (WT) response to NTHi was restored. Ebmeyer et al.[15] also noted a potentiative interaction between allergic and bacterial OM, which depended upon the presence of mast cells.

The response of the ME to pathogen signaling. A significant proportion of host responses during OM are elicited by the interaction of specific signals with cell surface receptors. Receptors that directly recognize pathogen are increasingly recognized as critical to host defense and as central to apthogen-mediated inflammation. These pathogen-associated receptor proteins (PARPs) include the TLRs, Nod-like receptors (NLRs) and TGFβRs, which recognize a variety of pathogen-associated molecular patterns (PAMPs). The best-studied PARPs are the TLRs. Recognized by their homology to the *Drosophila* Toll receptor, the 10 human (and 13 mouse) TLRs have been shown to be important mediators of responses to pathogenic organisms. Because TLR senses a distinct repertoire of conserved microbial molecules, collectively they can detect most if not all microbes.[6] Downstream signaling from TLRs occurs via a family of adaptor molecules, especially MyD88 and TRIF, which via a series of intermediates can activate the MAPKs as well as NFκB, influencing the expression of many genes involved in inflammation, tissue proliferation, apoptosis and other processes.[1, 55]

A number of recent molecular studies have probed the role of TLRs in host responses during OM. For example, studies in knockout (KO) mice have shown that TLR2 and TLR4, signaling through MyD88, contribute to the initial pathogenesis of bacterial OM, although this contribution is relatively modest.[33, 49] The initial proliferation of the ME mucosa in the KO mice is similar to that seen in WT mice. However, the recruitment of leukocytes is reduced in TLR2-deficient animals. These receptors play a much greater role in OM recovery, and appear to be critical for bacterial clearance in OM. This leads to enhanced hyperplasia at times when OM in WT mice has resolved, presumably secondary to failure of bacterial clearance, and to persistence of OM for much greater periods of time than in WT mice. Hernandez et al.[31] and Leichtle et al.[49] found that when inoculated with NTHi in the ME, mice deficient in TLR2 and MyD88 remained infected for at least 3 weeks, while WT animals were culture-negative by day 5.

However, there is also evidence that TLR signaling contributes to early OM pathogenesis. A peptide that interferes with TLR signaling, by competing with the TLR adapter MyD88, reduced inflammation in a mouse model of OM induced by heat-killed bacteria,[54] while mice deficient in MyD88 show a slight reduction in mucosal hyperplasias 2 days after NTHi inoculation. [31]

The effect of TLR stimulation and MyD88 activation is often to induce the production of inflammatory cytokines, and especially TNFα. Leichtle et al.[48] found that mice deficient in TNFα showed an OM phenotype similar to that exhibited by TLR2- and MyD88-deficient mice.

The importance of pathogen signaling to OM human disease was recently supported by Emonts et al.[17] In a genome association study, they found that polymorphisms in the genes encoding TLR4 and TNFα are positively associated with recurrent OM.

*Host Gene Expression Profiles in OM*. The behavior of the ME during OM is ultimately controlled by the expression of both host and bacterial genes. Recent studies have sought to profile the expression of gene transcripts during OM using gene arrays. Chen et al.[10] reported gene expression profiles of early pneumococcal OM in the rat, using a 1176-transcript rat gene array. They observed upregulation of genes encoding cytokines, stress-related proteins and TFs at 12 hours, and apoptosis-related genes at 48 hours. Hernandez et al.[30] evaluated the expression of 55,000 transcripts in the mouse ME from 3 hours to 7 days after NTHi inoculation. They found that upregulated genes segregated into three waves of gene activation: 3-6 hours, 24 hours and 48-72 hours. These included genes related to inflammatory mediators/receptors (3-6 hours), leukocyte recruitment (24 hours) and tissue proliferation (48-72 hours). A group of downregulated genes were associated with development and lipid biosynthesis. These observations may lead to a greater understanding of genes that contribute to the pathogenesis and resolution of an acute OM episode.

*Role of Inflammatory Cytokines in OM.* A significant amount of molecular work was directed at the role of cytokines in OM. For example, using real-time PCR, Tong et al.[81] documented the up-regulation of TNFα, IL-1a, IL-1b, IL-6, IL-10 and iNOS by NTHi in the ME of the rat. As noted above, Leichtle et al.[48] evaluated experimental OM due to NTHi infection in mice lacking the TNFα gene. They found that OM is greatly prolonged in these mice, as was bacterial clearance. This was associated with defects in macrophage phagocytosis and intracellular killing, and changes in the expression of other cytokines. This suggests that TNFα may play a central role in regulating inflammation during OM. When TNFα was supplied to the ME or to macrophages, their behavior was only partly restored. Interestingly, most aspects of OM and macrophage interaction with bacteria could be restored by treatment with the chemokine CCL3, suggesting that TNFα acts via CCL3 for a significant proportion of its role in OM. Alper et al.[3] reported that polymorphisms in cytokine genes are predictive of OM frequency after upper respiratory viral infections, indicating the importance of these genes in human disease.

*ME Infection and Cholesteatoma.* Cholesteatoma grows aggressively, destroys the neighboring tissues or structures such as the ossicular chain and the ME cleft and causes complications such as hearing loss, brain abcess, facial palsy, etc. Clinically, acquired cholesteatoma is frequently associated with chronic OM, it is believed that ME infection promotes aggressive growth of cholesteatoma epithelium through inflammatory cytokines and mediators. What would be the candidate factor committing to growth of keratinocytes in cholesteatoma? A recent study suggested that inhibitor of DNA binding protein (Id1, an oncogene in promoting keratinocyte proliferation) is one of the candidate factors involved in the growth of keratinoctyes.[90] Hamajima et al.[24] demonstrated in a recent study that Id1 is involved in the hyperproliferation of keratinocytes, positively via the nuclear factor-kappa B (NF-κB)/cyclin D1 pathway which is linked to cell cycle progression and negatively via the $p16^{Ink4a}$ which is linked to cell cycle inhibition. Id1 significantly increased the transcription of NF-κB which, in turn, upregulated the expression of cyclin D1 and keratin 10 in keratinocytes. Specific NF-κB inhibitors (pyrrolidine dithiocarbamate, PDTC), or dominant-negative inhibitor (I kappa B alpha mutant, IκBαM) abrogated the Id1-induced cell proliferation and keratin 10 production whereas p65, a subunit of the NF-κB heterodimers and an enhancer of the NF-κB activity strengthened the Id1-induced cell proliferation and keratin 10 production. It was concluded in this study that Id1 contributed to hyperproliferation of keratinocytes via enhancement of cell cycle progression and removal of cell cycle inhibition and simultaneously increased keratin production.

*Molecular Studies of Pathogens in OM.* Despite the fact that the first free-living organism whose genome was sequenced was a rough derivative of a serotype d strain of NTHi (strain Rd or KW20), because serotype d strains do not cause disease, the information that could be gleaned from the Rd genome was not ideal in terms of contributing to our full understanding of the pathogenesis of this heterogeneous group of microbes in OM and other diseases of the respiratory tract. Thereby, since the last symposium, the partial genomes of two and the full genome of one of these clinical isolates of

NTHi have been determined and published.[61, 27] These investigators determined that whereas the gene content and order were similar between the clinical isolates and strain Rd, there were also a substantial number of genes not previously found in the *Pasteurellaceae* and there were some regions where the gene content and order were different from that found in strain Rd. These findings suggested that the genomes of the clinical NTHi isolates were a complex mosaic of strain Rd and non-strain Rd like features. The fully sequenced and annotated genome of strain 86-028NP, which was recovered from a pediatric patient undergoing tube insertion for chronic OM, revealed 280 ORFs present in this strain that were absent from the strain Rd genome. The genome of strain 86-028NP has been used to generate a strain-specific microarray probe set that was built on the commercially available oligonucleotide set for *H. influenzae* strain Rd, plus 548 additional custom-made 70-mer probes representing open reading frames from the OM isolate's genome that were either absent in strain Rd or that had insufficient similarity to strain Rd homologues. This microarray is currently being used to dissect the pathogenic mechanisms of *H. influenzae* strain 86-028NP in OM.[28]

A number of additional genomes of NTHi and of *Streptococcus pneumoniae* have been sequenced.[32, 34] In both cases, there was significant variation in the genes of different strains, indicating that a "core genome" is supplemented by a "supragenome" distributed across many strains, for each species. In the case of *S. pneumo*, a comparison of OM phenotype induced by 13 isolates was performed in chinchilla.[19] Wide variation in disease severity was observed, and variation occurred both within and between serotypes, illustrating the variability of bacterial genotypes.

SHORT-TERM GOALS

1. Additional studies of host gene expression in OM, including differences in the response to various pathogens.

2. Bioinformatic analysis of gene networks activated in the ME during OM, using gene array data.

3. Additional studies of pathogen gene expression during OM, including viruses.

4. Studies of the interaction of host and pathogen gene expression using both mouse and pathogen mutants and gene arrays.

5. Identify the gene targets and functional consequences of various cell signaling networks in ME cells.

6. Develop transfection and siRNA technologies for *in vivo* up- and down-regulation of genes in the ME, respectively.

7. Utilizate transgenic and mutant bacterial models to understand pathogenesis, virulence and biofilm formation.

8. Study regulatory sequences that target gene expression to the ME.

LONG-TERM GOALS

1. Understand how the complex cell signaling pathways and gene regulatory networks that are activated in OM interact to produce various outcomes in this disease (a meta-analysis, if you will, of the many separate studies of such pathways).

2. Translate molecular findings on cell signaling and gene regulation during OM into improvements in patient care.

3. Obtain chinchilla genome sequence.

## B. RECENT ADVANCES IN THE BIOCHEMISTRY OF OTITIS MEDIA

*Signaling Pathways Involved in OM Pathogens.* Watanabe et al.[86] showed that NTHi and TNFα, when present together, synergistically induce NF-kappaB activation via two distinct signaling pathways: NF-kappaB translocation-dependent and -independent pathways. Furthermore, Kweon et al.[47] demonstrated that NTHi and *S. pneumoniae* synergistically induce NF-kappaB-dependent inflammatory response via activation of multiple signaling pathways in vitro and in vivo. The classical IKKbeta-IkappaBalpha and p38 MAPK pathways were involved in synergistic activation of NF-kappaB via two distinct mechanisms, p65

nuclear translocation-dependent and -independent mechanisms.

*Cytokines.* The occurrence and role of cytokines in OM has been an active area of study since the last review. Kariya et al.[39] reported that IL-2, IL-4, IL-5 and IFN-g were present in significant fractions of adults OM effusions. IL-12 appeared the production of IL-2 and IFN-g, regardless of allergic status. Moreover, they observed that IL-4 was positively correlated with allergic rhinitis, and may have some impact on immunologic conditions. They also noted that IL-10 potentially affects the viscosity of ME effusions. While Skotnicka and Hassman[77] reported high levels of IL1-b, IL-6 and IL-10 in effusions from children with OME, they found that none of the cytokines were correlated with clinical parameters. Russo et al.[71] reported that IL-1 and IL-6 were elevated in ME effusions of children with chronic OM, while Nguyen et al.[62] observed elevated IL-4 in effusions of atopic children with OME. Iino et al.[35] observed elevated IL-5 in ME effusions from children with eosinophilic OM associated with bronchial asthma. A strong, positive correlation between elevated eosinophil cationic protein and IL-5 was also noted, suggesting that IL-5 may play a central role in the recruitment of eosinophils to the ME. Rezes et al.[69] compared immunoglobulin (Ig)-to-albumin (A) ratios with cytokine profiles in OM effusions. They noted that effusions with higher Ig/A ratios were more likely to show increased IFN-g and TNFa, while in effusions with lower Ig/A ratios, IL-4 and IL-10 predominated.

Maeda et al.[51] demonstrated that in the endotoxin-induced OM, the subepithelial space of ME mucosa was severely thickened with the infiltration of a large number of mononuclear cells expressing TNFα. Park et al.[65] found that treatment with soluble TNF receptor 1 reduced the severity of immune-mediated OM in a rat model, while Kim et al.[46] reported similar findings in endotoxin-induced OM. Tong et al.[81] reported that a mutation in the NTHi LOS processing gene htrB reduced cytokine production in experimental OM, when compared to WT NTHi. Jung et al.[37] found that a PAF antagonist decreases OM caused by killed NTHi in the chinchilla. Moon et al.[58] observed that ME epithelial cells release IL-1 alpha when stimulated by NTHi components and this cytokine acted in an autocrine/paracrine synergistic manner with NTHi to up-regulate beta-defensin 2. This synergistic effect of IL-1 alpha on NTHi-induced beta-defensin 2 up-regulation appeared to be mediated by the p38 MAP kinase pathway.

Several studies explored potential mechanisms of cytokine action in OM. Choi et al.[13] reported that IL-1b suppresses epithelial sodium channel expression and EnaC-dependent fluid absorption in a ME epithelial cell line, implicating this cytokine in the retention of effusion. The same group[14] earlier reported that IL-1b promoted ciliogenesis and mucin gene expression. Similarly Kerchner et al.[40] found that IL-6 promoted mucin production in a ME epithelial cell line.

A significant association between polymorphisms in cytokine genes and OM susceptibility has recently been documented. Patel et al.[67] compared otitis-prone and normal children, and found that polymorphisms of both the TNFa and IL-6 genes were significantly over-represented in the otitis-prone children. Emonts et al.[17] found a significant relationship between certain alleles of the TNFa, IL-6 and IL-10 genes and recurrent acute OM. These important data underscore the importance of cytokines in the prevention of OM.

*Arachadonic acid Metabolites*. Cho et al.[12] reported that both COX-1 and COX-2 are produced in the ME mucosa during endotoxin-induced OM in the rat, implicating prostanoids in the pathogenesis of OM. Yuksel et al.[89] found that nasal prostaglandin challenge increased gas exchange from the blood to ME, suggesting an increase in physiological demand upon the Eustachian tube for gas supply.

*Cathepsin.* Li-Korotky et al.[50] demonstrated that ME mucosa expression of a gene cluster encoding the lysosomal cysteine proteases, cathepsins B (Ctsb), L (Ctsl), and K (Ctsk), was modified after *S pneumoniae* challenge.

*Oxidative Stress and Antioxidants*. Oxidative stress is a common component of bacterial infection and inflammation, induced by the responses of phagocytic and other cells to bacteria, and by inflammatory mediators. Tong et al.[81] presented evidence that modifications of bacterial LOS by the NTHi htrB gene were important for the induction of

iNOS during OM, implicatitng LOS in free radical generation. Taysi et al.[80] measured lipid peroxidation in ME effusions of guinea pigs during experimental OM induced by histamine. They observed increased lipid peroxidation in ME effusions, and a compensatory increase in the levels of several antioxidant enzymes. Cemek et al.[9] reported that the levels of several antioxidant enzymes were increased in the serum of patients with acute OM, suggesting systemic, in addition to local, responses to free radical stress that originates in the ME. Free radicals can be expected to elicit strong responses from the cells of the ME mucosa. Martin et al.[52] observed that the addition of NO in LPS-induced OM increased the mucin concentration in ME fluid and increased mucosal thickness and inflammation in ME mucosa. It is suggested that NO may contribute to the pathogenesis of mucoid OM.

*Substance P.* Caye-Thomasen et al.[8] demonstrated that substance P was depleted in the rat ME mucosa in the hyperacute phase of acute OM. The release of substance P may be the trigger of the concurrent bone resorption and may further augment the inflammatory response to the bacterial colonization.

*Aquaporins*. Regulation of fluid is critical to the homeostasis of the ME, and to the generation of effusions during OM. Kang et al.[38] evaluated the expression of several aquaporins (AQPs) in the normal rat ME and Eustachian tube. They reported that AQPs 1, 4 and 5 were expressed. Immunocytochemistry indicated that AQP 1 was expressed by stromal fibroblasts, AQP 4 was found on the basolateral ciliated epithelial cells, while AQP 5 was observed on the apical surfaces of serous gland cells.

SHORT-TERM GOALS

1. More precisely define mucus components and their modifications in various types of ME effusions and nasoparyngeal secretions.

2. Understand mucin chaperones that occur in the ME and nasopharynx and their role in OM.

3. Explore the link between pathogens and pathogen combinations and the production of cytokines, chemokines and growth factors.

4. Explore combinatorial strategies for inducing the various phenotypes of ME cells from undifferentiated precursor cells.

5. Identify diagnostic and prognostic markers of different stages and varieties of OM using various proteomic methods.

LONG-TERM GOALS

1. Perform more detailed proteomic analysis of ME components and nasopharyngeal secretions during OM to explore post-translational processing of gene products.

2. Define the biochemistry of ME mucin degradation to aid in the development of treatments for mucoid OM.

## C. 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 this, there has been continuous work to develop additional and improved animal models for this condition. Unfortunately, there are still significant gaps in our animal models of OM. In particular, animal models of chronic OM that are similar to human disease remain elusive. However, several recent models have increased the severity and duration of OM in animal models.

Several existing rodent models of experimental OM have yielded valuable information regarding the pathogenesis of OM. Since the last symposium, Novotny et al.[63] expanded the utility of the chinchilla model to develop a methodology that allows direct, continuous biophotonic imaging of bioluminescent NTHi during experimental disease. This model, which is non-invasive and can detect bioluminescent NTHi resident within several microenvironments in the uppermost airway (nasopharynx, Eustachian tube and tympanum), required generation of a luciferase reporter construct in NTHi strain 86-028NP. Relative intensity of the detected luminescent signal allows for an approximation of bacterial load within this host over the experimental disease course. This

model system is being used to understand NTHi gene expression profiles *in vivo* during both colonization as well as frank disease induction in the ME.[53]

While nearly all upper respiratory tract viruses and some of the enteroviruses can predispose to bacterial OM, RSV is often acknowledged as a key, if not the most common viral co-pathogen of ME infections. Despite its prevalence however, the absence of a relevant animal model of RSV infection of the tubotympanum has severely limited our ability to understand the molecular mechanisms that underlie the ability of this virus to compromise its mammalian host and predispose to retrograde ascension of bacteria from the nasopharynx, through the Eustachian tube and into the ME. In 2005, Gitiban and colleagues[21] published a study wherein models of upper respiratory tract infection due to RSV were established and characterized in both the murine and chinchilla hosts. These models are currently being used to both better characterize RSV-induced infections of the uppermost airway as well as to assay the relative protective efficacy of RSV-derived vaccine candidates.

Furukawa et al.[20] developed a method for inserting the catheter of an osmotic minipump into the subepithelial stroma of the guinea pig ME mucosa. This was necessary since substance injected into the ME lumen do not reach the subepithelium, which is rich in cells and receptors for biological factors. This method allows the delivery of substances to those cell types and receptors, which are quite distinct from those of the mucosal epithelium.

Great progress has been made in developing multiple mouse models of OM. This species is challenging for OM research, due to its small size and its minimal ME volume, which averages less than 6 µl. The unexpanded ME is located deep within the head, making access for delivery of bacteria or other agents through the bullar wall more difficult than in the chinchilla, for example. Moreover, the relative patency of the Eustachian tube and its location near the floor of the tympanic cavity enhances the drainage of fluid from the ME if it is vented to the atmosphere, such as when substances are injected through the tympanic membrane. However, several groups have now had good success in inducing acute OM in mice, primarily through surgical access to the ventral bulla.[56, 15, 18, 33] This has allowed them to take advantage of the molecular resources available in this species, including gene expression arrays and genetically modified animals, several of which have been described above.

OM can be induced by pathogens or by introduction of inflammatory substances. Mouse strain differences have a significant effect upon induced OM, with Swiss–Webster mice being significantly resistant to bacterial OM, the BALB/c strain being quite susceptible and C57Bl/6 mice exhibiting intermediate responses. With these and other limitations in mind, natural or induced mutations offer the opportunity to study the role of an ever-increasing number of genes.[72]

A significant advance has been the screening of large N-ethyl N-nitrosourea (ENU) mutagenesis projects for hearing loss. Indeed, the discovery of the deaf mouse mutant *Jeff* (*Jf*), a single locus model for OM, in an ENU program in the United Kingdom underlines the potential of large-scale mutagenesis projects for the identification of OM mouse models.[25] The *Jf* mutant shows a significant conductive hearing loss by postnatal day 35 with fluid and pus in the ME cavity. *Jf* mice develop a chronic suppurative OM with severe inflammation of the mucoperiosteum. The Jeff locus maps to mouse chromosome 17. Further study demonstrates that *Jf* carries a mutation in an F-box gene, *Fbxo11*, a TF which is expressed in epithelial cells of the MEs from late embryonic stages through day 13 of postnatal life. In contrast to Jeff heterozygotes, *Jf* homozygotes show cleft palate, facial clefting and perinatal lethality. Fbxo11 is one of the first molecules to be identified, contributing to the genetic aetiology of OM.[26] Another group observed evidence consistent with an association between polymorphisms in *Fbxo11* and chronic OM with effusion/recurrent OM(COME/ROM) by genotyping 13 SNPs across the 98.7 kilobases of genomic DNAencompassing FBXO11.[75] Another mutant, *Junbo* (*Jbo*), with a very similar phenotype has been identified from the same ENU mutagenesis program. *Jbo* maps to chromosome 3, and recently a mutation in the gene encoding the TF *Evi1* has been shown to underlie the OM phenotype.[26] The same group identified the causal mutation, a missense change in the C-terminal zinc finger region of the TF Evi1. This protein is

expressed in ME basal epithelial cells, fibroblasts, and neutrophil leukocytes at postnatal days 13 and 21 when inflammatory changes are underway. The identification and characterization of the *Jbo* mutant elaborates a novel role for Evi1 in mammalian disease and implicates a new pathway in genetic predisposition to OM.[66]

ENU mutation screens can have weaknesses. The screen described above was restricted to dominant mutations, thus loss-of-function mutations are under-represented. Moreover, by analysis of the published data of randomly acting mutagens, a study showed that ENU-induced mutations identified in phenotype-driven screens were in genes that had higher coding sequence length and higher exon number than the average for the mouse genome. Data also showed that ENU-induced mutations were more likely to be found in genes that had a higher G + C content and neighboring base analysis revealed that the identified ENU mutations were more often directly flanked by G or C nucleotides. ENU mutations from phenotype-driven and gene-driven screens were predominantly A:T to T:A transversions or A:T to G:C transitions. Knowledge of the spectrum of mutations that ENU elicits will assist in positional cloning of ENU-induced mutations by allowing prioritization of candidate genes based on some of their inherent features.[4] Additional screening methods, such as recessive ENU screens[74] will also be productive.

In addition to the mouse models discussed above, a mouse model of OM was also used to identify the expression of defensins, which are antimicrobial peptides that play a major role in innate immunity. As the expression levels of beta-defensins 2-4 (mBD2, mBD3, mBD4) in tubotympanum were upregulated in experimental mice of OM, they may play a protective role in the pathogenesis of OM.[36] In other research, mice immunized with the *S. pneumoniae* D39 strain were employed to identify novel *S. pneumoniae* antigens by screening awhole-genome lambda-display library, and Spr0075 was identified as an expressed *S. pneumoniae* gene product, having an antigenic function during infection.[5]

Rivkin and colleagues[70] found that mucosal hyperplasia during OM was enhanced in lpr/lpr mice when compared to WT controls. In addition, the recovery of the mucosa was significantly delayed in Fas-deficient mice. The results suggest that Fas-mediated apoptosis plays a role in remodeling of the ME mucosa during OM-induced mucosal growth and that recovery of the mucosa during OM resolution involves apoptosis mediated by death ligands and receptors. In another study, the role of the plasminogen (plg)/plasmin system in spontaneous development of chronic OM was investigated by analysis of plg-deficient mice. Essentially all of the WT control mice kept a healthy ME status; whereas all the plg-deficient mice gradually developed chronic OM with varying degrees of inflammatory changes during an 18-week observation period. Five bacterial strains were identified in ME cavity exudates of six plg-deficient mice, suggesting that plg plays an essential role in protecting against spontaneous development of chronic OM.[18] Moreover, lysosomal neuraminidase deficiency may result in the alteration of ear morphology. External auditory canal obstruction, OM and ossicle changes may cause conductive hearing loss, and defects in lysosomal storage of neurons, stria vascularis, spiral limbus, Reissner's membrane and basilar membrane cells may contribute to sensorineural deafness.[22]

*Mucosal Cell Lines from Animal Models.* Because the number of OM animal models presently available is limited, some studies have investigated the interaction between pathogen and host cell or cell line. It is worth mentioning that a temperature-sensitive mouse ME epithelial cell line has been established for OM studies. The primary culture of ME epithelial cells was established from the ME mucosa of an Immortomouse derived from an SV40-bearing egg. The cultured cells were transduced by a temperature-sensitive large T-antigen mutant and cultured for >50 passages. Temperature-sensitive ME epithelial cell lines are essential for pathophysiologic studies of OM. The cell line is very useful for studying the pathogen-host interaction, receptor identification, signal transduction, cytokine/mucin production and cellular responses, especially for cell proliferation and differentiation.[83]

Establishment of ME epithelial cell lines has also been a useful step for studying mucin regulation and mucous cell differentiation in order to understand the disease mechanism of OM. In recent years, attention has been placed on

temperature-sensitive ME epithelial cell lines. So far, rat and mouse ME epithelial cell lines have been established for OM research.[82-84] These ME epithelial cell lines are very valuable in studying cytokine expression, mucin regulation, and mucous cell differentiation because the SV40 oncogene which immortalizes the ME epithelial cells is controllable by changing cell culture temperature from 33ºC to 39ºC. This makes ME epithelial cells act as normal ME epithelial cells, instead of oncogene-driven cells. With these cell lines, research for cellular differentiation events such as mucous cell differentiation becomes possible.

## SHORT-TERM GOALS

1. Standardization of phenotype determination in mouse models of OM.

2. Development of better animal models of chronic OM.

3. Development of better models of mucoid and serous OM (OME).

4. Development of models of conditional gene expression in the ME, including both site specificity and inducibility.

5. Study polymicrobial effects on airway and ME epithelial cells *in vitro*.

6. Identify the susceptibility of inbred mouse strains to induced OM, especially AJ, SNJ and DBA2J where resources for rapid localization of trait loci relative to C57BL/6 exist.

## LONG-TERM GOALS

1. Improve all of our existing animal models of OM, and continue to develop new models.

2. Use a diversity of animal models to study OM, to assure that differences between any one species and humans do not bias our data.

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# 5. MICROBIOLOGY AND IMMUNOLOGY

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## I. VIROLOGY

### A. VIRAL PATHOGENESIS

Most cases of acute otitis media (AOM) in children are preceded by a viral upper respiratory tract infection (URI). Viral URI induces development of AOM by generation of cytokines and various inflammatory mediators, which result in Eustachian tube (ET) dysfunction and increased adherence and colonization of bacteria in the nasopharynx.

Since the last report, investigators have continued to elucidate the inflammatory mechanisms that lead to development of AOM during a viral URI. Das and coworkers studied the proinflammatory effects of respiratory syncytial virus (RSV) infection by measuring the expression of various cytokines in human nasal epithelial cells after RSV infection alone or after subsequent tumor necrosis factor (TNF)-alpha stimulation.[28] RSV infection of the nasal epithelial cells resulted in significant accumulations of interleukin (IL)-6, IL-8, and RANTES when compared with findings in control samples. Significant increases in IL-8 gene expression has been demonstrated following RSV infection and TNF-alpha stimulation of the cells following well-established RSV infection induced marked increases in IL-6, IL-8, and RANTES.

RSV is one of the most important viruses in the context of AOM. However, despite its importance, the specific mechanisms by which RSV predisposes to AOM have been poorly studied, largely due to the absence of relevant animal models. Recently, Gitiban and coworkers demonstrated that murine and chinchilla hosts are susceptible to nasopharyngeal and ET infection by RSV following intranasal challenge.[38] They also observed that chinchillas are relatively permissive for RSV infection, showing signs of illness in addition to evidence of virus replication and virus-induced inflammation. Therefore, the chinchilla model of RSV infection may prove useful in further studies on the mechanisms of AOM development during RSV infection.

Influenza A virus is another key player in the development of AOM. Tong and coworkers used microarray technology to characterize the mRNA expression profile in human middle ear epithelial cells induced by influenza A virus.[122] They observed alterations of mRNA expression in 142 of approximately 12,600 genes at 24 h after influenza A virus infection. The most prominent genes with altered expression included interferon inducible genes, chemokine and cytokine genes, pro- and anti-apoptotic genes, signal transduction and transcription factors, cellular immune response, and cell cycle and metabolism genes. The results revealed several previously unknown alterations of host gene expression induced by influenza A viruses.

Peltola and coworkers determined whether the frequency and character of secondary pneumococcal infections differed depending on the strain of influenza virus that preceded bacterial challenge.[95] They first infected young ferrets with influenza viruses and then challenged them with pneumococcus. Influenza viruses of any subtype increased bacterial colonization of the nasopharynx. Nine of 10 ferrets infected with H3N2 subtype influenza A viruses developed either sinusitis or otitis media, while only 1 of 11 ferrets infected with either an H1N1 influenza A virus or an influenza B

virus did so. These data indicate that different strains of the same virus may differ in their relative ability to predispose to AOM. The study also demonstrates that the ferret animal model may be useful for further study of the mechanisms that underlie viral-bacterial synergism.

## B. EPIDEMIOLOGICAL AND CLINICAL ASPECTS

Epidemiological studies have provided ample evidence for a strong association between viral URI and AOM. Newly discovered respiratory viruses that may cause URI such as human metapneumovirus, bocavirus, and coronavirus Netherlands-63 (NL-63) have all been reported to also be associated with AOM.[60, 105, 124, 125] A study by Winther and coworkers confirmed past observations relating new episodes of OM to a concurrent viral-type illness.[127] These investigators followed 18 families with children aged 1-8 years throughout one respiratory season using parent-completed daily diaries and weekly examinations using pneumatic otoscopy for the diagnosis of OM. Overall, there were 82 new OM episodes, 40 (49%) of which were associated with URI in the individual child and 18 (22%) with a concurrent URI in a family member.

In a prospective study of two cohorts of children who were followed from 2 to 24 months of age in Finland, Nokso-Koivisto and coworkers showed that monthly rates of AOM coincided with those of viral URI.[88] These investigators examined nasopharyngeal and MEF specimens for ten respiratory viruses: adenoviruses, influenza A and B viruses, parainfluenza viruses 1, 2, and 3, RSV, enteroviruses, parechoviruses, and rhinoviruses. Picornaviruses (rhinoviruses, enteroviruses, and parechoviruses) were determined by reverse transcription PCR while antigen detection was used for the other viruses. AOM was associated with a specific virus in 54% of cases in the first cohort and in 67% in the second cohort. More than half of AOM cases were associated with picornavirus infections; rhinovirus was the most common virus detected (32-41%), followed by enterovirus (25%, sought in the second cohort only) and RSV (10%). All the other viruses were associated jointly with 8-10% of the AOM events. The relatively high rate of picornavirus detection, compared to other viruses in this study may have been, in part, from the differences in the virus detection method.

Sagai and coworkers studied hospitalized children with RSV infection.[107] Among 230 RSV-infected children, 120 (52%) had AOM; the rate was 73% among children younger than 2 years of age. RSV was detected in the MEF by antigen detection in 36 of 52 (69%) children in whom viral detection was attempted.

Some previous studies have suggested species-specific interactions between viruses and bacteria in the middle ear. Kleemola and coworkers reanalyzed their data to assess any specific viral-bacterial interactions in 529 children with AOM.[63] They did not find any support to the theory that respiratory infection caused by a given viral species would favor the growth of a certain bacterial pathogen in the MEF more than another. However, the numbers of positive viral findings for most viruses in their study were very small, which may have hampered the conclusions from that study.

The association between specific viruses and AOM bacterial pathogens in the nasopharynx has also been evaluated. Jacoby and coworkers studied Australian aboriginal children; rhinovirus infection was positively correlated with carriage of *S. pneumoniae*, *H. influenzae* and *M. catarrhalis*, and adenovirus with *M. catarrhalis*.[54] Pitkaranta and coworkers studied Finnish children who were otitis-prone; rhinovirus correlated positively with *M. catarrhalis* and *S. pneumoniae* but not with *H. influenzae*.[97] Further studies are needed to ascertain that specific viruses promote the colonization with specific bacteria, as well as the studies related to the mechanisms of interactions.

Investigations have continued on the effectiveness of influenza vaccines in prevention of OM. Hoberman and coworkers performed the study using an inactivated vaccine during two influenza seasons when influenza virus was only responsible for small proportion of respiratory illnesses in the community.[46] Vaccine efficacy in preventing influenza illness could not be shown in the second season. They reported no overall effectiveness of the vaccine for AOM. However, during the first season when the vaccine efficacy in preventing influenza illness was 66%, they were able to show efficacy of the vaccine against influenza-associated AOM. Ozgur and coworkers reported from Turkey that

influenza vaccine was effective in reducing AOM and OME episodes in children during influenza season.[92]

Live-attenuated influenza vaccine has been shown to reduce incidence of febrile OM,[11] although its side effects include nasal congestion and runny nose. In a recent study comparing relative efficacy of live-attenuated and inactivated influenza vaccines, Ashkenazi and coworkers reported superior relative efficacy of the live-attenuated vaccine; however, rhinitis, rhinorrhea and otitis media were reported as more frequent adverse effects in live-attenuated vaccine subjects.[4]

## C. VIRUSES IN THE MIDDLE EAR FLUID

Several previous studies using viral culture and viral antigen detection have detected respiratory viruses in approximately 20% of the MEF samples from children with AOM. Patel and coworkers summarized the results of several virologic studies of AOM conducted in Galveston, Texas, over a 10-year period.[94] In these studies, detection of viruses in the MEF was based mainly on viral culture; antigen detection was additionally used for RSV. Among 566 children with AOM, RSV was the most common virus identified in the MEF; it was found in 16% of all children and 38% of virus-positive children.

The increased use of PCR has substantially increased the rates of viral detection in the MEF of children with AOM. Further, the use of PCR techniques to detect "difficult-to-grow" viruses such as rhinoviruses has recently questioned the conventional concept about the relative prevalence of different viruses in the MEF. Because rhinoviruses are the most frequent viruses causing URI, it is not surprising that the use of appropriate detection methods for these viruses have disclosed their great importance in AOM. Recently, Nokso-Koivisto and coworkers used PCR for detection of rhinoviruses, enteroviruses, and parechoviruses in nasopharyngeal aspirates and MEFs from children with AOM; antigen detection was used for influenza A and B viruses, parainfluenza virus types 1, 2, and 3, RSV, and adenovirus.[88] Among a total of 1,416 AOM events, a virus was detected in the MEF in 579 (41%) cases. Rhinoviruses were found in 236 (17%), enteroviruses in 226 (16%), and parechoviruses in 3 (0.2%) cases. Overall, picornaviruses were found in 465 (33%) cases, and together they accounted for 80% of all virus-positive cases. Whether the relative high rate of picornavirus in the MEF was, in part, due to more sensitive detection method used still needs to be determined.

Bulut and coworkers studied the MEFs of 120 children with AOM using RT-PCR for several viruses.[20] They detected respiratory viruses in 39 (32.5%) of the samples, with RSV being the most commonly found virus, followed by rhinovirus, coronavirus, and influenza A virus. Monobe and coworkers used multiplex RT-PCR to detect influenza A and B viruses, parainfluenza virus types 1, 2, and 3, rhinovirus, and adenovirus in 93 MEF specimens from 79 children with AOM.[81] They found one or more respiratory viruses in 39 (42%) specimens; RSV was the most frequently identified virus. Human metapneumovirus is a newly identified virus that is also difficult to culture. Using PCR-based techniques, this virus has been found in the MEF of several children with AOM.[117, 124]

The limited volume of the MEF obtained by tympanocentesis has made it difficult for detection of a broad variety of respiratory viruses by the sensitive PCR methods. To avoid this problem, Ruohola and coworkers set up to detect a broad range of viruses and bacteria in 79 young children with new onset (< 48 h) of otorrhea through a tympanostomy tube.[105] All children except one had a concomitant viral-type respiratory infection, and in 73% of cases the otorrhea had appeared within 24 h of study entry. Bacterial detection methods included culture, multiplex PCR, and broad-range PCR. Viral detection methods included viral culture; antigen detection for RSV, adenovirus, influenza A and B viruses, and parainfluenza virus types 1-3; and PCR for rhinovirus, enterovirus, coronavirus 229E and OC-43. PCR was also done on selected samples for RSV and influenza A and B viruses, human metapneumovirus, adenovirus, parainfluenza virus types 1-4, coronavirus NL63, and human bocavirus. The investigators found bacteria and/or viruses in the MEF of 76 (96%) of the 79 children. Bacteria were found in 73 (92%) cases and viruses in 55 (70%) cases. In 52 (66%) children, both bacteria and virus were found concomitantly in the MEF. Picornaviruses were detected in 41% of the samples (rhinovirus 20%, enterovirus 10%, and a nontypeable picornavirus 11%), and they accounted

for 60% of all viral findings. RSV was found in 14% and parainfluenza virus in 6% of the samples. These findings suggest that the majority of AOM cases are combined bacterial-viral infections, which may have substantial implications for the treatment of AOM. It must be noted, however, that the relative proportions of different viruses in this study do not necessarily indicate the true relative prevalence of these viruses in AOM since the prevalence of different viruses causing both URI and AOM varies by geographic location and from year to year.

The role of viruses in acute myringitis with concurrent AOM has been explored. Kotikoski and coworkers studied 119 Finnish children with acute bullous and hemorrhagic myringitis.[66] In bullous myringitis, respiratory viruses were detected in 27% of MEF samples of 82 cases while 28% of 37 cases of hemorrhagic myringitis were virus-positive. The virus distribution was similar to AOM and no specific respiratory virus could be identified to be the etiologic agent of acute myringitis.

In OME, recent studies using molecular techniques have shown virus nucleic acids in the MEF collected from children without acute symptoms. Chantzi and coworkers used RT-PCR to detect picornaviruses, influenza A & B, adenovirus and coronavirus OC43 and 229E from the MEF of 37 children.[24] They detected rhinovirus in 15 (41%), enterovirus in 1 (3%), and no other virus.

The role of herpesviruses in OM remains controversial. New reports continue to emphasize the need to consider these viruses whose pathogenetic mechanisms for acute or chronic OM development have not been explored. Bulut and coworkers studied 92 MEF samples of Turkish children with OME by PCR detection methods;[21] EBV was found in 13%, HSV in 8%, CMV in 5% and VZV in 3%. Yano and coworkers studied 495 Japanese children with AOM by virus culture and PCR of MEF and NPS samples and by serology;[131] 4.2% of children were identified to have CMV infection; 1.6% of MEF samples were positive by PCR.

Inner ear symptoms such as vertigo and sensorineural hearing loss, and facial nerve complications of AOM have explored with respect to viral etiology. Hyden and coworkers studied 20 Swedish children with these complications;[52] 4 children had evidence of virus infection by serology-VZV in 2, HSV in 1 and adenovirus in 1. Eight of the children had bacteria in the MEF.

### D. PROLONGED PRESENCE OF VIRAL NUCLEIC ACIDS IN THE RESPIRATORY TRACT AND MIDDLE EAR

Because of the close link between URI and AOM, virus detected in respiratory samples of children with AOM is generally considered the AOM associated virus. Virus detected in the MEF is considered OM the causative virus. Recently, using PCR assay, investigators have found virus RNA/ DNA in respiratory specimens of children who had no symptom of URI or AOM;[87, 126] some virus nucleic acids may have prolonged presence in the respiratory tract.[128] Therefore, when PCR detects virus DNA or RNA from the respiratory tract or in the MEF, the virus may be the cause of acute infection as well as recent infection. Further studies are required for better understanding of clinical significance of prolonged presence of respiratory virus DNA/ RNA in URI and OM.

## II. BACTERIOLOGY

Significant progress has been made in understanding the pathogenesis of disease caused by nontypeable *Haemophilus influenzae* since the 2003 Symposium.[10] Each of the studies cited below has significantly enhanced our understanding of the disease process associated with *H. influenzae* infection. As a whole, the studies demonstrate the power of functional genomics to address questions that would have been very difficult to answer just a few years ago.

