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Title: Creating an Otitis Media Research Network in Aboriginal Medical Services in Australia

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Introduction: Australian Aboriginal and Torres Strait Islander peoples have one of the highest rates of complicated otitis media in the world. Best practice research and knowledge translation requires that research is done in close partnership with Aboriginal communities. Objective: To describe key factors in the successful establishment of the Aboriginal Medical Service otitis media research network. Methods: Two clinical trials examining management of otitis media in Aboriginal and Torres Strait Islander children are currently being run in 6 Aboriginal Medical Services (AMSs) nationally, commencing with the WATCH (Watchful waiting for Aboriginal and Torres Strait Islander Children) trial in 2014 and the addition of the INFLATE (autoinflation for otitis media with effusion) trial in 2017. Five AMSs have left the research network over this time due to inability to recruit and organisational changes. Process evaluation has been undertaken to understand and enhance the running of the trials and the network. This comprises thematic analysis of committee meeting minutes and interviews with site-based research officers, carers of participants, healthcare providers at sites and community reference group members. Results: We will present the findings of our process evaluation which relate to the enablers and challenges of establishing an otitis media research network in this context. Challenges to establishing a network include balancing research with clinical imperatives, organisational changes in AMSs and lower than expected numbers of children presenting to services with otitis media. Key enabling factors relate to relationship building, research staffing, funding models, flexible governance, clinical research which is aligned with community priorities, and two-way capacity building. Conclusion: An otitis media research network in Aboriginal Medical Services has long been needed. We have demonstrated that this is feasible and our ongoing successful recruitment demonstrates sustainability. Consideration of the specific enablers and challenges in this context is important to success.

Title: Nasopharyngeal Microbiome Analysis in Healthy and Otitis-Prone Children: Focus on History of Spontaneous Tympanic Membrane Perforation

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Introduction: The etiology of acute otitis media is ascribable to bacteria that colonize the upper respiratory tract and become more virulent under favorable circumstances. Recurrent acute otitis media has relevant effects, in terms of direct/indirect costs and antibiotic misuse. The most common complication of acute otitis media is otorrhea, whose epidemiology has been proved different from not-complicated acute otitis media and whose clinical management is often more challenging as soon as it becomes recurrent. Recently several trials have focused on nasal probiotic therapies, in order to prevent colonization by the most common otopathogens, but unfortunately results are still poorly clear. Objective: To compare nasopharyngeal microbiome of children with recurrent acute otitis media (otitis-prone children) to the one detected in healthy controls; to compare nasopharyngeal microbiome of otitis-prone children without otorrhea to otitis-prone children with recurrent otorrhea. Method: During winter 2016-17 nasopharyngeal swabs were collected from 112 children (mean age 3.5 years, SD ±1.5) including 36 healthy children, 39 otitis-prone children without otorrhea and 37 otitis-prone children with recurrent otorrhea. DNA was subsequently extracted and 16S rRNA gene V3-V4 regions were PCR amplified and sequenced using Illumina Mi Seq technology. Results: A higher relative abundance of Dolosigranulum and Corynebacterium genera was detected in the nasopharynx of healthy children (15.1% and 8%) in comparison with otitis-prone group without otorrhea (8.8% and 4.7%) and otitis-prone group with otorrhea (5.3% and 3.7%). On the other hand, Staphylococcus, Alloiococcus and Bifidobacterium were detected more often in otitis-prone children (2.4%,1.9% and 1.3%) than in the healthy group (0.7%, 0% and 0.4%). In all groups, the most abundant genera were Moraxella, Streptococcus and Haemophilus, followed by Dolosigranulum and Corynebacterium. Dolosigranulum and Corynebacterium showed a co-occurrence pattern with positive correlation in all groups, with negative correlation with Streptococcus and Haemophilus. Conclusions: To our knowledge, this is the first study comparing nasopharyngeal microbiota in not-complicated otitis-prone children to the one detected in otitis-prone children with recurrent otorrhea. Moreover, our study provides a characterization of the upper respiratory tract microbiome in children who experienced recurrent otorrhea. Although our data did not achieve statistical significance, we believe that this topic is worthy of further in depth-analysis, as it could potentially explain the clinical and epidemiological differences between complicated or not complicated otitis-prone children. In line with previous studies, our study identifies Dolosigranulum and Corynebacterium to be

more abundant in the healthy group, strengthening the hypothesis that these two genera could be fundamental elements of the healthy respiratory tract microbiome.

Title: Tubomanometry may describe Passive Properties of Eustachian Tubes in Ears with Intact Tympanic Membranes

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Introduction: The Forced Response test (FRT) is one of the few Eustachian tube (ET) function (ETF) tests that provides information on the passive and structural properties of the ET. Positive pressure is applied to the middle ear through a non-intact tympanic membranes (TM) until the ET passively opens (opening pressure or OP) and establishes a directional airflow towards the nasopharynx (NP). Tubomanometry (TMM) is a simple in-office test of ETF that can be used in both intact and non-intact TMs. It delivers a controlled bolus of air into the nasal cavity at 30, 40, or 50 mbar and uses the para-tubal muscle assistance during a swallow to open the ET and allow the movement of air from the NP to the middle ear. Based on this knowledge, the authors hypothesize that the OP measured during the FRT test will generally be greater than those in TMM due to the contribution of active function in the latter test and expect that the OP measured on FRT and TMM will not be correlated. Objective: To determine if the TMM OP can be used as a new parameter of passive ET properties. Methods: This is a retrospective exploratory cross-sectional study in a group of 14 children tested at a specialized ETD Clinic between 5/6/2013 to 9/11/2018 who underwent FRT and TMM tests. OPs of FRT at 11ml/min and 23ml/min (FRT11, FRT23) were compared to the opening pressures of TMM at 30, 40, and 50mbar (TMM30, TMM40, TMM50). OPs in TMM were defined as the NP pressure at the moment of ET opening during swallows. Data were analyzed using student's t-tests and Cohen's d effect size. Results: The age range of the population was 7.8-18.5 years (mean= 13.1 ± 3.5 years), 9 were males and 13 were white. Data for 15 non-intact ears were analyzed. ET OPs in FRT11 and FRT23 were highly correlated ($r=0.824$, $p<0.001$), hence data from only FRT23 was used to compare to TMM data. OPs of TMM are correlated, though the relationship weakened with increasing applied pressures (TMM30 and TMM40: $r=0.605$, $p<0.001$; TMM30 and TMM50: $r=0.268$, $p=0.055$). FRT23 OPs was significantly greater than TMM30 OP ($p=0.01$) and was also greater than both TMM40 ($p=0.127$, Cohen's $d=0.574$) and TMM50 OPs ($p=0.830$, Cohen's $d=0.079$). Conclusions: Overall, FRT OPs were greater than those observed in TMM and supports the initial hypothesis that the para-tubal active muscular function serves to decrease ET OP. Though we were unable to show a significant difference between the OPs of FRT23 and TMM40, effect size analysis suggests that, with a larger sample size, this difference would become more apparent. However, the OPs of FRT23 and TMM50 would continue to be statistically similar, meaning that the OP of TMM50 is likely the most accurate proxy for OP of FRT23. Since this study was done in patients with ETD, further studies will be carried out in individuals with normal ETF to elucidate these initial findings.

Title: Management of acute respiratory tract infections including acute otitis media in Danish general practice

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Objective: Antibacterial resistance is increasing worldwide. A reduction in the use of antibiotics is a key to reduce this problem. Acute respiratory tract infections (ARTI) and acute otitis media (AOM) are common reasons for prescribing antibiotics even though they most often are self-limiting especially in childhood. Antibiotics are only recommended when certain diagnostic criteria are met. This study analyses the associations between patient- and general practitioner (GP) characteristics and antibiotic prescribing for children and adults with AOM and examines to what extent Danish guidelines for AOM management are fulfilled. Method: All 2,118 GPs in the Northern, Southern and Central regions of Denmark were invited to participate in this quality improvement study (APO-method). The APO-method comprised 1) registration of symptoms, examinations, findings and antibiotic treatment in patients with symptoms of an ARTI during a four-week winter period in 2017/2018, 2) feedback with group discussion and work-shops 3) new registration of ARTI patients, 4) feedback with discussion. Associations were analysed by means of multivariate logistic regressions. For each patient, the GP's tendency to prescribe antibiotics was assessed as the proportion of the GP's other patients in the audit who had an antibiotic prescribed. Results: In total, 164 (7.7%) GPs participated in the registration of 6,104 patients. Some 1,264 patients (20.7%) had a lower respiratory tract infection, 2,031 (33.3%) a simple cold, 1140 (18.7%) had upper respiratory tract infection diagnosed inclusive 425 (7%) patients with AOM of whom 310 (72.9%) were 7 years or younger, and 233 (75.2%) of the children were prescribed an antibiotic, most often penicillin V (60.0%). Antibiotic prescribing was associated with fever, otorrhea, poor general condition, and with the GPs' overall tendency to prescribe antibiotics. Considerable variation was observed in the GPs' tendency to prescribe antibiotics. Conclusion: Danish GPs often prescribe antibiotics for AOM even though the diagnostic criteria are not met, however with large variation. Antibiotic treatment of AOM depends as much on GP characteristics as on patients' symptoms and signs. We find needs for improvement in Danish GPs management of otitis media.

Title: Pain Management in Acute Otitis Media: a Qualitative Study of Parents' Views and Expectations

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Introduction. Earache is a key symptom of acute otitis media (AOM). For unclarified reasons, parents tend to be cautious about administering analgesics to their children, potentially leading to suboptimal management of AOM symptoms. We aim to understand parents' views and expectations of pain management in childhood AOM. Methods. Qualitative study alongside a cluster-randomized controlled trial (PIM-POM study) aimed at optimizing pain management in childhood AOM. We purposefully sampled 14 parents of children diagnosed with AOM by their general practitioner (GP), who were recruited to the trial between November 2017 and May 2018. Semi-structured interviews were held at home in the first two weeks after trial enrollment. Interviews were audio-recorded, transcribed and analyzed thematically. Results. Parents experienced difficulties in recognizing earache and other symptoms of an ear infection. They consulted the GP for a diagnosis, for reassurance and for management advice. Parents shared that, prior to consultation, they had insufficient knowledge of the benefits of correctly dosed pain medication at regularly scheduled intervals. Parents valued GP's advice on pain management and were happy to accept pain medication as standalone therapy, provided that the GP explained why antibiotics would not be needed. Parents' views and expectations of pain management in AOM were shaped by previous experiences of AOM within their family; those with a positive experience of pain medication are more likely to use it in subsequent AOM episodes. Conclusions. Parents of children with AOM consult the GP to help cope with uncertainties in recognizing symptoms of AOM and receive management advice. It is important that GPs are aware of parents' lack of understanding of the role of pain medication in managing AOM, and that they address this during the consultation.

Title: Enhancing Otitis Media Clinical Trials through Qualitative Research

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Introduction: The WATCH (Watchful waiting for Aboriginal and Torres Strait Islander children with acute otitis media) and INFLATE (autoinflation for otitis media with effusion) trials are multi-centre clinical trials examining otitis media management. Embedded within both trials is a program of qualitative research which aims to explore treatment acceptability and the experience of taking part in and conducting the trial, from the perspectives of carers, health professionals, researchers and services. Qualitative research can improve the implementation of clinical trials and understanding of the results. Objective: To outline examples of the qualitative research being undertaken within the WATCH & INFLATE trials, and discuss the challenges and benefits of such qualitative research. Methods: We interviewed parents/carers, health professionals and researchers about their experiences of the trial and their views on the otitis media treatment approaches, as well as site-based research officers and community reference groups. Interviews were audiotaped and transcribed verbatim. N-Vivo was used to assist with data management, and coding of data was shared between the team, bringing diverse perspectives and increasing the trustworthiness of the analysis. We used a framework approach to analysis, suitable for large team projects. Results: We present two examples of our qualitative research. Firstly, we examined how our acute otitis media (AOM) symptom scales functioned within the WATCH trial, to extend and explain our quantitative analysis of the reliability of the tools. We found the AOM-Faces Scale was preferred by research officers and carers over the AOM-Severity of Symptoms Scale, however, more training was needed to ensure consistent application. Interviewees were concerned that as a ‘right now’ tool it was not able to capture children’s fluctuating symptoms. Secondly, we explored carers' attitudes to different treatment choices for AOM to better understand recruitment challenges. We found that carers had varying and sometimes strong views on whether antibiotics should or should not be used for AOM, nevertheless consented to participate. Sometimes this was related to not understanding the randomisation process but, in general, carers chose to participate if they trusted the doctor and research officer, or if they supported the research goals, regardless of their AOM management preferences. Qualitative research within ear health trials in Aboriginal Medical Services enhances engagement with community and site-based research officers. Fixed protocol requirements may limit change to qualitative findings; however, learnings can be incorporated into future trials. Conclusion: Qualitative research is enhancing the otitis media clinical trials in our setting and provides an opportunity to build the whole research team’s capacity to undertake research with Aboriginal Medical Services.

Title: NTHI and M. catarrhalis Released from a Polymicrobial Biofilm by Antibodies against NTHI Type IV Pili are Hypersensitive to Antibiotic Mediated Killing

Authors: E. M. Mokrzan[1], L. A. Novotny[1], K. L. Brockman[1], L. O. Bakaletz[1,2]

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Objective: Otitis media (OM) is often polymicrobial, with nontypeable Haemophilus influenzae (NTHI) and Moraxella catarrhalis frequently co-cultured from clinical specimens. The chronic and recurrent nature of OM is due to bacterial biofilms in the middle ear; therefore, strategies to eradicate these biofilms are needed. We have focused our vaccine development efforts on PilA, the majority subunit of the NTHI Type IV pilus, which is important for adherence, colonization, motility and biofilm formation. Antibodies against a recombinant, soluble form of PilA (rsPilA) disrupt and prevent the formation of NTHI biofilms in vitro. Moreover, immunization with rsPilA both prevents and resolves experimental NTHI-induced OM. Epidemiological studies show that M. catarrhalis is rarely found as a sole OM pathogen and instead often co-infects with NTHI, which suggests a symbiotic relationship that facilitates polymicrobial OM. Thus we hypothesized that vaccine strategies that are effective against NTHI-induced OM might also mediate a collateral, albeit indirect, benefit in terms of resolution of polymicrobial OM. Here we explored the effects of anti-rsPilA exposure on polymicrobial biofilms formed by these two predominant OM pathogens. Method: We established biofilms formed by NTHI + M. catarrhalis at temperatures typical of the nasopharynx (34°C) or middle ear (37°C), exposed them to anti-rsPilA, then quantitated how many of each species was disrupted from the biofilm and now present in the supernatant. Results: Notably, anti-rsPilA dispersed both NTHI and M. catarrhalis from biofilms at 34°C and at 37°C. With the knowledge that bacteria newly-released from biofilms often display greatly enhanced antibiotic sensitivities, we then examined the relative sensitivity of newly-released NTHI and M. catarrhalis to first-line antibiotics used to treat OM. When newly-released NTHI were exposed to trimethoprim-sulfamethoxazole, we observed significantly more killing at 34°C and at 37°C than for NTHI in broth suspension ($p<0.05$). Similarly, we observed significantly more killing of newly-released M. catarrhalis exposed to clarithromycin than M. catarrhalis in broth at both temperatures ($p<0.01$). These data demonstrated that anti-rsPilA-mediated dispersal of bacteria from residence in a biofilm resulted in significantly increased antibiotic sensitivity in both the newly-released NTHI and M. catarrhalis. Conclusion: The results of this study revealed that immunization with rsPilA could induce antibodies that disrupt a dual-species biofilm despite specifically targeting only a single pathogen. Moreover, when needed, immunization with rsPilA could offer a new and potentially powerful defense against the growing threat of antibiotic resistance by combining the power of active immunization with the killing activity of traditional antibiotics but now effective at a markedly reduced dose. Funded by NIH-R01-DC003915.

Title: Rapid Gene Regulation by nontypeable Haemophilus influenzae Impacted Sensitivity to First-Line Antibiotics Used to Treat OM

Authors: S. D. Stacey[1,2], K. L. Brockman[1,2], J. M. Atack[3], M. P. Jennings[3], S. D. Goodman[1,2], L. O. Bakaletz[1,2]

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OBJECTIVE: Chronic and recurrent otitis media (OM) is difficult to treat with antibiotics, in part because OM pathogens form biofilms in the middle ear. These biofilms are 1000 times more resistant to antibiotics than free-living bacteria. In addition to biofilm formation, nontypeable Haemophilus influenzae (NTHi) has evolved a powerful mechanism to adapt to and persist within its' human host termed the 'phasevarion' (phase variable regulon). NTHi uses the phasevarion to rapidly and reversibly regulate the expression of multiple genes. Phasevarion switching results in two genetically identical but phenotypically unique subpopulations (e.g., 'ON' and 'OFF') of a single NTHi strain. Subpopulation diversity increases the adaptability and survivability of NTHi in its' human host. When exposed to conditions similar to chronic OM, temperatures ≥37°C and alkaline pH, the phasevarion has an influence on biofilm formation by NTHi. Due to the unique biofilms formed by 'ON' and 'OFF' subpopulations, we hypothesized that there were also likely differences in the antibiotic susceptibility of the biofilm-resident NTHi. This study investigates the role of the phasevarion in the relative susceptibility of NTHi to antibiotics in any of three unique lifestyles: planktonic, biofilm-resident, and those newly released from a biofilm due to the action of any of several known cues/agents. METHODS: To study the phasevarion, genetically modified variants, termed 'locked ON' or 'locked OFF,' were assayed. These locked variants cannot phase-vary which allows us to separate the two phenotypic subpopulations and assess how NTHi uses the phasevarion to respond to biological cues without the confounding issue of ongoing phase-variation. These variants were tested for sensitivity to commonly prescribed antibiotics for OM, ampicillin, cefdinir, or trimethoprim/sulfamethoxazole. Antibiotic susceptibility for planktonically grown bacteria was tested via use of the published MIC_{90} of the antibiotic. Biofilms were grown in conditions similar to a healthy middle ear (37ºC, pH 7) or those common during chronic OM (37ºC, pH 9). Due to the resistant nature of biofilms, to see differences in susceptibility between locked ON vs. OFF variants, biofilms were treated with 1000X MIC_{90}, then architectural differences (via confocal microscopy) and biofilm resident bacterial viability were determined. The antibiotic susceptibility of bacteria newly released from a biofilm via exposure to antibodies that target the type IV pilus adhesin, PilA, or the DNA-binding protein, integration host factor, each of which is known to disrupt an NTHi biofilm, was also tested. RESULTS: There was no difference in the antibiotic sensitivity between locked ON or locked OFF variants when grown planktonically. Regardless of pH, biofilm-resident bacteria of the locked OFF variant were twice as sensitive to ampicillin than the locked ON counterpart. However, antibiotic susceptibility of ON and OFF variants newly released from a biofilm is likely to provide data of

greatest interest. CONCLUSIONS: To date, our data demonstrate that in conditions similar to chronic OM, NTHi utilizes the phasevarion to modify both biofilm formation and resistance to antibiotics. A complete understanding of the NTHi phasevarion is crucial to develop efficient therapies and possibly prevent infections by this organism and as such, our work in this area continues. Support NIH/NIDCD R01DC015688