### A. PATHOGENESIS OF *HAEMOPHILUS INFLUENZAE* DISEASE

In an effort to identify potential virulence determinants expressed by NTHI during progression of disease, Mason and colleagues constructed a GFP-promoter trap system to monitor bacterial gene expression using a differential fluorescence induction enrichment strategy.[74] After screening 16,000 clones in a chinchilla model of experimental

OM, this group isolated 44 that contained unique gene fragments and encoded proteins of known or as-yet unknown function. Twenty-six of these clones were confirmed by quantitative RT-PCR to have increased gene expression *in vivo* over that demonstrated *in vitro*. These data provided insight into the response of NTHI as it senses and responds to microenvironmental cues present in the middle ear during OM and identified the *sap* (sensitivity to antimicrobial peptides) operon of NTHI as a genetic region of interest.

The products of the *sap* operon were subsequently shown to be important to the ability of NTHI to resist effectors of innate immunity.[73] Moreover, *sapA* gene expression was shown to be upregulated by NTHI in the middle ear of the chinchilla host and a non-polar *sapA* mutant was both more sensitive to killing by antimicrobial peptides (APs) than was its parental isolate, as well as significantly attenuated in ability to either colonize the NP or cause OM. SapA is believed to function as the periplasmic solute binding protein of an ABC transporter in NTHI and was later shown to directly bind a recombinant chinchilla AP called beta-defensin-1.[72] Further, exposure of NTHI to AP *in vitro* resulted in increased expression of the *sap* operon by NTHI and the putative Sap transporter ATPase protein, SapD, was shown to be required for AP resistance as well as for potassium uptake by NTHI. Collectively, these data suggested that Sap system-mediated resistance to APs depends on both Sap-dependent transport of APs and restoration of potassium homeostasis. NTHI thus requires a functional Sap system in order to both mediate survival as well as pathogenesis *in vivo*.

During the course of colonization and infection, NTHI must be able to withstand oxidative stress caused by any of several reactive oxygen species produced by both other co-pathogens as well as its human host. Harrison et al. used an NTHI-specific DNA microarray that contained the 1821 ORFs of strain 86-028NP to identify 40 genes that are up-regulated during oxidative stress due to exposure of NTHI to hydrogen peroxide.[44] Using an isogenic *oxyR* mutant, this group also identified a subset of 11 genes that were transcriptionally regulated by OxyR, a global regulator of oxidative stress. Interestingly, $H_2O_2$ exposure also induced OxyR-independent upregulation of expression of genes that encoded components of multiple iron utilization systems, suggesting that NTHI must carefully balance levels of intracellular iron in order to minimize the damaging effects of oxidative stress during colonization and disease. Further, these data suggested that regulatory pathways in addition to those governed by OxyR are involved in iron utilization by NTHI.

NTHI is known to express multiple adhesins that have been described and characterized in detail by several laboratories. However, since the last symposium, it has been shown that NTHI also expresses a functional type IV pilus that allows it to demonstrate 'twitching' motility.[7] The expression of a type IV pilus, or Tfp, by NTHI was dependent on expression of *pilA* and *comE*, as mutants expressed either no Tfp or expressed unusual, large, bulbous membrane-bound structures that contained fibrous material, respectively. The deduced amino acid sequence of the single *pilA* gene from 13 low passaged clinical NTHI isolates demonstrated extensive conservancy, suggesting that if this structure is expressed *in vivo*, the Tfp pilin protein could serve as a potential new target for vaccine development. This notion is particularly intriguing given the fact that Tfp expressed by other human pathogens are typically involved in multiple functions including the uptake of foreign DNA (or competence), adherence, biofilm formation and pathogenesis. Further defining the biological function(s) of NTHI Tfp should help to enhance our understanding of NTHI pathogenesis.

NTHI can bind to a variety of receptors on the host cell membrane due to its expression of multiple adhesins. In 2006, Avadhanula and colleagues demonstrated that in addition to its known ability to adhere to CEACAM1, CEACAM 3, PAF receptor, fibronectin, laminin and respiratory tract mucins, NTHI could also adhere to intercellular adhesion molecule 1 (ICAM-1).[6] Adherence to ICAM-1 was mediated by the OMP P5-homologous adhesin and incubation of NTHI with respiratory epithelial cells increased ICAM-1 expression fourfold. These data suggested that adherence of NTHI to respiratory epithelium upregulates expression of one of its own cognate receptors. The authors concluded that blocking interactions between NTHI P5 and ICAM-1 might be an effective strategy for reduction or prevention of NTHI colonization and/or disease. In a follow-up study, this same group showed that several respiratory tract viruses (RSV, HPIV3 and

IAV) augmented the adherence of nontypeable *H. influenzae* and *S. pneumoniae* by increasing the expression of known receptors for these bacteria in a viral species- and cell type-dependent manner.[5]

## B. PATHOGENESIS OF *STREPTOCOCCUS PNEUMONIAE* DISEASE

Significant progress has been made in understanding the pathogenesis of disease caused by *Streptococcus pneumoniae* since the 2003 Symposium.[10] Studies have ranged from genome-based analyses to sophisticated molecular studies of individual genes and proteins and their interactions with host cells. Each of these studies cited has increased our understanding of this major human pathogen both in the setting of otitis media and with other diseases caused by this organism.

*Formation of Biofilms by Streptococcus Pneumoniae.* Allegrucci and coworkers used a continuous-culture biofilm system to characterize biofilm development of 14 different *S. pneumoniae* strains representing at least 10 unique serotypes.[1] Biofilm development was found to occur in three distinct stages: initial attachment, cluster formation, and biofilm maturation. While all 14 pneumococcal strains displayed similar developmental stages, the mature biofilm architecture differed significantly among the serotypes tested. Overall, three biofilm architectural groups were detected based on biomass, biofilm thickness, and cluster size. The biofilm viable cell counts and total protein concentration increased steadily over the course of biofilm development, reaching approximately 8 x $10^8$ cells and approximately 15 mg of protein per biofilm after 9 days of biofilm growth. Proteomic analysis confirmed the presence of distinct biofilm developmental stages by the detection of multiple phenotypes over the course of biofilm development. Protein identification revealed that proteins involved in virulence, adhesion, and resistance were more abundant under biofilm growth conditions.

In a subsequent study, this same group characterized colony morphology variants from *Streptococcus pneumoniae* serotype 3 biofilms.[2] The colony variants differed in colony size and their mucoid appearance on blood agar. A small nonmucoid variant (SCV) emerged during the initial attachment stage of *S. pneumoniae* biofilm formation and dominated over the course of biofilm growth. Mucoid variants appeared at later biofilm developmental stages. The reduction in colony size/mucoidy correlated with a decrease in capsule production and an increase in initial attachment. The large mucoid variant formed flat unstructured biofilms, failed to aggregate in liquid culture, and adhered poorly to solid surfaces. In contrast, SCVs autoaggregated in liquid culture, hyperadhered to solid surfaces, and formed biofilms with significant three-dimensional structure, mainly in the form of microcolonies. The variants showed similar antibiotic resistance/susceptibility based on a modified Kirby-Bauer test and when grown as biofilms. However, antimicrobial treatment of *S. pneumoniae* biofilms altered the colony variant's distribution and mainly affected the most interior areas of biofilm microcolonies. The findings suggest that *in vitro* biofilm formation of *S. pneumoniae* serotype 3 coincides with the emergence of colony variants with distinct genotypic and phenotypic characteristics.

Moscoso and coworkers also reported on recent studies in which they identified the initial steps of biofilm formation by pneumococcus during growth on abiotic surfaces such as polystyrene or glass.[82] Unencapsulated pneumococci adhered to abiotic surfaces and formed a three-dimensional structure about 25 microns deep, as observed by confocal laser scanning microscopy and low-temperature scanning electron microscopy. Choline residues of cell wall teichoic acids were found to play a fundamental role in pneumococcal biofilm development. The role in biofilm formation of choline-binding proteins, which anchor to the teichoic acids of the cell envelope, was determined using unambiguously characterized mutants. The results showed that LytA amidase, LytC lysozyme, LytB glucosaminidase, CbpA adhesin, PcpA putative adhesin, and PspA (pneumococcal surface protein A) mutants had a decreased capacity to form biofilms, whereas no such reduction was observed in phosphocholinesterase (Pce) or CbpD putative amidase mutants. Moreover, encapsulated, clinical pneumococcal isolates were impaired in their capacity to form biofilms. In addition, a role for extracellular DNA and proteins in the establishment of *S. pneumoniae* biofilms was demonstrated.

*Pneumococcal Virulence Factors.* In addition to the capsular polysaccharide and adhesion molecules of *Streptococcus pneumoniae*, a number of other molecules have been identified as having critical roles in virulence. Among them are the proteins known as pneumococcal surface protein A (PspA), hyaluronidase (Hyl), pneumolysin (Ply), autolysin (LytA), pneumococcal surface antigen A (SpsA), choline binding protein A (CbpA), and neuraminidase (NanA). Phase variation in the colonial opacity phenotype of *Streptococcus pneumoniae* has also been implicated as a significant factor in bacterial adherence, colonization, and invasion in the pathogenesis of pneumococcal otitis media. Since the last Symposium, work has continued in further defining the role of several of these factors in virulence and also in defining the role of other previously uncharacterized molecules. A few of these studies are highlighted below.

The 1999 and 2003 Conference Reports highlighted studies demonstrating the importance of the colonial morphology, either transparent or opaque, in mediating the adherence properties and virulence characteristics of *Streptococcus pneumoniae*.[10] However, the molecular mechanisms responsible for the observed differences remain incompletely characterized. Recent work has begun to address this issue. One recent study examined the contribution of *S. pneumoniae* opacity variants to the induction of proinflammatory mediators in vivo using the rat otitis model.[70] Both the opaque and transparent phenotype variants induced a significant up-regulation in gene expression for interleukin-1alpha (IL-1α), IL-1β, IL-6, IL-10, tumor necrosis factor alpha, and inducible nitric oxide synthase (iNOS) compared to saline sham-inoculated controls. At 24 h post-challenge, opaque-variant challenged animals demonstrated a significant increase in gene expression for IL-1α IL-1β, IL-6, IL-10, and iNOS relative to animals inoculated with the transparent variant. These results suggested that the opaque variant may be more efficient at survival and multiplication within the middle ear space, resulting in the accumulation of more inflammatory cells and the enhanced expression and production of inflammatory mediators. However, when the data were normalized to account for differences in middle ear bacterial titers, the transparent variant was the more potent inducer of inflammation, triggering the accumulation of more inflammatory cells and substantially greater fold increases in the expression and production of inflammatory mediators.

In another study King and coworkers used microarray analysis to define differences between isogenic transparent and opaque strains.[61] Twenty four open reading frames were identified that demonstrated increased expression in transparent variants, including 11 predicted to be involved in sugar metabolism. A single genomic region contained seven of these loci including the gene that encodes the neuraminidase, NanA. In contrast to earlier studies, the investigators found no contribution of NanA to adherence of *S. pneumoniae* to epithelial cells or to colonization in an animal model. However, they did observe NanA-dependent desialylation of human airway components that bind to the organism and may mediate bacterial clearance. Targets of desialylation included human lactoferrin, secretory component, and IgA2 that were shown to be present on the surface of the pneumococcus in vivo during pneumococcal pneumonia. The efficiency of desialylation was increased in the transparent variants and enhanced for host proteins binding to the surface of *S. pneumoniae*. Because deglycosylation affects the function of many host proteins, the authors speculated that NanA may contribute to a protease-independent mechanism to modify bound targets and facilitate enhanced survival of the bacterium.

In a subsequent study, this same group reported on their investigations of other glycosidases expressed by *Streptococcus pneumoniae*.[62] The organism produces three surface exoglycosidases: the neuraminidase, NanA, a beta-galactosidase, BgaA, and a beta-N-acetylglucosaminidase, StrH. As noted above, the proposed functions of NanA include revealing receptors for adherence, affecting the function of glycosylated host clearance molecules, modifying the surface of other bacteria coinhabiting the same niche, and providing a nutrient source. However, it remains unclear whether the desialylation activity of *S. pneumoniae* can further deglycosylate human targets through the activity of BgaA or StrH. In this study, the investigators demonstrated that NanA, BgaA and StrH act sequentially to remove sialic acid, galactose and N-acetylglucosamine and expose mannose on human

glycoproteins that bind to the pneumococcus and protect the airway. Both BgaA and NanA contributed to the adherence of unencapsulated pneumococci to human epithelial cells. These studies suggest that BgaA and StrH also contribute to pneumococcal colonization and/or pathogenesis.

A variety of other recently or newly identified molecules continue to be the subject of continued investigation for their roles in pathogenesis. In another recent study, Dawid and coworkers investigated the contribution of the blp locus, encoding putative bacteriocins and cognate immunity peptides, to intraspecies competition.[29] These investigators sequenced the relevant regions of the blp locus of a type 6A strain able to inhibit the growth of the type 4 strain, TIGR4, in vitro. Using deletional analysis, they confirmed that inhibitory activity is regulated by the function of the response regulator, BlpR, and requires the two putative bacteriocin genes blpM and blpN. Comparison of the TIGR4 BlpM and -N amino acid sequences demonstrated that only five amino acid differences were sufficient to target the heterologous strain. Analysis of a number of clinical isolates suggested that the BlpMN bacteriocins divide into two families. A mutant in the blpMN operon created in the clinically relevant type 19A background was deficient in both bacteriocin activity and immunity. This strain was unable to compete with both its parent strain and a serotype 4 isolate during cocolonization in the mouse nasopharynx, suggesting that the locus is functional in vivo and confirming its role in promoting intraspecies competition.

Successful colonization of the upper respiratory tract by *Streptococcus pneumoniae* is an essential first step in the pathogenesis of pneumococcal disease. However, the bacterial and host factors that provoke the progression from asymptomatic colonization to invasive disease are yet to be fully defined. In another recent study, Ogunniyi and coworkers investigated the effects of single and combined mutations in genes encoding pneumolysin (Ply), pneumococcal surface protein A (PspA), and pneumococcal surface protein C (PspC, also known as choline-binding protein A) on the pathogenicity of *Streptococcus pneumoniae* serotype 2 (D39) in mice.[91] Following intranasal challenge with D39, stable colonization of the nasopharynx was maintained over a 7-day period at a level of approximately $10^5$ bacteria per mouse. The abilities of the mutant deficient in PspA to colonize the nasopharynx and to cause lung infection and bacteremia were significantly reduced. Likewise, the PspC mutant and, to a lesser extent, the Ply mutant also had reduced abilities to colonize the nasopharynx. As expected, the double mutants colonized less well than the parent to various degrees and had difficulty translocating to the lungs and blood. A significant additive attenuation was observed for the double and triple mutants in pneumonia and systemic disease models. Surprisingly, the colonization profile of the derivative lacking all three proteins was similar to that of the wild type, indicating virulence gene compensation.

Pneumococcal adherence and virulence factor A (PavA) is displayed to the cell outer surface of *Streptococcus pneumoniae* and mediates pneumococcal binding to immobilized fibronectin. PavA, which lacks a typical gram-positive signal sequence and cell surface anchorage motif, is essential for pneumococcal virulence in a mouse infection model of septicemia. In recent report, Pracht and coworkers examined the impact of PavA on pneumococcal adhesion to and invasion of eukaryotic cells and on experimental pneumococcal meningitis.[98] In the experimental mouse meningitis model, the virulence of the pavA knockout mutant of *S. pneumoniae* D39, which did not show alterations of subcellular structures as indicated by electron microscopic studies, was strongly decreased. Pneumococcal strains deficient in PavA showed substantially reduced adherence to and internalization of epithelial cell lines A549 and HEp-2. Similar results were obtained with human brain-derived microvascular endothelial cells and human umbilical vein-derived endothelial cells. Attachment and internalization of pneumococci were not significantly affected by preincubation or cocultivations of pneumococci with anti-PavA antisera. Pneumococcal adherence was also not significantly affected by the addition of PavA protein. Complementation of the pavA knockout strain with exogenously added PavA polypeptide did not restore adherence of the mutant. These data suggest that PavA affects pneumococcal colonization by modulating expression or function of important virulence determinants of *S. pneumoniae*.

Lactoferrin is an important component of innate immunity through its sequestration of iron, bactericidal activity, and immune modulatory activity. Apolactoferrin (ALF) is the iron-depleted form of lactoferrin and is bactericidal against pneumococci and several other species of bacteria. In previous work, it had been observed that lactoferricin (LFN), an 11-amino-acid peptide from the N terminus of lactoferrin, is bactericidal for *Streptococcus pneumoniae*. Strains of *S. pneumoniae* varied in their susceptibility to ALF. Lactoferrin is bound to the pneumococcal surface by pneumococcal surface protein A (PspA). Using mutant PspA(-) pneumococci of four different strains, one recent report noted that PspA offers significant protection against killing by ALF.[111] Knockout mutations in genes for two other choline-binding proteins (PspC and PcpA) did not affect killing by ALF. PspA did not have to be attached to the bacterial surface to inhibit killing, because the soluble recombinant N-terminal half of PspA could prevent killing by both ALF and LFN. An 11-amino-acid fragment of PspA was also able to reduce the killing by LFN. Antibody to PspA enhanced killing by lactoferrin. These findings suggest that the binding of ALF to PspA probably blocks the active site(s) of ALF that is responsible for killing.

*Genomic Studies of Streptococcus Pneumoniae.* The information provided by the genomic sequences of bacterial pathogens opens the field of pathogenesis research to a number of very powerful research techniques. These methods are only beginning to be applied to *Streptococcus pneumoniae*, but even in the relatively short period since the last Research Conference, important new genomics-based research has been published. Several of these studies are highlighted below.

Pettigrew and coworkers utilized a molecular epidemiological approach involving genomic subtraction of the *S. pneumoniae* serogroup 19 middle ear strain 5093 against the laboratory strain R6.[96] Resulting subtraction PCR (sPCR) products were used to screen a panel of 93 middle ear, 90 blood, 35 carriage, and 58 cerebrospinal fluid isolates from young children to identify genes found more frequently among middle ear isolates. One probe, designated P41, similar to a hypothetical protein of *Brucella melitensis*, occurred among 41% of middle ear isolates and was found 2.8 and 1.8 times more frequently among middle ear strains than carriage, blood, or meningitis strains, respectively. sPCR fragment H10, similar to an unknown *Streptococcus agalactiae* protein, was present in 31% of middle ear isolates and occurred 3.6, 2.8, and 2.6 times more often among middle ear isolates than carriage, blood, or meningitis strains, respectively. Further studies will be needed to define the precise role of these genes in otitis media pathogenesis.

In another recent study, Obert and coworkers determined the genetic composition of 42 invasive and 30 noninvasive clinical isolates of serotypes 6A, 6B, and 14 by comparative genomic hybridization.[89] Comparison of the present/absent gene matrix identified a candidate core genome consisting of 1,553 genes (73% of the TIGR4 genome), 154 genes whose presence correlated with the ability to cause invasive pneumococcal disease, and 176 genes whose presence correlated with the noninvasive phenotype. Genes identified in this study were cross-referenced with the published signature-tagged mutagenesis studies which served to identify core and invasive disease-correlated genes required for in vivo passage. Among these, two pathogenicity islands, region of diversity 8a (RD8a), which encodes a neuraminidase and V-type sodium synthase, and RD10, which encodes PsrP, a protein homologous to the platelet adhesin GspB in *Streptococcus gordonii*, were identified. Mice infected with a PsrP mutant were delayed in the development of bacteremia and demonstrated reduced mortality versus wild-type-infected controls. Finally, the presence of seven regions of diversity correlated with the noninvasive phenotype, a finding that suggests some of these regions may contribute to asymptomatic colonization.

Shen and coworkers recently reported the results of their comparative genomic studies.[112] Eight low-passage-number *Streptococcus pneumoniae* clinical isolates, each of a different serotype and a different multilocus sequence type, were examined. Comparative genomic analyses were performed with these strains and two *S. pneumoniae* reference strains. Individual genomic libraries were constructed for each clinical isolate with an average insert size of approximately 1 kb. A total of 73,728 clones were picked for arraying, providing more than four times genomic coverage per strain. A subset of

4,793 clones were sequenced, for which homology searches revealed that 750 (15.6%) of the sequences were unique with respect to the TIGR4 reference genome and 263 (5.5%) clones were unrelated to any available streptococcal sequence. Hypothetical translations of the open reading frames identified within these novel sequences showed homologies to a variety of proteins, including bacterial virulence factors not previously identified in *S. pneumoniae*. The distribution and expression patterns of 58 of these novel sequences among the eight clinical isolates were analyzed by PCR- and reverse transcriptase PCR-based analyses. The unique sequences were nonuniformly distributed among the eight isolates, and transcription of the genes in planktonic cultures was detected in 81% (172/212) of their genic occurrences. All 58 novel sequences were transcribed in one or more of the clinical strains, suggesting that they all correspond to functional genes. Sixty-five percent (38/58) of these sequences were found in 50% or less of the clinical strains, indicating a significant degree of genomic plasticity among natural isolates

*Host Immunity to Streptococcus Pneumoniae.* Antibodies to capsular polysaccharide (PS) are protective against systemic infection by *Streptococcus pneumoniae*, but the large number of pneumococcal serogroups and the age-related immunogenicity of pure PS limit the utility of PS-based vaccines. In contrast, cell wall-associated proteins from different capsular serotypes can be cross-reactive and immunogenic in all age groups. Since the last Symposium, a number of groups have reported on studies characterizing the role of a number of pneumococcal surface proteins in host immunity and evaluating their potential as vaccine antigens.

Gor and coworkers evaluated three pneumococcal proteins with respect to relative accessibility to antibody, in the context of intact pneumococci, and their ability to elicit protection against systemic infection by encapsulated *S. pneumoniae*.[39] Sequences encoding pneumococcal surface adhesin A (PsaA), putative protease maturation protein A (PpmA), and the N-terminal region of pneumococcal surface protein A (PspA) from *S. pneumoniae* strain A66.1 were cloned and expressed in *Escherichia coli*. Using flow cytometry, the investigators demonstrated that PspA was readily detectable on the surface of the pneumococcal strains analyzed, whereas PsaA and PpmA were not. Consistent with these observations, mice with passively or actively acquired antibodies to PspA or type 3 PS were equivalently protected from homologous systemic challenge with type 3 pneumococci, whereas mice with passively or actively acquired antibodies to PsaA or PpmA were not effectively protected. These experiments support the hypothesis that the extent of protection against systemic pneumococcal infection is influenced by target antigen accessibility to circulating host antibodies

Green and coworkers describe studies in which they identified a 20-kDa protein that has significant homology to a nonheme iron-containing ferritin protein from *Listeria innocua* and other bactoferritins as pneumococcal protective protein A (PppA).[41] These investigators expressed and purified recombinant PppA (rPppA) and evaluated its potential as a vaccine candidate. The antibodies elicited by purified rPppA were cross-reactive with PppA from multiple strains of *S. pneumoniae* and were directed against surface-exposed epitopes. Intranasal immunization of BALB/c mice with PppA protein and either a synthetic monophosphoryl lipid A analog, RC529AF, or a cholera toxin mutant, CT-E29H, used as an adjuvant reduced nasopharyngeal colonization in mice following intranasal challenge with a heterologous pneumococcal strain. PppA-specific systemic and local immunoglobulin G (IgG) and IgA antibody responses were induced. The antisera reacted with whole cells of a heterologous *S. pneumoniae* type 3 strain. These observations indicate that PppA may be a promising candidate for inclusion in a vaccine against pneumococcal otitis media.

As noted above, *Streptococcus pneumoniae* neuraminidase has been implicated as a virulence factor in the pathogenesis of pneumococcal otitis media. In recent work, Long and coworkers assessed the potential of neuraminidase as a vaccine candidate in an animal model of infection.[69] Native neuraminidase was partially purified from cultures of *S. pneumoniae* by serial chromatography with DEAE-Sepharose and Sephacryl S-200. Recombinant neuraminidase, a 3,038-bp fragment of the neuraminidase A (nanA) gene, was cloned into the pET-28b vector and then expressed at high levels in *Escherichia coli*. Chinchillas were immunized

subcutaneously with either the gel-purified native or recombinant neuraminidase, and all responded with elevated titers of antineuraminidase antibody in serum. Immunization with neuraminidase resulted in a significant reduction in nasopharyngeal colonization as well as in the incidence of otitis media with effusion.

Another group recently reported on the protective efficacy of immunization of mice with PdB (a pneumolysin toxoid), PspA, PspC (CbpA), PhtB, and PhtE in an invasive-disease model.[90] The antigens were administered in alum adjuvant, either alone or in various combinations. Protection against intraperitoneal challenge with virulent type 2 and 6A strains was assessed in two murine strains. The investigators found that in some situations, different individual proteins gave the best (and worst) protection. However, in many cases, a synergistic/additive effect was seen by using multiple proteins even where the individual proteins showed little value by themselves. For instance, the median survival times for mice immunized with combinations of PdB and PspA, PdB and PspC, or PspA and PspC were significantly longer than those for mice immunized with any of the single antigens. The combination of PdB, PspA, and PspC appeared to offer the best protection

Shah and coworkers recently reported on studies of the vaccine potential of one of the polyamine transporter proteins.[110] *Streptococcus pneumoniae* contains genes for a putative polyamine ABC transporter which are organized in an operon and designated potABCD. Polyamine transport protein D (PotD) is an extracellular protein which binds polyamines and possibly other structurally related molecules. PotD has been shown to contribute to virulence in both a murine sepsis model and a pneumonia model with capsular type 3 pneumococci. The protective efficacy of recombinant PotD was evaluated by active immunization and intravenous challenge with capsular type 3 pneumococci in CBA/N mice. Immunized mice had 91.7% survival following lethal pneumococcal challenge, compared with 100% mortality in the control group. Immunized animals had high-titer anti-PotD antibodies following three immunizations with alum. Protection in a sepsis model was also seen after passive administration of rabbit antiserum raised against PotD ($P < 0.004$). These results suggest that antibodies to PotD confer protection against invasive disease and that the protein should be studied further as a potential vaccine candidate for protection against invasive pneumococcal infections.

Finally, Roche and coworkers recently described studies in which live attenuated vaccines were assessed for their ability to provide protection in an animal model of infection.[101] These investigators examined the safety and protection induced by live attenuated strains of *S. pneumoniae* containing combinations of deletions in genes encoding three of its major virulence determinants: capsular polysaccharide (*cps*), pneumolysin (*ply*), and pneumococcal surface protein A (*pspA*). Both the *cps* and *ply/pspA* mutants of a virulent type 6A isolate were significantly attenuated in a mouse model of sepsis. These attenuated strains retained the ability to colonize the upper respiratory tract. A single intranasal administration of live attenuated vaccine without adjuvant was sufficient to induce both systemic and mucosal protection from challenge with a high dose of the parent strain. Immunization with *cps* mutants demonstrated cross-protective immunity following challenge with a distantly related isolate. Serum and mucosal antibody titers were significantly increased in mice immunized with the vaccine strains, and this antibody is required for full protection, as muMT mice, which do not make functional, specific antibody, were not protected by immunization with vaccine strains.

## C. MICROBIAL ECOLOGY

*Bacterial Interactions in the Nasopharynx.* There are at least four mechanisms responsible for maintaining and controlling the nasopharyngeal flora: 1) bacterial-nasopharyngeal mucin interactions; 2) bacterial interference, a situation in which a commensal organism may inhibit the colonization and replication of pathogenic bacteria; 3) mucosal secretory immunity (IgA); and 4) receptor analog competition (for example, cranberry juice interfering with the attachment of bacteria to the mucosal surface by binding mannose on the bacterial surface and preventing *E.coli* from binding to bladder mucosa). When the normal nasopharyngeal microflora is disturbed by external factors, it may be altered and, as a result, infections

of the upper respiratory tract such as otitis media and sinusitis may develop. Among those factors that may impact the microflora are: 1) viral infection, 2) allergy, 3) overuse of antibiotics, 4) lack of specific secretory IgA, 5) inherited traits that affect the synthesis of specific secretory IgA and IgG subclasses, and 6) the use of probiotics that compete with pathogenic microflora of the nasopharynx.

The mucociliary system of the upper and lower respiratory tract is a critical, non-specific pathway for the elimination of bacteria and other particulate matter from these anatomic sites. The interactions between resident bacteria and the mucins of the upper and lower respiratory tracts have been a major focus of research work by a few dedicated laboratories. One report by Bernstein and Reddy suggests that bacterial-mucin interaction displays a wide array of structures.[12] The mosaics of carbohydrate chains contained in airway mucins can be viewed as having an important role in trapping the inhaled microorganisms before they reach the surface of the airway epithelial cells and therefore have an important role in the defense of the respiratory mucosa.[67] Bernstein and coworkers have reported that nontypeable *Haemophilus influenzae* and *Moraxella catarrhalis* adhere to human purified nasopharyngeal mucin and human middle ear mucin via a limited number of specific outer membrane proteins.

There have been no published studies on the interaction of *Streptococcus pneumoniae* and purified mucin. Such information would be of potential value for devising strategies to prevent colonization of this pathogen to nasopharyngeal mucin in vivo. In a recent study, Bernstein and collaborators used an overlay assay with purified radiolabeled mucins of the upper and lower respiratory tracts and the four major pathogens of the upper and lower respiratory tracts.[14] The investigators found that *Moraxella catarrhalis* bound to purified nasopharyngeal mucin with a single outer membrane protein of approximately 57 kDa. The binding of the mucins by *Streptococcus pneumoniae* proteins appeared to involve two low molecular weight proteins of 17.5 and 20 kDa. Interestingly, *S. pneumoniae* did not bind to human tracheal bronchial mucin. Nontypeable *Haemophilus influenzae* bound to purified human nasopharyngeal mucin via outer membrane proteins P2 and P5. Confirmation that P2 and P5 were the principal mediators of the *Haemophilus influenzae* interactions came from experiments in which the parent strains and their respective isogenic mutants that lacked the outer membrane proteins were compared. Binding of microorganisms to mucins is a prerequisite for the physiological clearance of inhaled bacteria. In addition, this interaction might also be the first step in microbial colonization if mucociliary activity in the nasopharynx is not functioning properly.

The American Academy of Microbiology in 2005 defined a "probiotic" as "live microorganisms, which when administered in adequate amounts confer a health benefit on the host." In theory, benign microorganisms can be used to combat pathogenic microorganisms and the disease they cause. Thus, they could also conceivably be used to prevent infectious diseases and immune dysfunction. This concept has been known for over 100 years and has been used to alter the gut microbiota. Furthermore, probiotic organisms may have the potential to interact with both the innate and acquired immune systems with possible benefits to the host. A commensal bacteria can be instilled into the nasopharynx and inhibit the colonization and replication of potential pathogens. The role of viridans streptococci (*Streptococcus oralis*) in the prevention of colonization with nontypeable *Haemophilus influenzae* and *Moraxella catarrhalis* was recently investigated in an adenoidal organ culture system.[12]

The adenoids from 100 patients who were undergoing adenoidectomy for either hypertrophy or recurrent otitis media were examined. *Streptococcus oralis* uniformly inhibited colonization with nontypeable *Haemophilus influenzae* or *Moraxella catarrhalis* over a 24-hour period of incubation in adenoidal organ culture. More recently, the same group demonstrated bacterial interference of both penicillin-sensitive and penicillin-resistant *Streptococcus pneumoniae* by *S. oralis* in an adenoid organ culture. The results indicated that some strains of *S. oralis* inhibit the growth of the most serious potential pathogens in the nasopharynx.[13]

*In vivo* studies with intranasal instillation of viridans streptococci in otitis-prone children have been described in two investigations from Sweden. A group from Gotenburg studied the ability of

colonization of the nasopharynx with α-streptococci to inhibit the growth of otopathogens in children with recurrent acute otitis media.[102] At three months, 42% of the children given the streptococcal spray were healthy and had normal tympanic membranes compared to 22% of those given placebo. The authors concluded that selected bacteria such as α-streptococci have the ability to inhibit the growth of common middle ear pathogens and may be used to protect against recurrent acute otitis media and secretory otitis media in children. In contrast to this work, similar studies at Umea University in Lulea showed no significant changes in the nasopharyngeal flora when using a nasal spray of α-streptococci.[119] Thus, these two groups came to different conclusions concerning the efficacy of nasal spray using interfering bacteria.

Nasopharyngeal colonization by potential pathogens is also inhibited by specific secretory IgA. Kodama and coworkers studied the cellular immune responses to nontypeable *Haemophilus influenzae* in adenoids recovered from young in children. Specifically, they measured the lymphocyte blast transformation and antibody secretion in response to the P6 outer membrane protein. In the lymphocyte transformation assay, adenoidal lymphocytes recovered from children with an otitis history had a significantly lower reponse to P6 than did cells from non-otitis prone children. Furthermore, the numbers of IgM- and IgA-secreting cells were significantly lower in otitis children than in those of non-otitis prone children. These data suggest that P6 protein is a target for the adenoid cellular immune response and failure to recognize P6 may be one of the causes of recurrent otitis media.[64]

Finally, sugars such as sialic acid, which is usually the terminal sugar of purified mucins of both middle ear and nasopharyngeal origin could theoretically be used for blocking the bacterial adhesins of potential pathogens in the nasopharynx. This is a common method used to interfere with *E. coli* colonization of bladder such as when cranberry juice recommended for those with recurrent urinary tract infections. It would be interesting to speculate on the role of sialic acid, the major terminal sugar in purified middle ear and nasopharyngeal mucin in inhibiting adherence to mucus in the nasopharynx of otitis-prone children.

*Antimicrobial Resistance and Otitis Media Pathogens.* Bacteria are found in 50%–90% of cases of acute otitis media with or without otorrhea.[15] *Streptococcus pneumoniae* and *Haemophilus influenzae* are the leading pathogens responsible for acute otitis media (AOM) and frequently colonize the nasopharynx.[15, 47] These two pathogens had been almost uniformly susceptible to β-lactam antibiotics and AOM caused by them had been easily treated with oral antimicrobial therapy. However, antibiotic resistant bacteria, especially penicillin-resistant *Streptococcus pneumoniae* (PRSP), have become one of the major causes of persistent otitis media.[47] Antimicrobial resistance in *H. influenzae* has also changed significantly during the last 20 years where, until relatively recently ampicillin, was considered the drug of first choice for treatment *H. influenzae* [37] otitis media.

Two well-known mechanisms of resistance to ß-lactams in *H. influenzae* have been reported. One is the production of either TEM-1 or ROB-1 type ß-lactamase.[100, 123] The other mechanism mediates the resistance in so-called ß-lactamase negative ampicillin resistant (BLNAR) strains. This latter resistance is mediated by a decreased affinity of the bacterial penicillin binding proteins (PBPs) for the ß-lactams and is caused by conformational changes secondary to genetic mutations.[77-79] Recent studies have revealed that increasing resistance to ß-lactams in BLNAR strains is correlated with mutations in the *ftsI* gene encoding PBP3, a protein which mediates septum peptidoglycan formation.[26, 93] The substitutions in the *ftsI* gene have been classified into three groups: group I, His is substituted for Arg-517 (Arg517His) near the KTG motif; group II, Lys is substituted for Asn-526 (Asn526Lys) near the KTG motif; and group III, three residues (Met-377, Ser-385, and Leu-389) near the SSN motif are replaced by Ile, Thr, and/or Phe (Met377Ile, Ser385Thr,and/or Leu389Phe, respectively), in addition to the replacement of Asn526Lys. Isolates with intermediate ampicillin resistance are commonly found in groups I and II, and isolates in group III are associated with a higher level of ampicillin resistance.

Resistances of *S. pneumoniae* to ß-lactams results from stepwise alterations in the high molecular weight penicillin binding proteins (PBPs) and the reduction of their binding affinity for ß-lactam antibiotics. Among the several PBPs, PBPs 1A, 2X,

and 2B have transpeptidase activity and contain the conserved amino acid motif of SXXK, SXN, and KT(S) G in an active serine residue. *S. pneumoniae* acquires exogenous low affinity genes and genetic mutations that alter PBP affinity for ß-lactams.[9, 59, 71, 83]

*Anti-microbial Resistance in Streptococcus Pneumoniae. S. pneumoniae* is the most important pathogens for AOM and 20-35% AOM cause by *S. pneumoniae*. The high incidence of penicillin resistant *S. pneumonia*e (PRSP) strains has recently become a global issue. Resistant strains are appearing in all parts of the world. Recently reported country-specific rates of PRSP are as follows: 54.8% in Korea, 43.2% in Hong Kong, 38.6% in Taiwan, 71.4% in Vietnam, 29.3% in Japan, 12% in the United States, and 2% in Germany.[47, 114] Almost 75.8% of characterized strains from Japan had mutations in penicillin binding proteins 1a, 2b and 2x (PBP) and were classified into seven genotypic classes based upon PCR studies. Variants of several PBPs, including proteins encoded by *pbp1a*, *pbp2x*, and *pbp2b,* were seen*:* (i) penicillin-susceptible *S. pneumoniae* (PSSP) isolates had no abnormal *pbp* genes (24.2%), (ii) genotypic penicillin-intermediate *S. pneumoniae* (gPISP) isolates had only an abnormal *pbp2x* gene [gPISP (*2x*)] (*26*%), (iii) with only an abnormal *pbp1a* gene [gPISP (*1a*)](0.1%) (iv) with only an abnormal *pbp2b* gene [gPISP (2b)](2.2%) (v) gPISP isolates with abnormal *pbp1a* and *pbp2x* genes (2.8%), (vi) gPISP isolates with abnormal *pbp2x* and *pbp2b* genes (2.2%), and (vii) genotypic penicillin-resistant *S. pneumoniae* (gPRSP) isolates with three abnormal *pbp* genes (*38.5*%). Almost 94.5% strains had abnormal ppb 2x gene mutations. The $MIC_{50}$ and $MIC_{90}$ of the strains with mutations in the three *pbp* genes to PCG were ≥ 2μg/ml, whereas strains without mutation in either *pbp* genes were ≤ 0.03μg/ml and 0.06μg/ml respectively. The $MIC_{50}$ and $MIC_{90}$ of the strains with mutations in *pbp2x* were 0.06μg/ml and 0.125μg/ml respectively. On the other hands, the $MIC_{50}$ and $MIC_{90}$ of strains with mutations in two type of *pbp* genes (*pbp1a* and *pbp2x*, *pbp1a* and *pbp2b* or *pbp2x* and *pbp2b*) strain varied ranged between 0.125 μg/ml and 0.5μg/ml and 0.5 μg/ml to 4 μg/ml respectively.

*S. pneumoniae* resistance to macrolide has also been a big concern and the rates of resistance were 70-80% in Japan, 92.1% in Vietnam, 86% in Taiwan, 80.2% in Korea, 76.8% in Hong Kong, and 30% in US.[36, 47, 48] The majority of the strains had *mefA* (*32.5*%) or *ermB* (*34%* ) and *mefA* and *ermB* (3.4%) gene-mediating macrolide resistance. Susceptibilities to clarithromycin of strains with *mefE* gene, *ermB* gene and both were 1-4 μg/ml, >64 μg/ml and > 4 μg/ml, respectively. Macrolide resistance genes were highly identified among penicillin non-susceptible strains (PISP+PRSP).[47, 48]

PRSP causes three times higher incidence of persistent AOM compared to PSSP. Serotypes 19F, 23F and 6 are most prevalence serotypes followed by serotype 3, serotype 9V, serotype 7F all over the world. The increasing threat of PRSP is a great concern worldwide and most the strains have also been getting multi-drug resistant. Although conjugated pneumococcal vaccine can reduce the colonization of *S. pneumoniae* in AOM patients, the efficacy of vaccine in AOM patients still remains controversial.

*Antimicrobial Resistance in Haemophilus Influenzae. H. influenzae* is the second leading pathogen that causes AOM of children. Most of AOM is caused by nontypeable *H. influenzae* (NTHi). Since the first reports of ampicillin resistant strains of *H. influenzae* in 1974 from the US, the major mechanism of resistance has been considered to be production of either TEM-1 or ROB-1 types of ß-lactamase.[30, 31, 121] The prevalence of ß-lactamase producing strains has increased progressively in the US from 15.2 % in 1983-1984, to 36.4 % in 1994-1995 and 31.3 % in 1997-1998.[30, 31] The beta-lactamase non-producing ampicillin resistant (BLNAR) strains were isolated at low frequency in 1980s but the BLNAR strains have increased rapidly to 19.5 % of the total in the 1990s.[99] With more recent data, it was reported that the rate of BLNAR was 58.1% in Korea, 37% in Japan, 0-33% in Europe, 4-10.1% in the US.[30, 31, 49, 56, 121]

*Antimicrobial Resistant Pathogens in Upper Respiratory Tract Infections in Japan.* In 2003, the Japan Society of Infectious Diseases in Otolaryngology conducted the 4th nationwide surveillance of pathogens responsible for upper respiratory tract infectious disease to define the current status of antimicrobial resistant pathogens. According to susceptibility criteria for *H. influenzae* to ampicillin published by the National Laboratory

Standard institute (CLSI), *H. influenzae* isolates included 61.0 % susceptible strains (MIC ≤1 μg/ml), 37 (14.0 %) intermediately resistant strains (MIC =2 μg/ml) and 66 (25.0 %) resistant strains (MIC ≥4 μg/ml). Five strains produced TEM type ß-lactamase. These included 3 (1.2 %) strains with mutations in *ftsI* gene (gBLPACR: genetically ß-lactamase producing amoxicillin-clavulanate resistant) and 2 (0.8 %) strains without mutations in *ftsI* gene gBLPAR (genetically ß-lactamase producing ampicillin resistant). Based upon PCR-based genotyping, 172 (65.1 %) isolates had mutations in *ftsI* gene without producing ß-lactamase (gBLNAR: genetically ß-lactamase nonproducing ampicillin resistant). They were 98 (37.1 %) strains with group I/II mutations in variable mutated region (Group I/II gBLNAR) and 74 (28.0 %) strains with group III mutations in highly mutated region (Group III gBLNAR). The remainder of the 87 (33.0 %) isolates were gBLNAS (genetically ß-lactamase non-producing ampicillin susceptible) strains with mutations in neither the *ftsI* gene nor *bla* gene. The Group III gBLNAR strains showed resistance to both penicillin and cephalosporin. Among the 61 gBLNAR strains with mutations in *ftsI* gene, 6 clones were identified. As the MIC to AMP increased, the frequency of the clonal dissemination increased. Six (25 %) strains among the 24 strains with an MIC to AMP 4 μg/ml, 6 (23.0 %) strains among 26 strains with MIC to AMP 8 μg/ml, and 7 (63.6 %) strains among strains with MIC to AMP ≥16 μg/ml showed similar PFGE patterns. PBP gene mutated *H. influenzae* not only demonstrated resistance to ampicillin but also had reduced susceptibility to cephalosporins. The high prevalence of gBLNAR strains of *H. influenzae* should be taken into account when treating the upper respiratory tract infectious diseases.

## III. IMMUNOLOGY

### A. INNATE IMMUNITY AND OTITIS MEDIA

Since the last symposium, the role of innate immunity in providing protection against otitis media has become of an area of increased interest for several laboratories. Because the chinchilla is often used as a rodent host in which to study the pathogenesis of both viral and bacterial OM, one group has begun to identify and characterize mucosal antimicrobial peptides (AP) expressed in the uppermost airway of this host. The first such AP characterized was chinchilla beta defensin-1 (cBD-1), a ~ 5 kDa cationic member of the defensin family that demonstrated significant homology with human beta defensin- 3 (hBD-3).[43] cBD-1 is 77% identical to hBD-3 and is expressed at specific sites in the chinchilla host, including the tongue, Eustachian tube, trachea, lung, nasopharynx and skin. Recombinant cBD-1 had bactericidal activity and killed all three pathogens most commonly associated with OM as well as *Candida albicans*. The second chinchilla AP identified was a member of the cathelicidin family and, in keeping with the nomenclature used for other rodent derived cathelicidins, was named chinchilla CRAMP (or cCRAMP). cCRAMP is 46% identical to human CAP18 (or LL-37) and recombinant cCRAMP selectively killed NTHi, *M. catarrhalis* and *S. pneumoniae* suggesting the presence of unique AP resistance mechanisms in these bacterial species.[76] Unlike cBD-1, cCRAMP mRNA was detected in virtually all tissues evaluated, similar to what has been reported for human LL-37.

To investigate the role that viruses play in terms of dysregulating the innate immune response operative in the uppermost airway, these investigators showed that incubation of chinchilla middle ear epithelial cells (CMEEs) with influenza A virus led to an ~50% reduction in cCRAMP mRNA levels, while RSV or adenovirus only minimally affected cCRAMP transcript abundance. In contrast, incubation of CMEEs with RSV reduced cBD-1 message levels by ~40%, whereas neither influenza A nor adenovirus induced a change in transcript abundance. Each of the bacterial pathogens most commonly causing OM also had an effect on transcription of genes encoding cBD-1 or cCRAMP, with *M. catarrhalis* being the only one tested that induced downregulation of both cCRAMP and cBD-1 mRNA. NTHi or *S. pneumoniae* infection induced a ~50% or ~175% increase in cCRAMP mRNA abundance, respectively; whereas the pneumococcus reduced the level of cBD-1 mRNA detected by ~53%. Collectively, these data suggested that pathogen-mediated dysregulation of expression of effectors of innate immunity in the uppermost airway may contribute to the disease course of OM.

## B. LYMPHOCYTE BIOLOGY OF THE MIDDLE EAR

Since the last Symposium, there have only been limited new data published on lymphocyte trafficking to the middle ear. Although it is thought that B cells derived from the tonsils and adenoids seed the upper respiratory tract; including the salivary glands, lacrimal glands, nasal mucosa in chronic sinusitis and middle ear mucosa in otitis media, there have been no studies on T cell trafficking.

There have been several earlier reports suggesting that P-selectin glycoprotein ligand-1 (PSGL-1) present on Th1 but not Th2 cells binds to P selectin and directs migration into inflamed skin.[16] Borges and coworkers demonstrated that murine Th1 cells but not Th2 cells are selectively recruited into inflamed sites of delay-type hypersensitivity (DTH) reaction to the skin. The migration was blocked by monoclonal antibodies to P- and E-selectin. These authors demonstrated that Th1 cells bind to P-selectin via PSGL-1 and that only this glycoprotein ligand was detectible by affinity isolation with a P-selectin-Ig fusion protein. This was the first *in vivo* study demonstrating the importance of PSGL-1 for mouse leukocyte recruitment.

Since this earlier work, other investigators have demonstrated the presence of PSGL-1 on the surface of both Th1 and Th2 lymphocytes. However, only the Th1 lymphocytes migrate to sites such as the lung, nasal mucosa, nasal polyp mucosa and lacrimal gland when P-selectin is the counter receptor on the surface of the endothelium.[45] Most important is the work Symon and coworkers who investigated P- and L-selectin mediating binding of T cells to chronically inflamed human airway endothelium using nasal polyp endothelium.[118] Their work suggested that memory T cells bind to nasal polyp epithelium via P- or L-selectin using the PSGL-1 ligand.

Kawauchi and coworkers recently used fluorescence-activated cell sorter analyses to investigate $CD3^+$, $CD4^+$ cells that bind P-selectin. These investigators found that the only subset of $CD3^+$, $CD4^+$ cells that could bind P-selectin were Th1 cells. They studied 18 patients with massive nasal polyposis and found that the average number of Th1 cells varied from 3.1 to 50.6 with an average of 34.7 +/- 18. The number of Th1 cells that bound P-selectin was significantly higher in lymphocytes derived from nasal polyp tissue than in those from peripheral blood mononuclear cells, reflecting the higher percentage of CD3+, CD4+ Th1 cells capable of binding P-selectin that can be found in nasal polyps.

This type of work will need to be done with middle ear mucosal tissue to investigate T cell trafficking from either the peripheral blood or the tonsils and adenoids to the middle ear during acute otitis media. These types of studies should help determine the origin of T-cells that reach the middle ear mucosa and the importance of specific binding of PSGL-1 on Th1 cells with either P-selectin or E-selectin on the vascular endothelium of middle ear venules.

## C. CLINICAL IMMUNOLOGY OF OTITIS MEDIA

Otitis media is the most common disease seen in childhood. *Streptococcus pneumoniae*, nontypeable *Haemophilus influenzae*, and *Moraxella catarrhalis* are the most frequent pathogens and about 35-40%, 30-35%, and 10-15%, respectively, of all episodes. Protection against disease due to these pathogens may depend on pathogen-specific antibody. In the case of *S. pneumoniae*, protective antibody has been thought to be directed mainly toward capsular polysaccharide antigens.[19] Capsular polysaccharides of *S. pneumoniae* are type specific and poorly immunogenic in children younger than 2 years old.[33, 65] Some pathogen-specific antibodies may be directed against protein immunogens such as pneumococcal surface protein A (PspA) of *S. pneumoniae*, P6 of nontypeable *H. influenzae*, and UspA of *M. catarrhalis*.

In studies from Yamanaka and coworkers, it was found that otitis-prone children were not unusually vulnerable to infections except those resulting in otitis media.[130] This fact seems to refute the presence of a broad immunologic deficit in the children. However, children who had recurrent episodes of otitis media caused by *S. pneumoniae*, or nontypeable *H. influenzae* did not mount a normal response to PspA, PCP-$IgG_2$, and P6 during the episodes and failed to have a secondary immune

response on repeated challenge.[50, 108, 109] It is likely, therefore, that these children will not respond adequately to immunization with PspA or P6 vaccines. Otitis-prone children also fail to respond appropriately to pneumococcal antigens and thus may not be immunized effectively with a vaccine for otitis media that contains pneumococcal polysaccharides. Selective immunologic derangements in otitis-prone children may therefore be wider than previously believed. Effective active immunoprophylaxis against otitis media will be possible only when the mechanism of the immunologic defect in otitis-prone children is understood.

*Immune Response to PspA of S. Pneumoniae in Children with Acute Otitis Media.* A number of recent publications described the importance of PspA in both disease production and immunity. PspA is attached to the surface of the pneumococcus by the C-terminal end of the molecule, and much of the immune response elicited by immunization in animals is directed against the N-terminal alpha-helical portion of the molecule.[75] The PspA gene is expressed in all strains of pneumococci, regardless of their capsular serotype.[27] Antibody responses to PspA in animals protect against sepsis and nasopharyngeal colonization.[129] Although PspA is a heterologous protein, there is a high degree of serologic cross-reactivity among different PspA molecules from the two major families of PspA.[18] A single recombinant PspA protein is capable of inducing protection against pneumococcal strains of diverse capsular serotypes and different PspA serotypes in animal models.[18] Thus, it is hypothesized that a single PspA protein may be able to provide protection against multiple diverse strains of *S. pneumoniae*.[18]

Immune responses to PspA in the sera of various age groups in the general population and in the nasopharynges of 30 children monitored from birth through 1 year of age were evaluated.[108] IgG was the dominant serum antibody to PspA. In the first 2 years of life, comparable amount of IgM and IgG antibodies were observed. In the older persons, IgG antibodies to PspA predominated over IgM antibodies. The level of IgA antibodies to PspA in serum remained low during the first 2 years of life. Although IgA was the dominant antibody to PspA in airway secretions, it was detected in a minority of the children. Even the majority of the children previously colonized with *S. pneumoniae* lacked antibody to it in their secretions. A decline in PspA IgG antibody concentration was noted in sera from adults, and this was reflected in a similar decline in the proportion of total IgG represented by PspA-specific IgG. Epidemiologic studies with *S. pneumoniae* indicate that acquisition of the strain and length of colonization decrease with increasing age, suggesting that maturation of the immune system in some way plays a role in controlling colonization patterns.[40]

The antibody response to PspA was evaluated in children with acute otitis media due to *S. pneumoniae*.[109] The age of the children had a range of 4-32 months. The mean IgG, IgM, and IgA antibody responses to PspA in sera from children at the acute and convalescent stages were 4864 versus 5831ng/mL, $p<0.05$, 1075 versus 3752 ng/mL, $p<0.05$, and 67 versus 93 ng/mL, non significant, respectively.

Studies of natural immunity to pneumococcal infections have focused almost exclusively on antibodies directed against the capsular polysaccharides. Although the introduction of conjugate vaccine has given satisfactory protective responses to polysaccharides in young children and raised expectations regarding a capsular-based vaccine, there are still more than 90 individual types of capsule in the pneumococcus. A single protein immunogen capable of eliciting protective antibodies would be attractive when compared with the need to include multiple polysaccharides in a vaccine for young children. This study showed that majority of children responded to an infection by *S. pneumoniae* by making antibody to PspA. Nevertheless, the specific antibody to PspA may not always protective to middle ear infections of *S. pneumoniae* due to several factors. Mucosal antibodies, expected to be those most crucial for protection at the respiratory surface, might not be parallel to serum antibodies. Moreover, it is quite possible that anti-PspA antibodies elicited during otitis media may be protective against invasive disease even if protection against otitis media is not always achieved.

*Antibody Response to Pneumococcal Capsular Polysaccharides (PCP) in Normal and Otitis-Prone Children.*[50] In healthy children, the total IgG2 level was lowest at 6 months of age. The level increased

until 2 years of age and then gradually decreased until 4 years of age. Thereafter, at 4-5 years, it increased again. Anti-PCP IgG2 was lowest at 6 months of age. The level increased until 2 years of age and decreased at 3 years of age. Thereafter, at 4-5 years, it increased again. Among the otitis-prone children, five of 36 otitis-prone children (13.9%) showed subnormal levels of total IgG. Thirteen of 27 otitis-prone children (48.1%) showed sub-normal levels of anti-PCP IgG2 antibody. The number of children with subnormal levels of total IgG2 was not higher in the otitis-prone group than in the normal group ($p = 0.1484$). However, the number of children with subnormal levels of anti-PCP IgG antibody was significantly higher in the otitis-prone group than in the normal group (anti-PCP IgG2, $p < 0.01$).

*Immune Responses to the P6 Protein of Nontypeable H. Influenzae.* Nontypeable *H. influenzae* (NTHi) is frequently associated with recurrent and chronic episodes of middle ear disease.[22] One of the major outer membrane proteins of NTHI, P6, is highly conserved among strains, is antigenically stable, serves as a target for bactericidal antibody, and has been proposed as a possible candidate for vaccine formulation.[84, 85] In work by Yamanaka and coworkers, the serum antibody response to P6 was studied in otitis-prone and normal children by ELISA.[130] At birth, serum anti-P6 IgG antibody was found at almost the same level as in adults, whereas no IgM or IgA antibodies specific for P6 were detected. Anti-P6 antibody levels in the three isotypes studied were lowest at 6 months of age and rose significantly after 2 years; IgG levels peaked at 10 years, whereas IgM and IgA peaked at 6 years. In every age group, IgG antibody specific for P6 was in the highest concentration among the three isotypes. Anti-P6 IgG antibody was detected in all individuals in each age group; however, IgM antibody specific for P6 was detected in all individuals older than 6 years of age, and IgA antibody specific for P6 was detected in all individuals only after 10 years of age.

Antibody levels to P6 measured in convalescent sera of children with acute otitis media exceeded those in acute-phase sera in 60%. When sera obtained during the acute and convalescent periods were screened for bactericidal antibody, ten acute-phase sera possessed bactericidal antibody and 10 did not; all convalescent-phase sera had bactericidal antibody. When the paired sera were divided into two groups, dependent on the presence or absence of bactericidal antibody in the acute period and then analyzed for antibody to P6, a significant rise in anti-P6 antibody was detected in the group initially lacking bactericidal antibody.

In this same investigation, anti-P6 antibody levels were measured longitudinally in 30 otitis-prone and 13 healthy children on 93 and 32 occasions, respectively. The age at time of sampling varied between 1 and 92 months. Antibody levels increased sevenfold in the normal group for 36 months, in comparison with less than threefold in the otitis-prone group for 48 months. The levels of antibody in the normal group were significantly higher than those in the otitis-prone group after the age of 18 months. In general, individual antibody levels in otitis-prone individuals did not have an age-dependent rise. Furthermore, children who experienced two or more episodes of otitis media caused by nontypeable *H. influenzae* had no anamnestic antibody response to P6. Immunoglobulin IgM and IgA antibody responses to P6 in otitis-prone children reached a plateau after 18 months of age, and the anti-P6 IgM antibody level remained below the adult serum level even after 4 years of age. Differences between otitis-prone and normal children were not statistically significant.

## D. ALLERGY AND ITS RELATIONSHIP TO OTITIS MEDIA

Modern immunologists have agreed on a categorization system for sinusitis and asthma based on their knowledge of T-cell activity and basic immunology. Since we are dealing with chronic inflammation of similar mucosa in the ear it would be appropriate to use the same standard of classification based on immunologic findings as has been adopted in the nomenclature guidelines published by both the European and American Academies of Allergy and Clinical Immunology in 2001 and 2003 respectively for the inflammation found in patients with asthma or sinusitis as being either “allergic or non-allergic.”[57] It seems a logical extension to suggest that otitis media should also be categorized in an identical fashion. Under the allergic category these academies sub-classified the

inflammation in these target organs of disease as being either "IgE or non-IgE mediated disease."

This categorization takes into account the difference between IgE mediated and non-IgE mediated allergic responses. The immediate IgE mediated response involving mast cells, B cells and the release of IgE is totally distinct from the non-IgE mediated response that involves the eosinophils. This helps explain why certain drugs and certain allergy tests may or may not be effective. The immediate response with mast cells causes the release of histamine. This leads to immediate sneezing, watery eyes and a runny nose. Antihistamines may work – but only for the immediate response. Asthma is a late phase disease and is managed with inhaled steroids and anti-leukotrienes.

Physicians know that the middle ear is similar to the sinuses and lung in that it is lined by pseudo-stratified, ciliated, columnar epithelium. This epithelium must respond presumably the same in the middle ear as it does everywhere else. Simplistically, all that has been made known about allergic inflammation of the mucosa in the nose as regards sinusitis, and in the lung as regards asthma should be able to be extrapolated to the mucosa in the middle ear. Recent studies support this thinking. It is important to make a distinction between acute otitis media and chronic otitis media, either of which may also present as an episode of otorhea through a perforation or tympanostomy tube. These are two distinct diseases. Acute otitis media will clear in 80% of cases in 3 days. Only 17% of AOM will proceed to OME. Of those with effusion present for 3 months, only 26% will resolve spontaneously in six months.[104]

Direct proof that allergy contributes to chronic OME and/or other manifestations of chronic middle ear disease is best done by a double-blind placebo-controlled trial. None have been published. Furthermore, objective information on the allergic status of OME patients is lacking in all available databases. The three academies of Pediatrics, Family Practice, and Otolaryngology-Head and Neck Surgery all agreed in 2004, in published guidelines,[103] that indeed "the middle ear mucosa is capable of allergic a response." That conclusion was derived from a variety of papers that have established that all mediators needed for an allergic response by the mucosa are indeed present. The guidelines note the lack of appropriate treatment studies and therefore make no recommendation regarding allergy management as a treatment for OME. A recent clinical review suggests that "the relation between allergy and OME will remain controversial until well controlled clinical studies are conducted documenting that in select populations anti-allergy therapy is efficacious in preventing or limiting the duration of OME."[80] This same shortcoming is identified in the conclusions of each of the past International Symposia for 20 years. Perhaps this remains unaddressed because otologists are unsure of allergy and allergists are unfamiliar with otologic problems.

OME is a multifactorial disease, of which allergy is only one risk factor. Parental smoking, day care classrooms larger than six students, asthma, and viral upper respiratory infection are also known to predispose one for OME. Yet allergy adds unique co morbidity. Allergy magnifies any of the previous named risks by a factor of 2 to 4.5 times that seen in non-allergics.[115] There is increasing evidence to support the concept of a "unified airway" for respiratory tract allergy in general. The nose, lung and middle ear function as a unified respiratory organ. Braunstahl[17] did a brilliant study where he took non-asthmatic children who had allergic rhinitis and did nasal provocation with pollen. He then measured by bronchial washings the inflammatory cells in the lungs of these non-asthmatics and found that the nasal provocation stimulated the lung to also produce an allergic inflammatory response with increased eosinophils etc. He concluded that the upper respiratory tract responded as one unified airway. This has become a keystone concept among all immunologists.

Hamid in Montreal looked at the cells in the middle ear fluid and took biopsies of middle ear mucosa, and at the same time did biopsies of the nasal pharynx mucosa just next to the Eustachian tube orifice.[86] He specifically measured eosinophils, CD-3 T-cells, IL-4 and messenger RNA for IL-5, both in the mucosa from the middle ear as well as from the opening of the Eustachian tube in the posterior pharynx of the same patient. He discovered that the inflammatory profile of middle ear effusion correlated with that in the nasal pharynx or upper airway as these mediators were elevated in both locations. He concluded that the middle ear may

behave in a "similar manner to the lungs under allergic inflammatory insults" and that the "middle ear may be included in the United Airways" concept. Jang demonstrated both RANTES and ECP were significantly elevated in MEE and suggested an association of allergy with the disease.[55]

What exactly is dysfunctional in Eustachian tube dysfunction? Rarely one will encounter a patient with dystonia of the velo-pharyngeal muscles. Most children with cleft palate have chronic OME until the cleft palate itself is repaired. Some of the children go on to continue to have effusion problems and require tubes on occasion - it is unknown if this subgroup is atopic. Other rare causes of ET dysfunction include nasopharyngeal carcinoma, post radiation stenosis, and ciliary immotility problems associated with extremely rare congenital diseases, including Kartagener syndrome. But what of the vast majority of patients with chronic middle ear disease? It has recently been shown that "there are no substantial differences in Eustachian tube function between ears that develop OME recurrence and ears that do not."[116] Her group measured Eustachian tube function in ears of children who develop recurrent middle ear disease and those who had no recurrence. She demonstrated that there was no substantial difference in the ability of the children in either group to open or equalize the pressure in their middle ears. That is, the Eustachian tube function in both children who have recurrent disease and those who do not is the same. It is also a myth that the Eustachian tube will grow to normal size as children mature, as there is no difference in the size of either the isthmus or pharyngeal portion of the ET in children with OME versus normals.[106] Therefore most cases of chronic middle ear disease are not the result of a permanent dysfunction, or distortion, nor physical abnormality of the Eustachian tube in these children. This chronic disease has less to do with the size of the Eustachian tube or its maturity than it does with the underlying inflammatory forces acting upon it.

The most common mechanisms that lead to Eustachian tube dysfunction remain to be either adenoid obstruction and/or tubal edema from allergic or infectious inflammation. Exhaustive review of the literature by the otolaryngology staff of University of Pittsburgh, citing 209 references, notes that "evidence that allergy contributes to the pathogenesis of OME is derived from epidemiologic, mechanistic, and therapeutic lines of investigation." They note that "allergen induced Eustachian tube dysfunction subverts the normal mechanisms of gas exchange into the middle ear and sets the stage for development of negative pressure and transudation of fluids into the middle ear." Another review of the most recent medical literature concurs, noting that "in patients with OME in which allergy may be a contributing factor, appropriate allergy treatment of avoidance of particular allergens, medications, and immunotherapy may be indicated." A recent review states "it may be prudent to screen every child with OME for allergic rhinitis and ultimately to manage those with allergic inflammation differently to nonatopic individuals with OME."[120]

In-vitro and in-vivo evidence indicates that like asthma and allergic rhinitis, a Th-2 mediated allergic response is found in MEE in some OME patients. A brilliant study by Hardy described a new rat model in which the middle ear becomes an actual target organ for allergy.[42] He demonstrated that exposure to transtympanic allergen induced both Eustachian tube dysfunction as well as formation of middle ear effusion. Antigen challenge of pollen in the nose of humans will produce ET obstruction, but not in placebo patients. It should be noted that none of these patients developed effusion and middle ear fluid. This might be because of patient selection and/or the short length of duration of exposure. A recent experiment by Ebert showed that pre-treatment with immunomodulatory oligonucleotides can prevent OVA-induced Eustachian tube dysfunction in a rat model.[34] This demonstrates a potential new way to manage OME and certainly shows that the middle ear is an immunologicaly active site.

Ebmeyer studied the role of mast cells in the pathogenesis of bacterial inflammation in middle ear disease.[35] He compared mast cell deficient mice with normal mice and used adoptive transfer of mast cells into their middle ears to study normal middle ear response to infection. He also examined the effect of bacteria and allergens alone or in combination in the ears of mice sensitized to allergen ovalbumin. He showed a definite role for mast cell degranulation in the pathology of middle ear disease induced by either bacterial infection or allergen exposure. Most importantly, the combined exposure

to allergens and bacteria in allergic animals led to a chronic middle ear lesion and significantly delayed recovery as compared with nonsensitized mice. This suggested a role of mast cells in the development and propagation of chronic disease in allergic sensitized animals.

Hamid noted that a minimal persistent inflammation is present in human mucous membrane among patients with mite and pollen allergy, and reiterates that it involves ICAM-1, a major receptor for human rhinoviruses. He found this in allergic patients and not in nonallergic controls.[86] Amin showed that viral infections in children stimulated ICAM – an inflammatory precursor and attractant of eosinophils and neutrophils only in allergic children.[3] Viral URI in normal children did not have the same effect. Thus the scientific correlation that supports what we observe when our atopic children get a URI. The virus triggers the persistent sub-clinical inflammation, which is constantly present in the mucosa of allergic children, to initiate an asthmatic attack, sinus infection, or exacerbation of their middle ear disease.

Smirnova and colleagues have also demonstrated that the cytokines present in middle ears play a key role as molecular regulators of middle ear inflammation.[113] These cytokines can switch the acute phase of inflammation to the chronic stage and induce molecular and pathologic processes leading to the histologic changes demonstrated in ears with chronic fluid and effusion. This work is extremely important. As complex as her presentation is, it explains the immunologic basis for why some ears following an acute episode can change seemingly spontaneously into developing chronic effusion. Another paper explained how middle ear effusion which has a high level of neutrophils – usually associated with bacterial infection – can be present in the effusion of atopics.[51] So infection itself can indeed be a stimulus. However, again referring to Rosenfeld's work, only 17 percent of children with acute episodes go on to develop chronic middle ear effusions. What is different about this select 17 percent? Are they atopic?

Additional interesting articles from around the world have looked at the relation of allergy to middle ear disease. One from Greece that looked at two groups of children aged one to seven.[23] One group of eighty-eight children had chronic OME versus another group of normal children with no middle ear disease. They did skin prick testing to look at allergy symptoms and found a much higher incidence of allergy in the children with chronic middle ear disease than in the controls. The researchers concluded that allergy is an independent risk factor for developing OME. A study in Mexico again looked at Eustachian tube function in children younger than eleven years old.[68] They evaluated eighty children with rhinitis and positive skin tests for dust, corn and cockroach. (Remember this was a study in Mexico and these are very common local allergens.) They found that 15 percent of the children with positive skin prick tests had abnormal tympanograms indicating very poor Eustachian tube function. This group was compared to fifty controls whose skin testing was negative for the same three allergens. One hundred percent of the controls had normal type A tympanograms. Thus it seems that allergy itself is an increased risk for children with rhinitis to have difficulty opening their Eustachian tubes.

Two studies out of Turkey looked at the role of allergy as an etiology of OME. Doner looked at a group of twenty-two children who required a M&T and adenoidectomy, but had no recurrence of their middle ear disease.[32] Eight percent of them had positive skin testing. This compared to a group of twenty-two children who likewise had an initial M&T and adenoidectomy, but then had recurrent middle ear effusions, which required repeat placement of tympanostomy tubes. The group requiring repeated surgery had 38 percent positive skin prick testing. This indicated a huge statistical difference. They concluded that allergy seems to be a major contributing factor for recurrent disease.

Dr. Kakizaki's group in Japan looked at fifteen patients who had asthma and chronic middle ears disease versus fifteen asthmatics with no middle ear disease.[53] They showed that the mediator IL-5 that is essential for a Th-2 allergic response and responsible for the accumulation of eosinophils triggering the same kind of allergic response we see in the lungs of asthmatics, is also present in the ears of children with chronic OME. This inflammatory mediator was not present in the normal controls. They concluded therefore that the IL-5 responsible for the eosinophil inflammation in these middle ears is produced

locally in the ear itself. This suggests that the allergic reaction occurs in the middle ear and again supports the idea that the middle ear is itself capable of an allergic response. His work supports allergy functions as a CAUSATION of middle ear disease, not merely a correlation factor.

A study by Keles's group looked at fifty-nine OME patients versus twenty-six controls.[58] They measured interferon gamma (a cytokine associated with a Th-1 response) and found that it was lower in the middle ears of the otitis patients, while serum IL-4 and IgE (cytokines associated with a Th-2 atopic response) was significantly higher in effusion patients than in controls. They therefore concluded that the T helper cell polarization suggested allergy as etiology of the middle ear effusions.

Few treatment studies are available. A study from Greece challenged patients with asthma and co-existing OME with leukotriene antagonists.[8] They found that "fifteen (60%) of the children receiving inhalers and monteleukast and nine (36%) of those receiving only inhalers were found free of OME after 30 days of therapy. Thus, it may be concluded that a statistically significant beneficial effect on the clinical course of OME resulted from the addition of monteleukast to the treatment of children with co-existing asthma and OME." Given that this antagonist affects the late phase allergic response, it suggested that allergy might be important in the pathophysiology of OME as well as asthma.

A cohort study involving seventy-four patients undergoing allergy immunotherapy treatment compared results to twenty-four control patients who chose no therapy. All 98 patients (100%) with intractable middle ear disease presenting with chronic effusion, chronic draining perforations or tubes, and/or draining mastoid cavities were proven by intradermal testing to be atopic. Immunotherapy significantly improved, if not completely resolved chronic OME in 91.2% of diseased ears. All children resolved on therapy. Most patients resolved within 4 months and have remained free of disease while on allergy immunotherapy for 2 or more years. All 24 (100%) patients who refused therapy failed to resolve their disease. Response to allergy IT was significantly superior to conventional treatment ($p<0.001$). This study appears to show that patients with OME 1) are almost universally atopic and 2) resolve with immunotherapy. This data supports the hypothesis that OME is an allergic disease.

Proof of the hypothesis that chronic OME is an allergic disease requires three steps. First, establish a relevant, associated, objective diagnosis of atopy in patients with persistent effusion or middle ear drainage. This requires using the most objective diagnostic tests available. Most studies on atopy and otitis media use prick testing which has a recognized 40-50% false negative rate, or RAST in vitro testing which has a similar false negative rate.[25] Intradermal skin testing as done by our AAOA colleagues remains the gold standard.

Secondly, establish an association of allergic immune-mediated histochemical reactivity within the effusion itself. This was done most recently by Hamid[86] and Jang[55] and other studies reviewed earlier. The evidence is conclusive enough to warrant acceptance in current guidelines on otitis media of the paradigm that "the middle ear mucosa is similar to that of the rest of the upper respiratory tract and is itself capable of an allergic response."[103] Finally, one must satisfy the principles of Koch's postulate and demonstrate a diminished incidence of OME in patients whose allergy has been appropriately treated. This is supported by the study on leukotriene antagonists and one using immunotherapy. This is the area recognized by the previous 4 International Symposium as most needed and yet it remains most in need of further confirmation.

In chronic otitis media, as in chronic sinusitis, for each individual case of "infection" the particular organism is of utmost importance. Yet, the bacteriology tells nothing about the pathophysiology of the disease itself. It is the host's immunologic status that holds the key to understanding the underlying mucosal response that is responsible for the immunologic processes that produce his/her sinusitis, asthma or middle ear disease.

## SHORT- AND LONG-TERM RESEARCH GOALS

Many research goals identified in the 2003 Report have been addressed in some detail during the past 4 years. However, further studies to increase our understanding are still needed. Areas

for proposed future study represent logical extensions of some of the more recent investigations. Short-term research goals that should be pursued over the next few years include the following.

## VIROLOGY/MICROBIOLOGY

1. Uniform and sensitive methods for the detection of a broad spectrum of viruses should be promoted in order to better document the association between viral infection and AOM.

2. The role of various inflammatory mediators and their mechanisms of action in the pathogenesis of AOM following viral URI need to be further studied.

3. Detailed mechanisms of viral-bacterial interaction on the mucosal level need to be explored. Specifically, the types of bacterial attachment receptors that are up-regulated on virally-infected epithelium need to be identified.

4. Further studies should be performed to evaluate if specific viruses interact or promote the colonization of specific bacteria.

5. 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 needs to be studied. Similarly, the impact of viral load in the middle ear on disease severity and outcome needs to be studied.

6. The clinical relevance of positive findings and prolonged presence of viral nucleic acids in the MEF and nasopharynx needs to be further elucidated.

7. The relative significance of specific URI causative viruses, including herpesviruses in AOM pathogenesis needs to be further studied.

8. Further studies on the prevention of AOM by means of prevention of viral URI should be performed.

9. Further evaluation of the relevant adherence factors for each of the common middle ear pathogens and identification of their corresponding cell surface receptors. Although substantial progress has been made in this area, a number of questions remain.

10. Examination of those microbial factors critical for virulence of pathogens in the middle ear needs to be continued. The availability of the entire genomic sequences of several of the major middle ear pathogens as well as the development of powerful new genetic tools for investigation of pathogenesis should make the next four years a particularly productive time.

11. Continued monitoring of antimicrobial susceptibility patterns of the major ME pathogens on a worldwide basis and additional studies of the microbiology of acute and chronic OM in developing countries of the world. Information in this area is critically needed as we attempt to apply current knowledge in antimicrobial treatment and vaccine development to the rest of the world.

12. The potential contribution of sequestered bacteria in the middle ear space, either in intracellular locations or in the form of biofilms needs to be further clarified.

## IMMUNOLOGY

1. The source and trafficking of lymphocytes to the tubotympanum from other distant mucosal sites (homing and compartmentalization) should be more extensively studied to enhance the clinical application of mucosal vaccination to protect the middle ear from infection.

2. The interactions between Toll-like receptors (TLRs) expressed on epithelial cells, fibroblasts, inflammatory cells (neutrophils and macrophages), and lymphocytes in middle ear cavity and TLRs ligands such as virus antigens and bacterial degradation products should be clarified.

3. The various cytokines produced by middle ear epithelial cells or immunocompetent cells should be identified in relation to TLR signals and assessed for their role in the defense of the tubotympanum as well as in the pathogenesis of persistent otitis media with effusion.

4. Innate immunity and acquired immunity in middle ear cavity should be further investigated by new molecular techniques. Their interaction within and between the middle ear and inner ear remains to

be investigated, particularly in view of inner ear involvement after middle ear microbial infection.

5. The role of adenoids and tonsils as the inductive sites for the mucosal immunity should be explored further and related to the functional aspects of the systemic immune response. These studies should be carried out in human materials and animal experiments, as well, in order to combine and contrast information obtained from complementary systems.

6. The importance of the various signal transduction pathways (NF-kappa B, Myd88, MAP-kinase) should be clarified in relation to the production of various cytokines (chemokines, proinflammatory cytokines, Th1 and Th2 type cytokines).

7. The ability of various drugs such as macrolides or antihistamines should be evaluated for their ability to down-regulate the important signaling pathways that induce an inflammatory response in the middle ear and nasopharynx.

8. Identify and characterize the presence and distribution of toll-like receptors on the array of cell types present in the middle ear.

9. Use molecular and genetic techniques to better characterize the innate and adaptive immune responses present in the middle ear: signal transduction, cytokine and chemokine patterns, antigen presentation.

10. Evaluate the immunogenetics of otitis media to determine whether there is a genetic predisposition to a particular pathogen, tropism to particular tissue receptors, or a genetic basis for responders versus non-responders for certain vaccines.

11. Define the potential of NALT-like tissues as sites for induction of an immune response.

12. Investigate the ability of various chemo- or immunotherapeutic or prophylactic agents to modulate the immune response or to modify specific signaling pathways critical to the development of inflammation in the middle ear.

13. Encourage studies to establish the true incidence of atopy among patients with otitis media with effusion in all its presentations – chronic effusion, and chronic suppurative otitis media presenting with a draining tube or perforation.

14. Encourage additional treatment studies looking particularly at drugs that specifically target the late phase allergic response such as steroids, anti-leukotriene, or immunotherapy itself.

## LONG-TERM GOALS

1. Attain sufficient understanding of the factors responsible for microbial virulence and disease pathogenesis to develop specific preventive and therapeutic strategies.

2. Put advances in basic immunologic understanding of disease into relevant changes in clinical approach to otitis media.

3. Define the immunologic defects responsible for disease in the otitis-prone child.

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# 6. VACCINE

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## RATIONALE FOR PREVENTION OF OTITIS MEDIA

The impact of middle ear infections and acute otitis media (AOM) on children and society is substantial and warrants efforts at prevention. AOM is highly prevalent in children worldwide with subsets of children who exhibit frequent recurrences (otitis prone).[1-4] It is the most commonly diagnosed disorder in children less than 5 years of age. Twenty percent of children fulfill the criteria for being otitis prone (3 episodes within 6 months or 4 within a year) and suffer from conductive hearing loss that persists for weeks to months following episodes of AOM.[5] In rural Kentucky, Pittsburgh and Tennessee 1.8% to 2.2% of children received pressure equalization tubes (PET) by one year of age and 4.2% to 5.8% received PET during the second year of life.[2, 3, 6] These studies identify a substantial ongoing burden of middle ear disease in a significant proportion of children. These children are characterized by early onset of AOM, male gender, absence of breast feeding and exposure to cigarette smoke.[7] In addition to frequency, the persistence of middle ear fluid after AOM results in moderate conductive hearing loss that has potential implications for speech and language development.[8, 9] Morbidity associated with ROM and persistent middle ear effusion is widely recognized in indigenous populations with persistent perforation, chronic suppurative OM, and hearing loss widely reported.[10-14] In developed countries, the impact on hearing, language and cognitive development is less clear.[15, 16] Subjectively, recurrent AOM negatively impacts on the quality of life of children proportional to its severity and is concerning to their caregivers. Brouwer reported that families of children with recurrent OM report a similar reduction in quality of life as those of children with asthma.[17]

AOM remains the most common reason for prescribing antimicrobial agents to children, accounting for more than 25% of antibiotic prescriptions.[18, 19] This quantity of antibiotic prescriptions contributes substantially to the ongoing evolution of resistance among respiratory tract pathogens.

## FEASIBILITY OF PREVENTING OTITIS MEDIA BY VACCINATION

The pathogenesis of OM is complex, most often involving acute infections with respiratory viruses and nasopharyngeal colonization with bacterial pathogens. Several respiratory viruses replicate effectively in the middle ear mucosa. Such viral replication in the middle ear can result in local mucosal damage, edema of the Eustachian tube that further compromises its function especially in young children, increased susceptibility to bacterial colonization and active mucosal replication and, under certain situations, viral infections may diminish host defenses to invading bacterial pathogens. Each of these factors, particularly in otitis prone children, contribute substantially to increased disease burden and represent significant challenges for vaccine development.

The earlier Vaccine Panel Reports (1999, 2003) identified the possibility for successful immuno-

prophylaxis against OM[20] detailing: 1) early experience with pneumococcal conjugate vaccine (PCV) showed protective immune responses by 7 months of age and a reduction in type specific pneumococcal middle ear infections;[21] 2) passive protection against pneumococcal OM from prophylactic administration of human immune serum globulin[22] in high risk children; and 3) strain-specific protective immune responses develop following AOM due to NTHi.[23, 24] Four years later, we have a pneumococcal conjugate vaccine licensed for the prevention of vaccine serotype specific pneumococcal OM. A second prototype pneumococcal vaccine containing protein D of *Haemophilus influenzae* has been shown to have demonstrable efficacy against both pneumococcal and *Haemophilus* related OM.[21, 25, 26]

Data from post marketing studies of PCV and clinical trials of an n-11 valent pneumococcal polysaccharide-protein D conjugate vaccines have provided further insights into the prospects for an effective vaccine against otitis media. PCV7 has demonstrated greater than a 70% decline in invasive pneumococcal disease and a more modest decline in episodes of AOM and insertion of pressure equalization tubes (PET).[6, 27-30] Estimates from New York and Tennessee insurance claims report the decline in episodes in the 11 to 20 percent range and 16 to 23 percent for PET.[29] The more modest decline in episodes of AOM (compared to invasive disease) reflects both a decrease in vaccine efficacy against type specific infection, as well as an increase in disease due to nonvaccine serotypes.[21] The vaccine results in reduction in carriage of vaccine type pneumococci with complete replacement (in the nasophayrnx) with non vaccine serotypes.[31, 32] Nonvaccine serotypes have demonstrated themselves as competent otopathogens, however more data about their ability to produce middle ear disease relative to vaccine serotypes is still needed.[33, 34] The replacement of vaccine serotypes in the nasopharynx in vaccinated populations is also seen in children less than 6 months of age suggesting the potential for reduction in AOM in children less than 6 months of age as well if non-vaccine serotype demonstrate less capacity to produce AOM.[35]

A phase 3 clinical trial of an 11- valent pneumococcal capsular polysaccharide conjugated to Protein D was completed in 2005.[26] A 52% reduction in type specific pneumococcal OM and a 35% reduction in episodes of OM due to NTHi were observed in the vaccine cohort demonstrating that a multivalent vaccine enhances the reduction in clinical episodes of AOM. A better understanding of mechanisms resulting in breakthrough is needed to provide insights in the development of future vaccines.