Title: Rapid Gene Regulation by nontypeable Haemophilus influenzae Resulted in Altered Adherence to Respiratory Tract Epithelial Cells and Mucus

Authors: S. D. Stacey[1,2], K. L. Brockman[1,2], J. M. Atack[3], M. P. Jennings[3], L. O. Bakaletz[1,2]

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OBJECTIVE: Nontypeable Haemophilus influenzae (NTHi) is a pathobiont of the human respiratory tract. NTHi attaches to and colonizes the human nasopharynx via a wide array of adhesive proteins, or adhesins, and resides therein as a commensal organism. However, NTHi is also one of the three major pathogens of middle ear infection (otitis media; OM), and is the major causative agent of chronic OM, recurrent OM, and OM in which treatment has failed. The environments of the nasopharynx and middle ear are somewhat unique and NTHi has developed a powerful mechanism to successfully adapt to and persist in these unique microenvironments termed the 'phasevarion,' for phase variable regulon. Phasevarions allow NTHi to rapidly and reversibly regulate the expression of multiple gene products. Phasevarion switching results in two genetically identical but phenotypically unique subpopulations (e.g., 'ON' and 'OFF'). The capacity for diversification markedly increases the adaptability and survivability of NTHi in its' human host. While phasevarions have been identified in the genomes of multiple human mucosal pathogens, the role they play in adherence has not yet been fully characterized. We showed that NTHi regulates expression of several adhesins via its phasevarion, thereby we hypothesized that this outcome would also influence adherence to respiratory tract epithelial cells and mucus. We investigate whether phasevarion regulation affects the ability of multiple NTHi clinical isolates to adhere to cells and mucus of the respiratory tract. METHODS: To study the effects of the phasevarion, genetically modified variants, termed 'locked ON' or 'locked OFF,' of four NTHi clinical isolates, which possess four of the five phasevarions most commonly associated with OM, were assayed. These locked variants are unable to phase-vary which allows us to separate the two phenotypic subpopulations. We can also assess how NTHi uses the phasevarion to respond to biological cues or conditions without the complication of ongoing phase-variation. Variants were assayed for relative ability to adhere to mucus harvested from human airway epithelial (HAE) cells or to the apical surface of both HAE and chinchilla middle ear epithelial (CMEE) cells. RESULTS: We observed that the ability of NTHi to adhere to mucus, HAEs, or CMEEs varied significantly among the tested isolates, and that differential regulation of adhesins by these four phasevarions affected how well each NTHi strain adhered to the tested targets. CONCLUSIONS: To date, we know that the phasevarions of NTHi regulate expression of gene products that affect pathobiology; for example, response to oxidative stress, resistance to opsonization and therefore phagocytosis by host immune cells, and biofilm formation. However, ours is the first study to investigate the possible effects the phasevarion has on the ability of NTHi to adhere to human airway epithelial cells and the mucus they produce. The phasevarion is a potent tool that NTHi has evolved to adapt to

variable microenvironments and persist in its' human host. A complete understanding of the phasevarion of NTHi will reveal how NTHi causes disease, and aid in the development of more efficient therapies and cures. Support: NIH/NIDCD R01DC015688.

Title: Immunization with a Bandaid Protects Against Experimental Otitis Media Due to nontypeable Haemophilus influenzae

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Objective: We've developed a strategy for immunization that involves placement of vaccine formulations on to a bandaid which is then affixed to the skin. This simple regimen, called transcutaneous immunization (TCI), has potential to be equally effective as injectable vaccination, yet may be less expensive, encourage greater compliance and promote widespread distribution to regions without ready access to healthcare. Herein, we fully characterized TCI to resolve active experimental otitis media (OM) due to nontypeable Haemophilus influenzae (NTHI), reveal the molecular mechanisms that underlie the significant efficacy achieved and further, demonstrate boostability and durability against an additional episode of OM. Methods: TCI was performed by placement of bandaids behind the outer ears of chinchillas. Formulations consisted of an immunogen called 'chimV4' which targets two critical adhesins expressed by NTHI: the Type IV pilus (involved in NTHI adherence, motility and biofilm formation) and outer membrane protein P5 (important for NTHI adherence), mixed with a potent adjuvant called 'dmLT' or dmLT alone. Bandaids were placed on days 0 and 7. We characterized the maturation of B-cells within the nasal-associated lymphoid tissue (NALT) as an immune inductive site after completion of a primary immunization series and upon receipt of a boosting bandaid 60 days later. In a second study, chinchillas were immunized by bandaid as before, then challenged on day 14 by direct inoculation of the middle ear with NTHI to induce active OM. To demonstrate prevention of a subsequent episode of OM, middle ears were inoculated with NTHI a second time 60 days later and resolution of active disease determined by video otoscopy and quantitation of bacterial burden. Results: TCI by bandaid induced a significant increase in long-lived memory B-cells and chimV4-specific antibody-secreting cells in the NALT ($P \leq 0.05$), compared to cells collected from animals administered dmLT, an outcome that was further augmented upon receipt of a boosting bandaid 60 days later ($P \leq 0.05$). Moreover, the immune response established after a TCI was durable, as upon a second challenge with NTHI animals administered chimV4+dmLT eradicated NTHI from the middle ear and resolved signs of inflammation within 3 days, compared to animals administered dmLT only ($P \leq 0.001$). Conclusions: Our collective data prove that TCI by bandaid to administer a dual NTHI adhesive protein immunogen is a highly effective therapeutic and also preventative strategy against NTHI-induced OM. The immune response shaped by TCI can be recalled and expanded, and a single immunization series was satisfactory to protect against an additional episode of OM, all characteristics that are essential to a successful vaccine and delivery strategy. Importantly, the simplicity of TCI with a bandaid has tremendous potential to expand the reach of vaccines against OM to underserved regions.
Support:NIDCD/NIH R01 003915.

Title: Antibody Against a Novel Chimeric Peptide Immunogen that Targets a Bacterial DNA-Binding Protein Integral to Biofilm Structural Integrity Resolves Experimental Otitis Media

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Objective: The chronic nature of many diseases, including otitis media (OM), is largely due to the formation of bacterial biofilms. An abundant component of the biofilm is bacterial extracellular DNA, arranged in a lattice and maintained by the DNABII family of bacterial DNA-binding proteins. One member, integration host factor (IHF) binds to biofilm DNA via its DNA-binding 'tip' regions, and thus the amino terminal ('tail') region is exposed. As a consequence, the natural immune response is directed against the exposed tail region of IHF, however we've shown that antibody directed to this region does not disrupt biofilms. In contrast, antibody that targets the occluded tip regions of IHF induces significant biofilm disruption in vitro and collapses biofilms in the middle ear during experimental OM. Herein, we designed a novel chimeric peptide immunogen that specifically re-routes the immune response to target the protective tip regions of IHF and compared the therapeutic potential for anti-tip chimer peptide antibodies versus anti-native IHF protein to disrupt bacterial biofilms in vitro and in vivo. Method: NTHI biofilms were formed in the middle ears of chinchillas prior to delivery of IgG-enriched rabbit anti-IHF tip chimer peptide, anti-native IHF, or as negative controls, IgG from naive serum or against a peptide that targets the non-protective tail region of IHF, directly into this anatomical site. The relative bacterial load, amount of NTHI biofilm and mucosal inflammation within each middle ear was assessed 1 day after receipt of the second dose of antibody, and again a week later. In vitro, NTHI biofilms were established and incubated with the aforementioned antibodies for 5 to 120 min and biofilm disruption examined. Result: Animals administered anti-tip chimer peptide or native IHF had 4-log fewer NTHI in their middle ears 1 d after receipt of the 2nd dose of antibody, compared to controls ($P \leq 0.05$). Also, significantly less biofilm was observed ($P \leq 0.001$) and 50% less biofilm remained in ears treated with anti-tip chimer peptide compared to anti-native IHF. In vitro, biofilms incubated with anti-tip chimer peptide had 50% less biomass compared to anti-native IHFafter 5 min ($P \leq 0.05$). As proof, whereas immunization of chinchillas with native IHF induced the production of antibodies directed primarily toward the non-protective tail region (as occurs during natural disease), delivery of tip chimer peptide induced a complete shift in antibody response toward the protective tip region with rapid resolution of disease, as by design. Conclusion: Our data revealed that a therapeutic approach for biofilm disruption is achieved by targeting an essential structural component, the DNABII proteins. The continued refinement of candidate immunogens and delivery strategies has tremendous potential as highly effective and broadly applicable means to reduce the burden of chronic diseases, including those under the spectrum of OM. NIH R01 DC011818.