These studies continue to support the hypothesis that despite the multifactorial spectrum of OM, induction of protective antibody may "tip the balance" in favor of the host and against the occurrence of OM. The challenge then remains to identify appropriate antigens and present them to the human host in such a way as to generate protective immune responses in the nasopharynx and/or middle ear.[36, 37]

## INCREASED RECOGNITION OF THE ROLE OF BIOFILMS IN OTITIS MEDIA

Over the past 4 years, substantial progress has been made in understanding the importance of biofilm formation and its potential role in OM.[38-42] Hall-Stoodley et al. directly detected biofilms in the middle ears of children with OME or recurrent OM. They further demonstrated the presence of *H. influenzae, S. pneumoniae* and *M. catarrhalis* in these biofilms. This was the first demonstration of bacterial biofilms in human middle ears. As vaccines for OM are developed and tested, it will be important to consider the propensity for biofilm expression by the targeted bacteria and evaluate the effectiveness of putative vaccines in preventing such biofilm formation.

## CHARACTERISTICS OF AN IDEAL VACCINE FOR OTITIS MEDIA

The apparent impact of the inclusion of the NTHI antigen, protein D, as the carrier protein in the development of pneumococcal conjugate vaccine has refocused efforts on the selection of appropriate antigens, carrier protein and the delivery systems for effective vaccine development. It is clear that development of new vaccines for OM must take into account the following specific considerations: 1) candidate vaccines must be

comprised of the 'right' antigens (i.e., immune responses generated will prevent the disease); 2) the antigens must be broadly conserved among strains of the bacterial species being targeted; 3) potential combination vaccine formulation must preserve the immunogenicity of each component vaccine antigen, such that protective antibody and/or cellular responses will be elicited in the population at risk (e.g., infants and toddlers), and such responses will persist for sufficient time to provide protection through the risk period; 4) the vaccines should not potentiate inflammation in the middle ear regardless of the mechanism and 5) the vaccines should not induce any other collateral damage such as perturbing the normal mucosal flora in a clinically significant manner. More specifically, they should not allow or promote colonization by other non vaccine pathogens.

Since the last report, discernable progress has been made, but deficiencies remain in our understanding of the innate and acquired immune mechanisms in the middle ear in relation to developing more effective therapeutic and prophylactic treatments for OM. The use of intranasal influenza virus vaccine in children has rekindled interest in mucosal vaccines, however, mucosal delivery as an approach for bacterial pathogens associated with AOM has not progressed sufficiently towards formulations for any specific vaccine. Although there have been advances in adjuvant and/or carrier system development, and about responses induced by delivery at different sites, many of the issues presented in the last report still remain to be resolved.

## VACCINE DELIVERY

Mucosal routes of delivery of vaccines, especially via oral, nasal or inhalation may be the most relevant alternative approaches to injectable (systemic) vaccine delivery. Recently, Lu and Hickey reviewed the adjuvants, aerosol-generation technology and pharmaceutical formulations specific for pulmonary immunization. This noninvasive approach appears to be relatively safe and low-cost, yet despite these benefits and advances on the technologic side, it has not progressed significantly for adoption in new or existing vaccines.[43] Effective oral immunization with peptide and protein vaccines requires formulations that protects the antigen from digestion and enables enhanced uptake in a manner that induces an effective immune response. Many delivery systems and adjuvants, including inert and live vectors, have been evaluated for oral vaccine delivery. Despite many approaches and some promising developments, bacterial vaccines for the respiratory tract and middle ear have not to date progressed to the level wherein they've generated a specific vaccine product.[44]

Intranasal (IN) immunization induces mucosal immune responses in the middle ear as well as ET. Suenaga et al.[45] and Kodama et al.[46] investigated lymphocyte subsets, mRNA of cytokines, and induction of antigen-specific IgA-producing cells in the middle ear mucosa and ET in mice. They found that P6-specific IgA-producing cells were markedly increased in the middle ear mucosa and ET by IN immunization with P6 together with cholera toxin. Further, *in vitro* stimulation with P6 of purified CD4(+) T cells from the middle ear and ET of immunized mice resulted in the proliferation of CD4(+) T cells that expressed Th2 cytokine mRNA such as IL-5 and IL-10. These results indicate that both middle ear and ET function as mucosal effector sites and may play a role in preventing middle ear infection following IN vaccination.

In IN immunization, nasal associated lymphoid tissue (NALT) especially in the rodent models is considered to be an inductive site of immune responses, as is gut-associated lymphoid tissue (GALT) in oral immunization. Shikina et al.[47] demonstrated the expressions of a series of IgA isotype class switch recombination-related molecules, including activation-induced cytidine deaminase, I-alpha-C micro circle transcripts, and Iα-C micro circle transcripts in NALT, but not in nasal mucosa, indicating that NALT plays an important role as an inductive site in IN immunization. On the other hand, the organogenesis of NALT is different from that of other inductive sites such as GALT. Fukuyama et al.[48] demonstrated that initiation of NALT organogenesis is independent of the IL-7R, LTβ-R, and NIK signaling pathways, but requires the Id2 gene and CD3(-)CD4(+)CD45(+) cells. They also found that the initiation of NALT organogenesis is independent of CXCL13, CCL19, and CCL21. However, the expression of these lymphoid

chemokines is essential for the maturation of NALT micro-architecture.[49] It should be however pointed out that humans don't have NALT. Instead, in man, human nasopharyngeal tonsils and the Waldeyer's ring function as a similar inductive site for immune responses.

The development of effective and safe mucosal adjuvant(s) is essential for the delivery of vaccines via the mucosal routes. Native cholera toxin (nCT) is commonly used as a potent mucosal adjuvant in animal experiments, however, nasal application of nCT has potential toxicity for human and for the central nervous system (CNS). Although mutants of cholera toxin (mCTs) have been developed that show mucosal adjuvant activity without toxicity, it still remains unclear whether these mCTs will induce CNS damage. To help overcome these concerns, new double mutant CTs (dmCTs) have been developed.[50] To date, these newly developed dmCTs retain strong biological adjuvant activity without CNS toxicity. Kataoka et al.[51] assessed a cDNA vector for Flt3 ligand (FL) for its potential to enhance mucosal immunity. IN immunization with OVA plus DNA plasmid with FL gene induced increased levels of OVA-specific antibody responses in mucosal sites and enhanced the number of activated lymphoid dendritic cells in NALT. These newly developed mucosal adjuvants might be effective and useful for clinical application of mucosal vaccine against OM, though their safety must be examined by clinical trials.

## MATERNAL IMMUNIZATION AGAINST AOM

The capsular polysaccharide-based vaccine has been successful against invasive pneumococcal disease but demonstrates limited efficacy against AOM. Thereby, particularly for protection in the first few months of life, maternal immunization should be explored as a strategy.[52-54] In a mouse model of maternal IN immunization with the NTHi antigen P6 and CTB, anti-P6 specific IgG, IgA and IgM were present at birth and maintained during the nursing periods in sera of P6-immunized mothers.[55] The predominant IgG subclass was IgG2b in both sera and breast milk, followed by IgG1 and IgG2a. The offspring from P6-immunized mothers had anti-P6 specific IgG in their sera at birth, with the predominant IgG subclasses in these breast-fed offspring being IgG2b followed by IgG1 and IgG2a, with the levels of specific IgG1 increasing dramatically after birth until it became the predominant subclass. Low or moderate levels of polysaccharide-specific maternal antibodies in mice immunized with pneumococcal polysaccharides conjugated to tetanus protein (Pnc-TT) provided protection in neonatal and infant mice.[56] A study presented at this conference showed that maternal IN immunization with PspA from *S. pneumoniae* reduced nasal carriage of pneumococci and prolonged survival against a lethal challenge dose in the offspring.[57]

Immunization of pregnant women with the 23-valent pneumococcal polysaccharide vaccine resulted in a significant rise in antibody to the polysaccharides varying from 3.3- to 9.1-fold for individual serotypes. The level of polysaccharide-specific antibody in cord blood was significantly higher in the vaccine group, demonstrating transfer from mother to infant to provide enhanced protection.[58, 59] The recent paper by Healy and Baker[60] reviewed the evidence that supported passage of protective antibody to infants and the potential to use this as a strategy for preventing infection in infants at risk of contracting vaccine-preventable illnesses. The main barriers appear to be fear of association with birth defects. Despite this, there is strong evidence that maternal immunization would be a feasible approach, particularly for providing early protective immunity to high risk children.

## *STREPTOCOCCUS PNEUMONIAE* VACCINE CANDIDATES

*Pneumococcal Capsular Conjugate Vaccines.* The clinical efficacy of pneumococcal conjugate vaccines against AOM in children who were vaccinated as infants (three doses between 2-6 months), followed by one dose at the age of 12-15 months, had been studied in four randomized trials.[21, 26, 61, 62] There was a follow-up of all children, up to the age of 24-27 months. The first two trials in American and Finnish children using PCV7, in which pneumococcal polysaccharides were conjugated to a mutant non-toxic diphteria toxin, showed a modest reduction of AOM episodes of 6-7%.[21, 61] Children prone to develop recurrent

AOM episodes seemed to benefit most from the conjugate vaccine, with 9-16% fewer children experiencing recurrent AOM. With respect to the isolation of *S. pneumoniae* in middle ear fluid, the Finnish study showed a 34% reduction in overall pneumococcal AOM (95% CI, 21% to 65%), a 57% protection (95% CI, 44% to 67%) from AOM caused by pneumoccal serotypes included in the vaccine, and a 51% protection (95% CI, 27% to 67%) from AOM caused by cross-reactive pneumococcal serotypes. However, the overall effect on AOM episodes was hampered because the number of episodes due to non-vaccine serotypes increased by 33 % (95% CI, -80% to 1%), confirming the pathogenicity of these non-vaccine serotypes.

The third trial (also performed in Finland) of a 7-valent conjugate vaccine composed of pneumococcal antigens linked to a meningococcal outer membrane protein, showed no reduction of overall AOM episodes.[62] In this study, the reduction of AOM episodes due to pneumococci was 25% (95% CI, 11 to 37%), while the reduction of AOM due to vaccine-type pneumococci was similar to PCV7 at 56% (95% CI, 44% to 66%). This vaccine failed to show protection against cross-reactive serotypes, however no replacement by non-vaccine serotypes was found.

The fourth study in Czech infants assessed the efficacy of a vaccine that contained polysaccharides from 11 different *S. pneumoniae* serotypes each conjugated to *Haemophilus*-derived protein D. In contrast to the first two studies with PCV7, the overall reduction of AOM episodes of 34% (95% CI, 21% to 44%) was far more prominent. Episodes of vaccine type pneumococcal AOM declined by again a similar 58% (95% CI, 41% to 69%), episodes of AOM due to cross-reactive pneumococcal types by 66% (95% CI, 22% to 85%), whereas, no change in episodes of AOM due to other non-vaccine serotypes was found. An efficacy of 35% (95% CI, 2-57%) was also shown against episodes of AOM caused by *H. influenzae.* This finding, and probably a selection of more severe cases of AOM, may have contributed to the higher efficacy of this conjugate vaccine against overall AOM episodes compared to studies using PCV7. Long term follow-up after vaccination with PCV7 revealed an efficacy for prevention of tympanostomy tube placement of 24% by 3.5 years in the American trial and 39% by 4 to 5 years in the Finnish trial.[25, 63]

Since PCV7 was introduced for routine use in infants and toddlers in The United States in 2000, a 20% reduction in OM visits have been described.[30] Furthermore, the rate of pneumococcal AOM fell by 24-39% for severe or treatment-resistant AOM in post-marketing studies.[64-66] Children born in Tennessee or New-York in 2000-2001, were less likely to develop frequent OM by 17 and 28% respectively, and also PET insertions declined by 16 and 23% as compared with a pre-vaccination birth cohort.[6] Importantly, however, these observations represent an association in time and not necessarily a casual relation study. AOM rates do fluctuate in time, as was shown in previous epidemiological studies. These results match or exceed the results of the randomized, controlled trials and have had important implications on the cost-effectiveness analysis for PCV7.

Based on the results of the pre-licensure trials in The United States and Finland which showed greater efficacy of PCV7 against recurrent AOM, in the year 2000 the American Academy of Pediatrics recommended immunization with PCV7 in all children < 6 years of age with a history of recurrent or severe AOM, as well as in children with tympanostomy tubes due to recurrent AOM. The question whether the results obtained in children vaccinated at infancy and who were followed up to the age of 2 years may be extrapolated to children who are older at the time of vaccination and who have already had recurrent episodes of AOM was addressed by Dutch and Belgium clinical trials.[67, 68] In both randomized controlled trials in children aged 1-7 years, who were vaccinated with PCV7 followed by a booster vaccination with a 23-valent pneumococcal polysaccharide vaccine 7 months later, vaccination failed to reduce the number of AOM episodes. In the Dutch study, the per-protocol analysis after complete vaccination even showed a small but significant increase in AOM episodes (rate ratio 1.29, 95% CI 1.02-1.62). The different outcomes of the studies in healthy infants and those in older children with a history of recurrent AOM may be explained for the greater part by differences in age at which vaccination was first given and the otitis-prone condition of the children included in the Netherlands-Belgium studies. In the infant studies, PCV7 vaccination might prevent or delay

nasopharyngeal acquisition of the most frequent pneumococcal serotypes and, as a consequence, prevent or delay pneumococcal AOM until a later age. As such, this may prevent an early AOM episode, which is a known risk factor for developing recurrent AOM. Furthermore, after pneumococcal vaccination in the otitis-prone children, *S. aureus* was isolated significantly more often from spontaneously draining ears. Based on this finding, others found that NP carriage of *S. aureus* in children appears to be inversely related to the carriage of pneumococcal vaccine serotypes. Finally, otitis prone children showed a lower IgG antibody response to pneumococcal serotype 6B and this finding, together with unfavorable genetically determined factors in innate and adaptive immunity, may hamper vaccine efficacy in this for AOM high-risk group of children.[71]

In conclusion, for prevention of pneumococcal AOM in general, and to protect children from developing recurrent AOM, it is crucial to start pneumococcal conjugate vaccinations early in life, at least before 12 months of age, and preferably before the first AOM episode has occurred. There is no evidence for efficacy of PCV7 in the management of AOM episodes in children older than 12 months of age with a history of recurrent AOM. The role of a polysaccharide booster is still debatable, because in the Dutch study, high antibody titers against serotypes that were included in the polysaccharide vaccine and not in the conjugate vaccine, did not influence NP pneumococcal replacement.

*Pneumococcal Protein Antigens.* Limitations of the current vaccine formulations include the high cost of production, serotype-specific protection, and the potential for replacement disease. Improved and/or alternative strategies would be protein-based vaccines, targeted to conserved proteins. These can be engineered at lower cost, would elicit T cell dependent immune responses, and provide serotype independent protection.[72, 73] Several pneumococcal proteins have been investigated for their use in component vaccines.[72, 73] Key antigens include a mix of surface exposed, cytoplasmic and secreted components (Table 1). Pneumococcal surface protein A (PspA) and pneumolysin (Ply) are the most extensively studied, but other novel protein antigens have recently been identified.[72, 73] Genome sequencing efforts have added significantly to our knowledge of conserved proteins, variable genomic elements and strain diversity. Thus, more information is now available to assist with the selection of preferred vaccine candidates.

*PspA.* PspA is expressed by all pneumococci and was shown to elicit protection in the rat OM model.[74] The α helical domain of PspA is the target of protective antibodies, however, PspA is antigenically variable, being divided into 3 families and 6 clades. Current work aims to identify 2-3 clades for coverage of the majority of important strains. Recombinant antigens from the C-terminal 104 amino acids and 115 N-terminal amino acids of a clade 5 PspA elicited some cross protection in mice.[75]

*Ply.* A pore-forming toxin produced by most of the 90 *S. pneumoniae* serotypes that is cytotoxic in its native form activates host inflammatory responses. Vaccine development has focused on production of nontoxic forms of Ply. A single amino acid deletion, ΔA146 Ply, prevents pore formation in erythrocytes[76] and retains the immunogenicity of native Ply without inflammatory effects *in vivo*. Mice immunized with the Ply mutant survive infection longer when challenged with a lethal dose of TIGR4.[76] As a potentially improved vaccination strategy, deletions were made in capsular polysaccharide (*cps*), *ply*, and *pspA* in a type 6A *S. pneumoniae* isolate.[77] IN administration with these mutant strains showed that a double *ply/pspA* mutant was attenuated and the *cps* mutant was avirulent in a mouse sepsis model. The attenuated strains also induced mucosal protection from challenge with the parent strain with the *cps* mutant eliciting cross-protection against a type 4 strain.[77]

*Pili.* Pili have recently been described in *S. pneumoniae.*[78, 79] Recombinant pilus subunits, RrgA, RrgB, and RrgC, were used to immunize BALB/c mice either alone or in combination[80] resulting in lower bacteremia and higher survival rates when challenged with TIGR4. However, pili are not present in all strains and are antigenically variable, so more work is needed to define the distribution and diversity of the pilus subunits among pneumococcal strains.

*Pht/Php/BVH.* Three laboratories independently named the pneumococcal histidine triad proteins, a group that contains a histidine triad motif that is repeated several times. They are called either pneumococcal histidine triad (Pht),[81] pneumococcal histidine protein (Php)[82] or BVH.[83] PhtA is the same as BVH11-3; PhtB is identical to PhpA and BVH-11; PhtD is the same as BVH-11-2; and PhtE is identical to BVH-3.[73] Immunization with a chimera comprising the C-terminal regions of BVH-3 (PhtE) and BVH-11 (PhtB or PhpA) induced protection in murine models of sepsis and pneumonia.[83]

TABLE 1. POTENTIAL VACCINE ANTIGENS OF *STREPTOCOCCUS PNEUMONIAE*.

| **Antigen** | **Molecular Mass** | **Putative function** | **Conservation among strains** | **References** |
|---|---|---|---|---|
| PspA | 65-kDa | Surface exposed, complement binding | Heterogeneous and conserved regions | 75, 93, 158-160 |
| Ply, PdB | 53-kDa | Cytolytic toxin | Conserved | 76, 93, 161, 162 |
| Pilin subunits RrgA, RrgB, and RrgC | 93, 66, and 40-kDa respectively | Adhesion | Antigenically heterogenous, not present in all strains | 80 |
| Pht, Php, or BVH | 77-88- kDa | Histidine triad proteins, function unknown | Heterogeneous and conserved regions | 83, 93 |
| NanA | 100-kDa | Cleavage of sialic acid | DNA sequence variation | 84, 85, 163 |
| PiuA and PiaA | 34 and 37 respectively | Iron uptake transporters | Highly conserved | 87 |
| PsaA | 35-kDa | Manganese and zinc transport | Highly conserved | 90, 164 |
| PppA | 20-kDa | Homologous to bactoferritins | Highly conserved | 92 |
| L7/L12 | 14-kDa | Ribosomal protein | Highly conserved | 165 |
| GAPDH | 34-kDa | Transferring binding and ADP ribosylating enzyme | Conserved | 165 |
| Phosphoryl-choline | 184 kDa | Structural component, adherence, mimicry | Highly conserved moiety | 166 |

*NanA.* Recombinant neuraminidase A (NanA) immunization gave a 33% reduction in the incidence of OME in the chinchilla model.[84] Recombinant NanA has been shown to eliminate carriage of pneumococci from the NP of chinchillas earlier than from controls.[85]

*PiuA/PiaA.* The iron uptake ABC transporters PiuA and PiaA were shown to protect mice from systemic pneumococcal infection,[86] elicit effective serum antibodies in the respiratory tract[87] and combined IN immunization resulted in greater survival when challenged with *S. pneumoniae.*[87]

*PsaA.* The highly conserved protein, pneumococcal surface antigen A (PsaA),[88] has homology with viridans group streptococcal proteins,[89] attracting concern over potential disruptions to the normal flora from cross-reactivity. IN immunization with a CTB-PsaA fusion prevented pneumococcal colonization of mice and did not significantly change the oral or NP flora.[90] This is not an indication that the same lack of effect will occur in humans. It has been shown that PsaA and a putative protease maturation protein A (PpmA) may not be accessible to circulating antibodies and may not protect well against systemic infection,[91] highlighting questions about the significance of PsaA as a vaccine candidate.

*Phosphorycholine (ChoP).* is expressed on the cell surface of many commensal and pathogenic bacteria in the nasopharynx. ChoP is being investigated as a potential vaccine target for both *S. pneumoniae* and *H. influenzae* vaccine, and is discussed in more detail under NTHI vaccine candidates.

*Other Candidates.* A novel 20-kDa protein pneumococcal protective protein A (PppA); the L7/L12 ribosomal protein, and a glyceraldehyde-3-phosphate dehydrogenase (GAPDH). IN immunization of BALB/c mice with rPppA protein decreased NP colonization.[92] The L7/L12 ribosomal protein and GAPDH protected mice in mucosal and parenteral immunization models[74, 89] (and unpublished).

*Combinations.* In animal model studies, no single protein has been shown to elicit protection comparable to conjugate vaccines, so a combination antigen approach may be required. Various combinations of PhtB, PhtE, PspA, pneumococcal surface protein C (PspC), and pneumolysin toxoid (PdB) were used in both BALB/c and CD1 mouse models against serotype 2 and 6A strains and shown to provide superior protection above the individual antigens.[93] Three antigens, PdB, PspA, and PspC, provided the best protective combination. In addition to the antigen combination, the challenge serotype and the mouse strain also affected the results. These results provide significant support to the concept of a multi-component vaccine.

## NONTYPEABLE *HAEMOPHILUS INFLUENZAE* VACCINE CANDIDATES

*"Proof of Principle" of Vaccines for H. Influenzae Otitis Media.* Perhaps the most exciting progress in the last 4 years was the demonstration of partial protection with an *H. influenzae* antigen in a clinical trial. In a study performed in the Czech Republic, Prymula and colleagues[26] tested the efficacy of an 11-valent vaccine in which pneumococcal capsular polysaccharides were conjugated to protein D, a conserved outer membrane protein of *H. influenzae*. This randomized, prospective, placebo controlled trial, demonstrated 35% efficacy for *H. influenzae* OM. This was the first demonstration of an effective vaccine antigen for *H. influenzae* OM in a clinical trial. Although the level of 35% efficacy will need improvement, this important result provides evidence of the feasibility of developing an effective vaccine for *H. influenzae* OM. Including more than one *H. influenzae* antigen in a vaccine formulation would be a rational approach to increasing the level of protection observed.

*Distinguishing H. Influenzae from H. Haemolyticus in Cultures:* In a study that has broad implications in the performance and interpretation of results of vaccine trials, Murphy and colleagues[94] have shown that methods used routinely in clinical microbiology laboratories worldwide do not accurately distinguish *H. influenzae* from non haemolytic *H. haemolyticus*. They further showed that *H. haemolyticus* is not a pathogen but, rather, is a common commensal in the human upper respiratory tract. It is important to interpret results of studies of NP colonization in the context of this

observation wherein a substantial proportion of strains reported as *H. influenzae* are actually *H. haemolyticus*. Furthermore, as the effect of putative vaccines on NP colonization by *H. influenzae* is assessed, it will be important to use laboratory methods that accurately identify this microbe.

*Putative Vaccine Antigens - An Update.* The following sections, and Table 2, provide a brief summary of progress made in the last 4 years to identify and develop putative vaccine antigens for NTHI-induced OM.

TABLE 2. POTENTIAL VACCINE ANTIGENS OF NONTYPEABLE *HAEMOPHILUS INFLUENZAE*.

| Antigen | Molecular Mass | Putative function | Conservation among strains | References |
|---|---|---|---|---|
| HMW 1<br>HMW 2 | 120-125 kDa | Adhesins, Homologous to FHA of *Bordetella pertussis*, | Heterogeneous and conserved regions | 95-97, 167-169 |
| Hia | 115 kDa | Adhesin, Hsf in type b strains | | 98-101 |
| Hap | 155 kDa | Adhesin, IgA protease-like | | 102 |
| P2 | 36-42 kDa | Porin, binds mucin, Most abundant surface antigen. | Heterogeneous and conserved regions | 109 |
| P5 | 27-35 kDa | Adhesin, binds mucin, OMP A like protein | Heterogeneous and conserved regions | 103, 104 |
| LPD | 42 kDa | Glycerophosphodiesterase | Highly conserved | 26, 112, 113 |
| P4 | 30 kDa | Phosphomonoesterase | Highly conserved | 114-117 |
| HtrA | 46 kDa | Heat shock protein | | 111 |
| OMP 26 | 26 kDa | Translocation of OMPs and LOS, Skp family | | 104, 170 |
| P6 | 16 kDa | Cell wall integrity | Highly conserved | 55, 115, 118-126 |
| Lipooligo-saccharide | 2.5-3.3 kDa | Endotoxin | Antigenically heterogeneous | 126, 127, 171 |
| Phosphoryl-choline | 184 Da | Adherence, host cell mimicry | Highly conserved moiety | 128, 166 |
| Type IV pilin protein (PilA) | 15.6 kDa | Adherence, biofilm formation, competence and twitching motility | Highly conserved | 129, 130 |

*HMW1/HMW2 Family.* Winter and coworkers reported on the functional activity of human antibodies specific for the HMW1/HMW2-like proteins using an *in vitro* complement-dependent opsonophagocytic assay.[95] This study demonstrated that human antibodies specific for the HMW1/HMW2-like proteins are opsono-phagocytic and that they recognize epitopes shared by the HMW proteins of unrelated strains. In a follow-up study, these same investigators measured the opsono-phagocytic activity of high-titer anti-HMW1/HMW2 chinchilla immune sera against a panel of homologous and heterologous NTHI strains.[96] They found that antibodies against the HMW1/HMW2-like proteins are opsono-phagocytic for both homologous and heterologous strains and suggest that common epitopes recognized by functionally active antibodies exist on the HMW1/HMW2-like proteins of unrelated NTHI strains. Further, the two binding domains of HMW1 and HMW2 have been localized in regions of maximal sequence dissimilarity (40% identity, 58% similarity). Recently, Guifre and coworkers[97] demonstrated two distinct clusters for the HMW1-like and HMW2-like core-binding domain sequences.

*Hia Family.* The Hia autotransporter is an adhesive protein that promotes adherence to respiratory epithelial cells. Hia adhesive activity resides in two homologous binding domains, called HiaBD1 and HiaBD2. Interesting new observations on structural characterization and elucidation of mechanisms of translocation of the adhesin have been made in the last 4 years.[98-101]

*Hap Family.* The ability to immunize with a fragment of the autotransporter protein Hap to protect against experimental NTHI infection was examined.[102] Liu and colleagues immunized mice with recombinant proteins corresponding to the C-terminal region of Hap(S) and demonstrated that the C-terminal region of Hap(S) is capable of eliciting cross-reacting antibodies that reduce NP colonization, suggesting possible use as an NTHI vaccine antigen.

*OMP P5 (Also Called P5 or OMP P5-Homologous Adhesin).* Is one of several adhesins expressed by NTHI. In 2003, Novotny and Bakaletz[103] demonstrated a hierarchical pattern of immunodominance among four predicted surface-exposed regions located in the N-terminal half of P5, with region 4 being the most immunodominant epitope and thus inducing the greatest immune recognition in chinchillas and children during experimental or natural disease, respectively. However, whereas antibody to region 4 was not protective, refocusing this natural but non-protective immune response towards subdominant region 3 resulted in significant protection against ascending OM in a chinchilla model. These data suggested that region 4 serves as a highly immunodominant but non-protective 'decoying' epitope and thus facilitates the ability of NTHI to evade the host's immune response. Moreover, these findings supported the continued development of P5 region 3-derived vaccine candidates.

*OMP 26.* A 26 kDa outer membrane protein, has also been actively pursued as a vaccine candidate. In a study designed to further evaluate the efficacy of OMP26 as well as that of P5-derived vaccine candidates, Kyd and colleagues[104] evaluated these candidates in reciprocal animal model systems. Briefly, OMP26, whose efficacy had largely been demonstrated in rat models of mucosal immunization and OMP P5, whose efficacy had been evaluated primarily in chinchilla models of parenteral immunization were now evaluated by the reciprocal route of immunization and using alternative measures of determining relative pathogenesis and protection. Both immunogens were found to induce high-titered and specific immune responses in the heterologous animal model system and each was found to be highly efficacious, yielding protection in a second rodent host. These observations provided support for the continued development of both OMP26- and P5-derived peptides as candidate vaccinogens.

*OMP P2.* P2 is the most abundant surface protein, comprising approximately half of the protein content of the outer membrane. Antibodies to P2 are protective in the chinchilla model of OM and in the infant rat model of *H. influenzae* type b infection.[105, 106] Furthermore, P2 is the target of potentially protective human serum bactericidal antibodies.[107, 108] However, a limitation of P2 as a vaccine antigen is marked sequence heterogeneity in several surface exposed loops resulting in strain specific immune responses. Neary and Murphy[109] evaluated peptides corresponding to antigenically conserved surface-exposed loops of P2 as a

potential vaccine approach. Antibodies directed at a conserved motif in loop 6 showed bactericidal activity against 13 of 20 strains of nontypeable *H. influenzae*. These results suggest that conserved regions of P2 are potential vaccine antigens.

*Htr Protein.* Htr is a heat shock protein with serine protease activity that has been shown to induce protective responses in the chinchilla model of OM.[110] Alonso et al.[111] genetically fused Htr of *H. influenzae* to filamentous hemagglutinin of *Bordetella pertussis*. Mice challenged with *B. pertussis* that was engineered to express recombinant Htr-FHA induced serum antibody responses to Htr. It will be important to determine whether this formulation induces protective responses.

*Protein D.* All strains of *H. influenzae* express a highly conserved surface lipoprotein called lipoprotein D. As noted above, an 11-valent vaccine consisting of pneumococcal polysaccharides conjugated to protein D of *H. influenzae* was tested in a clinical trial.[26] The vaccine was composed of recombinant delipidated protein D conjugated to pneumococcal polysaccharides. Efficacy of 35% was observed for *H. influenzae* OM, providing evidence for the first time in a human trial that vaccination is capable of protecting against *H. influenzae* OM. Analysis of serum samples from children in this trial will provide the valuable opportunity to identify correlates of protection. Such observations would substantially advance the field of vaccine development and testing for NTHI.

In one such study to address the issue of identifying a correlate of protection, Novotny et al.[112] passively immunized chinchillas with serum from children in this clinical trial. Passive immunization conferred approximately 34% protection against ascending *H. influenzae* OM in a viral-bacterial co-infection model. The similarity in the level of protection in children and in the chinchilla model support this model as one that may predict outcomes of clinical trials and thus act as a correlate of protection. More such studies to further elucidate correlates of protection should be an area of priority to guide vaccine development.[113]

*OMP P4.* P4 is an ~30 kDa surface protein that is highly conserved among strains and induces bactericidal antibodies in experimental animals. Since the last update, Hotomi et al.[114] demonstrated that IN immunization with recombinant P4 induced mucosal antibodies, bactericidal antibodies and resulted in clearance of *H. influenzae* from the NP of mice. In a second study, IN immunization of mice with a combination of P4 and P6 along with the *M. catarrhalis* antigen UspA2 reduced nasal colonization by *H. influenzae.*[115] These two studies advance our assessment of P4 by demonstrating that mucosal immunization is a potentially fruitful approach.

P4 is also an enzyme with phosphomonoesterase activity. Green et al.[116] used site-directed mutagenesis to abrogate enzymatic activity. This enzymatically inactive protein was still fully capable of generating potentially protective bactericidal antibodies. Finally, Ou et al.[117] reported the crystallization of P4 which will facilitate structural analysis of this promising vaccine antigen.

*Outer Membrane Protein P6.* Five new studies from four different research groups all showed that mucosal immunization with P6 induced protective responses in mice or rats using various endpoints of protection.[55, 118-121] This new work adds to the overwhelming body of evidence that P6 induces protective immune responses in multiple animal model systems via multiple routes of immunization.

Several studies elucidated the role of this important putative vaccine antigen in pathogenesis and immunity. Construction and analysis of a mutant deficient in P6 revealed that P6 is important in bacterial cell wall integrity.[122] Chen et al.[123] showed that P6 up regulates transcription of mucin genes. Berenson et al.[124] showed that P6 is a potent inducer of human macrophage pro inflammatory cytokines. Finally, McMahon et al.[125] demonstrated that P6 contains a single immunodominant T cell epitope that is important in protective immune responses induced by immunization of mice with P6.

*Detoxified Lipooligosaccharide (dLOS).* LOS is a virulence factor as well as a vaccine component. The dLOS based-protein conjugates from NTHi have been investigated for years as candidate vaccines against NTHi and one of them, dLOS-tetanus toxoid (TT), confirmed as a safe and immunogenic vaccine in healthy adults. Efforts have been made to further improve the conjugate

vaccines by including a new P6 protein carrier. The resulting dLOS-P6 conjugates elicited both anti-LOS and anti-P6 IgG antibodies in mouse and rabbit models. Similar to previous dLOS-protein conjugates, the rabbit antisera elicited by the dLOS-P6 showed bactericidal activity against the homologous strain and three of five major serotype strains.[126] In addition, IN immunization with the dLOS-TT conjugate demonstrated that the vaccine could elicit both mucosal and systemic immunity against NTHI and provided protection in a mouse model of bacterial clearance in NP and lungs. Further kinetic studies of humoral and cellular immune responses to the IN immunization with the dLOS-TT revealed useful information as effective mucosal vaccines against NTHI infections.[127]

*Phosphorylcholine (ChoP).* Phosphorylcholine (ChoP) is an antigenic epitope of NTHi LOS, which is not only related to bacterial virulence but also to host protection. This structure is also a common component of a wide variety of human pathogens. Human ChoP-specific antibody (IgG2) recognizes ChoP on the LOS of *H. influenzae* and on the lipoteichoic acid of *S. pneumoniae.*[128] The human antibody was effective against some clinical isolates of NTHi and isolates of several common serotypes of *S. pneumoniae* in *in vitro* killing assays. In a recent study, BALB/c mice immunized IN with ChoP-keyhole limpet hemocyanin (KLH) plus cholera toxin (CT) showed increased ChoP-specific IgM in serum and IgA in nasal wash and saliva.[126] The salivary IgA antibodies reacted to most strains of *S. pneumoniae* and *H. influenzae* tested. Furthermore, the clearance of *S. pneumoniae* and *H. influenzae* from the nasal cavity was significantly enhanced. Thus, anti-ChoP specific immune responses might help to prevent upper airway infections caused by *S. pneumoniae* and *H. influenzae*.

*Type IV Pili.* In 2005, Bakaletz et al.[129] demonstrated that NTHI expressed a type IV pilus (Tfp) that was dependent upon the products of the *pilA* and *comE* genes and that Tfp expressed by NTHI strain 86-028NP are functional, mediating twitching motility. In a follow-up study,[130] this group showed a critical role for NTHI Tfp in adherence and colonization of the mammalian upper respiratory tract.[41] These observations, combined with a demonstration that all clinical isolates examined to date contain a single well-conserved *pilA* gene, suggested that the PilA protein might serve as an interesting target for vaccine development efforts. Studies to demonstrate the immunogenicity and protective capability of NTHI type IV pilin-derived vaccine candidates were presented at this symposium.[131, 132]

## *MORAXELLA CATARRHALIS* VACCINE CANDIDATES

*Should Vaccine Development for M. Catarrhalis Proceed?*: *M. catarrhalis* causes a substantial proportion of AOM and is present in the middle ear of many children with OME. Furthermore, a recent study by Revai et al.[133] demonstrated that the frequency of NP colonization by *M. catarrhalis* in children with OM has increased with the widespread use of the pneumococcal conjugate vaccine, suggesting that the incidence of *M. catarrhalis* OM may increase. Reduction of OM due to *S. pneumoniae* and *H. influenzae* resulting from new vaccines may result in a further increase in the incidence of OM due to *M. catarrhalis*. Therefore, in order to have the greatest impact on reducing OM overall, effort and resources should be devoted to development of *M. catarrhalis* vaccine antigens so they can be included with *S. pneumoniae* and *H. influenzae* antigens to be tested in clinical trials. In this way, the likelihood that large expensive trials that will be required to test vaccines for OM will not have to repeated separately or subsequently with *M. catarrhalis* vaccines.

*Protective Immune Responses to M. Catarrhalis.* Since the last update,[20] little progress has been made in defining the elements of protective immune responses to *M. catarrhalis* OM. Some progress regarding protective immune responses to *M. catarrhalis* in adults with chronic obstructive pulmonary disease (COPD) has however been made recently, and perhaps some of these observations may guide such work in the clinical setting of OM.[134-136] Adults with COPD who acquire *M. catarrhalis* clear the bacterium efficiently and develop strain- specific protection from reacquisition of the same strain. Patients make serum IgG and/or sputum IgA responses to surface antigens of their homologous strains. The development of a sputum IgA response is

associated with reduced symptoms of exacerbation, suggesting that a mucosal immune response may have a protective effect. Characterizing the elements of a protective immune response to *M. catarrhalis* infection should be given high priority. Identifying a correlate of protection for *M. catarrhalis* is another area of research that should be given high priority.

*Candidate Vaccine Antigens.* An area of substantial progress over the last several years has been the identification of putative vaccine antigens of *M. catarrhalis*. Table 3 lists vaccine antigens that have been demonstrated to induce potentially protective responses which, for the purpose of this update, is defined as either enhanced clearance in the mouse pulmonary challenge model or induction of bactericidal antibodies. The antigens listed in Table 3 are ready for production to be tested in human trials. In addition to these established antigens, Murphy and colleagues presented new data at this meeting in which they employed a genome mining approach to identify five new antigens that induce protective responses.[137] Table 4 lists antigens that have characteristics to suggest that they may represent good vaccine antigens but that have not yet been demonstrated to induce potentially protective responses.

TABLE 3. CANDIDATE VACCINE ANTIGENS OF *M. CATARRHALIS* THAT INDUCE POTENTIALLY PROTECTIVE IMMUNE RESPONSES.

| **Antigen** | **Molecular mass** | **Putative Function** | **Conservation among strains** | **Evidence of Protection** | **References** |
|---|---|---|---|---|---|
| UspA1 | 88 kDa (oligomer) | Adhesin, binds laminin | Heterogeneous and conserved regions, phase varies | Bactericidal, mouse pulmonary clearance | 172-180 |
| UspA2 | 62 kDa (oligomer) | Binds complement components and laminin | Heterogeneous and conserved regions | Bactericidal, mouse pulmonary clearance | 115, 173-176, 178, 180-185 |
| MID/Hag | 200kDa | Adhesin, binds IgD | Heterogeneous and conserved regions | Mouse pulmonary clearance | 173, 174, 179, 186-196 |
| TbpB/OMP B1 | 80-85 kDa | Binds transferrin | Heterogeneous and conserved regions | Bactericidal, Mouse pulmonary clearance | 197-199 |
| CopB | 80 kDa | Iron uptake | Present in ~70% of strains. Five sequence types. | Bactericidal, Mouse pulmonary clearance | 143 |
| OMP CD | 45 kDa (~60 kDa) | Porin, binds mucin, adhesin, OMP A-like protein | Highly conserved | Bactericidal, mouse pulmonary clearance | 142, 200-203 |
| dLOS | 2.5 - 4 kDa | Endotoxin | Three serotypes | Mouse pulmonary clearance | 140-142, 204, 205 |

TABLE 4. CANDIDATE ANTIGENS OF *M. CATARRHALIS* WITH CHARACTERISTICS OF VACCINE ANTIGENS.

| Antigen | Molecular mass | Putative Function | Conservation among strains | References |
|---|---|---|---|---|
| McmA | 110,000 kDa | Adhesin | Unknown | 206 |
| MhaB1, MhaB2 | 184 kDa 201 kDa | Adhesin, filamentous hemagglutinin-like | Present in 63% of strains; conservation not known | 144 |
| McaP | 66 kDa | Adhesin, phospholipase B | Highly conserved | 207, 208 |
| OMP E | 50 kDa | Fatty acid transport | Highly conserved | 209-211 |
| M35 | 36.1 kDa | Porin | Conserved with one variable loop | 212 |
| OMP G1a | 29 kDa lipoprotein | Copper transport | Highly conserved | 213, 214 |
| OMP G1b | 29 kDa | Unknown | Highly conserved | 213, 215 |
| OlpA | 24 kDa | Opa-like protein | Highly conserved | 138 |
| OMP J | 19 kDa and 16 kDa | Opa-like protein | Exists in 2 forms | 216 |

*OlpA.* An interesting new 24 kDa outer membrane protein called OlpA was discovered this year by Brooks and colleagues.[138] OlpA has homology to Opa proteins adhesins of *Neisseria* however, in contrast to *Neisseria* Opa proteins, the *M. catarrhalis* OlpA is quite conserved among strains and does not appear to have adhesin function.

*LOS.* Peng et al.[139] constructed a mutant of *M. catarrhalis* that is completely devoid of lipooligosaccharide by knocking out the gene responsible for the first step of lipid A biosynthesis. The mutant is viable, shows reduced adherence to epithelial cells but increased sensitivity to killing by normal human serum. Of interest, the mutant showed markedly reduced toxicity and induced bactericidal antibodies and protection in the mouse pulmonary clearance model after SQ immunization with whole bacteria. Since the last update, Gu and colleagues have constructed detoxified LOS vaccines of serotypes B and C and demonstrated that they induce protective responses.[140, 141] Therefore, dLOS preparations have now been made for each of the three LOS serotypes. In addition, the *M. catarrhalis* surface proteins OMP CD and UspA1 have been used as carrier proteins for the dLOS preparations.[142] It will be important to assess whether dLOS vaccines induce protective responses against many strains.

*CopB.* Liu et al.[143] recently showed that there is significant diversity in what appears to be the major immunodominant epitope of the CopB protein, which could have a negative effect on the vaccine potential of this OMP.

*FHA-like Protein.* Two groups have identified a locus in *M. catarrhalis* that encodes a large adhesin protein that is homologous with the filamentous hemagglutinin (FHA) of *Bordetella pertussis*.[144, 145] Although the gene is not present in all strains, this protein deserves further investigation as a potential vaccine antigen by virtue of its similarity to FHA which is a component of acellular pertussis vaccines.

## VIRAL VACCINES

*Influenza A Virus Vaccines.* The consideration of viral vaccines in the prevention of AOM is based on ample evidence that viral infection plays a crucial role in the initiation of events that finally lead to development of bacterial AOM. Prevention of the preceding viral infection could reduce substantially the incidence of bacterial AOM.

At present, influenza vaccine is the only commercially available vaccine for the control of respiratory virus infections, but it might be one of the most effective viral vaccines for prevention of AOM. A recent prospective 2-year study among outpatient children demonstrated that AOM developed as a complication in approximately 40% of children younger than 3 years of age and in 20% of children aged 3-6 years.[146]

The current preparations licensed for use in young children are inactivated split or subunit virus vaccines. These vaccines are administered IM and they can be given to children older than 6 months of age. In June 2003, a nasally administered live attenuated influenza vaccine was approved in the United States. For safety considerations, however, until recently the use of this vaccine was restricted to persons aged 5-49 years, and therefore could not be used in young children in whom the incidence of AOM is highest. In September of 2007, the U.S. Food and Drug Administration expanded the approved use of this vaccine to include children as young as 2 years old without history of asthma or recurrent wheezing.

Since the last report, two studies have assessed the efficacy of inactivated influenza vaccine in preventing AOM. Hoberman et al.[147] carried out a randomized, double-blind, placebo-controlled 2-year study among 786 children aged 6-24 months. During the second season, 70% of the children had received at least one dose of pneumococcal vaccine. Overall, no significant differences were observed between the vaccine and placebo groups in the proportions of children with at least one episode of AOM during the entire 2-year study. However, the primary analysis was hampered by the fact that there was so little influenza activity during the second year of the study that even the efficacy of influenza vaccine against influenza infection *per se* could not be shown. When the analyses were limited to the first year when there was substantial influenza activity, the efficacy of influenza vaccine against influenza-associated AOM was 62%.[148] Ozgur et al. evaluated the efficacy of inactivated influenza vaccine in preventing AOM in children 6 to 60 months of age who attended day care.[149] This study was a prospective, single-blind study including a total of 119 children (61 vaccinees and 58 controls). During the influenza season, 8 (13%) vaccinated children and 16 (28%) control children had at least one episode of AOM, which translated into vaccine efficacy of 51% against AOM.

Although live attenuated influenza vaccine is not currently approved for children younger than 5 years of age, a recent study by Belshe et al.[150] suggested that this vaccine might afford even more protection against AOM than the inactivated influenza vaccine. In this trial, a total of 8,475 children 6 to 59 months of age were enrolled to receive intranasally either a refrigeration-stable formulation of cold-adapted trivalent live attenuated influenza vaccine or trivalent inactivated vaccine in a double-blind manner. Overall, the live attenuated vaccine was superior to the inactivated vaccine in preventing influenza infection, as demonstrated by 54.9% fewer cases of culture-confirmed influenza in the group that received live attenuated vaccine than in the group that received inactivated vaccine (153 vs. 338 cases, $P<0.001$). The superior efficacy of live attenuated vaccine was observed for both antigenically well-matched and drifted viruses. The relative efficacy of the live attenuated vaccine in preventing OM was 50.6% when compared with the inactivated vaccine; the main difference occurred with influenza infections caused by strains that were antigenically dissimilar to the strains included in the vaccine. This study did not include a placebo arm, and thus it is impossible to draw any conclusion about the actual efficacy of either vaccine to prevent AOM in comparison with unvaccinated children.

An obvious inference of the influenza vaccine trials is that effective vaccines against other major viruses predisposing to AOM, especially against RSV, could also afford a substantial benefit. Nonetheless, in developing live viral vaccines to be used for prevention of AOM, it is important that such vaccines are sufficiently attenuated so as not to induce the development of AOM as an adverse event of the vaccination.

*Respiratory Syncytial Virus Vaccines*. RSV is undoubtedly one of the major viruses predisposing a child to AOM,[151] and therefore an effective vaccine against RSV might have a substantial impact on the incidence of AOM in infants and young children. Although the seriousness of RSV illness and the need for an RSV vaccine are widely recognized, the development of vaccines against this virus has been hampered by the unfortunate experiences associated with the use of formalin-inactivated RSV vaccine in the 1960s. Further obstacles in RSV vaccine development include difficulties associated with development of animal models, the immunologic immaturity of neonates as the target population, the potential confounding of immunogenicity caused by maternal antibodies, and the existence of two subgroups of RSV.[152] Despite several different vaccine formulations that have been studied (e.g., live attenuated vaccines, subunit vaccines with or without adjuvants, and virus-vectored or DNA vaccines), no RSV vaccine candidate has yet entered wide-scale clinical trials.

Only live attenuated vaccines have been investigated in young seronegative infants - the most important target group for an RSV vaccine. Recently, Karron et al. reported development of a new recombinant live attenuated RSV vaccine.[153] The vaccine appeared sufficiently attenuated and well tolerated in infants. The study also showed that modern recombination technology can be used effectively to create new vaccine candidates. Further studies will be required to demonstrate the clinical efficacy of this vaccine in preventing RSV illness and RSV-associated AOM.

Maternal immunization has been considered as an alternative approach of preventing RSV infections in infants. Munoz et al. studied the safety and immunogenicity of an RSV fusion protein-2 vaccine in 35 pregnant women in their third trimester and their offspring.[154] The vaccine was safe and well tolerated by the women, and all 35 children were born healthy. The infants were followed during their first RSV season for occurrence and severity of respiratory illnesses, and no increase in the frequency or morbidity associated with respiratory illnesses was observed in the infants of vaccine recipients.

*Parainfluenza Virus Vaccines.* Several types of parainfluenza virus vaccines have been developed and tested in animal models, but primarily only human live attenuated parainfluenza virus type 3 (PIV3) and bovine PIV3 vaccines have undergone clinical evaluation. Both of these vaccines have been previously shown to be satisfactorily attenuated, immunogenic, and phenotypically stable in infants and young children. Madhi et al.[155] studied the transmissibility, infectivity, and immunogenicity of a live attenuated, cold-passaged, IN administered PIV3 vaccine that has been previously tested extensively in phase I and II trials in humans. A total of 80 subjects were enrolled in playgroups in which the vaccinees were in close contact with seronegative placebo recipients. No child fulfilled the criteria for transmission of the vaccine virus. The results demonstrated that the attenuated virus was less infectious than wild-type human PIV3, which could pave way for further clinical development of this vaccine.

Greenberg et al.[156] conducted a phase II trial to assess in young infants the safety, tolerability, infectivity, and immunogenicity of an IN administered bovine PIV3 vaccine. The study included 192 healthy 2-month-old infants. The safety profile of the vaccine was similar to that of placebo, except for fever that was more common in the vaccine recipients.

*Passive Prophylaxis.* Passive administration of antibodies has been proposed as one way to prevent AOM in children. Epidemiologic studies have suggested that high levels of transplacentally acquired antibodies protect infants from severe RSV infections, and a recent survey strongly indicated that maternal antibodies in breast milk may protect the child from enterovirus infections.[157] However, a previous trial assessing the efficacy of intravenously administered RSV-enriched immune globulin in prevention of AOM did not produce convincing data, and it is obvious that this approach is not feasible in everyday clinical practice.

Palivizumab is a recombinant humanized monoclonal antibody directed against the F glycoprotein of RSV. It has been shown to reduce RSV-related hospitalizations in premature children. However, palivizumab does not prevent RSV infection *per se* nor has it been shown to reduce the incidence of AOM.

## BARRIERS TO PROGRESS

*Technology.* The technical features that need to be resolved in the development of safe and effective bacterial and viral vaccines of importance in prevention of OM are outlined in prior sections. Although vaccines directed at viral pathogens and the major bacterial pathogens [pneumococcus, non-typeable *H. influenzae* (NTHI) and *M. catarrhalis*] are of the most value in prevention of AOM in infants and children, these vaccines would also be of value for adults with recurrent and severe sinusitis, AOM, as well as infectious pneumonia in older adult populations and in high-risk groups such as chronic obstructive pulmonary disease patients. In addition to the technical issues associated with development of specific vaccines are generic issues relevant to dose sparing strategies, use of adjuvants, assessment of different routes of administration and other features that facilitate use of vaccines to prevent disease in individuals and communities.

*Difficulty of Clinical Trials of Efficacy and Safety of Vaccines.* An otitis media vaccine could be based on an already available pneumococcal vaccine with added antigens for *S. pneumoniae*, NTHI and *M. catarrhalis,* rather than separate pneumococcal, NTHI, and *M. catarrhalis* vaccines. The addition of antigens to the available pneumococcal conjugate vaccines would have many formulation and stability issues, and would require determination of optimal dose and schedule, in addition to assurance that the NTHI and *M. catarrhalis* antigens are individually safe and effective and do not affect the efficacy of the pneumococcal conjugate vaccines. Alternatively, the new *S. pneumoniae,* NTHI and *M. catarrhalis* antigens could be combined in a separate vaccine. In addition to prevention of disease, an OM vaccine would need to prevent or greatly reduce carriage of the pathogen as has been demonstrated for the pneumococcal conjugate vaccine (PCV7). Preliminary clinical trials of vaccine candidates evaluation could look at impact on carriage (natural or following nasal challenge) as a possible surrogate endpoint.

Clinical trials of promising vaccines would require adherence to criteria for AOM as provided by authoritative groups such as the American Academy of Pediatrics and the American Academy of Family Physicians. The trial would also require a microbiologic endpoint for episodes of AOM that occur after immunization. The microbiology of the episode would be provided by an aspirate of middle ear fluid (tympanocentesis) at least for the first episode of AOM subsequent to immunization. Ethics panels would need to be assured of the need for the safety of tympanocentesis. It is likely that a study incorporating tympanocentesis would get consent only from parents of children with severe or recurrent disease. Thus, the development of an OM vaccine will face substantial technical hurdles and will be a long and expensive process. Manufacturers would need to be assured that such a vaccine was economically feasible and supported in an already crowded vaccine schedule for infants in The United States.

*Perception of Otitis Media as a Disease of Importance.* Recent studies concluding that AOM is a self-limited disease that may be managed by observation rather than antimicrobial therapy have resulted in a general impression among the public and some investigators that OM is a benign disease and that children do not suffer. If the disease does not require treatment, the perception is that it is a disease of limited importance. The results of the studies that indicate OM need not be aggressively managed are believed by many to be flawed: there was an absence of appropriate diagnostic criteria, thus enrolled children may not have had AOM; children enrolled in the studies were usually older than two years of age and had minimal signs (the majority afebrile) that would suggest, again, a group of children likely to have less disease or no disease. In the study of Little and colleagues, a difference of one day of pain or fever extended by delayed treatment was not appreciated as being of importance to the investigators (who suffered no pain).

The Epidemiology panel for this Post-Symposium Research Conference has identified current data about the continued incidence of AOM and the cost to parents and the health system of management. Investigators need to continue research into the appropriate role of therapy for accurately diagnosed OM so that the perception of OM as a benign disease that may be observed rather than treated can be dispelled.

*Government Funding.* The NIH and other government sources of funding have a multitude of

areas of medical need competing for limited funds, and as such, investigation of AOM has not been a high priority for the funding agencies. Investigators and interested individuals must be advocates for funding research in OM and its prevention so that NIDCD and other Institutes can provide consistent support needed for infrastructure, basic science and clinical programs necessary for vaccine development and clinical trials.

*Industry.* Vaccine development timings and costs have increased significantly. However, the documentation of the vaccine protective effects and mechanisms represent a major question that needs to be addressed from preclinical research to clinical efficacy studies. Investigators need to continue to monitor the epidemiology and microbiology of OM and develop cost-effective models that can provide incentives for industry to invest in costly research and development of vaccines that prevent OM.

*Intellectual Property and Proprietary Rights.* Since much of research in large and small biotechnology firms is proprietary and the information is unavailable to the community of investigators until patent protection becomes available, there may be waste of labor, time and money in achieving common goals. The intellectual property rights issue may be resolved by *agreement at onset* of how much antigens, adjuvants and other components of the vaccine are valued and how royalties are to be divided among the participants in the program.

*Lack of Insight Into Prevention of Otitis Media by Immunization.* Lack of understanding of the immunology of acute and chronic OM continues to be an obstacle to progress in development of effective vaccines. The immunology of OM has been extensively studied over the past 5 decades but we still lack insight into the qualities of antigens that would provide an optimal systemic and local protective response. Experience with one conjugate pneumococcal vaccine (PCV7) identifies some of the perplexities of vaccine protection against OM: PCV7 had more than 90% efficacy against invasive pneumococcal disease due to vaccine serotypes but was only 52% effective in prevention of AOM episodes due to the same vaccine serotypes. In addition there was an increase in AOM due to non-vaccine pneumococcal serotypes and NTHI in children immunized with PCV7. On the contrary, an 11-valent polysaccharide-proteinD conjugate vaccine (PCV11), which showed 57.6% efficacy in protection of AOM episodes due to vaccine types did not show an increase in AOM due to non-vaccine pneumococcal serotypes. Moreover, this vaccine which showed 35.3% efficacy against AOM episodes due to NTHI.[26] This result demonstrates that protection against NTHi is feasible and that animal models such as the chinchilla model of OM which showed the protection of PCV11 against NTHI OM are important tools that could help in developing new vaccines.[112] Other questions remain unanswered to date. Is it possible that some children with risk features for severe and recurrent AOM will not be protected by this or other vaccines – or is it that our understanding of prevention of OM by immunization is incomplete? Moreover, a better understanding of the role of biofilm in AOM and recurrent otitis media would allow the scientific community to best design prevention strategies.

## SHORT-TERM AND LONG-TERM GOALS

1. Industry-academic-government partnerships, with a specific focus on the development of vaccines for OM should be strongly encouraged in order to evaluate promising vaccine candidates in clinical trials. A multi-center approach should be emphasized since it is unlikely that any one site would have all the required skills in OM epidemiology, microbiology and immunology necessary to study viral and bacterial pathogens and measure correlates of protection.

2. Governments should take leadership in developing programs that support the training of investigators in the conduct of OM clinical trials and PI's should similarly submit training grants to achieve this goal.

3. Increased public and private funding is needed to support translational (bench to bedside) research to get vaccine candidates into clinical studies quickly.

4. Mucosal delivery systems should continue to be developed for all OM vaccine candidates and the usefulness of new vaccine adjuvants (with a particular focus on adjuvantation appropriate for infants) for both mucosal and parenteral delivery, as well as alternative novel delivery systems (i.e., transdermal), should be explored to enhance the immune response to vaccine antigens.

5. Maternal immunization with multiple vaccine candidates should continue to be developed as a powerful approach to early infant OM prevention.

6. Rapid microbe-specific diagnostic methods should be further developed to test OM vaccine outcomes. A long-term goal is to develop non-invasive microbe-specific diagnostic methods.

7. The continued development of multiple animal models of experimental OM useful in studying mechanisms of vaccine-induced middle ear protection (i.e., to provide details of immune responses generated by immunization) and of animal models for genetic factors that might influence the host immune response should be a priority. These models are expected to provide a link in which to study similar parameters in children.

8. With regard to viral vaccines:

a. Industry and federal programs should be encouraged to measure OM outcomes in the evaluation of all respiratory tract viral vaccine candidates.

9. With regard to *Streptococcus pneumoniae* vaccines:

a. Currently, phase 3 trials of 10-, and 13-valent pneumococcal vaccines are underway in infants and toddlers. These vaccines have been developed primarily to increase coverage against invasive disease in all areas of particular need. However, because they do not cover the majority of potentially replacing non-vaccine serotypes at the mucosal level, it is doubtful whether these vaccines will increase efficacy against AOM in infants.

b. More interesting, are the encouraging results of the Czech study in which the pneumococcal polysaccharides are conjugated to *Haemophilus*-derived protein D. It has to be confirmed, in a randomized trial in infants, if such a vaccine has a better efficacy against AOM episodes as compared with a 7-13 valent vaccine in which the polysaccharides are coupled to $CRM_{197}$. With respect to such a study, it must not be forgotten that *S. pneumoniae* and *H. influenzae* isolated from the middle ear during an episode of AOM originate from the NP, so sampling from both sites should occur during the follow-up process during an episode of AOM. By doing so, interactions with other bacteria like *S. aureus* can also be studied.

c. Combining conjugate vaccines with protein vaccines or viral vaccines could potentially offer more benefit for the prevention of AOM in infants and toddlers.

10. With regard to nontypeable *Haemophilus influenzae* vaccines:

a. Development of GMP methods to produce P6, P4, P5 (region 3-derived) peptides, OMP 26 and detoxified LOS should be pursued so that clinical trials can proceed.

b. Further preclinical evaluation of HMWs, conserved regions of P2, Hap, Hia, type IV pilin (PilA) and phosphoryl choline should continue.

c. The momentum from the observation that protein D induced partial protection from *H. influenzae* OM in a clinical trial should be seized by investigators, vaccine development companies and government agencies by vigorously pursing clinical testing of other antigens. The rationale is that if a single antigen induces 35% protection, then a higher rate of protection may be achieved by including multiple antigens in the formulation.

11. With regard to *Moraxella catarrhalis* vaccines:

a. Active efforts toward vaccine development for *M. catarrhalis* should continue.

b. Several antigens should proceed toward production and evaluation for human trials. UspA2 and OMP CD are particularly promising.

c. Identification and characterization of additional vaccine antigens of *M. catarrhalis* should be actively pursued.

d. *M. catarrhalis* antigens should be included in vaccine preparations for clinical trials as soon as feasible. This approach will avoid the necessity of repeating expensive trials with *M. catarrhalis* antigens separately.

12. With regard to all OM microbial pathogens:

a. Proteomics, genomics and molecular methodologies should be utilized to identify and evaluate novel vaccine candidates.

b. Efforts to identify *in vitro* correlate(s) of protection from OM due to NTHI, *S. pneumoniae* and *M. catarrhalis* should be a priority. Such correlates of protection would facilitate vaccine development substantially. It is expected that recent clinical trials will facilitate the generation of such correlates.

c. The use of NP culture as a surrogate for protective efficacy should be considered as the ability to conduct tympanocentesis within clinical trials becomes increasingly limited.

d. Continued microbiological surveillance must be a priority as vaccine use, licensure of new vaccine(s) and new vaccine candidate development efforts evolve.

e. Evaluation of antigen combinations and combination vaccines should begin with the long-term goal of a multi-pathogen OM vaccine.

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# 7. TREATMENT

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## INTRODUCTION

Evidence-based medicine (EBM) is the conscientious, explicit, and judicious use of current best evidence in making decisions about the care of individual patients.[1] The Panel on Treatment convened on June 7-8, 2007 to identify and summarize research and clinical audit on questions about treatment published during the preceding 4 years (July 2003 through June 2007), with an emphasis on the categories of evidence quality. The literature search included MEDLINE, the Cochrane Database, and symposium abstracts,[2] and was supplemented by additional articles identified by panel members after review of each document.

The initial literature search identified 286 potential articles, which was reduced to 240 after excluding narrative (nonsystematic) review articles and letters to the editor. A final data set of 176 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.

It is customary within evidence-based medicine (EBM) to distinguish various levels of evidence. For present purposes, however, we slightly adapted one existing hierarchy[3] and added a separate category of healthcare delivery research, which is a growing body of work handling issues that interleave with treatment studies.

- Level 1: randomized controlled trial (RCT) or systematic review (meta-analysis) of RCTs
- Level 2: controlled prospective study (cohort, outcomes, case-control, or ecological) or systematic review of controlled prospective studies
- Level 3: controlled retrospective study (case-control) or systematic review of controlled retrospective studies
- Level 4: uncontrolled case series
- Level 5: expert opinion without explicit critical appraisal, or based on physiology, bench research, or first principles
- Level 6: non-peer reviewed abstract of RCT from the immediately preceding international symposium on OM

The panel was well aware that the hierarchy of designs did not capture all elements of quality (e.g., sample size and appropriateness of factors controlled for), and that a detailed review of all possible biases and confounding factors was impossible. Where comments of an evaluative nature appear it is usually because a particularly dangerous or common failing has occurred, or to provide a link to later research recommendations. Absence of such comment does not mean that the panel necessarily viewed a study favorably, and in appraising both our digests and the original reports readers should apply methodological criteria, being wary of small studies or vaguely defined outcome measures.

The included studies are grouped under the following major headings: Acute Otitis Media (AOM) General Treatment, AOM Observation, AOM Antibiotics, Recurrent AOM, Otitis Media with Effusion (OME), and Tympanostomy Tubes,

Tympanostomy Tube Otorrhea, and Chronic Suppurative Otitis Media (CSOM). Within each section, relevant articles are grouped by level of evidence, concluding with Healthcare Delivery Research. The panel acknowledged that this would be imperfect for some studies on or overlapping boundaries, but that it offered the most useful basis of search without elaborate indexing.

We have included the goals for future research related to OM treatment identified by Panel members at this and past Research Conferences. We hope this document will stimulate appropriately conducted research into these disease entities, because many basic questions remain unanswered on AOM, OME, and CSOM.

## ACUTE OTITIS MEDIA: GENERAL TREATMENT

*Level 1: RCT or Systematic Review of RCTs.* Chonmaitree et al.[4] Double-blind trial of 179 children aged 3 months to 6 years with AOM randomized to single-dose, intramuscular ceftriaxone and either chlorpheniramine maleate (0.35 mg/kg/d) and/or prednisolone (2 mg/kg/day) or placebo. Five-day treatment with antihistamine or corticosteroid, in addition to antibiotic, did not improve AOM outcomes. Corticosteroid use was associated with temporary normalization of tympanometric findings. Antihistamines prolonged middle-ear effusion (MEE) by about 40 days and should be avoided.

Foxlee et al.[5] Cochrane systematic review that assessed the efficacy of topical analgesia for AOM in 4 randomized trials. One trial showed significant pain reduction of 25% for anesthetic drops vs. placebo after 30 minutes, and three trials (all with one common co-author) showed naturopathic drops superior to topical anesthetic 15-30 minutes after instillation at 1 to 3 days after diagnosis. The reviewers conclude that existing evidence is insufficient to determine the efficacy of topical analgesics for AOM.

Hautalahti et al.[6] Double-blind trial of 663 healthy children in daycare randomized to xylitol 3 times daily vs. placebo for AOM prevention during the respiratory infection season. About 29% of children had at least one AOM episode, with no difference between groups.

Renko et al.[7] Randomized trial comparing amoxicillin with cefuroxime-axetil in 90 children with AOM. MEE resolution, as measured by a normal tympanogram, occurred after a median of 7.5 days, and 69% were effusion-free by 14 days. The median time to a normal tympanogram was 5 days after unilateral AOM, and 19 days after bilateral. The median duration of effusion did not differ between the two treatments (8 vs 7 days, P=0.7).

Sarrell et al.[8] Double-blind trial of 180 children aged 5 years or older with AOM randomized to four groups: naturopathic herbal extract ear drops with or without oral amoxicillin; and anesthetic ear drops with or without oral amoxicillin. Each group had a statistically significant improvement in ear pain over the course of the 3 days. Patients who were given ear drops alone had a better response than patients who were given ear drops together with amoxicillin. Both drops reduced pain after 15 and 30 minutes the first day of treatment. Nonetheless, pain was mostly (80%) self-limited and could be explained simply by the time elapsed.

*Level 2: Controlled Prospective Study.* Hamre et al.[9] Multinational prospective study of 1016 consecutive outpatients aged 1 month or older, consulting an anthroposophic (n=715) or conventional physician (n=301) with a chief complaint of acute sore throat, ear pain, sinus pain, runny nose or cough. Anthroposophic medicine is a holistic and salutogenetic approach that focuses on ensuring the conditions for health are present in a person. Anthroposophic treatment had more favorable outcomes, lower antibiotic prescription rates, less adverse drug reactions, and higher patient satisfaction.

Hotomi et al.[10] Prospective study of 308 children aged 9 months to 8 years with AOM, classified as severe (n=277) vs. non-severe (n=31). Severe AOM was managed with amoxicillin and 60% of children required a change in antibiotic. Non-severe AOM was initially managed without antibiotics and 52% of children eventually received antibiotics. Symptoms improved at the same rate for both groups by day 5. Poorer outcomes occurred in younger children and those with pathogens, especially penicillin-resistant *Streptococcus pneumoniae*.

Nomura et al.[11] Prospective study of 59 children with AOM assigned to oral antibiotics and myringotomy (n=36) or oral antibiotic alone (n=23). OME occurred in 17% of children treated with myringotomy vs. 44% of those with antibiotic alone, early recurrence of AOM in 31 vs. 35%, and recurrent AOM in 25 vs. 13%. Progression of OME was significantly less frequent after myringotomy (P=.036).

Wustrow et al.[12] Prospective study of 390 children aged 1 to 10 years with AOM treated conventionally vs. alternatively (plant-based tinctures and homeopathic potencies). The alternatively treated children received fewer antibiotics (14 vs. 81%, P<.001), but also had less severe otoscopic findings and clinical symptom ratings at baseline.

*Level 4: Uncontrolled Case Series.* Monobe et al.[13] Case series of 61 children with AOM examined in outpatient clinics. One month after treatment 52% had MEE and 23% had recurrent AOM; 23% had more than 3 recurrences in 6 months. Age under 2 years and persistent effusion were related to developing recurrent AOM. Absence of group nursing was related to early recurrence. Clinical outcomes were unrelated to bacterial species isolated from the effusion or nasopharynx.

*Level 6: Symposium Abstract.* Hyashi.[14] Case series of 146 Japanese children with AOM treated according to 2006 local guidelines, using a severity score based on symptoms and tympanic findings. Adherence to guidelines occurred in 84% of the cases, and 91% of cases had good outcomes.

*Healthcare Delivery Research.* Akkerman et al.[15] Cross sectional study of 438 AOM consultations for adherence to a Dutch national guideline by 146 general practitioners. Seventy-one percent of prescribing decisions adhered to the guideline. Under-prescribing of antibiotic, which occurred in 11%, was associated with shorter symptom duration, fewer signs of inflammation, and reduced illness severity. Over-prescribing, which occurred in 18%, was associated with age under 2 years, more severe illness, and perception that the parent expected an antibiotic.

American Academy of Pediatrics.[16] Multi-disciplinary, evidence-based clinical practice guideline from the American Academies of Pediatrics and Family Practice outlining appropriate use of watchful waiting as an initial management option for uncomplicated AOM. This is the first national United States guideline to offer observation for selected children. Optimal candidates for observation are those aged 2 years or older with non-severe AOM and assurance of follow-up. Recommendations are also made about diagnosis, pain relief, and antimicrobial selection for initial therapy and treatment failures.

Cohen et al.[17] Prospective study of 1906 nasopharyngeal cultures in French children aged 6 to 24 months with AOM, obtained by 89 pediatricians, to assess the impact of the 7-valent pneumococcal vaccine (PCV7) and judicious antibiotic use from 2001 to 2004. The proportion of PCV7-vaccinated children increased yearly while the proportion of children who received antibiotics within 3 months of enrollment decreased. Overall pneumococcal carriage decreased by 16% and carriage of PCV7 serotypes by 35%. The prevalence of penicillin-resistant strains (PRP) also decreased. Risks for PRP carriage were lowest in immunized children who had not received antibiotics, and highest in unimmunized children who had received antibiotics.

Francis et al.[18] Prospective study of adherence by primary care physicians to a locally developed OM practice guideline, adapted from the 1999 Centers or Disease Control and Prevention's treatment recommendations. Pediatricians' performance after introducing the guidelines was better than that of internists and family physicians.

Pulkki et al.[19] Case series of 3059 Finnish children with AOM managed by primary care clinicians to assess analgesic use. Analgesics were prescribed or recommended in only 10.4% of cases, in contrast to treatment guidelines.

Razon et al.[20] A multicenter study of over 4000 clinic visits before-and-after one-day seminar on diagnosing and treating respiratory tract infections in children based on CDC recommendations. The seminar reduced prescribing for all forms of upper respiratory tract infection except sinusitis. For OM it improved adherence to CDC recommendations guideline (odds-ratio 1.8).

Reuveni et al.[21] Case series of adherence to an Israeli AOM guideline by primary care physicians managing 590 Israeli children under age 48 months. Uncomplicated AOM accounted for 26% of antibiotic use and had 55% guideline adherence. For complicated AOM, antibiotic use and adherence were 51% and 54%, respectively. The value of such adherence studies can be limited where the clinical reasons for non-adherence cannot be, or are not, elicited.

Sanz et al.[22-23] Cross-sectional study of AOM and medical treatment in over 12,000 children aged 14 years or younger from 5 countries (Spain, France, Bulgaria, Slovakia and Russia). Despite the general agreement of most guidelines, wide differences in the treatment of uncomplicated AOM in children were observed. Although some eastern countries are high users, in this sample, eastern countries used fewer antibiotics, and western countries tended not to follow recommendations for first-line antibiotic selection. Factors other than guidelines and evidence-based medicine appeared to influence decisions. The main areas for improvement detected were high use of mucolytics, prescription of aspirin in potential or established viral infections, overuse of antibiotics and identification of specific patterns of incorrect antibiotic prescription and clinical entities associated with each location.

Spiro et al.[24] Children aged 6-35 months who presented to a pediatric emergency department with either fever or upper respiratory infection symptoms were randomly assigned to either the Tymp Aware (N=341) or Tymp Unaware (N=340) group, which determined whether or not the 27 participating physicians would be blinded to the patient's tympanogram during the encounter. AOM was diagnosed in 27% and 26% of the groups, respectively, while OME was diagnosed in 6% and 3%, respectively. In 2.8% of the Tymp Aware encounters, physicians indicated that tympanometry altered the treatment plan. Of patients for whom antibiotics were prescribed for OM, 14% had normal tympanograms for both ears. Tympanometry did not appear to change diagnoses or prescribing behavior of the physicians.

Vayalumkal and Kellner.[25] Cross-sectional survey of Canadian pediatricians and family practitioners regarding their practice, skills and attitudes towards tympanocentesis in managing children with AOM. Questionnaires were mailed to a random group of 498 physicians across Canada in 2002; the overall response rate was 56%. Only 4% of responding pediatricians and family physicians had received training in tympanocentesis but none were currently performing the procedure.

Woolley and Smith.[26] Retrospective case note audit comparing emergency department prescribing practices in Bristol with published AOM guidelines. Review of 50 children aged 3 months to 11 years showed that 86% received antibiotics even though only 52% had documented AOM. All antibiotics were prescribed below recommended dosage.

## ACUTE OTITIS MEDIA: OBSERVATION AS FIRST-LINE MANAGEMENT

*Level 1: RCT or Systematic Review of RCTs.* Le Saux et al.[27] Double-blind trial of 512 Canadian children with AOM aged 6 months to 5 years randomized to amoxicillin 60mg/kg/d vs. placebo for 10 days. In contrast to other placebo-controlled AOM trials most children were young (mean age 1.6 years) and had moderate (81%) or severe (13%) illness. At 14 days 84% of the children receiving placebo and 93% of those receiving amoxicillin had clinical resolution of symptoms (absolute difference -9%, 95% CI -14 to -3%). Amoxicillin also reduced pain and fever on days 1-3 (number need to treat, 10). Although amoxicillin was found superior to placebo, the effect was modest (number need to treat, 11) and delaying treatment was associated with clinical resolution in most children.

McCormick et al.[28] Investigator-blinded study of 223 children aged 6 months to 12 years with non-severe AOM randomized to immediate antibiotics (ABX) or watchful waiting (WW). Sixty-six percent of subjects in the WW group completed the study without antibiotics. Immediate ABX was associated with faster symptom control and with decreased numbers of failures (overall 16% difference, higher in children under age 2 years), but with increased adverse events (12 vs. 5%) and a higher percent nasopharyngeal carriage of multi-drug resistant *S. pneumoniae* (89 vs. 60%). Parental satisfaction was similar with both approaches.

Pshetizky et al.[29] Parents of 81 children (aged 3 months to 4 years) with AOM diagnosed at a family practice in Israel were randomized to receive an educational intervention on diagnosis and treatment of AOM or to a control group with no intervention. The educational intervention decreased antibiotic administration from 63 to 37%. Lower maternal education was the only factor associated with eventually purchasing an antibiotic. In children with AOM, a brief explanation by the family physician to the child's parents about the disease and the expected spontaneous recovery could decrease antibiotic use by approximately 50%.

Spiro et al.[30] Assessor-blinded trial of 283 Connecticut children aged 6m-12y (28% under age 2 years), with clinically diagnosed AOM in an emergency room, randomized to "wait and see prescription" (WASP) vs. immediate antibiotic (92% amoxicillin) according to a United States treatment guideline. Antibiotics were eventually used by 87% in the immediate group vs. only 38% in the WASP group (a 49% reduction). There was no significant difference between the groups in subsequent fever, otalgia, or unscheduled visits for medical care. Children managed with watchful waiting had less emesis at 4-6 days (1.2 vs. 1.5 days, P=.02), less diarrhea at 4-6 days (8 vs. 23%, P<.01), and less diarrhea at 11-14 days (12 vs. 24%).

*Level 2: Controlled Prospective Study.* Little et al.[31] One-year follow-up (by questionnaire at 3 and 12 months) of a cohort of 315 children aged 6 months to 10 years with AOM randomized to immediate- vs. delayed-antibiotics (48 to 72 yours) at the parents discretion. Delaying therapy did not significantly increase the risk of earache and poor scores on function scale at 3 or 12 months in the overall population. Immediate antibiotic, however, resulted in less episodes of earache at 3 months in children with recurrent AOM (2 or more prior episodes).

Merenstein et al.[32] Cross-sectional survey to examine how 466 parents, attending 2 family practice offices in Maryland, responded to 1 of 3 clinical vignettes. These were assigned at random to parents, and represented doctor-patient-communication styles on a continuum from paternalistic (doctor gives immediate antibiotic) through two shared decision models: (a) shared-decision with prescription of acetaminophen and safety-net antibiotic prescription; and (b) safety-net prescription without recommendation. Parents who received the paternalistic-model vignettes were more likely to say they would use antibiotics than those who received a shared-decision making vignette. Conversely, parents' satisfaction was greater in the shared decision groups (93% and 84%) than in the paternalistic-model group (76%).

*Level 4: Uncontrolled Case Series.* Marchetti et al.[33] Prospective case series to evaluate a wait and see strategy in 1287 Italian children aged 1 to 14 years with AOM diagnosed clinically by 169 non-validated primary pediatricians. Antibiotic use was associated more frequently with the presence of fever plus a red and bulging tympanic membrane and male sex. Of the 1099 children eligible for symptomatic treatment only (no otorrhea, no recurrent AOM), 67.6% and 65.1% recovered without antibiotic treatment and no complications within 3 and 30 days of follow-up, respectively.

Siegel et al.[34] Prospective case series to evaluate a safety-net antibiotic prescription for Cincinnati children aged 1 to 12 years with uncomplicated, non-severe AOM. Children with temperature above 101.5ºF (38.6ºC) or AOM in the past 3 months were excluded. At time of diagnosis pain medication was prescribed and an antibiotic prescription was given with instructions to be filled if symptoms did not resolve after 48 hours. Out of 175 children (mean age 5 years) followed-up 5 to 10 days after enrollment, only 55 (31%) had filled the antibiotic prescription. Parents' satisfaction to pain medication was high: 78% declared that pain control was effective and 63% that they would treat future AOM with analgesics and no antibiotics.

*Healthcare Delivery Research.* Coco.[35] Cost-effectiveness analysis of treatment options for AOM in a primary care setting, which compared amoxicillin 5 days, amoxicillin 7-10 days, and watchful waiting. Initial management with observation or amoxicillin were both considered reasonable economic approaches in managing AOM.

Finkelstein et al.[36] Cross-sectional survey of 2,054 parents and 160 Massachusetts community physicians to determine current use of initial observation of AOM and acceptability to families. The survey was conducted before implementing the

2004 U.S. AOM guideline.[16] Most physicians (62%) reported at least occasionally using initial observation, but few used it frequently (6%). Many parents had concerns regarding this option, but acceptability was increased among those with more education and those who felt included in medical decisions.

Fischer et al.[37] Cross-sectional survey in New York of 84 physicians and 654 parents in an emergency department setting about awareness and acceptability of observing AOM without initial antibiotics. Potential side effects of antibiotics were not known by 61% of parents and 72% were unaware of AOM observation as a management option. Most parents and physicians were comfortable with observation and willing to consider selective use of antibiotics.

Vernacchio et al.[38] Questionnaire-based survey to determine primary care physicians' knowledge and practice regarding the 2004 AOM guidelines[16] in the United States (response rate 59% of 480 sent surveys). Most (90%) had read the guidelines. Pneumatic otoscopy was used 50% or more of the time by only 39%. Observation was considered a reasonable option by 88%, but used only 10% of the time (median), even without concerns for complications. For severe AOM, antibiotic choices failed to adhere to the guideline more often (82%) than for treatment failure (72%) or mild afebrile AOM (31%).

## ACUTE OTITIS MEDIA: ANTIBIOTICS

*Level 1: RCT or Systematic Review of RCTs.* Arguedas et al.[39] A multicenter, open label, double tympanocentesis study designed to evaluate the role of high dose cefdinir (25 mg/kg/day) once daily for 10 days in 447 children (74% age 2 years or younger) at risk for recurrent or persistent OM. The bacteriological response of high dose cefdinir against penicillin non-susceptible *S. pneumoniae* was below expected, as well as marginal against *H. influenzae*. Multivariate analysis showed that the most important driver for bacteriological and clinical failures was low antimicrobial susceptibility.

Arguedas et al.[40] Multicenter, double blind, single tympanocentesis (day 1) study comparing high dose amoxicillin for 10 days versus a single dose of azithromycin at 30 mg/kg/day for uncomplicated AOM. In this study of 313 children, most of whom were less than 2 years of age (83%), a bacterial pathogen was isolated in 68% of patients. Clinical efficacy was comparable for patients treated with azithromycin (80%) vs. amoxicillin (83%). Adverse events were more common with high dose amoxicillin, particularly gastrointestinal complaints.

Arguedas et al.[41] Multicenter, open clinical trial (phase 2) of children at high risk of having a resistant middle ear fluid pathogen. Children were treated with gatifloxacin for 10 days and followed for 3 to 4 weeks before entering a long phase study (12 months) study to evaluate for osteoarticular problems. Cure was achieved in 88% of 198 clinically evaluable patients, with similar outcomes for patients younger or older than 2 years of age. No selection of resistance to gatifloxacin was detected among nasopharyngeal pathogens. No arthropathy was observed during the study or the 12 month follow up.