Title: Contact with Host Respiratory Tract Epithelial Cells Upregulates NTHI Expression of the Vaccine Candidate Antigen PilA

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Objective: The Type IV pilus (Tfp) of nontypeable Haemophilus influenzae (NTHI) plays a major role in bacterial adherence/colonization, motility and biofilm formation, processes that contribute significantly to the pathogenesis of NTHI-induced otitis media (OM). The major protein subunit of Tfp, PilA, is a promising vaccine immunogen for use against OM due to NTHI. As such, it is important to understand how microenvironmental cues encountered in the nasopharynx (34°C) and middle ear (37°C), the sites of NTHI asymptomatic colonization and infection during OM, respectively, affect the expression of this vaccine candidate. Here, we examined how epithelial cells influenced NTHI Tfp expression. Method: We used an NTHI reporter construct in which GFP expression is driven by the pilA promoter, and monitored relative fluorescence intensity over time to estimate pilA promoter activity and by inference, Tfp expression. NTHI were incubated in epithelial cell culture medium (CCM) alone or together with human airway epithelial cells. Results: CFU of NTHI incubated in CCM only remained at or above initial values for 12 h, during which time pilA promoter activity did not increase. In contrast, NTHI incubated in CCM with epithelial cells increased in both CFU/well and pilA promoter activity over time at both 34°C and 37°C ($p<0.05$ after 4 h). Furthermore, the changes in pilA promoter activity were not solely due to bacterial growth, since qRT-PCR revealed significantly greater pilA gene expression by NTHI incubated with cells vs. CCM only ($p<0.0001$). These data suggested that microenvironmental cues provided by the epithelial cells led to increased expression of Tfp. Because NTHI require heme iron for survival and have multiple systems for scavenging iron from host cells, we hypothesized that heme iron obtained from the epithelial cells might be one of many potential factors that contributed to the observed increase in Tfp expression. Accordingly, we incubated NTHI in a defined iron source medium (DIS) with or without 2 µg heme/ml and monitored pilA promoter activity. NTHI grown in DIS with heme displayed pilA promoter activity that increased steadily during stationary phase growth at 34°C and at 37°C, whereas promoter activity did not increase in NTHI incubated in DIS without heme. These results support the hypothesis that epithelial cells stimulated NTHI Tfp expression in part by providing a source of heme iron. Conclusion: These data suggest that vaccine-derived antibodies induced by immunization with PilA can effectively target NTHI during both colonization in the nasopharynx and infection in the middle ear, and strongly support the utility of PilA as a vaccine immunogen for the prevention or therapeutic treatment of OM due to NTHI. Funded by NIH-R01-DC003915.

Title: The Transcriptional Landscape of the In Vitro Murine Middle Ear Epithelium

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Introduction: Current knowledge suggests that otitis media (OM) is caused by an unrestrained response of the middle ear epithelium to an exogenous trigger, often a pathogen. The mechanisms underpinning epithelial remodelling in OM remain unclear but they result in the development of an abnormal mucociliary epithelium that contributes to the development of characteristic exudates. Research into the pathogenesis of OM is limited because of difficulties in accessing appropriate samples but murine models are often employed. To complement these studies we recently described a novel in vitro model of mouse middle ear epithelial cells (mMEECs) cultured at an air liquid interface (ALI). In this model cells isolated from dissected bullae, undergo mucociliary differentiation into the varied epithelial cell populations (ciliated and secretory) seen in the native middle ear cavity. The model offers the possibility to understand the processes underpinning the development of OM in a genetically tractable manner. Objective: To invetigate genome wide gene expression profiles of mMEECs during differentiation to better understand the biology of this in vitro model of the murine middle ear. Methods: We used gene expression array analysis on triplicate samples of mMEECs cells cultured at the ALI. mMEECs isolated from dissected bullea were placed into culture and when confluent were differentiated at an ALI. We compared the gene expression profiles of original (uncultured) middle ear cells, confluent cultures of undifferentiated cells (day 0 of ALI) and cells that had been differentiated for 7 days at an ALI. MIcroscopy and PCR was used to validate findings in mMEECs and in mouse tissues. Results. Multidimensional scaling analysis showed that samples from each set grouped together. >5000 genes were differentially expressd among the three groups of cells. Approximately 4000 genes were differentially expressed between the original cells and day 0 of ALI culture. The original cell population was shown to contain a mix of cell types, including contaminating inflammatory cells and reticulocytes that were lost on culture. Approximately 500 genes were upregulated during differentiation. These included some secretory genes (Lyz1, Lyz2 – the most induced, Muc5b) and some enzymes (Aldh1a1, Cyp2a5) but most were associated with the process of ciliogenesis. Conclusion. Our in vitro model of differentiated murine middle ear epithelium exhibits a trancriptional profile consistent with the mucocilary epithelium seen within the middle ear. Knowledge of the transcriptional landscape of this epithelium will provide a basis for understanding the phenotypic changes seen in murine models of OM.

Title: Eradication of Biofilms on Tympanostomy Tubes with Acetic Acid Treatment; An In Vitro Study

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Introduction: Bacterial biofilm is believed to play a part in posttympanostomy tube otorrhea. Biofilm is known for its increased tolerance to antibiotics. Acetic acid has shown promising antibiofilm effect. Objective: To evaluate the eradicative effect of acetic acid on biofilm grown on sterile tympanostomy tubes by in vitro studies. Methods: Biofilms of Pseudomonas aeruginosa and Staphylococcus aureus were grown on sterile tympanostomy tubes for 24 hours. The tubes were treated with acetic acid solutions at various concentrations for 24 hours. Main outcome was viability of bacteria after treatment. Presence of consistently attached biofilm was examined on selected tympanostomy tubes with confocal laser scanning microscopy. Both pH-adjusted and non-pH-adjusted media solutions were applied as control groups. Results: Complete eradication of P. aeruginosa biofilm was obtained with 0.50 % v/v acetic acid. Biofilm of S. aureus was eradicated with 1.25 % v/v acetic acid. Low pH value alone led to decreased growth of already established biofilm but not eradication. Conclusion: Acetic acid showed an eradicating effect on biofilm established on sterile tympanostomy tubes in vitro.

Title: Waiting to Hear: The Impact of Otitis Media with Effusion (OME) on Quality of Life of Aboriginal and non-Aboriginal Children from Urban, Regional and Rural Areas of New South Wales, Australia.

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Introduction: Little is known about the effects of chronic otitis media on the quality of life of children living in the urban, regional and rural areas of NSW. With long waiting lists and distances to travel for specialist ENT treatments, the impact of OME can be underestimated, resulting in hearing loss, speech impairment and psychological stress. Aboriginal children experience higher prevalence of chronic ear disease than their non-Aboriginal counterparts. Exact OM prevalence rates are difficult to determine. In our study cohort of 59 children to date, 47% of the children referred for ventilation tube (VT) surgery in two NSW public hospitals identified as Aboriginal. This is a disproportionately higher number compared with the non-Aboriginal population, given that Aboriginal communities comprise 5% of the population in the geographical regions covered by the health service. Objective:To investigate how parents perceive the impact of OME on their child's quality of life (QoL) before and after the insertion of VTs. Method: The Otitis Media–6 (OM-6) questionnaire was chosen because of its wide acceptance among other OM research groups and for its brevity and ease of use in the hospital ward setting. Parents were approached to participate in the QoL study on the day of their child's surgery in either of two hospitals, a large metropolitan and a smaller non-metropolitan hospital. They completed the OM-6 and a brief demographic questionnaire, which were self-administered and required approximately ten minutes for completion. A follow-up phone call between 4-6 weeks after surgery, from a research co-ordinator, facilitated administration of a post-surgical OM-6 to the parent. Results: The OM-6 and demographic questionnaires proved a convenient and efficient combination for the collection of data in the hospital setting. The OM-6 showed the domains of hearing loss, speech impairment and caregiver concerns scored highly for Aboriginal and non-Aboriginal children referred for VT surgery. These scores were greatly reduced at the follow-up OM-6, completed by 65% of participants. Conclusion: The study gave NSW parents of children living with OM a means to describe the high impact it has on the quality of life of children, especially on the degree of hearing loss and speech impairment experienced before surgery. The project was suitable for both Aboriginal and non-Aboriginal families who travelled from metropolitan, regional and rural areas for the surgery. The project benefitted greatly from the participation of Aboriginal families. Providing valuable insights into patients' well-being, parental concerns and the

efficacy of specialist ENT surgical intervention, the data demonstrates that further research could inform future directions in health service policy.

Title: Non-Invasive, Cross-Sectional Optical Middle Ear Imaging Compared with Acoustic Measurements during Otitis Media

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Objective: Non-invasively characterizing middle ear conditions during otitis media (OM) is often intrinsically challenging due to the anatomical location of middle ear. Although wideband acoustic immittance (WAI) assesses middle ear function by measuring the sound conduction over a range of frequencies, its lack of structural information has made it challenging to determine the effects of various middle ear conditions on WAI during OM in vivo. Furthermore, standard otoscopy only allows for surface visualization of the tympanic membrane without any quantitative characteristics of the middle ear or middle ear effusions (MEEs). The purpose of this study is to: (1) quantify physical characteristics (presence, relative turbidity, and amount) of MEEs in vivo using optical coherence tomography (OCT), a non-invasive optical imaging technique, and (2) correlate WAI measurements with quantitative characteristics determined from cross-sectional OCT images. Method: A total of 21 subjects (average age of 7 ± 4 years) visiting an outpatient pediatric clinic were recruited under an approved IRB protocol. A total of 29 ears (normal: 18, OM with effusion: 8, and acute OM: 3 ears, based on pediatrician's diagnosis) were included in the study. Standard otoscopy, OCT, tympanometry, and WAI measurements were collected in a clinical setting. OCT images were analyzed to assess the presence of a MEE and/or middle ear biofilm, type of MEE (relative turbidity based on the amount of scattering), and amount of MEE fluid (relative fluid level). These OCT metrics were then utilized to categorize subject ears into: no MEE (control), biofilm without a MEE, serous-scant, serous-severe, mucoid-scant, and mucoid-severe MEE groups. The power absorbance of each group was correlated to evaluate statistical significance at $\alpha=0.05$. Results: The presence of a MEE decreased the power absorbance. The power absorbance from 2-5 kHz showed large variance across the groups, suggesting the dependence on both the type and amount of MEEs. The mucoid MEE group showed significantly less power absorbance at 1.65 kHz ($p=0.04$) and 2.65 kHz ($p=0.01$), when compared with the serous MEE group. The greater amounts of MEE fluid significantly decreased the power absorbance, especially at higher (>2 kHz) frequencies. The lower amounts of MEE fluid did not significantly affect the power absorbance, as expected. Conclusion: A portable, handheld OCT-otoscope can non-invasively determine physical characteristics of the middle ear and MEEs during OM. Quantitative cross-sectional OCT images can not only provide additional structural information about the middle

ear space, but can also be utilized to better understand abnormal WAI measurements from the early- to late/severe-stages of OM. Further investigations to correlate acoustic measurements with other physical characteristics of middle ear conditions in vivo are needed.

Title: Deciphering Deafness in Down Syndrome: Finding the Otitis Media Gene

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Introduction: Down syndrome (DS) is a common chromosomal abnormality involving full or partial trisomy of human chromosome 21 (Hsa21). DS has a variety of phenotypes, including craniofacial defects and learning difficulties. 96% of DS children also suffer from hearing loss due to fluid accumulation in the middle ear cavity (Otitis Media with Effusion (OME)), which further compounds their learning difficulties. The standard treatment for chronic OME, grommet insertion, is not possible for children with DS due to their increased susceptibility to infection. Therefore, we need to understand how they are genetically predisposed to OME to allow the development of effective therapies for hearing loss in DS. The mouse orthologues to the genes from Hsa21 are present on mouse chromosomes 16 (Mmu16), 10 (Mmu10) and 17 (Mmu17). Our collaborators have developed mouse models of DS with full or partial duplication of the homologous regions to Hsa21. Upon determining the OM phenotype of these mice, we found that half of the Dp9Tyb mice and all of the Dp5Tyb mice have OM. The Dp5Tyb mice are of particular interest as they only have 12 genes present in 3 copies. Objective: To investigate which of these 12 genes plays a role in the OM phenotype of Dp5Tyb mice. Method: We designed primers for the genes in the Dp5Tyb region and tested their fidelity on wild-type (WT) mouse tissues known to express these genes. PCRs were then carried out on RNA extracted from WT middle ear epithelial cells (ECs) to study the expression of the genes in the middle ear. Based on the known role of these genes we selected two proteins encoded by Hsa21 genes, ERG and ETS2, to look for protein localisation by immunohistochemistry (IHC) on control tissue and head sections from Dp5Tyb and WT mice. A western blot was carried out to identify the presence of ERG and ETS2 in lung lysate then WT middle ear EC lysate. Results: The PCR results showed that Erg and Ets2 are expressed in WT middle ear ECs. IHC indicated that ERG is localised in very few middle ear ECs in WT mice, but more positive cells were seen in Dp5Tyb mice. ETS2 was expressed much more strongly than ERG in the middle ear EC lining of both WT and Dp5Tyb mice. In addition, ETS2 positive cells were seen in the middle ear cavity fluid of Dp5Tyb mice with OM. The preliminary data from the western blot analysis showed high levels of both proteins in WT lung lysate and low levels, especially for ERG2, in the middle ear EC lysates. Conclusion: The expression data for the 12 Dp5Tyb genes will help us understand their role in the development of OME. The next step will be to analyse the genes in the Dp9Tyb region using the same methods. Identifying the gene(s) which in three copies result in the development of OM could allow therapies to be developed to

treat OME in DS children, improving their hearing and therefore their ability to learn and interact with their surroundings.

Title: Otitis Media in Down Syndrome: Towards Identification of the Gene for OM Using Mouse Models

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Objective: Down syndrome (DS) is caused by an extra copy of some or all of the genes of human chromosome 21 (Hsa21). This condition results in a number of phenotypes one of which is hearing loss as a result of the development of otitis media with effusion (OME). Method:To understand better the genetic basis for OME in DS we are currently studying mouse models of DS at the MRC Harwell Institute. Results:The orthologs to the genes from Hsa21 are spread in three regions on the mouse genome located on chromosomes 10 (Mmu10), 16 (Mmu16) and 17 (Mmu17) and we have undertaken detailed studies of a large number of mice carrying genome duplications across larger and smaller segments of regions syntenic to Hsas1. Mutants with duplication of the genes from Mmu10 (Dp2Yey) and Mmu17 (Dp3Yey) revealed no middle ear inflammation. However, mice with duplication of the genes from Mmu16 (Dp1Tyb) all had bilateral OME, suggesting that this region contains the gene(s) that contribute to the OME phenotype. We tested the mice for hearing loss by performing click-evoked auditory brainstem response (ABR). At two months of age, the Dp1Tyb carriers had elevated mean thresholds (+ 27.5 dB SPL) compared with wild-type mice. In addition assessment of the inner ear of the mice by scanning electron microscopy and histology revealed no indication of a sensorineural element to the hearing loss. These results suggested that the Dp1Tyb mutants have conductive hearing loss. Further examination revealed that they also had thickened middle ear mucosa, exudate rich in neutrophils and macrophages, intact tympanic membranes and craniofacial defects. To narrow down genes which in three copies result in OME, we first examined the phenotypes of duplication mice, Dp2Tyb, Dp3Tyb and Dp9Tyb, each of which comprises a separate segment of Dp1Tyb. While we detected low levels of OME in Dp9Tyb mice compared to Dp1Tyb mice and no OME in Dp2Tyb mice, we observed substantial OME in Dp3Tyb mice. We have proceeded to examine mice with duplication of smaller segments of the Dp3Tyb region for OME - Dp4Tyb, Dp5Tyb and Dp6Tyb – and have found that only Dp5Tyb mice had OME. The Dp5Tyb region contains only 12 genes. We are currently analysing the expression data of these 12 genes in the middle ear and are crossing the Dp5Tyb carriers to single gene knockouts to see if the OME phenotype is rescued in the double mutants. Conclusion:The identification of the causative gene(s) from the Dp5Tyb region will lead to a better understanding of the mechanisms leading to middle ear inflammation in DS and potentially aid the development of new therapeutic approaches for this condition.

Title: Otitis Media and Primary Ciliary Dyskinesia: Impact of Disease

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Objective: Primary ciliary dyskinesia (PCD) is a ciliopathic, autosomal recessive genetic disorder that causes defective mucociliary clearance throughout the respiratory tract. We wish to explore the impact of otitis media (OM) in this population and: 1) Analyze the frequency of OM. 2) Evaluate the frequency of OM complications and surgical interventions. 3) Establish a generalizable treatment paradigm for this cohort. 4) Explore the interaction of causal genotypes and clinical otologic outcomes. Method: A retrospective chart review was performed using a patient registry at the PCD Center of an academic tertiary children's hospital. Demographic, clinical and audiologic information were analyzed. Genetic Information was analyzed for known causal PCD mutations, and relationships were evaluated between these mutations and clinical outcomes. Results: Fifty-four subjects were identified. Age at diagnosis was <2 years (n = 16, 29.6%), 2-5 years (n = 12, 22.2%), >5-18 years (n = 23, 42.6%), or unknown (n = 3, 5.6%), with male/female ratio of 35.2%/64.8%. Otitis media with effusion was diagnosed in 51 subjects, thirty-six (66.7%) of whom underwent pressure equalization (PE) tube placement. Of these, 28 (77.8%) received multiple sets of PE tubes, and 26 (72.2%) developed otorrhea. Four patients needed tympanoplasty and two received mastoidectomy. Four patients required 6 ear-related admissions for intravenous antibiotics. In 19 patients with audiometric data, hearing loss was present in 32 ears, 24 of which was conductive in nature. The mean speech reception threshold with hearing loss was 23.2dB on the right, and 22.4dB on the left. Genetic test results were available in 27 subjects, in whom 23 (85.2%) had biallelic mutations in a PCD gene. Conclusion: PCD patients are greatly afflicted by OM, majority of whom receive multiple sets of PE tubes. Given the preponderance of otorrhea but rarity of other complications, PE tube should be used judiciously.