Block et al.[42] Randomized, investigator-blinded, multicenter center to compare the efficacy of 5 days of cefidinir (14 mg/kg/day divided into 2 doses daily) to 10 days of amoxicillin-clavulanate (45/6.4 mg/kg divided into 2 doses daily) for AOM in 425 children aged 6 months to 6 years. The satisfactory clinical response rate at end of therapy was comparable for cefdinir versus amoxicillin/clavulanate (88 vs. 85%; 95% CI -4.9, 9.3). In a subsample of patients 6-24 months old who had received conjugated pneumococcal vaccination, Cefdinir showed an apparent trend for higher efficacy than amoxicillin/clavulanate (92 vs. 77%; P = 0.019) and had fewer side-effects (24% vs. 38%; P = 0.002).

Block et al.[43] Multicenter, investigator-blinded, randomized study of the safety profiles and efficacy of 5-day courses of cefdinir (7 mg/kg q12h for 5 days) and azithromycin (10 mg/kg once on day 1, then 5 mg/kg once daily on days 2-5) in 357 children aged 6 months to 6 years with AOM. After 7-9 days clinical cure rates were comparable between the cefdinir and azithromycin groups (86% and 83%); there was no difference between subjects who had received PCV7 and those who did not. There were also no differences in tolerability, missed days of work and daycare, health-care

utilization, parents' satisfaction, ease of use, taste, and compliance.

Bowlware et al.[44] Pharmacokinetic study in 37 children with AOM randomized to standard dose (14 mg/kg) or to high dose (25 mg/kg) cefdinir. Blood samples were obtained at different time intervals for maximal plasma concentrations and area-under-the-curve values. Based on Pk/Pd parameters, high dose cefdinir was suggested to be ineffective against penicillin-nonsusceptible *S. pneumoniae* isolates.

Casellas et al.[45] Multicenter, randomized, evaluator blinded study comparing the efficacy of high dose amoxicillin-sulbactam vs. amoxicillin-clavulanic acid for 10-day treatment of 247 patients with non-recurrent AOM. Clinical efficacy at Days 12-14 and 28-42 was comparable. *S. pneumoniae* and *H. influenzae* isolated from nasopharynx were highly sensitive to both drugs.

Cifaldi et al.[46] Investigator-blinded, multicenter study of parent-reported outcomes for 367 children aged 6 months to 6 years with AOM randomized to cefdinir vs. amoxicillin-clavulanate. Based on parental assessment, cefdinir was easier to administer and tasted better than amoxicillin/clavulanate; children who received cefdinir also experienced less vomiting and had greater compliance. There were no differences between groups in work or daycare missed.

Dunne et al.[47] Children aged 6 months to 12 years (mean 3.5) with AOM were randomized to 3 day azithromycin (10 mg/kg/day, n=188) or 10 days of standard dose co-amoxiclav (n=185) treatment. Clinical success at day 10 was 83% and 88%; cure rate at day 28 was 74% and 69% in the children treated with azithromycin and co-amoxiclav, respectively. Signs of AOM were seen less frequently in azithromycin- than co-amoxiclav-treated children at day 28. Adverse events were seen in 11% of azithromycin patients compared with 20% on co-amoxiclav (P = 0.014).

Garrison et al.[48] Double-blind trial of standard- vs. high-dose amoxicillin for uncomplicated AOM in children. No differences were found in clinical failures at 3-4 days or in overall mean duration of illness (3 days). The lack of bacteriologic data combined with short follow-up may account for the negative findings.

Guven et al.[49] Single-blind, single-tap, trial of 180 children with AOM randomized to azithromycin vs. standard-dose amoxicillin-clavulanate for 10 days No difference in clinical success was found, but pathogen-specific resolution rates were higher for *S. pneumoniae* and *H. influenzae* with amoxicillin-clavulanate.

Hoberman et al.[50] Multicenter, double blind trial of 730 children aged 6 to 30 months with AOM randomized to high-dose amoxicillin-clavulanate for 10 days vs. azithromycin for 5 days. For the 494 children with pathogens at baseline tympanocentesis, clinical success rates at the end of therapy were 91% vs. 81%, favoring amoxicillin-clavulanate (P<.01). Similar results were obtained for bacterial eradication (94% vs. 70%, P<.001). Other studies from this group consistently have shown the same bacteriological and clinical results.

Rovers et al.[51-52] Meta-analysis using individual patient data from RCTs to assess the natural history of AOM and the incremental benefit of antibiotics. The odds rations (ORs) favoring a prolonged course of AOM (pain, fever, or both at 3-7 days) were having age under 2 years (OR 2.1, 95% CI 1.5-2.9) or bilateral AOM (OR 1.7, 95% CI 1.2-2.4). Similarly, antibiotics were most beneficial in children under age 2 years with bilateral AOM and for children of any age with AOM and otorrhea. Age alone did not predict antibiotic efficacy.

Toltzis et al.[53] Children with AOM were randomized to amoxicillin, cefprozil, ceftriaxone, or azithromycin. Among 1009 enrollees, nasopharyngeal colonization by *S. pneumoniae* was found at baseline in 24%, of which 41% were penicillin-nonsusceptible. Colonization by nonsusceptible pneumococci was unaltered during the observation period in all treatment groups, with no detectable differences among groups at each visit. In contrast, there was a substantial reduction in colonization by penicillin-susceptible *S. pneumoniae*, most notably in subjects treated with amoxicillin at 10-12 days.

Wang et al.[54] Open trial of 110 children with AOM randomized to standard-dose amoxicillin-clavulanate for 10 days vs. single-dose ceftriaxone. No difference was found in clinical cure, but conclusions are limited by open design, no tympanocentesis, small sample size, and intent to

treat analysis that only included patients completing at least 3 days of oral therapy.

*Level 2: Controlled Prospective Study.* Aggarwal et al.[55] Multicenter, prospective clinical trial of cefaclor versus low dose amoxicillin plus clavulanate in children with AOM (mean age 5 years). Clinical success at end of therapy was 98% with cefaclor vs. 85% with amoxicillin clavulanate (P<.05). The good results seen with cefaclor may not reflect efficacy, but instead a result of studying older children (with high spontaneous resolution) and not including relapses in the outcome.

Block et al.[56] Multicenter, investigator-blinded study of 318 children aged 6 months to 6 years with AOM randomized to cefdinir vs. high-dose amoxicillin-clavulanate for AOM. No differences were found using intent to treat analysis, but per protocol cure rates were 82% for cefdinir vs. 90% for amoxicillin (P=.045). These results may differ from the double tympanocentesis study performed by Arguedas and co-workers[39] because there was no tympanocentesis (at baseline or during therapy), children were older, high risk children were excluded, and there was no late follow up to detect relapses.

*Level 4: Uncontrolled Case Series.* Al-Shawwa and Wegner.[57] Review of 6 cases of AOM with otorrhea secondary to community-acquired methicillin-resistant *Staphylococcus aureus*. All were refractory to oral antibiotic or fluoroquinolone ear drops, but all were sensitive to trimethoprim-sulfamethoxazole and gentamicin sulfate.

Babin et al.[58] Case series of 31 children with persistent AOM despite antibiotic therapy for 48 hours. Fourteen (36%) were culture negative; bacteria most frequently identified were *H. influenzae* and *S. pneumoniae* (57% had reduced susceptibility to penicillin). Failures may have been related to antibiotic resistance or other factors such as viral infection.

Dunne et al.[59] Multicenter, case series of 242 children with AOM treated with single-dose azithromycin. Clinical cure occurred in 85% by day 28, but was only 64% for children with *H. influenzae* at baseline tympanocentesis.

Garbutt et al.[60] Serial microbiological prevalence surveys. Between 2000 and 2004, bacteriological samples were acquired in children less than 7 years of age with acute upper respiratory infections in community pediatrician's offices in St Louis, Missouri to estimate the local prevalence of *S. pneumoniae* nonsusceptible to penicillin and amoxicillin after widespread use PCV7. The prevalence of nonsusceptible *S. pneumoniae* fell from 25 to 12%, prompting a recommendation for standard-dose amoxicillin as first-line AOM therapy in those who have received 3 or more doses of PCV7.

Garbutt et al.[61] Cross-sectional study of the prevalence of *S. pneumoniae* on nasopharyngeal swabs in 212 children under age 7 years with AOM managed by community pediatricians. Although 48% had isolates that were non-susceptible to penicillin, less than 5% were characterized as non-susceptible to amoxicillin. Therefore, the authors recommend standard-dose amoxicillin therapy in their community. This elegant study highlights the value of nasopharyngeal cultures as surrogates for the susceptibility of AOM pathogens in studies of clinical policy, even though nasopharyngeal profiles may be poor for identifying ear pathogens in the individual child.

Gupta et al.[62] Case series of 334 children aged 6 months to 12 years with AOM treated with cefprozil for 10 days. Clinical cure was observed in 96.6%. Results are limited by lack of controls and no reporting on the susceptibility testing of isolated pathogens.

Piglansky et al.[63] Prospective case series of 50 children aged 3 to 22 months with culture-positive AOM treated with high dose amoxicillin (80 mg/kg/d) for 10 days. Overall middle-ear fluid eradication was 82%, but fell to 62% in patients with beta-lactamase positive *H. influenzae*. Clinical success was observed among 94% of the study participants, and amoxicillin was considered a reasonable first-line agent in their community.

Soley et al.[64] Prospective case series of 89 children aged 3 to 48 months with AOM treated with trimethoprim sulfamethoxazole. Clinical success was 69% for culture-positive children versus 91% for culture-negative children at baseline tympanocentesis (P = 0.03). Results indicate that this antimicrobial should not be use for treating children with AOM.

*Level 5: Expert Opinion without Explicit Critical Appraisal or Based on Bench Research.* Clawson et al.[65] In-vitro study of faropenem, a carbapenem antibiotic available in Japan since 1991, tested against 1,188 middle ear fluid pathogens. Faropenem was the most active beta-lactam antibiotic against *S. pneumoniae* and *H. influenzae*, with activity against *Moraxella catarrhalis* and *Streptococcus pyogenes*.

Nicolau et al.[66] In vitro study of cefprozil pharmacokinetics in 53 children aged 6-48 months with AOM. Comparison of plasma and middle-ear fluid concentrations predicted higher clinical failure rates for penicillin-non-susceptible *S. pneumoniae* or *H. influenzae*.

*Healthcare Delivery Research.* Bauchner et al.[67] Cluster randomization study at 12 pediatric practices (6 educational intervention and 6 control sites), with main outcomes adherence to the CDC recommendations for AOM and a subsequent antibiotic prescription for AOM within 30 days after diagnosis. Overall adherence was about 74%, and did not improve with intervention. After controlling for clustering according to site and covariates, children who were not treated in adherence to the CDC recommendations for both episodes had 1.60 times the odds of a subsequent prescription within 12 days, compared with those treated in adherence at both episodes.

Garbutt et al.[68] Review of AOM diagnosis and antibiotic prescribing by 29 Missouri pediatricians. Compliance with CDC diagnostic guidelines was low both by chart review (38%) and self-report (41%). Antibiotic choice was more adherent to CDC guidelines for a new AOM (chart review 68%, self report 100%) than for a treatment failure (chart review 63%, self report 83%) and recurrent disease (50%). Reasons for noncompliance were overuse of broad-spectrum antibiotics (28%) and subtherapeutic dosing (26%).

Gueylard et al.[69] Willingness to pay for short-course vs. conventional antibiotic therapy for AOM was assessed by telephone interview of 562 parents of recently treated children. Parents were willing to pay more with increasing household income, increasing number of AOM episodes during the previous year, and adverse treatment effects. The results for expected predictors provided some validation of the technique and the focal result was greater willingness to pay (by a small factor of 1.19) for a single dose compared to 3 daily doses of antibiotic.

Pichichero.[70] Before-and-after survey of antibiotic prescribing preferences for US physicians (2190 pediatricians, 360 general practitioners, and 273 otolaryngologists) in relation to a one-day workshop that discussed 1999 CDC AOM guidelines. Adherence to guidelines for antibiotic selection significantly increased from about 75% to 90% for all physician categories. Generalizability of results from physicians who self-select to attend a workshop to the general population is unknown.

Quach et al.[71-72] Review of over 60,000 case records in an insurance database to examine conformity of AOM prescribing practice to a local guideline recommending amoxicillin as first line and amoxicillin-clavulanate or cefprozil as second. Overall adherence was 42%. Compared with general practitioners, pediatricians were almost as likely to prescribe in conformity, but otolaryngologists were 50% less likely to prescribe a recommended antibiotic. Azithromycin had a higher rate of failure in the first few days than others, but lower when totalled up to 30 days, an inconsistency lacking a coherent explanation.

## RECURRENT ACUTE OTITIS MEDIA

*Level 1: RCT or Systematic Review of RCTs.* Arrieta et al.[73] Multicenter clinical trial of 304 children (66% under age 2 years) with recurrent or persistent AOM randomized to high-dose azithromycin (20 mg/kg daily for 3 days) vs. high-dose amoxicillin-clavulanate. Baseline tympanocentesis yielded a pathogen in 55% of subjects. Clinical success (84-86%) at 12-16 days was comparable for the two treatment arms, as was the per-pathogen clinical efficacy against *S. pneumoniae* and *H. influenzae*. More clinical and bacteriological data with this novel regimen of azithromycin are needed to evaluate the impact of this high dose particularly against pump efflux azithromycin *S. pneumoniae* resistant isolates.

Le et al.[74] Double blind trial of 383 children aged 1 to 7 years with recurrent OM randomized to PCV7 followed by either the 23-valent pneumococcal polysaccharide vaccine or hepatitis A or B vaccines. No differences in OME

prevalence were noted between groups at up to 26 months, possibly because pneumococcus comprises only a portion of bacteria leading to AOM, at both episode and child level not all AOM leads to OME, and some susceptible children may not generate immune responses, whether naturally or artificially.

Mills et al.[75] Clinical trial of 57 children aged 6 months to 6 years with recurrent AOM (at least 3 in past 6 months) randomized to routine pediatric care with or without osteopathic manipulation. Adjusting for baseline AOM frequency, intervention patients had 0.14 fewer episodes of AOM per month (P=.04), less surgery (P=.03), more mean surgery-free months (6.0 vs. 5.3, P=.01), and more normal tympanograms. This was a well- performed study, but the high drop-out makes it difficult to conclude whether the manipulation makes a difference.

Sáez-Llorens et al.[76] Multicenter trial of 413 children aged 6 months to 7 years with recurrent AOM and/or AOM antibiotic treatment failure in past 14 days randomized to gatifloxacin vs. standard-dose amoxicillin-clavulanate suspension for 10 days. Clinical cure at the end of treatment was 90% for gatifloxacin and 84% for amoxicillin-clavulanate group (P≥.05); gatifloxacin was associated with higher cure rates in children younger than 2 years. Cure rates by pathogen and adverse events were comparable.

Sher et al.[77] Multicenter clinical trial of 354 children aged 6 months to 7 years with recurrent OM or AOM treatment failure randomized to gatifloxacin vs. high-dose amoxicillin-clavulanate for 10 days. Tympanocentesis was optional and performed in 116 children. Gatifloxacin was at least as effective and well-tolerated as amoxicillin-clavulanate. Importantly, no selection of quinolone resistance was observed in the study population (nasopharyngeal swabs were performed at baseline, during therapy, and post-therapy) and no arthropathy was observed.

*Level 4: Uncontrolled Case Series.* Arguedas et al.[78] Multicenter open label trial of 205 children (80% under age 2 years) with, or at high risk for, persistent or recurrent OM treated with baseline tympanocentesis then levofloxacin for 10 days. Clinical success occurred in 94% of children. Bacterial eradication was 88%; however, eradication of *S. pneumoniae* was inferior to that observed with gatifloxacin in similar studies. There were no serious adverse events.

Leibovitz et al.[79] Prospective case series of 160 children aged 6 to 48 months with recurrent or nonresponsive AOM treated with baseline tympanocentesis and gatifloxacin suspension for 10 days; 80% of patients completed treatment of which 70% had bacteria identified in the middle-ear fluid. The rate of clinical success was 90% and bacterial eradication was achieved for 98% of pathogens. Adverse events were recorded in 13% of patients: vomiting, diarrhea, and maculopapular rash, but no arthropathy.

Pichichero et al.[80] Secondary analysis of four clinical trials that included 867 children with recurrent AOM or AOM treatment failure treated with gatifloxacin. Two trials used amoxicillin-clavulanate as a comparator. Clinical efficacy of gatifloxacin was 88-89% and no arthropathy was found during 1 year follow-up.

## OTITIS MEDIA WITH EFFUSION

*Level 1: RCT or Systematic Review of RCTs.* Arick and Silman.[81-82] Clinical trial on 94 children aged 4-11 years with persistent OME and hearing loss randomized to twice-a-day home treatment with a modified Politzer device with controlled air pressure and flow for 7 weeks vs. no treatment. Hearing sensitivity was within normal limits in at least one ear in 85% of subjects using the device vs. 32% of controls. In a follow-up study,[82] the Politzer device was used for 7 to 9 weeks in 32 subjects from the initial trial with persistent OME. After therapy, hearing was normal for 72% of ears. Lack of masking, limited follow-up, and no data on OME resolution limit the validity of these results.

Cengel and Akyol.[83] Clinical trial on 122 children aged 3-15 years, on a waiting list for adenoidectomy and/or ventilation tube placement, randomized to intranasal mometasone furoate monohydrate vs. no treatment. Patients were evaluated at 0 and 6 weeks. Resolution of OME was significantly higher in the study population than in the controls (42% vs. 14%, P<.001). Adenoid size was reported as decreasing in 67% of patients in the

study group but the pathological relevance of this is unclear.

Combs.[84] Clinical trial on 67 children aged 2-12 years with OME randomized to 30 days of montelukast sodium vs. placebo. Of the participants evaluable after 4 weeks 17 of 29 (58%) in the montelukast group were effusion-free by tympanogram and acoustic reflectometry compared to 5 of 31 (16%) subjects in the placebo group ($P<0.003$).

Ingels et al.[85] Secondary analysis of 187 Dutch children with persistent OME randomized to tympanostomy tubes vs. watchful waiting. Compared to non-intervention controls, those receiving tubes had more otorrhea and were prescribed antibiotic more often. The incidence of earache and fever did not differ.

Koopman et al.[86] Clinical trial of 208 children with bilateral OME randomized by ear to laser myringotomy vs. ventilation tube insertion. Mean patency rates were 2.4 weeks for myringotomy vs. 4.0 months for tubes. Mean success rates were 40% for myringotomy vs. 78% for tubes. This particular overall comparison is consistent with past findings on tubes vs myringotomy alone. However the report lacks randomization details and systematic reporting and interpretation of covariates, so in the absence of untreated controls, claims about adenoidectomy and baseline OME risk factors having particular affinity for one or other treatment arm are not valid.

Lesinskas.[87] Clinical trial of 198 Lithuanian adults with OME randomized to middle-ear inflation daily for 10 days, with or without amoxicillin, vs. a control group. Treatment effectiveness at 60 days (by otoscopy, audiometry, and tympanometry) was 51% for inflation only, 59% for inflation plus antibiotic, and 11% for controls. Prognostic factors included mastoid pneumatization, paranasal sinus disease, previous OME history, patient age, and baseline otoscopy.

Paradise et al.[88-90] Follow-up analysis of a clinical trial in 429 otherwise healthy children (mean age 15 months) with persistent OME (mostly intermittent and unilateral) randomized to prompt vs. delayed (6 to 9 months) tympanostomy tube insertion. Prompt surgery did not improve cognitive, language, speech or psychosocial development at age 4 years for randomized children (n=397), and there was no correlation between cumulative effusion duration and developmental outcomes for children with lesser degrees of OME (n=234) that did not qualify for study entry. Randomized children were again tested at age 6 years (n=395) and no benefit of early tube insertion was detected. Last, the randomized children were evaluated at age 9 to 11 years (n=391), and no differences were found between the prompt vs. delayed tube groups on 48 developmental measures. These results argue against strictly duration-based criteria for tube insertion in otherwise healthy children identified by monthly screening, but cannot be generalized to referral-based populations or to children with delays, disorders, or syndromes that affect speech, language, learning, or development.

van Heerbeek et al.[91] Clinical trial of 161 children aged 2 to 8 years with persistent bilateral OME were treated with tympanostomy tubes. One half of the subjects were assigned to additional vaccination with PCV7 at 3 to 4 weeks before, and a pneumococcal polysaccharide vaccine 3 months after, tube insertion. The overall recurrence rate of bilateral OME was 50%. Pneumococcal vaccinations induced 5- to 24-fold increases in geometric mean IgA and IgG titers but did not affect recurrence of OME.

*Level 2: Controlled Prospective Study.* Balatsouras et al.[92] Prospective, controlled study of 50 asthmatic children with bilateral OME treated with either budesonide and terbutaline inhalers, with or without a leukotriene inhibitor (montelukast). At the end of treatment for 30 days, 15 (60%) of those receiving the montelukast were effusion-free by pneumatic otoscopy, tympanometry and audiology (parameters not specified) compared to 9 (36%) of those receiving only the inhalers.

Bozkurt and Calguner.[93] Prospective controlled study of 35 children with persistent serous OME treated with adenoidectomy plus carbon dioxide laser myringotomy vs. ventilation tube insertion. The recurrence rate of 21% at 6 months was similar for the two groups, but no real conclusion can be drawn from this seriously under-powered study. The authors prefer laser myringotomy for serous

OME, but use ventilation tubes for recurrences or when thick mucoid effusion is present.

Hassmann et al.[94] Clinical trial of 109 children with OME nonrandomly assigned to laser myringotomy, laser myringotomy plus tube insertion, or classical myringotomy plus tube insertion. After a minimum follow-up period of 1 year, the recurrence rate was lowest when a tube was placed (regardless of myringotomy technique) and highest with myringotomy alone. Use of the laser did not affect tympanic membrane healing.

Ino et al.[95] Prospective trial of 38 patients (43 ears) diagnosed with eosinophilic OM. Twenty-four patients had a steroid suspension (triamcinolone acetonide) instilled into the middle ear and Eustachian tube while 14 had topical steroid ointment applied to the tympanic membrane. Instillation improved otorrhea and hearing level compared to controls.

Kanemaru et al.[96] In-vitro and prospective, controlled study of 101 patients with OME evaluating the effect of 5-fluorouracil (5-FU), an anti-neoplastic agent, on cell proliferation and myringotomy patency. The in vitro study confirmed suppression of cell proliferation. Mean time to myringotomy closure was 8.1 days without 5-FU and 20.5 days after a single dose. No adverse events occurred.

Zanetti et al.[97] Prospective, controlled study of 40 children and adults aged 13-76 years with chronic OME non-randomly treated with diode laser-assisted myringotomy (n=28) vs. "cold" insertion of ventilation tubes (n=22). No pain or complications were observed. Myringotomy patency was 7 to 25 days, hearing improved immediately, and recurrence of OME was observed in 92% of the ears within 1 month from closure of perforation. In contrast, ventilation tube patency was 126 to 301 days, and recurrence of OME was observed in only 24%.

*Level 4: Uncontrolled Case Series.* Jang and Park.[98] Pharmacokinetic study of 25 children aged 1-13 years with chronic OME treated with a single does of cefprozil 15 mg/kg. Cefprozil concentration exceeded the minimum inhibitory concentration for most common pathogens and the authors consider it a first line drug for cefprozil susceptible agents when antibiotics are required for chronic OME.

Khan et al.[99] Case series of 87 patients with OME treated medically and followed for 18 to 24 months. Improvement occurred in 34% but 66% eventually required surgery. OME was associated with rhinosinusitis and hypertrophic adenoid.

Lin et al.[100] Case series of 54 children (73 ears) with OME treated with carbon dioxide laser myringotomy. The mean myringotomy patency was 18 days (range 1-5 weeks) with 73% resolution of OME.

MRC Multicentre OM Study Group.[101] Secondary report of hearing outcomes and ventilation tube status for children aged 3.5 to 7 years with bilateral chronic OME and hearing loss who had tube insertion in the TARGET trial. The duration of at least one functioning tube (Shephard, short-stay) was almost double that of both remaining functioning (40 vs 21 weeks), suggesting that in cases with fluid recurrence, asymmetric hearing may be experienced for a material proportion of the period of efficacy. The prevalence of tube infection in this persistent and severe OME caseload was low (1%).

Nakagawa et al.[102] Case series of 12 subjects with intractable OME with eosinophils (>10% in middle ear secretions), treated with antihistamines, leukotriene receptor antagonist, and a topical steroid. Bone conduction hearing levels at 4 and 8 kHz were poorer than at lower frequencies, and response at 8 kHz improved after treatment.

Prokopakis et al.[103] Case series of 88 patients aged 3-14 years who had laser-assisted tympanostomy without tube insertion for OME for 8 weeks or longer despite 3 weeks of systemic antibiotic. Average myringotomy patency time was 2.6 weeks (range 1-7 weeks). Ears with a thick tympanic membrane and/or glue-like effusion had a patency time of 2.1 weeks vs. 3.1 weeks in ears without these findings. Relapse by 2 months was seen in 45% of ears, and 41% of parents were dissatisfied.

*Level 6: Symposium Abstract.* Casselbrant et al.[104] Clinical trial of 98 children aged 24-47 months with bilateral OME for 3 months or longer, or unilateral OME for 6 months or longer, or recurrence after extrusion of prior tympanostomy tubes randomized to myringotomy and tubes vs. adenoidectomy plus myringotomy with or without

tubes. Adenoidectomy with or without tube insertion did not alter time with effusion compared to tube insertion alone over the 18 month follow-up period.

Daniel et al.[105] Follow-up study of children in the United Kingdom TARGET trial found a close correlation between results on extensive clinical follow-up (all centers, 1 to 5.5 years) and intensive follow-up (single largest center, minimum 6 years) in terms of the number of consultations and operations reduced by adenoidectomy. For both designs, the odds-ratio was less than 0.5, a material reduction.

Haggard et al.[106] A benefit of ventilation tubes for OME on development in older, but not younger, children was found in the TARGET trial. For developmental outcomes there was a significant interaction (the appropriate test for claiming a difference) between treatment with ventilation tubes and age; children under age 60 months showing no benefit, in agreement with previous trials, but older ones showing benefit.

*Healthcare Delivery Research.* Rosenfeld et al.[107] A multi-disciplinary, evidence-based clinical practice guideline from the American Academies of Pediatrics, Family Practice, and Otolaryngology – Head and Neck Surgery described optimal management of OME in children aged 2 months through 12 years with or without developmental disabilities. Pneumatic otoscopy is recommended for diagnosis, and clinicians are advised to distinguish the child with OME who is at risk for speech, language, or learning problems from other children with OME and more promptly evaluate hearing, speech, language, and need for intervention in children at risk. Hearing tests are recommended for effusions persisting 3 months or longer. An individualized approach to surveillance and surgery is proposed. The guideline specifically recommended against population-based screening for OME in healthy children, and against use of antibiotics, steroids, antihistamines, or decongestants for routine management.

## GENERAL OTITIS MEDIA AND TYMPANOSTOMY TUBES

*Level 1: RCT or Systematic Review of RCTs.* Kinnari and Jero.[108] Clinical trial of 170 patients receiving tympanostomy tubes randomly assigned to receive an. albumin coated tube on one side. Follow-up was performed for 9 months to detect otorrhea, early occlusion, and duration of ventilation if excessive bleeding during operation. Albumin coated tubes had fewer sequelae, less adhesion to the tube surface, and fewer tube occlusions.

Lous et al.[109] Systematic review of the effect of tympanostomy tubes found children treated with tubes spent 32% less time (95% CI, 17 to 48%) with effusion during the first year of follow-up. Tubes improved hearing with 9dB (95% CI, 4 to 14 dB) after the first 6 months. In otherwise healthy children with long-standing OME and hearing loss, early insertion of tubes had no effect on language development or cognition. Intubated ears had an additional risk for tympanosclerosis of 0.33 (95% CI 0.21 to 0.45) one to five years later.

Nguyen et al.[110] Clinical trial of 63 children with recurrent AOM or chronic OME randomly assigned to tubes with or without adenoidectomy. Children were followed at least 1 year after surgery. For the 34 children with adenoid abutting the torus tubarius failure was observed in 50% treated with tubes alone vs. 17% with tubes plus adenoidectomy ($P<.05$). For the 29 children with adenoid that did not abut the torus 38% failed with tubes and 40% with tubes plus adenoidectomy ($P=.92$). The authors claim that patients with adenoid abutting the torus tubarius are those who may benefit from an adjuvant adenoidectomy; however, they do not report the appropriate interaction test, which when performed is not significant. Also, overall status was not worse for children with abutting adenoid, which would appear a pre-requisite for regarding the abutting adenoid as a crucial issue in the efficacy of adenoidectomy

Ragab.[111] Clinical trial of 96 children aged 1-12 years with recurrent AOM or OME randomized to cold knife myringotomy or radiofrequency tympanostomy with or without topical mitomycin C application. Application of mitomycin prolonged patency of the tympanostomy from 3.5 weeks to 5.3 weeks in children with recurrent AOM, and from 3.5 to 7.0 weeks in children with OME. No complications were observed.

Wallace and Newbegin.[112] Clinical trial of 66 children treated with ventilation tubes randomized to follow-up by the otolaryngologist at 1 week and 1 month vs. 1 month only. The additional early follow-up visit had no impact on rates of otorrhea, tube patency, tube extrusion, or need for primary care consultation.

*Level 2: Controlled Prospective Study.* Palmu et al.[113] Cohort study of 756 children from the Finnish Otitis Media Vaccine Trial who had been vaccinated at 2, 4, 6, and 12 months of age with PCV or hepatitis B vaccine (control). A single follow-up visit was done at 4-5 years of age. During the vaccine trial (2-24 months of age), vaccine efficacy in preventing tympanostomy tube placement was only 4% (95% CI, -19 to 23%). From 2 to 4-5 years of age, vaccine efficacy was 39% (95% CI, 4 to 61%). In the hospital-based data of all children (N = 1490), vaccine efficacy was 44% (95% CI, 19 to 62%).

*Level 3: Controlled Retrospective Study.* Ahn et al.[114] Retrospective cohort of 423 Korean children after tube insertion for chronic OME with a nested case-control analysis to determine risk factors for receiving 3 sets of tubes (cases) compared to those undergoing just one procedure (controls). Factors associated with triple tubes included younger age at first procedure (4.1 vs 5.4 years), no concurrent adenoidectomy at initial procedure (80% vs 61%), shorter duration of ventilating tube (9.9 vs. 13.6 months), otorrhea within 1 month post-operatively (5.4% vs. 0.6%), and extrusion of ventilating tube within 3 months post-operatively (5.4% vs. 1.2%). Glue-like effusion at the time of surgery was not a risk factor.

Kadhim et al.[115] Population-based review of 51,373 Australian children under age 10 years who had tympanostomy tubes at least once from 1981 to 2004. Concurrent adenoidectomy (with or without tonsillectomy), performed in 29% of cases, significantly reduced the odds of requiring subsequent tubes (OR .61, 95% CI .52 to .72). The low complication rates for adenoidectomy makes it a potentially cost effective first line management option for OME.

Price et al.[116] Case-control study of adenoidectomy outcomes for 27 children with Down syndrome (cases) vs. 53 age- and sex-matched controls. More controls than Down syndrome patients had improvement in symptoms, including nasal obstruction (87 vs. 50%, p=.005), snoring (73 vs. 41%, p<.01), mouth breathing (84 vs. 41%, p<.001), and middle-ear disease (68 vs. 23%, p<.001). Down syndrome patients were 7.7 times more likely to have otorrhea after adenoidectomy.

*Level 4: Uncontrolled Case Series.* Adkins and Friedman.[117] Chart review of 82 children aged 2-15 years after removal of tympanostomy tubes. Overall 87% of perforations closed, unrelated to repair technique, duration of intubation, indication for removal, or demographic factors.

Cloutier et al.[118] Chart review of 190 patients aged 3-19 years with atelectasis of the tympanic membrane or adhesive OM treated with subannular tympanostomy tube insertion. Mean tube patency was 22 months, with complications of otorrhea (18%), perforation (8%), plugging (7%), and cholesteatoma (1.6%).

Cotter and Kosko.[119] Chart review of 47 children aged 0.5 to 15 years with recurrent AOM and chronic OME treated with laser-assisted myringotomy. Failures occurred in 54% of children with RAOM and in 63% with COME. Tympanostomy tubes were inserted in 57% of children and two had persistent perforation at 2 years follow up.

Deutsch et al.[120] Uncontrolled cohort of 251 children with recurrent AOM or chronic OME evaluated at 1, 2, 4, 8, and 12 weeks after laser-assisted tympanic membrane fenestration. At the 6 week follow up 97% of the fenestrations were not longer patent. Spot size of 2.4 and 2.6 mm had a higher rate of patency than 2.0 mm spot size at 3 weeks. Cure at 90 days was related to larger spot size for all patients and those with RAOM.

Duckert et al.[121] Chart review of 40 adults and children with atelectasis managed by composite cartilage tympanoplasty with a T-tube incorporated into the graft. The authors conclude the procedure is safe and effective based on 65% tube retention at least 4 years in adults and no cases of extrusion or permanent perforation.

Groblewski and Harley.[122] Chart review of 6 patients with tympanostomy tubes that medialized into the middle ear. The authors recommend

removal because half of the patients were symptomatic.

Haapkylä et al.[123] Case review of Finnish registry data for all children aged 16 years or younger who underwent surgery for OM in 2002. Large regional variations were observed in Finland despite nationally accepted guidelines.

Jassar et al.[124] Long-term follow-up of 37 patients who received subannular T-tubes, of which 26 were previously reported. The authors conclude a beneficial effect compared to traditional myringotomy incision based on an 8% perforation rate and 9% rate of tube reinsertion.

Kim et al.[125] Five-year follow-up study of children treated with Paparella II tympanostomy tubes for persistent OME after extrusion of short-term tubes. Median tube patency was 3.7 years, with 52% retained more than 5 years. Elective removal is recommended after 3 years because of increased otorrhea and perforation with longer retention.

Kujawski and Poe.[126] Case series of 56 patients (108 ears) with refractory Eustachian tube dysfunction (middle ear atelectasis or effusion) treated with laser Eustachian tuboplasty. Under general anesthesia dilation was achieved by vaporizing cartilage from the luminal posterior wall, then performing myringotomy for ventilation until packing in the tube was removed. Tuboplasty had "early promise" based on 65% normal middle ear aeration at 3 year minimum follow-up.

Lindstrom et al.[127] Chart review of 507 children, median age 23 months, with OM treated using Armstrong beveled tympanostomy tubes. Median time to extrusion was 15.5 months, with a 1.3% rate of persistent perforation.

Poetkler et al.[128] Chart review of 286 patients with OM treated with tympanostomy tubes. The mean improvement in the pure tone average hearing level was 14.8 dB in patients with OME, 9.5 dB for OME plus recurrent AOM, and 6.3 dB for recurrent AOM only. Patients with OME, with or without recurrent AOM, had greater hearing improvement that those with recurrent AOM only.

Praveen and Terry.[129] Prospective case series of 606 children with recurrent OME treated with tympanostomy tubes. Compared to children of non-smoking parents, children exposed to environmental tobacco smoke had earlier tube extrusion (median 59 vs. 86 weeks) and more post-extrusion myringosclerosis (64 vs. 20%). Smoke exposure was also related to narrow ear canals, otorrhea, attic retraction, and post-extrusion perforation.

Prokopakis et al.[130] Case series with 2 month follow-up of 108 adults (142 ears) with OME or eustachian tube dysfunction treated with laser-assisted tympanostomy. OME resolved after closure of the tympanostomy in 48% of patients, and eustachian tube dysfunction improved in 79%.

Puterman and Leiberman.[131] Chart review of 27 children aged 4 to 15 years with retained tympanostomy tubes treated with perforation debridement and Gelfoam plug after tube removal. All perforations closed without complications. Lack of concurrent controls precludes evaluation of efficiency of treatment.

Robertson et al.[132] Case series of safety and tolerability of phenol as topical anesthesia for tympanostomy tube insertion. No change in hearing occurred for 57 patients and 17 patients reported high satisfaction.

Timmerman et al.[133] Before-and-after survey of parents of 77 children with persistent OME treated with tympanostomy tubes. The investigators validated a Dutch translation of the OM-6 and showed moderate to large improvements after surgery for most children. "Response-shift-bias," a form of adaptation to a prevailing health state or disability, was present at the group level, and manifested as the parental tendency to underestimate the seriousness of hearing loss and overestimate the child's quality of life before surgery.

Tran et al.[134] Chart review of 449 children with sickle cell disease to assess incidence of tympanostomy tubes over 11.5 years of follow-up (mean 6.1). Only eight children received tubes, an event rate of 0.29/100 persons-years (lower than that of the general population).

Valtonen et al.[135] Prospective case series of 237 children aged 5 to 16 months with chronic OME or recurrent OM followed for 14 years after ventilation tube insertion. Repeated tympanostomy tube insertion was performed in 60%, more often in ears

with OME. Children whose ears were healed after 5 years did not benefit from further follow-up.

Witsell et al.[136] Before-and-after multicenter study of 272 children with OM, of whom 177 received tympanostomy tubes. Large improvements in disease-specific quality of life were seen on the OM-6 questionnaire up to 9 months after tube placement, but loss to follow-up increased from 27% at 3 months to 78% at 9 months.

Yilmaz et al.[137] Case series of 27 patients who had immediate repair of tympanic membrane perforations with a paper patch after removal of long-term tubes from 36 ears (mean intubation 50 months). Persistent perforation occurred in 17% of ears, and were most often anterior and marginal.

*Level 6: Symposium Abstract.* Rosenfeld et al.[138] Historical cohort study of outcomes after tympanostomy tube insertion in 229 children with OM, 55% of whom were at-risk for developmental delays or disorders. Parents of at-risk children were more likely to report a "much better" response a median of 2 years after tubes for their child's hearing (OR 2.14, P=.033), speech and language (OR 4.83, P<.001), and learning or school performance (OR 3.40, P<.001) when adjusted for age, gender, and baseline hearing levels.

*Healthcare Delivery Research.* Arason et al.[139] Cross-sectional survey of antimicrobial use and tympanostomy tubes in four Iceland communities, repeated 5 years after a prior survey. The prevalence of tube insertion was 34% and correlated positively with antimicrobial use for AOM. Communities with low antimicrobial use in 1998 had further declines by 2003, and high-use communities in 1998 increased their use. Parents in communities where antimicrobial use was lowest and narrow-spectrum drugs were used most often were least likely to favor antimicrobial treatment. It is unclear if there is a specific causal link or just a general dimension of interventiveness in physician and community attitudes, which may also change over time, but the association is striking.

Compliment et al.[140] A detailed, but probably not generalizable, managerial and financial case study comparing a new outpatient treatment suite and an outpatient surgery center with standard operating room to control the costs of placing ventilation tubes. They introduced detailed cost information but failed to provide a clear and fundamental structure and process definition of the difference between the outpatient suite and surgery center. The rather small cost advantage for the suite over the center ($280 per case) undermines the enthusiastic but obscure claims. The definite cost advantage relative to an operation room ($2130) agrees with studies in many forms of elective minor surgery.

Karevold et al.[141] Cross-sectional study comparing national registry data on OM surgery in all children between 0-16 years in Norway and Finland. Finland had much higher surgery rates compared to Norway (146.5 per 10,000 children vs.82.5, respectively). Rural areas in both countries had higher surgery rates compared to urban areas. Present guidelines do not ensure consistent management of children with OM.

Karevold et al.[142] Analysis of Norwegian registry data for all children 16 years or younger who had surgery for OM in 2002. The peak age for surgical treatment of OM was 5 years. Tympanostomy tubes were inserted in more than half of the children treated and the analysis showed considerable regional variation in the rates and in choice of surgical treatment.

Korzyrskyi et al.[143] Analysis of a health care database from Manitoba, Canada for all children 19 years or younger to study trends in antibiotic prescription. Antibiotic prescribing decreased from 1.2 prescriptions per child in 1995 to 0.9 prescriptions in 2001. Total antibiotic use declined for all respiratory tract infections, and was greatest for sulfonamides and narrow-spectrum macrolides. In contrast, use of broad-spectrum macrolides increased 12.5-fold. OM accounted for one-quarter of the use of the latter agents.