Title: Candidatus Ornithobacterium hominis: an Important but Under-Recognised Nasopharyngeal Bacterium?

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Objective: DNA-based studies have revealed the presence of a new nasopharyngeal bacterium recently named Candidatus Ornithobacterium hominis (OH). This uncultured bacterium has been detected at high relative abundance in NP microbiota datasets from around the world and was shown by a PCR-based study to be highly prevalent and persistent in a Thai paediatric population at high-risk of respiratory infection. Furthermore, the closest known OH relative (Ornithobacterium rhinotracheale) is a respiratory pathogen of birds. Although OH genomes can be derived from metagenomic data, isolates are needed to deepen understanding of the bacterium's role in human infections. The aim of this study was to isolate OH and determine its optimal culture conditions. Method: Culture was performed using biobanked NP swabs from four children (age 1-2 years) with chronic lung disease. All were OH-positive by 16S rRNA gene sequencing at 5-55% relative abundance. Colonies were screened using OH-specific PCR targeting the 16S rRNA and toxA genes. Presumptive OH colonies were confirmed using genome sequencing. Results: OH was successfully cultured from all four NP swabs. The isolate genomes had average nucleotide identity of 97.86-98.23% with draft genomes OH-22767 and OH-22803 (derived from metagenomic data), indicating they are members of the same species. Optimal primary culture was achieved using Tryptic Soy Agar with 5% Sheep Blood incubated in a microaerophilic atmosphere at 35°C for up to 5 days. Under these conditions, OH colonies ranged in size from 1-3 mm and were pleomorphic, glistening, grey and concave. All isolates were pleomorphic Gram-negative bacilli and were oxidase-positive and catalase-negative. All isolate genomes contained distinct lipopolysaccharide (LPS) biosynthesis clusters. All produced β-lactamase and contained genes encoding efflux pumps associated with multi-drug resistance. Conclusion: OH isolates were successfully recovered from all of the NP swabs. The optimal culture conditions included a microaerophilic atmosphere and prolonged incubation time; both conditions which are not part of standard culture conditions used to recover respiratory pathogens. Heterogeneity among the LPS cluster is suggestive of multiple capsular types, consistent with observations from earlier DNA-based studies. The presence of antibiotic resistance genes in all OH isolates suggests a potential capacity to indirectly affect treatments targeting pathogenic species. We propose a global study to more deeply assess OH diversity, distribution and prevalence and to determine whether this species may directly or indirectly contribute to OM pathogenesis.

Title: A2ML1 and Otitis Media: Novel Variants, Differential Expression and Relevant Pathways

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Objective: A genetic basis for otitis media is established, however the role of rare variants in disease etiology is largely unknown. Previously a duplication variant within A2ML1 was identified as a significant risk factor for otitis media in an indigenous Filipino population and in US children. Our aim was to identify (1) novel A2ML1 variants, (2) genes that are differentially expressed according to A2ML1 expression levels, and (3) A2ML1-related pathways that are relevant to otitis media. Method: In this report exome sequencing was performed using additional DNA samples from the indigenous Filipino population, Filipino cochlear implantees, and Finnish and Pakistani families with otitis media. Saliva samples from Filipino and Colorado participants were submitted for RNA-sequencing. For the Colorado patients with otitis media, correlation analysis according to A2ML1 levels and differential expression analysis using DESeq2 were performed. GO and KEGG pathways were identified using GAGE. Validation of differentially expressed genes was performed using qPCR. Results: Nine novel, damaging A2ML1 variants identified in otitis media patients were rare or low-frequency in population-matched controls. In the indigenous population, both gingivitis and A2ML1 variants including the duplication variant and c.4061+1G>C were independently associated with otitis media. Sequencing of salivary RNA samples from indigenous Filipinos demonstrated lower A2ML1 expression according to carriage of A2ML1 variants. Sequencing of additional salivary RNA samples from US patients with otitis media revealed differentially expressed genes that are highly correlated with A2ML1 expression levels. In particular RND3 is upregulated in both A2ML1 variant carriers and high-A2ML1-expressors. Conclusion: These findings support a role for A2ML1 in keratinocyte differentiation within the middle ear as part of otitis media pathology and the potential application of ROCK inhibition in otitis media.

Title: Surgical Outcomes of Ossiculoplasty Following Tympanomastoidectomy and Comparison with Polycel® and Titanium Prostheses

Authors: O. Choo[1], H. Kim[1], J. Jang[1], H. Park[1], Y. Choung[1]

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Objective: Polycel® or titanium materials are widely used as prostheses for ossicular reconstruction. In this study, we reviewed the surgical outcomes of ossiculoplasty following tympanomastoidectomy, and compared the results according to the materials for prosthesis. Method: Only patients with continuous large air-bone gaps after mastoidectomy (± ossiculoplasty) were included in the study. From January 2005 to December 2017, 221 patients received staged ossicular reconstruction after mastoidectomy by a single surgeon. Either Polycel® or titanium was used during the operation. Demographic data of the patients were collected retrospectively, and changes in hearing status before mastoidectomy, after mastoidectomy, and after staged ossiculoplasty were measured by pure tone audiometry and speech audiometry results. Multivariate analysis was also conducted to compare the effects of prosthesis materials. Results: Titanium prostheses also showed better surgical outcomes in partial ossicular reconstruction (PORP) (35.9% in titanium and 13.0% in Polycel®, p=0.005). Both Polycel® and titanium prostheses represented no difference in total ossicular reconstruction (TORP). In multivariate analysis, the titanium material showed better effect than Polycel® (OR 6.313, 95% confidence interval 2.227 – 17.897) in PORP. Older age and CWD mastoidectomy showed negative effects on the successful rate in PORP. In TORP, staged ossiculoplasty demonstrated better outcomes in multivariate analysis (17.259, 1.921 – 155.045). Conclusion: If considering PORP as ossiculoplasty after tympanomastoidectomy, titanium prosthesis can be recommended. If stapes head was missed at the first tympanomastoidectomy, consider staged ossiculplasty with TORP. Graft material itself may not that important in TORP.

Title: The Effectiveness of Ventilation Tube Insertion in Cleft Palate Patients

Authors: O. Choo, H. Kim[1], J. Jang[1], H. Park[1], Y. Choung[1]

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Objective: Hearing loss is a common complication in children with cleft palate (CP) due to recurrent otitis media with effusion affecting their hearing abilities. The increased risk for this complication is caused by Eustachian tube dysfunction. Thus, studies have suggested ventilation tube insertion (VTI) for CP patients. The purpose of this study was to identify the proper management of the middle ear in patients with CP to prevent hearing impairment and maintain sufficient middle ear space until the development of adequate E-tube function. Method: VTI was done simultaneously with CP surgery in 82 patients in Ajou University Medical center between 2005 and 2015. Postoperative follow up loss of 30 patients were excluded. A retrospective review of otology database (otoscopic examination, hearing evaluation, CP type, type of inserted ventilation tube (VT), post-operative complications, and prognosis) was performed. Results: A total of 52 patients (103 ears) were enrolled in the study. After VTI, complication was noted in 9 cases (8.7%) and recurrence in 29 cases (28.2%). The cases were divided into 3 groups according to the type of CP; submucosal(mild), incomplete(moderate), and complete(severe). Both the submucosal and complete CP group showed no statistical significance in complication or recurrence between the two different types of VT. In the incomplete CP group the recurrence rate was significantly higher in Type I VTI (39.0%) ($p=0.017$), and the complication rate was significantly higher in Type II VTI (23.3%) ($p=0.021$). Conclusion: We concluded that for the submucosal CP with palatoplasty alone and complete CP with a combination of palatoplasty and alveoloplasty, Type I VTI seems to be the treatment of choice. In cases of incomplete CP (moderate type) with palatoplasty alone, Type II VT may be a better option. In conclusion, during VTI for CP patients, consideration of the severity of CP and the type of CP surgery is important in regards to good prognosis and minimal complication.

Title: Extrusion rate and complications according to the type of ventilation tube: Multicenter registry study on the effectiveness of ventilation tube insertion in pediatric patients with chronic otitis media with effusion—Part II

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Introduction: Ventilation tube insertion for chronic otitis media with effusion is most commonly received operation in pediatric population. Various types of tube are used, but it is unclear as to what impact the tube shape and material have on extrusion and complication rates. Objective: This multicenter registry study aimed to investigate the effectiveness of ventilation tube insertion and the microbiology of otitis media with effusion in children. This part II study was conducted to evaluate the postoperative results especially according to the type of ventilation tube. Methods: Patients <15 years old who were diagnosed as having otitis media with effusion and received ventilation tube insertion were prospectively enrolled in 15 tertiary hospitals from June 2014 to December 2016. Follow-up data were collected until December 2017. After excluding patients with missing data, the data of 401 patients (727 operated ears) were analyzed among a total of 432 enrolled patients. The demographic data, surgical findings including the type of ventilation tube, and follow-up data were collected. Results: Average follow-up duration after tube insertion was 313 ± 238 days (range 3 to 1377 days). After excluding the results of long-lasting tubes (Paparella type II and T-tube; 13 ears), silicone tube (Paparella type I, 570 ears) showed significantly ($P<0.001$) more extended time to extrusion (average of 400 days) than titanium tube (Collar button type; 157 ears, average of 312 days).