Plasschaert et al.[144] Analysis of a Dutch primary care research database for children 13 years or younger. From 1995 to 2003, the overall general practitioner consultation rates for AOM and OME declined by 9% and 34%, respectively. Antibiotic prescribing rates, however, increased by 45% for AOM and 25% for OME. Increased prescribing may induce increased cost and antibiotic resistance.

Rovers et al.[145-146] Two papers from a dissemination impact study in the Netherlands make

essentially the same point for differing audiences about the ability of findings from a high quality clinical trial to influence clinical practice where there are strong vested interests. First, otolaryngologists were surveyed before and after publication and dissemination of a RCT showing negligible benefits of ventilation tubes on hearing, language development and quality of life in young mildly affected children. The high level of belief that there could not be complete recovery without treatment was shifted very little by disseminating the null results. Second, with a more formal analysis, the group showed that members of this profession were not Bayesian in their inference, failing to use the evidence to revise their prior beliefs in the way considered optimal within Bayes' Theorem. Such before and after studies of dissemination are valuable although they can be hard to interpret in detail, and apply if the possible reasons (e.g., through not accepting the general relevance of the trial result, whether rightly or wrongly) are not also elicited.

Uppal et al.[147] Cross-sectional survey of patient satisfaction with follow-up after tympanostomy tube insertion provided by nurses vs. physicians in the UK National Health Service. Follow-up by nurses produced higher overall satisfaction and less waiting time.

## TYMPANOSTOMY TUBE OTORRHEA (TTO)

*Level 1: RCT or Systematic Review of RCTs.* Dohar et al.[148] Multicenter, observer-masked, trial of 80 children aged 6 months to 12 years with acute TTO (less than 3 weeks) randomized to ciprofloxacin 0.3%/ dexamethasone 0.1% eardrops for 7 days vs high-dose oral amoxicillin/clavulanic acid for 10 days. Ciprofloxacin-dexamethasone reduced the overall duration of otorrhea (4.0 to 7.0 days) and resulted in better clinical response at day 18 (84.6% vs 58.5%; P=.01). Topical treatment was associated with fewer gastrointestinal adverse effects and complications from yeast infections.

Gilles et al.[149] Single-center, evaluator-blinded trial of 200 children aged 6 months to 12 years undergoing bilateral tympanostomy tube insertion randomized to ciprofloxacin 0.3%/ dexamethasone 0.1% eardrops vs. no treatment. Topical treatment significantly reduced the number of children with otorrhea (5 vs 39, P<.0001) or clinically diagnosed OM and effusion at the 2-week post-operative visit. Subjects with bilateral effusion at time of surgery had the greatest benefit (93% reduction of otorrhea).

Kocaturk et al.[150] Investigator-blinded trial of 280 children aged 3 to 11 years undergoing tympanostomy tube insertion randomized to one of four groups: (1) isotonic saline irrigation of the middle ear intra-operatively, (2) postoperative sulbactam-amoxicillin, (3) postoperative topical ofloxacin eardrops, or (4) no treatment (control). Purulent otorrhea was observed in 16% of patients receiving irrigation, 14% receiving oral antibiotic, 9% receiving eardrops, and in 30% receiving no treatment. There was no statistically significant difference among the 3 treatment groups, but all were significantly different from the control group. There was no significant relation between the type of effusion found at surgery and occurrence otorrhea.

Nawasreh and Al-Wedyan.[151] Clinical trial of 150 children aged 3 to 14 years who underwent tympanostomy tube insertion randomized to no antibiotic drops (control) vs. ciprofloxacin drops during surgery vs. ciprofloxacin drops during surgery and for 5 days after. Postoperative otorrhea was more common in controls than with either treatment (17 vs. 8%, P=.011). Treatment for 5 days was recommended for mucoid or purulent effusions.

Poetker et al.[152] Clinical trial of 306 children undergoing tympanostomy tube insertion randomized to one of 3 groups: (1) no postoperative otic drop (control group), (2) ofloxacin otic drops and (3) neomycin sulfate-polymyxin B sulfate-hydrocortisone otic drops (COS group). Intervention groups were less prone to post-operative otorrhea and "tube failure" (i.e., blocking or plugging) when MEE was present, compared to the control group (8-12% vs. 30%).

Roland et al.[153] Multicenter, single-blind, trial of 201 children aged 6 months to 12 years with acute TTO (less than 3 weeks) randomized to topical ciprofloxacin 0.3% eardrops, with or without dexamethasone 0.1% for 7 days. In the 167 evaluable, culture positive subjects, dexamethasone reduced the overall duration of otorrhea from 5.3 to 4.2 days (P=.004) and resulted in better clinical

response at day 8. Comparable outcomes were achieved at day 14.

Roland et al.[154] Multicenter, observer-masked trial of 599 children aged 6 months to 12 years with acute TTO randomized to ciprofloxacin 0.3%/ dexamethasone 0.1% eardrops for 7 days vs. ofloxacin 0.3% eardrops for 10 days. At the test of cure visit ciprofloxacin-dexamethasone had higher rates of clinical cure (90 vs. 78%) and bacteriologic success (92 vs. 82%). Median time to otorrhea cessation was less with ciprofloxacin-dexamethasone (4 vs. 6 days). Both preparations were safe and well-tolerated.

*Level 2: Controlled Prospective Study.* Odutoye et al.[155] Controlled prospective study of 35 patients who had a tympanostomy tube placed in one ear by a surgeon wearing sterile gloves and masks, and the other while wearing only clean non-sterile gloves. No difference in postoperative otorrhea rates were observed, but low statistical power limits validity.

*Level 4: Uncontrolled Case Series.* Antonelli et al.[156] Case series of 24 children aged 2 to 16 years with TTO, who had assay performed on the otorrhea to detect pepsinogen. Only 8 of 26 samples were positive for pepsinogen, at a lower concentration compared with serum, thus excluding a major role for gastric reflux in tube otorrhea in these children.

Kumar et al.[157] Case series of 488 children aged 6 months to 14 years who underwent tympanostomy tube insertion for OME, recurrent AOM, or both, over a 9-month period. One surgeon prescribed ciprofloxacin eardrops after surgery for 219 patients while another in the same practice used oxymetazoline drops for 269 patients; during surgery all MEEs were cleared with suction and saline irrigation of the ear canal. By 4 weeks post-operatively, the incidence of otorrhea was 7-10%, with no group differences.

Martin et al.[158] Case series of 166 children with otitis externa (25%) or TTO with fungal organisms on culture. Children were identified over a 7 year period from 1,242 who had culture of otorrhea at a tertiary care hospital. The most common organisms were Candida albicans or parapsilosis (67%) or aspergillus fumigatus (13%) Fungal infections were more frequent after the introduction of fluoroquinolone ototopical drops. Time to resolution ranged from 1 week to 9 months (median 3.8 weeks).

*Level 6: Symposium Abstract.* Granath.[159] Unmasked trial of 50 children under age 3 years who underwent tympanostomy tube insertion for recurrent AOM randomized to receive, in case of otorrhea, topical treatment with or without systemic antibiotics. No difference was found in group outcomes. Only 51 episodes of otorrhea were found, all apparently caused by pathogens present in the nasopharynx.

*Healthcare Delivery Research.* Roland et al.[160-161] Cost-effectiveness analysis of ofloxacin vs. ciprofloxacin-dexamethasone ear drops for treating AOM in children with tympanostomy tubes. Efficacy estimates were based on clinical trial outcomes and costs were based on complicated assumptions about treatment failures. Ciprofloxacin-dexamethasone was 4.5 times more cost-efficient than ofloxacin. This finding, however, needs to be approached with reservations about the assumptions, the scope and sensitivity of the conclusions, and the financial interests of the authors. In another study,[161] they reported the cost-effectiveness aspect of the modeling using otorrhea-free days as the outcome. The regimens were in fact close in both cost and effectiveness, ofloxacin being less cost-effective, (ratio between ratios of 1.15) which in another context might be taken as equivalence.

## CHRONIC SUPPURATIVE OTITIS MEDIA

*Level 1: RCT or Systematic Review of RCTs.* Couzos et al.[162] Multi-center, double-blind, trial in eight Aboriginal Community Controlled Health Services of 147 children aged 1-14 years with CSOM randomized to topical ciprofloxacin vs. framycin-gramicidin-dexamethasone (FGD) together with povidone-iodine ear cleaning. Resolution of otorrhea was significantly higher with ciprofloxacin compared to FGD (76 vs. 52%, P=.009). Tympanic membrane perforation and hearing levels did not change. *Pseudomonas aeruginosa* was the most common bacterial pathogen (48%).

Indudharan et al.[163] Prospective, randomized trial of topical antibiotic vs topical antibiotic-steroid combination for treating CSOM. A total of 152

ears in 135 patients with organisms sensitive to gentamicin were randomly assigned to gentamicin eardrops or gentamicin-steroid combination for 3 weeks. Clinical improvement was found in 87-88% and bacteriologic improvement 75-83%. Audiograms showed deterioration in bone conduction threshold above 5 dB at speech frequencies in 29% vs 30% of subjects, respectively. The authors conclude that adding a topical steroid does not improve CSOM outcomes or alter ototoxicity.

Jaya et al.[164] Double-blind trial of 40 subjects aged 10 years or older with CSOM randomized to topical 5% povidone-iodine vs. topical 0.3% ciprofloxacin for 10 days. No differences in "clinical improvement" (undefined) were found at each time-point. No ototoxic effects were noted; no deterioration of hearing was noted from pre-and post-treatment pure-tone audiograms.

Macfadyen et al.[165] Clinical trial of 427 Kenyan school children with CSOM randomized to topical ciprofloxacin vs. boric acid in alcohol. Resolution of otorrhea at 2 weeks was 59% with ciprofloxacin vs. 32% with boric acid (P<.001), with significant differences persisting at 4 weeks. Fewer adverse events were found with ciprofloxacin.

Minja et al.[166] RCT of various treatments for CSOM: dry mopping alone; dry mopping and boric acid in alcohol ear drops; and dry mopping, boric acid in alcohol ear drops and oral amoxicillin. Three hundred twenty-eight children aged 5-17 years in Dar es Salaam with CSOM in one or both ears were entered. At follow-up 3-4 months after beginning treatment, 31% of the subjects in the dry mopping group had dry ears, compared to 54% in the eardrop group and 56% in the eardrop with amoxicillin group. Hearing testing did not reveal any improvement in hearing levels.

van der Veen et al.[167] Clinical trial of 101 children aged 1-12 years with CSOM treated with a short-course of steroid-antibiotic eardrops and randomized to 6-12 weeks of orally administered trimethoprim-sulfamethoxazole vs. placebo. The prevalence of otorrhea in the antibiotic vs. placebo groups at 6 weeks was 28 vs. 53%, at 12 weeks 32 vs. 47%, and at 1 year 25 vs. 20%, respectively. Pure-tone hearing levels and health-related quality of life improved during the study but did not differ between the trimethoprim/sulfamethoxazole group and the placebo group. A 6-12 week course of antibiotic for CSOM is beneficial, but the effect disappears once the drug is discontinued.

*Level 2: Controlled Prospective Study.* Jang et al.[168] Clinical trial of 55 patients with otorrhea cause by methicillin-resistant *S. aureus* prospectively assigned to vancomycin eardrops vs. topical gentamicin 0.3% solution. Otorrhea was significantly reduced in 94% of the vancomycin group and only in 20% in the gentamicin group (P < 0.03).

Lancaster et al.[169] Prospective study of adherence to therapy for 36 adults and children with otitis externa or CSOM treated with Gentisone HC, Sofradex, or Otomize ear drops. The amount of Sofradex used differed statistically from the amount expected. There was a trend to overdose with Sofradex and Gentisone HC, due to differing delivery systems and drop viscosities.

*Level 3: Controlled Retrospective Study.* van der Veen et al.[170] Case-control study of predictive factors for development of CSOM in 480 children aged 1 to 12 years. Treatment with tympanostomy tubes was the most important prognostic factor (OR=121); other factors included having more than 3 upper respiratory tract infections in the past 6 months (OR=12), having parents with low educational level (OR=14), and having older siblings (OR=4.4). Independent predictors for CSOM after tympanostomy tube insertion were more than 3 episodes of OM in the past year (OR=4.9), attending day care (OR=3.6), and having older siblings (OR=2.6).

*Level 4: Uncontrolled Case Series.* Baba et al.[171] Cross-sectional study of 324 patients with CSOM, followed for 6 months or longer after tympanoplasty. Hearing improved in 73% of patients with poor preoperative hearing and 50% with good hearing. Tinnitus improved or resolved in 66%, vertigo in 30%, aural fullness in 86%, and otorrhea in 85%.

Bahmad and Merchant.[172] Histopathological study of ossicular grafts and implants in 50 surgical and 6 temporal bone cases. Autogenous malleus, incus and cortical bone grafts maintained their morphologic shape, size, and contour for at least 30 years. Cartilage grafts developed chondromalacia with loss of stiffness and tendency to resorption.

Synthetic prostheses of porous plastic (Plastipore, Polycel) elicited foreign body giant cell reaction with biodegradation of implants. Prostheses made of hydroxyapatite and Bioglass were lined by connective tissue and mucosal epithelium and partially resorbed.

Cruz et al.[173] Chart review of surgical therapy for 84 ears with CSOM. Forty-one ears with noncholesteatomatous COM underwent tympanomastoidectomy, 43 ears with cholesteatoma were managed according to the extension of the disease, closed mastoidectomy was indicated in 19 cases, and open mastoidectomy was performed in 24 ears. Good results were achieved.

Jang and Park.[174] Case series of 88 adults with recurrent otorrhea caused by CSOM unresponsive to topical ciprofloxacin. Ciprofloxacin-resistant *Pseudomonas aeruginosa* was isolated in all cases. Susceptibility to imipenem was 96%, followed by amikacin (56%), piperacillin/tazobactam (37%) and ceftazidime (32%).

Kashiwamura et al.[175] Case series of 48 patients (50 ears) with refractory chronic otorrhea due to various conditions treated with topical Burow's solution (13% aluminum acetate with a pH of 3.06) from 1-3 days per week for 1 day to 4 weeks. After treatment, 35 ears (70%) were considered cured and 10 (20%) were improved. In vitro susceptibility was also studied for various bacteria and fungi.

Katsura et al.[176] Case series of 17 patients aged 5-70 years with bilateral CSOM (from a larger series of 200 CSOM patients) treated with bilateral same-day tympanoplasty. The rate of successful perforation closure was 91% after simple underlay myringoplasty and 75% after conventional tympanoplasty.

Mak et al.[177] Prospective case series of 58 Aboriginal children, aged 5-15 years, with CSOM. Myringoplasty had a 49% success rate, defined as closure of perforation and normal hearing in the operated ear 6 or months after surgery. Success rates were unrelated to perforation size or active otorrhea at the time of surgery.

Rickers et al.[178] Case series of 47 children with CSOM (without cholesteatoma), long-term follow-up for 5 to 21 years. A dry ear was achieved in 94% for several years after tympanomastoidectomy. Revision mastoidectomy was needed in 13% and revision tympanoplasty in 21% of children.

Suzuki et al.[179] Prospective case series of topical ofloxacin therapy up to 4 weeks for otorrhea caused by CSOM (n=64) or acute exacerbation (AE) of chronic OM (n=173). Clinical response after 2 weeks was 39% for CSOM and 61% for AE, rising to 58% and 75%, respectively, after 4 weeks. Bacterial eradication at 2 weeks was 91%. Secondary fungal infection or serious adverse events did not occur. The authors recommend a 2-week course of initial therapy, with extension up to 4 weeks as needed.

Wang et al.[180] Before and after study of 77 Taiwanese patients with CSOM treated with tympanomastoid surgery. The issue whether all or none of these patients should receive operations could not be addressed, but results favored the policy of operating on wet ears.

## METHODOLOGY OF OTITIS MEDIA CLINICAL TRIALS

The current Panel concluded that the methodological considerations in clinical trials, as published in the previous Conference Report, still contain important challenges for the future. Therefore, the list of considerations has been updated with the addition of further goals set by current needs.

1. Clear definition of the condition(s) being studied is paramount. Standardized definitions of AOM, otitis media treatment failures, recurrent AOM, OME, and CSOM should be developed, refined, and used in clinical trials.

2. Variables that are known or reasonably suspected to affect outcome, such as age, gender, anatomic and environmental factors, and disease laterality, should be identified and managed in the experimental design and analysis by appropriate stratification or statistical adjustment. This means that most analyses have to be multivariable.

3. Entry and exclusion criteria should be clearly stated.

4. OM should be recognized as a disease continuum, with specification in a treatment trial of where the subjects fall within this continuum in severity, persistence, or both.

5. Concurrent control or comparison groups are mandatory to differentiate treatment effects from the favorable natural history of untreated AOM and OME.[181]

6. Rigorously defined measures of outcome, and cut-off criteria on these where category counts are adopted, are essential. This includes precise definition of timing or time-intervals.

7. Methods for retaining subjects and maximizing treatment compliance should be incorporated into study design. The retention and compliance should be fully documented and where compromised alternative analyses (e.g., with imputation of missing data) presented to reduce biases.

8. The analysis as randomized (by "intention-to-treat") is obligatory, although other analyses can be reported to address specific issues. Any exclusion of variables measured or of outcome time points must be fully specified and justified.

9. Where presence versus absence or the relative magnitudes of effects (e.g., in two conditions or with two treatments are contrasted) it should be made explicit whether this is purely descriptive, permitting only a v weak conclusion, or whether the appropriate statistical test (of interaction) has been performed and is significant, permitting a strong conclusion.

10. Economic costs of treatments and benefits, in terms of treatment efficacy, quality of life considerations, and developmental outcomes, will become increasingly important in an era of diminishing health care resources.

11. Ethical considerations are paramount in designing clinical trials; the high information value of the RCT has to be balanced in certain sensitive areas against an ethical and practical cost.

12. The magnitudes of important clinical benefits should be emphasized to define appropriate expectations for parents and health care providers. Mere statistical reliability (significance) of a finding should not be misrepresented as material clinical benefit.

13. Harms and adverse events should be reported in detail, and discussed in relation to potential benefits of the intervention.

14. Quality of reporting standards should be observed for RCTs,[182] meta-analyses of RCTs,[183] and noninferiority (equivalence) randomized trials.[184]

15. Data-sharing is encouraged, to facilitate individual patient meta-analyses.[51-52]

## FUTURE RESEARCH GOALS

### *Short-term research goals*

1. Improve diagnostic accuracy for AOM and OME.

2. Evaluate the role of parent-assisted identification of MEE cases, using tympanometry or acoustic reflectometry.

3. Identify children with AOM best suited for initial observation without antibiotics and define surrogate markers to prioritize the need for antibiotics in treating AOM.

4. Gather additional information on safety and outcomes of initial observation of AOM without antibiotics in children aged 6 months to 2 years.

5. Define and articulate initial management strategies for children with AOM observed without antimicrobials, including optimal pain control and duration of observation.

6. Compare MEE incidence based on initial AOM treatment (immediate antibiotic, delayed antibiotic, or no antibiotic).

7. Determine rate of biofilm formation in experimental models (e.g., chinchillas) of AOM treated with immediate antibiotic, delayed antibiotic, or no antibiotic.

8. Establish safety and efficacy of topical analgesic drops in children with AOM, especially in young children who have been excluded from existing studies.

9. Conduct prospective observational studies, preferably multi-site, to evaluate the impact of guidelines including an observation option guidelines in which clinicians' reasons for deviation from recommendation are acquired. Documentation and analysis of these reasons may both encourage general adoption (where not explicitly rejected as

inappropriate) and identify detailed aspects in need of revision.[185]

10. 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.

11. Develop alternative delivery systems of antimicrobial agents directly to the middle ear, especially with an intact tympanic membrane.

12. Define strategies and interventions for managing OME detected in neonates or young infants who fail their newborn hearing screen.

13. Conduct prospective studies to define the natural history and spontaneous resolution of TTO, and to identify prognostic factors, hence subgroups best suited for prophylaxis, initial observation, or symptomatic treatment.

14. Assess the efficacy of non-antimicrobial strategies for TTO, such as daily aural toilet, saline irrigation, and hydrogen peroxide.

15. Define risk vs. benefit profile of topical antimicrobials for TTO, including quinolones, especially regarding fungal superinfection and selective pressure on bacterial resistance.

16. Evaluate the efficacy of surgery vs. medical management vs. both for treating CSOM, including alternative medi al strategies.

17. Establish the role of adenoidectomy, gastroesophageal reflux management, and anti-allergy therapy in managing the child with refractory TTO or CSOM.

18. Evaluate the efficacy of immunomodulation of the nasopharyngeal mucosal immune system, such as by vaccination (bacterial, viral, systemic, and mucosal), pro- and pre-biotic agents, and commensal bacteria such as alpha hemolytic streptococcus, and the consequent effectiveness for reducing MEE prevalence after AOM.

*Long-term research goals*

1. Encourage international collaboration in designing and conducting clinical trials in OM through practice-based networks.[186]

2. Conduct international comparative studies of OM diagnosis (AOM, otitis media treatment failures, recurrent AOM, OME, CSOM) in relation to treatment with antibiotics or surgery, to analyze which parts of the OM continuum are treated and if this differs between countries.

3. Monitor shifts in bacteriological epidemiology caused by vaccination and determine their implications for antimicrobial use.

4. Gather additional information on selective pressure for bacterial resistance caused by antimicrobial therapy for OM and continue evaluating new ecologically “safe” antimicrobials in the context of bacterial resistance.

5. Determine whether vaccines against respiratory viruses are also effective in preventing OM.

6. Determine the efficacy of vaccines not yet adopted for universal prevention as treatment for OM cases definable by risk factors or adverse history.

7. Include intermediate and long-term outcome measures in OM treatment studies of medication vs. placebo, therapy vs. no therapy, and surgery vs. no surgery.

8. Detect surrogates that may allow reduction in the numbers of participants in clinical trials , so as to promptly differentiate “good” vs. “bad” antimicrobials for AOM.

9. Identify and evaluate surgical interventions for OM other than tympanostomy tubes and adenoidectomy.

10. Identify subgroups of children with OM who are likely to benefit most from surgery.

11. Conduct large, observational studies to define more precisely the stay-time (functional duration) of short-, medium-, and long-term tympanostomy tubes, and to document the impact of stay-times on OM recurrence. Define the harm vs. benefit ratio of tympanostomy tubes with different functional durations

12. Conduct randomized trials to determine the impact of tympanostomy tube stay-time on developmental outcomes in severely affected children with OME, including the trade-off with

adenoidectomy as a known extender of the benefit from short-term tubes.

13. Obtain additional information using observational studies and RCTs on the long-term impact of tympanostomy tubes on otherwise healthy children with OME.

14. Define watchful waiting and surveillance strategies for children who are not immediate candidates for surgery in OME.

15. Conduct well-designed RCTs with adequate statistical power to assess the efficacy of anti-allergy therapy, complementary and alternative therapies for AOM and OME.

16. Conduct well-designed RCTs with adequate statistical power to assess the efficacy of Eustachian tube auto-inflation for OME.

17. Establish the impact of tympanostomy tubes on developmental sequelae in children with special needs (Down syndrome, cleft palate, cerebral palsy, developmental delays).

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# 8. COMPLICATIONS AND SEQUELAE

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## INTRODUCTION

The Panel considered certain categories of complications and sequelae of otitis media (OM). They were organized according to the anatomic pathology from tympanic membrane (TM) to intracranial complications.

Published reports for the past 5 years were included for sequelae or complications ensuing from OM in childhood to adulthood.

## TYMPANIC MEMBRANE

Sequelae of OM manifested in the TM include atrophy, myringosclerosis, retraction, adhesion and perforation.

In a paper from the Dutch group[1] long-term consequences of tympanostomy tube treatment is reported. Three hundred fifty-eight subjects with a positive and negative history of OM or tympanostomy tube insertion derived from a birth cohort had been followed-up from preschool to adult age. TM abnormalities were investigated at ages of 8 and 18 years. The occurrence of myringosclerosis was 6 times as common in the tube treated OM ears (56%) compared to the non-OM ears (9%) and the frequencies were not changed throughout the follow-up period. In contrast the frequencies of atrophy, atelectasis and pars flaccida retractions diminished throughout the follow-up period. Atrophies were more common in tubulated ears compared to the nontubulad, 20% vs. 7%, but in contrast to myringosclerosis the occurrence of atrophies decreased with time. Atelectasis and retractions of pars tensa as well as the pars flaccida showed similar dynamics as atrophies but with less differences between the tubulated and non-tubulated ears.

In a study by Johnston LC et al.[2] on children with persistent MEE and tympanostomy tube treatment in the first 3 years of life the TM abnormalities were followed up at the age of 5 years. The children had been grouped in an early-treatment group, a late-treatment group and a non-trial group. One or more types of TM abnormalities in one or both ears, among children who received tubes, were 70.7%, 42.5% and 9.5%, respectively. Within the 3 groups, among children who received tubes, the proportions who had an abnormality of some type were similar, namely, 82.6%, 80.4%, and 83.3%, respectively. The corresponding proportions among children who had not received tubes were 15.4%, 19.3%, and 7.2%, respectively. Segmental atrophy and tympanosclerosis were the most common abnormalities found.

In a retrospective study by Ryding M et al.[3] on thirty-four patients, aged 16-25 years, with previous chronic OME persisting at least 6 years it was found that 76% of tube-treated exhibited TM abnormalities 18 years after surgery, compared to 0% in the control group. Similar figures for TM pathologic abnormalities after tube treatment was reported by Stenstrom et al.[4]

A Danish study[5] presented at the present symposium described in a 25-year follow-up that the pathology of the TM after treatment with grommets changes over time. While the occurrence of retractions decreases over time, atrophy increases and sclerosis remains grossly stable.

Tos and Cayé-Thomasen[6] proposed a new 6-staged classification for posterior pars tensa retractions, which is based on their long-term epidemiological studies on secretory otitis and eardrum pathology, as well as studies on the pathogenesis of cholesteatoma. A report by Awad and Pothier[7] suggests that digital photography of TM changes is considerably more accurate than the generally used clinical drawings and that its use should be encouraged.

Oktay et al.[8] evaluated the histopathological changes in central TM perforations caused by chronic otitis media without cholesteatoma. Twenty-nine temporal bones from 25 patients (13 male patients and 12 female patients) with central TM perforations; 18 chronic otitis media with perforation and 11 chronic otitis media with perforation caused by ventilation tubes and 30 aged-matched normal temporal bones were included in this study. An extension of the migration of stratified squamous epithelium at the inner surface of the TM was observed in 11 of the 29 perforations (38%). The thickness of TM was significantly different between the perforation groups and the control group. Of the 29 TMs, 13 (44%) had tympanosclerosis and 8 (28%) revealed papillary projections of squamous epithelium. Most of the TMs showed one or more signs of sequelae or persistent abnormalities such as tympanosclerosis, papillary projections, thickening, and ingrowth without significant differences whether caused by tympanostomy tube treatment or the disease *per se*.

The efficiency of a subannular tube insertion technique in a group of pediatric patients with adhesive otitis or severe atelectasis of the TM was evaluated in a retrospective nonrandomized case series.[9] The study group consisted of 190 patients (316 tubes) aged between 3 and 19 years (average 9 years old) and operated on between 1993 and 1999 by four pediatric otolaryngologists. The average follow-up was 53 months. The tubes remained in place for an average of 21.8 months, with fluoroplastic tubes lasting 17.8 months and Goode T tubes lasting 23.8 months. When used in children between 5 and 9 years of age and in cases of adhesive otitis, Goode T tubes showed statistically significantly better results than fluoroplastic tubes. The complications of this technique were otorrhea (17.7%), perforation (7.9%), a plugged tube (7.0%), and cholesteatoma (1.6%). The 5- to 9-year-old group and the reintervention group of patients showed statistically higher complication rates compared with all other groups. Sixty-four patients (128 tubes) were eligible for audiogram analysis, which showed a gain of 13.4 dB (speech reception threshold). The technique of subannular tube insertion is a safe and effective method for long-term middle ear ventilation in cases of adhesive otitis or severely atelectatic tympanic membranes or for patients with pathology related to dysfunction of the Eustachian tube.

The diffusion of gas across the TM in humans and animals is slow. However, structural changes caused by repeated TM perforations could affect gas diffusion rates. This possibility was evaluated by Felding et al.[10] using a chinchilla model. The study showed an increased rate of diffusive gas exchange across TMs that had been repeatedly perforated. This effect may be caused by a structural thinning secondary to scar formation and could have implications for middle ear pressure regulation in ears with a history of repeated myringotomies and/or tympanostomy tube insertions.

Of certain interest is the report of Ibekwe TS et al.[11] concerning persistent perforations among adults in West Africa. The majority of the perforations were caused by infections.

A study on surgical removal of tympanostomy tubes by Adkins and Friedman[12] included 92 pediatric patients. It was shown that the overwhelming majority of patients who undergo surgical removal of tubes will show complete tympanic membrane healing independent of technique at the time of removal, duration of intubation, patient age, or indication for removal.

Goode´s T-Tubes have a bad reputation because their residual tympanic perforation rate. Carignan et al.[13] reported on a modified Goode´s T-tube that had a comparable perforation rate to that of short-term tubes despite the tubes had been in place at an average of 2.9 years.

Stenfeldt et al.[14] analyzed the distribution of 3 collagen types; I, II and III, in healthy TMs, during healing of a perforation, and during infection with *Streptococcus pneumoniae*. In normal TMs Type II collagen was a main

constituent of the lamina propria of the pars tensa, whereas type I collagen was found mainly in the pars flaccida. Collagen types I and III were found at the insertion to the malleus handle and in the loose connective tissue surrounding the main collagen layer of the pars tensa. After myringotomy, collagen types I and III were found at the perforation border and around dilated blood vessels early in the healing phase. During infection, the collagen layer was thickened and stained strongly for type II collagen. Collagen types I and III were found in the edematous connective tissue around the main collagen layer and around dilated blood vessels. Three months after perforation or infection, all 3 collagens were present in the lamina propria of the TM. Extensive amounts of all 3 collagen types were present in the scar tissue in the TMs of rats that had undergone myringotomy during the presence of acute otitis media.

Several studies have investigated effects of various compounds on the healing of TM perforations in animal models. In a study by Eken et al.[15] the effects of insulin on the healing of acute traumatic TM perforations were tested in guinea pigs. The treatment showed an increased activity of the fibroblasts in lamina propria as well as an increased content of collagen. It was concluded that topical insulin treatment may be beneficial in the treatment of atrophic membrane, which is a sequel of perforation. Ramalho and Bento[16] studied the effect of epidermal growth factor (EGF) and pentoxifylline on subacute TM perforations in chinchillas and quantified the healing rate of such perforations treated with these drugs alone or in combination. EGF promoted healing of the TM perforations and the use of pentoxifylline did not. The combination of the two had no synergistic effect on the healing.

Ramahn et al.[17] investigated if the short-term healing scar that forms after experimental laser myringotomy will revert to a normal lamina propria in the long run.

Potassium titanyl phosphate laser myringotomy was made on one side of the TM in rats and the stiffness and strength of the healed TMs were measured. The interferometry readings showed a slightly reduced strength in the myringotomized and healed TMs. After half a year, still there were immense structural changes including an increased thickness over a wide area of the pars tensa with increased amounts of fibers. An obvious reorganization of the fiber layer was lacking. It was concluded that laser myringotomy causes profound, long-standing, or permanent structural changes in the lamina propria of the pars tensa, whereas the strength of the TM may become slightly reduced.

In a series of clinical studies Turkish groups[18-20] have tested various substances interfering with the formation of oxygen radicals on patients with chronic otitis media in attempts to hinder development of myringosclerosis. Also animal experiments have been performed to study if myringoscleros can be prevented.[21-23] Some positive results were obtained by administration of L-carnitine, vitamin E and alpha-tocopherol. In a study by Uneri et al.[24] it was shown that the vitamin E also reduced the formation of myringosclerosis in tympanostomy-treated TMs in humans. The authors however, concluded that further clinical studies consisting of a larger patient population are needed to bring about routine clinical use of antioxidants in myringotomy and VT insertion. The same group[25] investigated the effects of vitamin E-coated tympanostomy tubes in a series of rat TM perforations. The results indicated that vitamin E-coated tube insertion decreases the quantity of reactive oxygen species in TM after myringotomy and tympanostomy tube insertion.

In a study by de Carvalho Leal et al.[26] the influence of hypercalcemia in the development of tympanosclerosis in rats with or without acute otitis media was assessed. One group of rats was subjected to hypercalcemia status through calcium diet supplementation and the other used as a control group (normal calcium content diet). Both groups were subjected to induction of tympanosclerosis by inoculation of *Streptococcus pneumoniae* on the right middle ear only. The group subjected to hypercalcemia presented a prevalence of tympanosclerosis of 25% against 16.7% in the control group, presenting a relative risk of 1.27 ($p=0.72$). The results suggest that hypercalcemia may have an influence in the development of tympanosclerosis

In rats Raustyte et al.[27] investigated the calcification process in the formation of myringosclerosis by use of the expression of three bone modelling markers: osteopontin (OPN), osteoprotegerin (OPG) and osteonectin (ON). Calcium depositions were initially accumulated in the cytoplasm of macrophages and dispersed in the connective tissue layers of the pars flaccida and pars tensa. Late accumulation occurred in the lamina propria of pars tensa, more extensively in myringotomized ears. OPN expression was found early in inflammatory cells including especially macrophages and late in pars tensa fibrocytes. OPG expression was initially located to inflammatory cells and late to pars tensa fibrocytes and the inner basal membrane of pars flaccida. Some ears displayed a marked pars flaccida expression of ON in the connective tissue matrix on early days and at the inner basal membrane on later days. The latter cases were from myringotomized ears. Otherwise, no apparent differences of marker expression occurred between myringotomized and non-myringotomized animals. The authors concluded that osteopontin, osteoprotegerin and osteonectin are expressed by different cell types in the TM during calcification in association with AOM, with or without myringotomy. These molecules may accordingly play a role in the pathogenesis of myringosclerosis, in which macrophages and fibrocytes appear as potential major players.

A challenge in healing of TM perforations is to create an animal model for a chronic perforation. Kaftan et al.[28] made an attempt to inhibit the epidermal growth factor receptor (EGFR) which may arrest wound healing. The EGFR tyrosine kinase inhibitor (erlotinib) was administered. However, systemic application of erlotinib was not suitable for creating a chronic TM perforation in rat.

Hebda et al.[29] studied the effects of the ciprofloxacin-dexamethasone (CDX) combination applied ototopically after myringotomy on TM healing in rat ears with Eustachian tube obstruction (ETO) and unobstructed ears. Animals were randomized into three groups to receive no treatment or bilateral once daily ototopical treatment with balanced salt solution (BSS, vehicle) or CDX for 13 days. On day 14, TM perforation healing rates were 100% in all ears of untreated and BSS-treated animals, 89% (8/9) in CDX-treated obstructed ears, and 30% (3/10) in CDX-treated unobstructed ears ($P < .05$ vs. BSS). On day 28, 100% (5/5) of the CDX-treated unobstructed ears and 80% (4/5) of the CDX-treated obstructed ears were healed. Histology showed initial TM postmyringotomy thickening in all ears but no significant qualitative differences between groups on day 28. The authors concluded that myringotomy healing was transiently modulated by treatment with CDX but proceeded normally after CDX discontinuation. This early modulation might enhance middle ear drainage and middle ear concentrations of CDX when tympanostomy tube surgery is performed in patients with active OME and ETO, thus potentially reducing otorrhea and preventing or treating infection. It would not be expected to increase the risk of premature tube extrusion or adversely affect normal healing of the TM after the usual spontaneous extrusion.

The Umeå-group has proposed plasminogen to play an important role in different tissue remodeling processes such as wound healing and tissue regeneration after injuries including the healing of TM perforations.[30] It was shown that the healing of TM perforations is completely arrested in plasminogen-deficient mice, with no signs of any healing even 143 days after perforation. Inflammatory cells were recruited to the wounded area, but there were no signs of tissue debridement. In addition, removal of fibrin, keratinocyte migration and in-growth of connective tissue were impaired. This contrasts with skin wound healing, in which studies have shown that, although the healing process is delayed, it reaches completion in all plasminogen-deficient mice. The finding that keratinocyte migration and re-epithelialization were completely arrested in plasminogen-deficient mice indicates that plasminogen/plasmin plays a more profound role in the healing of TM perforations than in the healing of other epithelial wounds.

In studies by Rahman et al.[31] the long-term influence of embryonic stem cells on acute perforations and the effect of gelatin as a vehicle for applied stem cells were investigated. The possibility of teratogenic effects of the stem cells was also observed. Bilateral laser myringotomy

was performed in adult Sprague-Dawley rats. Stem cell treated ears did not show any enhanced healing of the perforation although a marked thickening of the lamina propria was observed compared with control group. After half a year the strength and the stiffness of the tympanic membrane was almost the same for both treated and untreated ears. No evidence of teratoma was found after half a year. The study suggests that the stem cells stimulate the proliferation of connective tissue and fibers in the lamina propria, possibly mediated by secreted substances, although the stiffness properties do not seem to be altered. The use of gelatin does not seem to enhance the healing process of the TM perforation.

## CHOLESTEATOMA

Based on epidemiological, clinical and immunohistochemical studies Sudhoff and Tos[32] proposed a four-step pathogenesis for cholesteatoma, combining the retraction and proliferation theory. The basic mechanism is the same as the same authors[33] earlier suggested for attic cholesteatoma. However the anatomical conditions especially the shape and severity of retractions around the incudostapedial joint and around the stapes differ from the attic cholestetatoma.

Tokuriki et al.[34] investigated the genes regulated in human cholesteatoma compared with normal skin tissue using complementary DNA arrays. The observed alteration in gene expression suggested a role in various mechanisms of pathogenesis in cholesteatoma.

Rochetti et al.[35] investigated the possible relationship between *Chlamydia pneumoniae* and the development of cholesteatoma. Tissue was studied in three different layers by polymerase chain reaction analysis. Four specimens contained *Haemophilus influenzae*, always in the external layer, whereas none contained *Mycoplasma pneumoniae*.

Kim et al.[36] identified upregulated proteins in human cholesteatoma in comparison with canal skin using proteomic analysis with 2D electrophoresis and matrix-assisted laser desorption and ionization time-of-flight mass spectrometry (MALDI-TOF MS). The authors concluded that proteomic analysis may be a powerful tool for the identification and characterization of many promising candidate proteins relating to cholesteatoma.

Ozturk et al.[37] determined the micronucleus (MN) frequency of acquired cholesteatoma tissue using an MN assay on 18 patients diagnosed as having chronic otitis media with acquired cholesteatoma and were divided into primary and secondary acquired cholesteatoma groups. These results indicate that there could be associations between MN frequency and acquired cholesteatoma and between MN frequency and complications.

Lee et al.[38] studied telomerase activity in cholesteatoma and its relationship with cellular proliferation and clinical findings of 40 patients. As a cellular proliferation index, expression of Ki-67 was measured by means of immunohistochemical staining. The clinical features did not show a relationship with either telomerase activity or the cellular proliferation index.

Kobayashi et al.[39] examined whether Vitamin D3 (VD3) could suppress matrix metalloproteinases (MMPs) production from cholesteatoma keratinocytes *in vitro*. Addition of VD3 into keratinocyte cultures caused the suppression of MMP and TIMP production, which was increased by LPS stimulation. This was dose-dependent. The present results showing the suppressive activity of VD3 on the production of MMPs, which are responsible for tissue remodeling, strongly suggest that VD3 would be a good candidate for an agent in the medical treatment of, or prophylaxis for, cholesteatomas.

Raynov et al.[40] investigated the presence of progesterone receptor (PGR) and oestrogen receptor (EGR) in human middle-ear cholesteatoma (MECh) tissues and to compare their expression between male and female patients. The preliminary experimental results give us ground to assume that female sex hormones may stimulate proliferation and affect differentiation of MECh keratinocytes.

Daudia et al.[41] investigated the matrix metalloproteinases (MMP) in 11 cholesteatoma specimens, ten deep meatal skin and ten

postauricular skin specimens with immunohistochemistry using monoclona antibodies antibodies to MMP-8 and MMP-13. Expression of MMP-8 and MMP-13 were found to be significantly higher in cholesteatoma compared to postauricular skin, suggesting that both MMP's are possible components of the destructive process seen in cholesteatoma.

Hwang et al.[42] evaluated localization and expression of the peroxidase proliferator-activated receptor (PPAR)gamma in cholesteatoma epithelium with reverse-transcription polymerase chain reaction performed on cholesteatoma tissues from 10 adult patients undergoing tympanomastoid surgery for middle ear cholesteatoma and on 10 samples of normal external auditory canal skin tissue. PPARgamma is up-regulated in the cholesteatoma epithelium compared with normal external auditory canal skin. These results suggest that PPARgamma may play an important role in the pathogenesis of cholesteatoma.

Tinling and Chole.[43] reported the sequence of gross and histopathologic change to the normal middle ear (ME), TM, and external auditory canal (EAC) during spontaneous gerbilline cholesteatoma development. They reported that cholesteatoma development followed the sequence of: 1) slightly thickened pars flaccida (PF) without ME effusion, 2) thickened PF with ME effusion, 3) continuous buildup of EAC debris, and 4) complete occlusion of the lateral EAC.

Yoshikawa et al.[44] investigated the role of fibroblasts in the pathogenesis of cholesteatoma on tissue specimens obtained from four patients using the human genome U133A probe array (GeneChip) and real-time polymerase chain reaction. These results suggested that fibroblasts may play a role in hyperkeratosis of middle ear cholesteatoma by releasing molecules involved in inflammation and epidermal growth.

Cho et al.[45] investigated the expression and localization of placenta growth factor (PlGF) within cholesteatoma on tissue samples from human cholesteatoma and normal auditory meatal skin were obtained from patients during surgery for cholesteatoma of the middle ear.

Ozturk et al.[46] investigated the status of c-MYC oncogene in primary acquired cholesteatoma in 15 patients using fluorescence in situ hybridization with a mixed DNA probe, which is specific for c-MYC located on 8q24 and chromosome 8 specific-alpha-satellite DNA probe. These findings suggest that the ability of hyperproliferation of primary acquired cholesteatoma might have been related to c-MYC copy number by deregulating c-MYC expression.

## OSSICULAR PROBLEMS

Ossicular problems due to either fixation or discontinuity or resorption can cause conductive or mixed hearing loss. In a retrospective study on 315 operated ears of 305 patients by Yusan[47] the predictive role of the audiometric Carhart's notch for the assessment of middle-ear pathology prior to surgical intervention was investigated. In patients with otosclerosis and tympanosclerosis, a Carhart's notch was seen at 2 kHz in 28 (93 per cent) patients but at 1 kHz in only two (7 percent). However, in patients with chronic otitis media, a Carhart's notch was seen at 1 kHz in 10 (55 percent) patients and at 2 kHz in eight (45 percent) patients. Otitis media with effusion, tympanosclerosis and congenital malformations should be considered in the differential diagnosis of a patient with a Carhart's notch seen on pure tone audiometry. A Carhart's notch at 2 kHz indicates stapes footplate fixation, whereas one at 1 kHz indicates a mobile stapes footplate; the footplate mobility can thus be predicted preoperatively.

## MUCOSAL SEQUELAE

Middle ear mucosal changes are other important sequelae of OM inducing hypertrophy and/or hyperplasia, adhesion, or tympanosclerosis. In a study[48] by the Umeå group the role of the plasminogen (plg)/plasmin system for the spontaneous development of chronic otitis media was investigated in plg-deficient mice. Whereas essentially all of the wild-type control mice kept a healthy status of the middle ear, all the plg-deficient mice gradually developed chronic otitis media with various degrees of inflammatory changes during an 18-week

observation period. Five bacterial strains were identified in materials obtained from the middle ear cavities. Morphological studies revealed the formation of an amorphous mass tissue and inflammatory changes in the middle ears of plg-deficient mice. Immunohistochemical studies further indicate a mass infiltration of neutrophils and macrophages as well as the presence of T and B cells in the middle ear mucosa of these mice. These results suggest that plg plays an essential role in protecting against the spontaneous development of chronic otitis media.

In a study by Sancovic S et al.[49] the number, distribution and degranulation frequency of mast cells were studied in the middle ear mucosa biopsies of patients with chronic otitis media. The number of mast cells increased in all areas of middle ear cleft in chronic inflammation of the middle ear mucosa. In chronic otitis media of atticoantral and tubotympanic type mast cells were present in 91.5% of samples, the cells with heparin granules were present in 62.2% and degranulation frequency was 37.8%. In secretory otitis media 33 mucosal samples were analysed and all samples contained mast cells with predominance of histamine granules and with a degranulation frequency of 81.8%. These findings indicate that mast cells have complex function in pathophysiology of chronic inflammation of the middle ear mucosa: it may take part in amplification of inflammation as well as in its limitation. The studies confirm what has earlier been stated in animal models concerning mast cells and middle ear inflammation.

## INNER EAR SEQUELAE

*Vestibular Disturbances.* In a study by Waldron et al.[50] on children, aged 6-10 years, with clinically and audiometrically proven OME, a universal effect on balance was shown. A reduced effect on optic fixation and a reduced proprioception was shown. Insertion of grommets normalized the situation. Another study by Engel-Yeger B et al.[51] in children with MEE was performed with Bruininks-Oseretsky Motor Performance Test. Children with MEE had poorer muscle strength than the control group, though not significantly. In a review on vertigo in children Niemensivu R et al.[52] described that balance disturbances may occur in children with otitis media.

*Hearing and Auditory Sequelae.* During this symposium Casselbrant M et al.[53] reported that behavioural hearing thresholds in the high frequencies, 12 kHz to 20 kHz, is significantly higher in children with histories of otitis media.

Lauritzen M-BG et al.[54] showed that the "Galker test of speech reception in noise" is a valid and reliable measurement of speech reception in noise. The method may potentially be useful to detect children who are disabled by otitis media in their daily communication.

Two reports on the same cohort of 429 children randomized to receive early or later tympanostomy tube treatment were reported by Paradise and colleagues.[55] Johnston et al.[2] reported that at the age of 6 years, hearing thresholds averaged 6.2, 6 and 4.3 dB in right ears of children who received early, later and no treatment with tubes, respectively. Paradise et al.[55] did not show any difference between otitis media children with early vs. late insertion of tympanostomy tubes with regards to hearing, speech, language and behavioural testing.

The MRC MultiCentre Otitis Media Study Group[56] reported on improvement in conductive hearing loss immediately and over time in 233 children aged 3.5 to 7 years enrolled in the TARGET trial (Trial of Alternative Regimens of Glue Ear Treatment) with persistent OME and bilateral hearing loss >= 20 dB HL, who had been randomized to receive either myringotomy and tubes alone or accompanied by adenoidectomy. Tubes that were functioning during follow-up assessment improved hearing an average of 12 dB, thus tubes did not completely alleviate the conductive hearing loss. Tubes functioned in both ears for only 12 weeks on average. The authors concluded that recurrence of effusion and residual viscous effusion coating the ossicles was the most likely explanation for remaining conductive loss even with functioning tubes.

Rovers et al.[57] conducted an individual patient data (IPD) meta-analysis on seven randomised controlled trials (n = 1234 children in all), focusing on interactions between treatment and baseline characteristics--hearing level (HL). The

effects of conventional ventilation tubes in children were small and limited in duration. Observation, watchful waiting, therefore seems to be an adequate management strategy for most children with OME. Ventilation tubes might be used in young children that grow up in an environment with a high infection load (for example, children attending day-care), or in older children with a hearing level of 25 dB HL or greater in both ears persisting for at least 12 weeks.

A meta-analysis of the effect of tube treatment on hearing levels was published by Lous et al.[58] The meta-analysis found that mean hearing levels improved by 9 dB (95% CI 4 dB to 14 dB) after the first six months, and 6 dB (95% CI 3 dB to 9 dB) after 12 months. The combined effect of tubes and adenoidectomy was an additional 3 to 4 dB improvement in hearing (95% CI 2 dB to 5 dB) at six months and about 1 to 2 dB (95% CI 0 dB to 3 dB) at 12 months. The benefits of tubes in children appear small and diminished during the first year.

Gravel et al.[59] reported on conventional and extended high frequency hearing thresholds, electrophysiological measures of auditory function, and higher order auditory processing measures in a prospective cohort of children in North Carolina and New York. Results showed an association between early life OME and extended high frequency hearing thresholds at school age and auditory brainstem responses which indicated a central conduction delay. No relationship was found between early life OME and higher order auditory processes at school age.

Two studies of auto-inflation were reported during this period. Arick and Silman[60] conducted a randomized, controlled trial with a modified Politzer device used in the home setting over a 7-week period in 94 children aged 4 to 11 years. At study's end, the hearing sensitivity of 73.9% of the treated ears was within normal limits, compared with only 26.7% of the control ears.

Perera et al.[61] reported a meta-analysis of randomised controlled trials that compared any form of autoinflation to no autoinflation in individuals with 'glue ear.' Improvement was found for the composite measure of tympanogram or audiometry at less than one month (Relative Risk of Improvement (RRI) 2.47, 95% confidence interval (CI) 0.93 to 6.58) and at more than one month (RRI 2.20, 95% CI 1.71 to 2.82), and no significant increase in risk, thus this may be considered a conservative treatment in children being watched for persistent OME.

## SPEECH AND LANGUAGE DEVELOPMENT

The years 2003-2007 were marked by a limited number of research endeavors that examined the developmental outcome of otitis media with effusion (OME), especially those that utilized prospective designs. There were a total of nine original research reports that examined the developmental sequelae of OME, of which five used a prospective research design. We are including research that has employed retrospective designs based on the following rationale. Although in the four retrospective reports, OME was retrospectively documented, the other variables were collected contemporaneously. Moreover, in these studies the hypotheses are well-stated, the outcomes are specific to the hypotheses being tested, and the measures appear to be carefully assessed. Nevertheless, we will weigh more heavily evidence from prospective studies. In addition to these nine studies, there were two reports that considered opinions of parents and professionals regarding sequelae and two research reviews that will be briefly described. Thus, we will summarize the findings of the reports that directly assessed children's development, concentrating on the prospectively designed studies, and will briefly describe the conclusions of the surveys and the reviews. Reports that included outcomes in different domains are discussed in each of the topic areas.

*Speech Perception and Production.* During the time period surveyed, there was only one prospective study that examined speech production. Paradise and colleagues[62] studied speech development as part of their longitudinal study that included a randomized cohort and an observational cohort. In the randomized trial, children with persistent OME had tympanostomy tubes inserted either promptly or up to 9 months later if the effusion persisted. The follow-up at 6-

years of age included 395 children on whom speech samples were collected during conversations. No differences in the percentage of consonants correctly produced were found between children who received tympanostomy tubes in the early vs. delayed treatment groups. Neither was there any association between percentage of time with OME and percent consonants correct in the observational sample of 233 children.

In addition, there were two research studies that used a retrospective design—one examining speech perception and one examining speech production. In a study of 5-year olds with varying histories of OME and SES, Nittrouer and Burton[63] found that the OME group used more immature strategy for speech perception than did the control group. Shriberg and colleagues[64] examined children with speech impairments who were 3 to 5.5 years of age, half of whom had histories of OME. Their analysis of conversational samples indicated that the OME group was more likely to use backing of obstruents (e.g., cable for table). As indicated above, results of such studies carry less weight in coming to a consensus regarding the impact of OME. However, their exploration of specific aspects of speech development that were hypothesized to be affected by OME suggests that these might be fruitful areas to examine in future prospective investigations.

In summary, the one prospective study of speech production reported a relation with early OME, whereas the retrospective study did find an association. However, the inclusion of a sample of children with speech delays in the retrospective study reported by Shriberg and colleagues[64] may be one reason why their findings differed from the Paradise study.[62] Moreover, the type of production deficit they described was rather subtle in contrast to the overall measure of consonant production in the Paradise study.[62]

*Language.* There were five studies detailing relations between OME and language development reported in the literature, of which two were prospective designs. The language outcomes were collected from infancy through school age. There were varied measures including standardized test scores, naturalistic language samples, and narrative retelling.

Vernon-Feagans[65] and her colleagues examined language development as a function of OME and childcare in a cohort followed since infancy. Naturalistic language samples were collected at 18, 24, and 36 months of age while children were playing with a familiar adult at their childcare center. Samples were coded for a variety of syntactic, morphological, and semantic markers of language development. Although there were differences in many of the language measures between children in high-quality and low quality child care, there were no main effects or interactions as a function of OME at any time point.

Paradise and colleagues[62] examined language at 6 years of age in both their correlational and randomized controlled studies described above. In the correlational study, they reported that children with a history of OME scored lower on a standardized measure of receptive vocabulary as well as number of different words and utterance length in a conversational sample even when demographic and hearing status were controlled. However, the percentage of variance explained was low. These researchers did not find that the early and late treatment groups differed on any language measures.

There were three studies that employed a retrospective design. Majerus and colleagues[66] examined a group of 8 year old children with histories of OME matched for age, gender, SES, and non-verbal cognitive ability with a group of children with negative OME histories. They found no differences between these groups in either receptive or expressive vocabulary skills. Winskel,[67] who compared 6-8 year old children with and without histories of OME matched for age, gender, and SES, reported differences between OME groups in word naming and definitions but not in receptive vocabulary or narrative skills. Finally, Nittrouer and Burton[63] examined comprehension of complex syntax in their sample, with a non-significant difference reported between OME positive and control groups.

In the research cited, a variety of language skills were measured using both standardized measures and naturalistic language samples. In the one prospective study to document any

association between OME and language skills, the variance explained was minimal. Hearing was examined in all of these studies and during the period when OME was documented in the prospective reports as well. However, hearing was not included as a dependent or mediating measure, although it is hearing loss which is hypothesized to affect children's language development. Feagans and colleagues[65] did report that hearing and OME were highly related, which was their justification for only using OME classification in the analyses. Based on these findings, there is limited evidence to conclude that early otitis media has a lasting impact on language skills.

*Phonological Processing.* There were four studies that considered measures of phonological processing of which only one was prospective. All but one of these studies involved school age children; the remaining study included preschool age children. A variety of different measures were used including rhyme judgment, syllable counting, syllable manipulation, phoneme detection, and initial sound recognition.

Paradise and colleagues[55] followed their cohorts who were part of the randomized trial and the representative correlational study when they were between 9 and 11 years of age. They failed to detect any relationship between OME and phonological awareness or rapid naming in either the randomized or the representative cohorts. In the three studies with OME that were retrospectively documented (Maejerus and colleagues,[66] Nittrouer and Burton,[63] and Winskel,[67] all described previously), the groups with histories of OME performed more poorly than the control samples on a variety of different phonological processing measures including rhyme judgment.

In summary, it may be premature to conclude anything about the effect of OME on phonological processing. Despite the failure to find an association in the large, carefully documented cohort of Paradise and colleagues,[55] it would be worthwhile to examine skills such as rhyme judgment in other prospective samples before concluding that there is no impact of OME on phonological processing. It would also be useful to examine in relation to hearing as that is the putative variable that is thought to affect phonological abilities.

*Cognition and Academics.* There were four investigations of cognitive and academic skills in relation to a history of OME that were published. There were three prospective studies, two of which were the follow-up studies of Paradise and colleagues.[55, 62] All reports involved school age children and the outcomes included IQ, reading, spelling, writing, and mathematics.

In the studies reported by Paradise and colleagues[55, 62] there was no association between intelligence and either timing of tube placement or the percentage of time with middle ear effusion at either 6 years or between 9 and 11 years of age. In their sample at 9-11 years of age, Paradise et al.[55] reported significant but low associations between the percentage of time with OME and both reading fluency (in 3rd grade children only) and spelling in the representative subgroup, but they failed to find any differences in these and other academic measures (i.e., word identification, word attack, reading comprehension, and writing). IQ scores were not found to be different between the groups randomized for tube insertion and there was no association between IQ and OME in the representative subgroup. A third report by McCormick, Johnson, and Baldwin[68] also included a prospectively documented cohort seen at 7 years of age. McCormick and colleagues administered a variety of measures of reading skills and mathematics. No relationship was detected between any academic achievement measure and percent of time with OME in the first three years of life; neither did they find any differences in academic achievement between extreme OME groups. In contrast, Winskel,[67] in whose sample OME was retrospectively documented, found group differences in reading fluency and comprehension as a function of OM.

Based on the findings of these studies, there is very limited evidence that OME is linked to intellectual or academic outcomes. In the one prospective study[55] to report a relationship between OME and academic outcomes, the investigators only found it in one of the two subgroups they were following and they indicated that although significant, the associations were

low. None of the studies considered hearing in relation to OME reducing the ability to interpret the lack of findings.

*Memory and Attention.* There were five studies examining outcome in these domains, three of which were prospective. Three reports included memory skills and two reports included attention. The children who were the focus of these investigations were all school aged.

Of the three studies reporting memory, only Paradise and colleagues[62] study was prospective. They administered a nonword repetition test to their 6-year old sample. No relationship was found between performance on this task and the percentage of time with OME, and contrary to expectation, the group randomized to delayed treatment repeated significantly more nonsense words than did the early treatment group. Majerus and colleagues[66] administered five different measures of verbal short-term memory and failed to find any association with OME. Only Nittrouer and Burton[63] reported that children with reported OME made more errors on two measures of verbal working memory than did the control group.

Two prospective reports included measures of attention. Paradise and colleagues[55] administered both visual and auditory continuous performance tests to their 9 to 11 year old sample and obtained ratings of inattention from parents and teachers. The continuous performance tasks involved attending to either verbal or visual material and responding when preceded by a valid cue; derived scores include inattention and impulsivity. No differences were detected between the groups randomized for treatment or between the children randomized and the observational cohort; nor was the percentage of time with OME associated with verbal or visual attention or on the ratings of children's inattention. The second study of attention was reported by Hooper and colleagues[69] who reported on their prospectively followed sample, initially recruited from child care centers. When in second grade, they were administered several measures of auditory attention as well as parent and teacher ratings of sustained attention. These investigators not only studied the percentage of time with OME during the first 4-years of life, but they also examined the percentage of time with hearing loss during the same time period. Neither OME nor hearing loss was found to relate to attention in the early school years, whereas the home environment and mother's education were related to several of the outcomes. However, there was an interaction reported between hearing loss and the home environment on one of the measures of auditory attention. Specifically, children with hearing loss from less responsive and supportive home environments demonstrated poorer attention than children with hearing loss from more responsive and supportive homes. In summary, there is a lack of evidence that OME has a negative impact on memory and attention in the school years. There is some evidence that hearing loss may have a synergistic effect with environment in the development of attention.

*Behaviour.* The only two studies that included behavioral functioning as an outcome were reported by Paradise and colleagues.[55, 62] They administered several questionnaires to parents and teachers regarding the children's behavior at both time points (i.e., 6-years and 9-11 years). At 6-years, there was no difference between the early and delayed treatment group on any measure of behavior. The authors report that there were some low, but significant correlations in the representative subgroup. At 9 to 11 years of age, Paradise et al. indicate that children who had delayed tube insertion had significantly fewer parent-reported problems than the early treatment group. The group not eligible for randomization had more favorable scores on a parent rating of impulsivity and on a teacher rating of impairment. In this representative subgroup, there were low but significant correlations between the percent of time with OME and behavioral outcomes, accounting for no more than 6% of the variance in behavior. In summary, there appears to be only a weak association between otitis media and behavioral adjustment.

*Parent and Professional Opinions about Sequelae of OME.* Two papers were published that queried different groups of individuals regarding the impact of otitis media on development. Sonnenschein and Cascella[70] asked a small group of pediatricians their opinions about the relationship between otitis media (both acute

and OME) and children's hearing and speech-language development. Results of their survey indicate that while these pediatricians thought that otitis media occurring in the first two years of life could impact speech-language development, they were less inclined to agree that otitis media would have an effect in general. Moreover, they felt that parents and childcare environments could lessen the impact of otitis media on speech-language sequelae. The second paper by Higson and Haggard[71] examined the differences between parents, teachers, and otolaryngologists in rating the importance of symptoms and developmental impact of OME. Among their findings, they report that the three groups differed on all four areas of impact (i.e., 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 tend to rate language-education impacts with decreasing importance as children age. The conclusions reached in both of these papers reflect findings in the research literature that indicate that by school age, there is little impact of OME on speech and language development.

*Summary.* In the past four decades, there have been a host of research endeavors examining the relationship between a history of OME and developmental outcomes. Although there have been considerably fewer studies that appeared in the literature from late 2003 through mid 2007, the conclusions reached are similar to those that have been reached previously; namely, findings indicate that OME has no more than a minimal impact on developmental outcomes. In the prospectively followed samples, only one found any association between OME and speech-language outcomes[62] the variance explained was minimal. Likewise, there was only one prospective study[55] in which there was a suggestion that OME may be related to academic outcomes. There were no prospective studies in which memory or attention was impacted by OME, although hearing loss was related only when the home environment was also considered. Finally, there were low but significant correlations between the percent of time with OME and behavioral outcomes reported in one prospective study.[55] On the other hand, there is some evidence from retrospective studies that specific measures of phonological processing, speech, and working memory may be associated with OME. In general, the studies reported here support the conclusions of the meta-analysis performed by Roberts and colleagues[72] and the published review based on data reported at an NIH conference.[73] Specifically, these conclusions were that there are at most very small negative relationships between OME or its associated hearing loss and children's development and that a history of OME may not be a substantial risk factor for later speech-language development or academic achievement. However, since hearing was inconsistently included as a variable in many studies, this conclusion should be interpreted cautiously. These conclusions are also consistent with the views of professionals and parents reported in surveys,[69, 70] many of whom did not believe OME would have long-term impacts on language or academic outcomes.

## FUTURE RESEARCH

In the previous post-symposium meeting in 2003,[74] 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 impact the relationship between OME and developmental outcomes, and to provide adequate information in research reports, including power for non-significant 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 utilized outcome measures to examine these effects. Populations included both middle-class and less advantaged groups, but only in one study was a clinical sample included.[64] 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 two studies—Hooper et al.[69] and Paradise et al.[55] Aside from SES, only Feagans et al.[65] and Hooper and colleagues[69] considered other environmental variables to account for development.

Our recommendations for future studies are to more consistently apply to 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 childcare experiences, as well as factors such as gender 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.* In a study by I Brook[75] the bacterial flora in acute mastoiditis has been investigated. The author states that the incidence of mastoiditis has decreased since the advent of antimicrobial agents. In the last decade, however, there has been a marked increased in the incidence of acute mastoiditis in several communities, sometimes in association with the growing resistance of pneumococci. *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Staphylococcus aureus* and *Haemophilus influenzae* are the most common organisms recovered in acute mastoiditis. Several recent studies demonstrated the predominance of *Pseudomonas aeruginosa* in this infection. However, because *P. aeruginosa* colonizes the ear canal it can contaminate specimens obtained through the non-sterile ear canal. *P. aeruginosa, Enterobacteriaceae, S. aureus* and anaerobic bacteria are the most common isolates in chronic mastoiditis. Anaerobes predominate in studies where adequate methods for their isolation are employed.

A retrospective study[76]on 37 infants, who were operated for acute mastoiditis during 2000-2004, were performed in Belgrade, Serbia and Montenegro. All patients had local and general symptoms. It was concluded that making a diagnosis of acute mastoiditis might not be easy since there are no specific symptoms. It was emphasized that acute mastoiditis should always be considered as a differential diagnosis in cases of prolonged acute otitis media with no improvement after 10 days of antibiotic treatment, in particular when accompanied with weight loss and general condition worsening.

The restrictive use of antibiotics for acute otitis media in certain countries has been claimed to increase the number of acute mastoiditis. This initiated a registry based study[77] with complete data on hospitalization for acute mastoiditis and cortical mastoidectomy in Norway during 1999-2005. Three hundred and ninety-nine Norwegian children aged 0-16 years were included. The incidence of acute mastoiditis in children below 2 years of age ranged from 13.5 to 16.8 per 100,000 during the study period. Corresponding numbers for children 2-16 years were 4.3-7.1 per 100,000 children. No incidence increase 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 group, 22% received surgery. Despite the introduction of restrictive Norwegian guidelines for antibiotic treatment of

acute otitis media in children aged 1 year and above, our data did not give evidence for an increase in acute mastoiditis.

Similarily a registry based study[78] in Italy, the "Ferrara" experience, between 1994 and 2005 could not confirm a real increase in the incidence of acute mastoiditis. The authors however stress that careful attention must be paid to the clinical assessment of children who are 2-years old or under with suspect acute meningitis, as they seem to be more exposed to the risk of clinical complications like meninigitis, meningo-encephalitis and sigmoid sinus thrombosis.

In contrast Benito and Gorricho[79] reported a progressive increase in the incidence of acute mastoiditis. These authors reviewed the cases of mastoiditis in the last 10 years (1996-2005) at The Niño Jesús University Children Hospital in Madrid to confirm the clinical impression, the bacteriology, treatment and evolution of the children and analyze the causes of this clinic situation. They studied 215 cases of mastoiditis (0.6-17 years), 67.4% less than 3 years old and 69.3% males. The number of cases every year was the double since 1999 with the same percentage of admissions in the Pediatric service, and the triple in 2005. The percentage of surgical treatment grew from 4.3% to 33% in the last years and to 70% in 2005. Most cases (80%) had received prior antibacterial agent therapy, but individual pathogens and current complications of periostitis or subperiosteal abscess formation were equally distributed between the two groups. *Streptococcus pneumoniae* was detected in 28.6% of the cases and *Staphylococcus aureus* in 16.3 %. A 53.7% of cases had negative cultures. The authors concluded that there is a progressive increase in the incidence of acute mastoiditis and an increase of the surgical treatments. Ten years ago the process was controlled with antibiotic therapy only, but now the number of interventions has been eight times the previous years. Most cases of acute mastoiditis have responded well to medical management alone. But if higher levels of resistance predominate, more severe forms of pneumococcal or other pathogen like *S. aureus* disease are likely to be seen, these would be less likely to respond to oral or parenteral antibiotic therapy, so, 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.

In a Danish study[80] sixty-seven patients with acute otitis media and thirty-nine patients with acute mastoiditis were studied. The overall bacterial flora found was dominated by *S. pneumococci*, all 100% susceptible to penicillin. However, *Staphylococcus aureus* was the primary bacterial pathogens cultured from patients treated with preadmission antibiotics. It is concluded that if a specimen from an AOM patient is obtained after the initiation of antibiotic treatment one should consider the possibility of the culture found being a result of the initial led treatment and not the causative AOM pathogen. The Danish data suggests that a restricted use of antibiotics in children with AOM may be associated with a higher incidence of acute mastoiditis. It was also found that a significant higher leucocytes count and CRP are found in the acute mastoiditis group compared to the AOM group.

It is now well accepted that most cases of acute mastoiditis can be successfully treated by parental antibiotics in combination with myringotomy. In a report by Spermo and Udovcic[81] it is however concluded that in coalescent mastoiditis, which has not responded to intravenous antibiotics within 48 hrs requires mastoid surgery. Studies by Zanetti and Nassif [82] showed that acute mastoiditis can fully recover with conservative treatment and myringotomy with tympanostomy tubes. Immediate surgical treatment is indicated for intracranial complications. A mastoidectomy with tympanoplasty is warranted in: (1) exteriorization, if the child is older than 30 months or >15 kg of weight, (2) intracranial complications (combined with a neurosurgical procedure as needed) and (3) cholesteatoma or granulation tissue.

In a study of Casula et al.[83] a fatal case of acute mastoiditis was obtained in a person with HIV infection. The mastoiditis was complicated by thrombosis of the sigmoid sinus and an intracerebral abscess caused by an unusual pathogen – *Nocardia asteroids*. Sulfonamides have remained the first-line agents for the management of *Nocardia* infections, but mortality

remains high in patients with intracerebral infection.

A few reports related to cochlear implantation concern children with otitis media. In a study by Lunz et al.[84] the risk for otitis media after cochlear implantation in otitis media (OM)-prone and non-OM-prone children was assessed. Onehundred and thirteen children were referred for cochlear implantation during the study period, and were implanted under the age of 7 years, 70 were classified as OM-prone and 43 as non-OM-prone. Postimplantation follow-up ranged from 6 to 75 months (average 35.5 months). During the first month after implantation 18 children suffered from acute otitis media, the vast majority of them (n=16) belonged to the OM-prone children (22.8% of this group) and 2 subjects belonged to the non-OM-prone children (4.6% of this group). During the late post-operative period 28 of the OM-prone children (40%) and 4 of the non-OM-prone children (9.3%) developed acute OM in the implanted ear. Eleven (9.7 %) of these cases, 10 belonging to the OM-prone (14%), and one belonging to the non-OM-prone group A (2.3%)) proved to be recurrent and therapeutically challenging. Three subjects developed acute mastoiditis without intracranial complications. Each episode of mastoiditis or otitis media was controlled conservatively without any need of surgical drainage of the mastoid. It is concluded that early referral led to early implantation, even in children susceptible to OM. The incidence of OM decreased after implantation in both groups, but was still significantly higher in the OM-prone group. Meanwhile, prior to CI it is not possible to predict the cases that become therapeutically challenging at a later stage.

In another retrospective study[85] from Israel the complication rate of cochlear implantation (CI) was evaluated and two different surgical approaches compared. The patients underwent CI between 1989 and 2003 and were followed-up for at least 18 months. The patients were divided into two groups according to the surgical technique that had been used for the implantation: the mastoidectomy with posterior tympanotomy approach and the suprameatal approach (without mastoidectomy). The incidence of complications following CI was compared between the two groups and between children and adults. Facial nerve paralysis, electrode misplacement, injury to the chorda tympani nerve and mastoiditis occurred only in the mastoidectomy with posterior tympanotomy approach group. Acute middle ear infection with or without mastoiditis emerged as the most common complication in both groups, followed by vestibular and wound problems. The suprameatal approach was demonstrated as being a good alternative technique to the classical surgery for CI.

*Facial Paralysis.* Facial paralysis is another serious but uncommon complication of OM. Nowadays, facial paralysis in relation to acute OM occurs at an estimated incidence of 0.005%, which should be compared to 0.5-0.7% in the pre-antibiotic era. A retrospective study by Popovtzer et al.[86] on children with facial palsy and acute OM showed antibiotic therapy and myringotomy to be the first-line procedures. Surgery should be employed in case of acute or coalescent mastoiditis, suppurative complications and lack of clinical regression.

In a retrospective study by Evans et al.[87] the causes and treatment of facial paralysis was characterized in pediatric patients. Thirty-five patients identified with partial or complete facial paralysis were evaluated between 1997 and 2003. A review of the medical records including sex, age, laterality, etiology, therapy, severity of paralysis according to House-Brackman (HB) six-point grading scale, duration, and degree of recovery was performed. The causes of facial paralysis were infectious (n=13), traumatic (n=7), iatrogenic (n=5), congenital (n=4), Bell's/idiopathic (n=3), relapsing (n=2) and neoplastic (n=1). Peak age distributions for both infectious and traumatic etiologies were bimodal: 1-3 and 8-12 years. Of the 13 infectious cases, 11 were associated with acute otitis media in combination with OME. Four (4/11) were bacterial-culture negative. Seven (7/11) were bacterial-culture positive, four (4/7) of which required prolonged, broth-medium culture. Bacteria cultured predominantly included *Staphylococcus* non-*aureus* species (5/7) and *Propionobacterium acnes* (3/7). One (1/13) was viral culture positive (*Herpes simplex* virus). All six patients who received intravenous steroids for OME-associated facial paralysis received the

doses within the first week of presentation and had complete recovery (HB I/VI); three of five patients who did not receive steroids had complete recovery. There were five iatrogenic cases; two (2/5) were planned surgical sacrifices and three (3/5) were complications of middle ear/mastoid surgery. Facial nerve function associated with infection returned in 0.5-2 months while, when associated with trauma, returned in 0.25-30 months. The study concluded that in infectious or traumatic facial paralysis, children aged 1-3 and 8-12 years are the primary groups involved. In acute otitis media alone or mixed with OME the culture-identified organisms may not be representative of traditional pathogens. Infectious facial paralysis averaged 1 month for recovery while traumatic facial paralysis averaged 9 months. Intravenous steroid therapy may improve the outcome. Recovery was complete (HB I/VI) in 8/10 infectious and in 4/6 of the traumatic cases.

Hydén et al.[88] described inner ear complications and and/or facial paralysis secondary to acute otitis media in 20 patients. Nineteen patients had inner ear symptoms. Eight of them had a unilateral sensorineural hearing loss and vertigo, three had vertigo as an isolated symptom and one, with bilateral AOM, had bilateral sensorineural hearing loss. Seven patients had a combination of facial palsy and inner ear symptoms (unilateral sensorineural hearing loss in three, unilateral sensorineural hearing loss and vertigo in two, bilateral sensorineural hearing loss and vertigo in one with bilateral AOM, and vertigo alone in one). One patient had an isolated facial palsy. Healing was complete in 11 of the 20 patients. In seven patients a minor defect remained at follow-up; a sensorineural hearing loss at higher frequencies in all. Only two patients had obvious defects, a pronounced hearing loss in combination with a moderate to severe facial palsy in one (House-Brackman grade 4), distinct vestibular symptoms and a total caloric loss in combination with a high-frequency loss in the other. Eight patients had positive bacteriological cultures from middle ear contents: *Streptococcus pneumoniae* in two, beta-hemolytic *Streptococcus* group A in two, beta-hemolytic *Streptococcus* group A together with *Staphylococcus aureus* in one, *Staph. aureus* alone in one and coagulase-negative staphylococci (interpreted as pathogens) in two. In the 12 patients with negative cultures, there was a probable bacteriological cause due to the outcome in SR/CRP and leukocyte count in five. In four patients serological testing showed a concomitant viral infection that was interpreted to be the cause; varicella zoster virus in two, herpes simplex virus in one and adenovirus in one. In three there was a probable viral cause despite negative viral antibody test due to normal outcome in SR/CRP, normal leukocyte count, serous fluid at myringotomy and a relatively short pre-complication antibiotic treatment period. Although the number of patients in this study is relatively low the findings show that inner ear complications and facial palsy due to AOM can be of both bacterial and viral origin. Severe sequelae were found only where a bacterial origin was proven.

The functional recovery in patients with facial paralysis due infective causes were reported in a retrospective study by Makeham et al.[89] The patients were identified from a database of 1074 patients with facial paralysis. One hundred twenty of the 150 patients identified as having facial paralysis due to an infectious disease caused by *Herpes zoster* oticus were excluded from the study. The remaining 30 patients were included in the study. Patients were treated both operatively and nonoperatively. Operative treatment included myringotomy and ventilation tube placement, cortical mastoidectomy, modified radical (canal wall down) mastoidectomy, petrous apicectomy, and lateral temporal bone resection. The House-Brackmann (HB) grade of facial function at 1 year after initial assessment was used to evaluate the outcome. The causes of facial paralysis were acute otitis media (n = 10); cholesteatoma (n = 10 [acquired, 7; congenital, 3]); mastoid cavity infections (n = 2); malignant otitis externa (n = 2); noncholesteatomatous chronic suppurative otitis media (CSOM; n = 2); tuberculous mastoiditis (n = 1); suppurative parotitis (n = 1); and chronic granulomatosis (n = 1). The patients with noncholesteatomatous CSOM who presented sooner after the onset of facial nerve symptoms had greater facial nerve recovery when assessed using the HB grade at 1 year. It is concluded that facial paralysis due to infective causes other than

*Herpes zoster oticus* is rare. Patients with noncholesteatomatous CSOM and FNP have a better outcome than those with facial paralysis due to cholesteatoma. Patients with facial paralysis due to acute otitis media tend to have a good prognosis without surgical decompression of the facial nerve being required.

In a case study[90] from Sweden a patient presented with facial palsy after an acute otitis media episode. The facial palsy was shown to be the first symptom of Wegener`s granulomatosis. The clues leading to diagnosis consist of the practioner´s suspicion of the disease, the use of appropriate serological measurements, and the histological confirmation. The early initiation of treatment leads to high rates of remission of an otherwise lethal disease.

## INTRACRANIAL COMPLICATIONS

Leskinen and Jero[91] published a paper on complications of acute otitis media.The retrospective chart review showed all pediatric patients treated for intratemporal and intracranial complications of AOM over a 10-year –period; 1990-2000. The only intracranial complication was an extradural abscess with meningitis. In a review by Leskinen[92] he summarizes that intracranial complications are encountered today only rarely in developed countries. Meningitis is found in 1-4% of the patients with AOM. Sinus thrombosis, intracranial abscesses and otic hydrocephalus are reported in 0 to 5%, 0 to 7% and 0 to 6%, respectively in children with acute otitis media. Encephalitis associated with AOM has been reported only occasionally during the past decade.

In a study by Redaelli de Zinis et al.[93] two cases of internal jugular vein thrombosis without sigmoid sinus thrombosis were described secondary to acute mastoiditis. This complication was successfully treated with anticoagulation therapy and antibiotics. The authors claimed that surgery should only be performed to eliminate the source of infection from the middle ear and mastoid.

In a study[94] from Ireland over a 7-year period showed twelve cases, 6-73 years of age. Five had brain abscesses, four had lateral sinus thrombosis and 3 had petrous apicitis. Eight were secondary to chronic otitis media and 4 were secondary to acute otitis media.

Miura et al.[95] reported six cases during a 2-year period in Brazil. The most common complication was meningitis, which was detected in all cases. Five of them had abscesses, three cases manifested as hydrocephalus. Lateral sinus thrombosis occurred in 2 patients.

In a retrospective study by Kim et al.[96] cochlear implantation in patients with a history of chronic otitis media was analysed. Four hundred eighteen cochlear implantations were investigated and nine patients who had chronic otitis media in the ear to be implanted were included. Of these, three showed active inflammation at presentation; the other six cases had undergone previous tympanomastoidectomy surgery and did not show active inflammation at presentation. Five patients with active inflammation or without an adequate soft tissue layer in the mastoid bowl underwent a two-stage procedure. Four cases who showed inactive inflammation and had an adequate tissue layer to protect the electrode array underwent a single-stage technique, although two of them showed dry TM perforation. No local or intracranial inflammation recurred.

## GOALS FOR FUTURE RESEARCH

Research goals for the sequelae and complications due to otitis media are listed as follows. The list resembles that of the Panel from 2003. Despite some of the goals have been addressed however, further studies to increase our understanding are still needed.

1. The sequelae of OM have to be better understood. The sequelae related to AOM, SOM and treatment should be differentiated, and studied separately.

2. The pathogenesis of TM atrophy has to be studied in experimental models in order to prevent or treat TM atrophy.

3. Tympanic membrane retraction pockets have to be studied further clinically and with the new animal models.

4. Pathogenesis of adhesive otitis has to be further studied to develop preventive strategies.

5. The pathogenesis of and myringosclerosis and tympanosclerosis needs to be clarified. Treatment methods to prevent these conditions have to be studied in terms of safety and efficacy.

6. The mechanisms of change in mastoid pneumatization secondary to OM should be investigated. Animal models should be developed to confirm this hypothesis.

7. The transient and permanent effects of OM in the vestibular system should be clarified.

8. The transient and long-term effects of inflammatory mediators of OM in the ME and the inner ear need to be studied further.

9. Though rare, the severe complications of OM needs to be further investigated. The potential underlying mechanisms; such as high resistance rates, overdiagnosis, or delayed treatment, need to be documented and clarified.

10. A need to develop consensus for the best and minimally invasive method of treatment for acute complication of OM such as mastoiditis, facial palsy, or intracranial involvement deciding when to perform surgery or myringotomy and antibiotics.

## PARTICULAR RESEARCH NEEDS FOR HEARING LOSS AND AUDITORY FUNCTION ASSOCIATED WITH OTITIS MEDIA WITH EFFUSION

1. There continues to be a dearth of developmental studies examining effects of OME on hearing as a function of onset, severity, and duration of hearing loss. A better understanding of the natural history of hearing loss including these variables is necessary in order to understand any potential impact of OME and how many children will be affected by various degrees and duration of hearing loss. Current research indicates that hearing thresholds should be routinely tested in children with OME rather than relying on parents´ judgments, even if formal questionnaires and parental education about symptoms are used.

2. Prospective studies are needed that investigate the required frequency of hearing testing over time to adequately describe the burden of hearing loss.

3. There is an urgent need for development of valid methods for describing longitudinal variations in hearing of children with OME over time.

4. Further research on sensorineural hearing loss using randomized controlled trials is needed to determine whether high-frequency sensorineural hearing loss is a result of the disease or of treatment with ventilation tubes.

5. Future studies of OME and development should include hearing level as a mediating variable.

6. Future studies should examine whether central auditory function is causally related to OME and, if so, how laboratory measures of complex auditory processes relate to functional ability to process speech in natural environments.

7. There is a need of validated questionnaires to determine the quality of life in children with SOM as this will influence the decision of tympanostomy treatment or not.

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