Silicone tube also showed a significantly longer time to recurrence of middle ear effusion than titanium tubes ($p<0.001$). The rate of tube otorrhea was 2.5% in the silicone tube, and 1.3% in titanium tube, which was not statistically significant. Persistent perforation after extrusion was found in 1.2% of silicone tube inserted ears, and none in titanium tube inserted ears. In revision ventilation tube ears, there was a significantly higher rate of persistent perforation than primary ventilation tube ears (3.2% vs. 0.3%) and also significantly higher rate of another revision tube surgery than primary tube ears (13.0% vs. 7.5%). Conclusion: Silicone tubes are significantly less prone to extrude early than titanium tubes. Although the rate of tube otorrhea and persistent perforation was higher in silicone tubes than titanium tubes, it was not statistically significant. Type of ventilation tube affects the time to extrusion and complication rates; therefore, we should choose an appropriate type of ventilation tube according to the patient's status.

Title: Ear and Hearing Outcomes from an Ear Bus Program in Western Australia

Author: H. L. COATES

Introduction Aboriginal and Torres Strait Islander (ATSI) children have the worst ear health of any community in the world. Chronic suppurative otitis media (CSOM) rates are up to 70% in some communities and on average children experience 32 months of otitis media in their first 5 years compared to three months in non-ATSI children. These high rates of middle ear disease and associated hearing loss significantly impact the health and education outcomes of these children. The Earbus Foundation of Western Australia is a children's charity that aims to reduce the incidence and impact of otitis media in ATSI and at – risk children in Western Australia. It operates in rural and remote Western Australia bringing together experts from education, health, culture and communities. Objectives To document the efficacy of an intensive ear health program delivered remotely by a mobile multidisciplinary team. Methods ATSI children are reviewed by a nurse audiometrist, audiologist and community Aboriginal health worker. If necessary children will have a targeted medical review by a nurse practitioner or General practitioner. Those children with middle ear pathology are seen by an otolaryngologist or have "store and send" video otoscopic images transmitted to the otolaryngologist together with a brief history and audiological findings. Medical treatment particularly for children with chronic suppurative otitis media includes intensive local treatment and referral for surgical management when necessary. Results Ear and hearing outcomes data of the children seen was collated over a 12-month period. Of 760 patients seen in the Pilbara region there was a reduction of 50% in hearing loss and otitis media referral rate. The prevalence of otitis media with effusion (OME) and CSOM was reduced by 66%. 1200 children seen in the Goldfields region had a reduction of 75 % in the otitis media referral rate. Hearing loss and the prevalence of OME and CSOM was reduced by 66%. Conclusion Intensive ear health management in remote and rural communities by utilisation of an ear bus with a multidisciplinary team resulted in a significant decrease in hearing loss and middle ear disease in ATSI children.

Title: The Molecular Microbiological profile of Otitis Media with effusion in Chinese Children

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Objective: Otitis media with effusion (OME) is one of the most common pediatric diseases worldwide. Some studies have found the diversity of microbiome in middle ear effusions from developed countries. But no microbiological studies of middle ear effusions from Chinese children with OME have been reported. The study aimed to characterize the middle ear and nasopharyngeal microbiological profile in children with otitis media with effusion and compare the microbial flora of nasopharynx from patients with otitis media with effusion and children without otitis media. Method:Middle ear effusions and nasopharyngeal swabs were acquired from 15 children undergoing ventilation tube insertion. Nasopharyngeal swabs from 15 patients without ear diseases were controls. Samples were analyzed using 16S rRNA sequencing on an IlluminaHiSeq2500 platform. Results:The total numbers of operational taxonomic units (OTUs) were similar among the middle ears (TM) and nasopharynx (TN) of OME subjects and nasopharynx of controls (CN). But the microbiota of TM was found to be dissimilar to that of the TN ($P = 0.001$), whereas the TN and CN microbiota were similar ($P = 0.06$) (Analysis of similarities). The middle ear effusion was dominated by Haemophilus (14.75%), Staphylococcus (9.37%), Streptococcus (5.76%), Halomonas (7.85%), Bacteroides (6.27%). The bacterial composition of nasopharynx in OME groups was dominated by Haemophilus (21.87%), Streptococcus (19.65%), Neisseria (5.8%), and Moraxella (5.16%). The bacterial composition of nasopharynx in control groups was dominated by Haemophilus (15.96%), Streptococcus (13.33%), Moraxella (12.28%), Neisseria (4.79%). The significant differential taxa between the nasopharynx and the middle ear in OME patients were Streptococcus, Moraxella and Neisseria. Conclusion:Our results confirmed the microbiota diversity of middle ear effusions in Chinese children. But the dissimilar microbiome composition between the nasopharynx and the middle ear question the theory that the nasopharynx serves as a reservoir microbiota of middle ear in children with otitis media with effusion.

Title: Effectiveness of a Multifaceted Intervention to Educate General Practitioners about Pain Management in Children with Acute Otitis Media: a Cluster Randomized Controlled Trial

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Introduction. Ear pain is the predominant symptom of childhood acute otitis media (AOM) and analgesics are the cornerstone of AOM management. Nevertheless, current evidence suggest that symptomatic management with analgesic management is suboptimal in daily practice, which in turn may lead to unnecessary discomfort, doctor consultation and antibiotic prescribing. Objective. To assess the effectiveness of a multifaceted intervention aimed at educating general practitioners (GPs) about pain management in children with acute otitis media (AOM). Methods. Pragmatic cluster randomized controlled trial with the GP practice as unit of randomization. 83 GPs and 11 GP trainees in 37 practices (intervention n=19; control n=18) across the Netherlands recruited 224 children with GP-confirmed AOM and ear pain (intervention n=94; control n=130) between February 2015 and May 2018. GPs in practices allocated to the intervention group received a blended GP educational program (online and face-to-face training). They were trained to explicitly discuss pain management with parents using an information leaflet, and prompted to prescribe analgesics (paracetamol, and ibuprofen in case of insufficient pain relief with paracetamol alone) in weight-appropriate dosage. GPs in the practices allocated to the control group provided usual care. The primary outcome was parent-reported mean ear pain score (scale 0-10) over the first three days. Results. Primary outcome data was available for 209 children (93.3%). Mean ear pain scores over the first three days were similar between groups (4.66 versus 4.36; adjusted mean difference -0.05, 95% confidence (CI) -0.93 to 0.83), whereas on these days analgesic use, and in particular ibuprofen, was higher in the intervention group than in the control group. Although children in the intervention group received fewer antibiotic prescriptions at the index consultation than those in the control group (mean rate 0.23 versus 0.39; adjusted rate ratio 0.67, 95% CI 0.43 to 1.04), the total number of antibiotic prescriptions during the 28-day follow-up was similar: mean rate 0.43 versus 0.47, respectively (adjusted rate ratio 0.98, 95% CI 0.69 to 1.39). Children in the intervention group consulted their GP more often for AOM-related complaints during follow-up: mean rate 0.70 versus 0.41 (adjusted rate ratio 1.73, 95% CI 1.15 to 2.62). Other secondary outcomes were similar in both groups. Conclusion. Our intervention aimed at educating GPs about pain management in children with AOM led to an increase in analgesic use, in particular ibuprofen, but this did not result in lower parent-reported ear pain scores. We therefore suggest not to routinely use ibuprofen in children with AOM.

Title: Global Prevalence of Antibiotic Resistance of Bacteria in the Nasopharynx and Middle Ear from Children with Acute Otitis Media: a Systematic Review.

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Objective: Acute otitis media (AOM) is one of the most common childhood infections and a prime reason for doctor consultations and antibiotic prescribing. With reports of an increase in antimicrobial resistance (AMR) rates globally, it is urgent to provide a systematic and current overview of AMR rates in childhood AOM across the world. To estimate the global prevalence of bacterial resistance of nasopharyngeal and middle ear isolates in children with AOM, and to investigate whether AMR rates vary over time and differ across countries with various antibiotic consumption levels. Method: Systematic searches of PubMed, EMBASE, and the Cochrane Library were performed from inception to June 20, 2018 using a broad search strategy. Two reviewers independently screened titles and abstracts of unique records for eligibility using pre-specified criteria. Next, the same reviewers independently reviewed full texts of potentially relevant articles for inclusion. Non-English studies, animal studies and those from which the full text could not be retrieved were excluded. All studies reporting any AMR data of nasopharyngeal and middle ear isolates from children with AOM were included. Risk of bias of included studies was assessed by the Cochrane 'Risk of bias' tool (for randomised controlled trials) or a quality assessment checklist (for observational studies) successfully applied in a similar review. Overall AMR rates of nasopharyngeal and middle ear isolates (S. pneumoniae, nontypeable H. Influenzae, M. Catarrhalis, S. Aureus, P. Aeruginosa, S. Pyogenes) to most commonly prescribed antibiotics will be presented according to the year(s) in which the study was conducted and to countries' antibiotic consumption levels (as provided by ESAC-Net Database and the Center for Disease Dynamics, Economics & Policy (CDDEP). Results: The electronic database searches yielded a total of 6,871 records. Removing duplicates left 3,767 unique records. After title/abstract screening, 268 potentially relevant articles remained for full text screening of which 87 were suitable for inclusion. Data extraction is currently ongoing and results will be presented at the conference. Conclusion: This systematic review will provide a current overview of global AMR prevalence of nasopharyngeal and middle ear isolates from children with AOM and will reveal whether AMR rates have increased over time and differ across countries with various antibiotic consumption levels.

Title: Machine Learning Approaches to Predict Haemophilus influenzae Associated Host Disease State, Host Tissue, and Gene “Dark Matter”

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Introduction: The characterization of genetic mechanisms of bacterial virulence remain elusive, particularly in species that have diverse phenotype presentation. For example, Non-typeable Haemophilus influenzae (NTHi) is a bacterium implicated in Otitis media and other disease states but can also colonize healthy human hosts. It is also isolated from various biogeographical locations, indicating an ability to successfully adapt to different environments, and present a variety of virulence phenotypes. Objective: Use the annotated genes from 993 whole genome sequenced (WGS) genomes of NTHi to predict host disease, and body site provenance using two machine learning algorithms. Explore genes both identified as important predictors and annotated as ‘hypothetical’ using deep learning neural networks to apply gene ontology (GO) terms. Method: WGS was performed on 993 genomes using multiple different sequencing platforms, though primarily using Nextseq 500 Illumina sequencing. Genomes were assembled using sequencer-appropriate software and automatic gene annotation was performed using Prokka. Pan-genome gene cluster analysis on all strains was performed with Roary. A matrix of 4,207 gene clusters was used as ‘features’ to predict strain isolates from host site, and disease state. Two algorithms used for class prediction were random forest (RF) and neural network (NN). All protein coding gene amino acid sequences were then examined with a deep learning neural network trained on the entire database of UnitProt to apply GO terms to all gene sequences. Results: Both Random Forests and Neural Networks performed significantly better than the ‘No Information Criteria’ in predicting either body site and disease state, though RF outperformed NN in all cases. Parameter tuning significantly improved RF prediction capability, with a maximum accuracy rate of 85% predicting the disease state of the patient. Initial results on a test group of strains (Moraxella catarrhalis) of the deep learning algorithm provided GO terms with 80% confidence to ~14% of ‘hypothetical’ gene annotations. Conclusion: We show that gene presence in NTHi strains provides adequate information to predict both ecological niches and associated disease state, up to an accuracy of 85%. Deep learning is shown to be a capable curative step in annotating genes of unknown function, applying GO terms to these annotations with a high degree of confidence. Further work is warranted both to explore additional methods and feature selection.

Title: Supragenome-wide Association Studies of Haemophilus influenzae Reveal Roles for Bacterial Nitrogen Metabolism and pH Homeostasis in Middle Ear Disease

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Introduction: Bacterial infections of the middle ear cause serious morbidity in children, and the human-restricted opportunistic pathogen non-typeable Haemophilus influenzae (NTHi) is a major culprit. Genomic diversity among strains is extremely high, which is reflected by the wide phenotypic variation among clinical isolates, as measured by in vitro assays of virulence and in animal models of pathogenesis. Thus, although host genetics and other factors like viral infection play major roles in disease risk, bacterial genetic diversity is also a crucial player. Objectives: Because gene content among NTHi strains is highly variable, we set out to identify NTHi genes whose presence or absence is associated with the incidence of otitis media. The results will inform the design of new interventions to control pathogenesis caused by this otherwise commensal member of the nasopharyngeal microbiome. Methods: We genome-sequenced hundreds of NTHi strains isolated from both healthy and acute otitis media (AOM) prone children from 6-36 months of age, from both nasal washes and middle ear fluid collected during AOM episodes. Curated genome assemblies and annotations were used to identify homologous gene clusters among strains, and orthology and paralogy were defined based on synteny. Maximum likelihood phylogenies were inferred from a concatenated codon-aware alignment of core protein-coding genes, and clonal lineages were assigned with a new algorithm that accounts for inter-strain recombination. We then conducted supragenome-wide association studies (SGWAS), whereby we found genes whose presence or absence was associated with health or disease. Results: After corrections that accounted for phylogenetic structure, we identified dozens of NTHi genes whose presence or absence was associated with isolation from: (a) middle ear fluid or nasal washes; (b) healthy visits or AOM episodes; (c) healthy or AOM-prone children; (d) AOM episodes followed by treatment failure or not. Two dominant themes emerged from examination of the top hits: (a) distributed gene absence was more often associated with disease than gene presence; (b) genes involved in nitrogen metabolism, polyamine transport, and pH homeostasis have strong associations with health or disease. We also found that NTHi strains associated with AOM episodes followed by treatment failure were not only enriched for beta-lactamase genes but depleted for a gene whose product is likely to inhibit recombination. Conclusion: SGWAS identified NTHi genes strongly associated with either commensalism or AOM virulence. Remarkably, most of the identifiable molecular pathways affected are plausibly involved in resistance to an alkaline environment, as may often be common in middle ear fluid. In combination with recent results that show distinct biofilm phenotypes for different NTHi strains at high pH, these results suggest new routes for mechanistic investigation and ideas for therapeutic intervention.

Title: Species-level bacterial community profiling of the otitis media microbiome using Pacific Biosciences sequencing of full-length 16S rRNA genes

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Introduction: Chronic otitis media with effusion (COME) pathogenesis is associated with bacteria adopting the biofilm mode of growth which makes them antibiotic resistant. The bacteria that cause acute OM are referred to as otopathogens, and include Streptococcus pneumoniae, non-typeable Haemophilus influenzae and Moraxella catarrhalis. These bacteria originate from the nasopharynx and are capable of migrating to the middle ear. Once in the ME they can cause acute or chronic disease. The role of other bacterial species in COME is, however, an open topic. Objective:To identify semi-quantitatively in an unbiased manner all of the bacterial species present in cohort of children ongoing myringotomy and tube placement for COME. Method:Seventy children between 5m and 16y of age were selected. One hundred thirty four middle-ear effusions (MEE) specimens were collected via Lukens traps with N-acetyl-L-cysteine (NAC) buffer. DNA extraction, full length 16S rRNA amplification, and multiplex DNA sequencing were conducted as described (Earl et. al. 2018). The PacBio Sequel demultiplexed FASTQ files were concatenated together for analysis through the MCSMRT pipeline. Reads with lengths 500> x >2000 bps and those mapped to the host genome were removed as well as reads missing both the PCR primers. The high-quality reads (with an expected error (EE) threshold of 1) were then dereplicated, clustered in OTUs, chimeras were identified and removed, and finally a species level OTU table was created. Results:Ninety-six samples out of 134 total (70.5%) were positive for the full length 16S rRNA PCR amplification and were sequenced using the Pac-Bio RSII or Sequel instruments. Raw data were filtered and analyzed using the MCSMRT pipeline. The samples with less than 500 total reads or less than 100 OTUs were removed. Finally, 88 samples (65% of original samples) from 55 patients were analyzed. For 35 patients both right and left ears were analyzed, and for 18 patients only a single ear was analyzed. In these samples we identified 126 species (OTUs). The most prevalent species were Alloiococcus otitis (26.5%), Staphylococcus pettenkoferi (18.8%), Haemophilus influenzae (12.8%), Turicella otitidis (12.1%), Moraxella catarrhalis (11.4%), Staphylococcus auricularis (10.4%), Pseudomonas aeruginosa (3.8%), Streptococcus pneumoniae (2.2%) and Staphylococcus simulans (1.9%). Correlations between patients, bacterial strains and sites will be presented and discussed.

Conclusion: It can be concluded by prevalence analyses that in addition to the accepted otopathogens that additional bacterial species including Alloiococcus otitis, Turicella otitidis, and Staphylococcus pettenkoferi are likely playing a role in the pathogenesis of COME. Further studies should be conducted to identify high probability virulence traits within the genomes of these pathogens to allow more efficient and successful treatment. Earl et al. 2018. Microbiome doi: 10.1186/s40168-018-0569-2.

Title: Global Gene Expression Profile of Middle Ear Mucosa in a Chinchilla Model of Pneumococcal-induced Otitis Media

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Objective: As the host's first line of defense, the ME mucosa (MEM) plays a pivotal role in initiating and maintaining the inflammatory response and determining the course of disease. Comprehensive characterization of global gene expression within the MEM following infection with OM pathogens may aid in understanding the molecules and pathways associated with the host immune response during OM and their roles in disease progression or resolution. In this study, we characterized the host immune response during different phases of OM by profiling global gene expression in chinchilla ME mucosa following pneumococcal infection. Methods: Chinchilla were infected by trans-bulla injection with Streptococcus pneumoniae. Noninfected chinchillas were set as control. ME mucosal cells were harvested on days 3 and 17 post-infection. Extracted RNAs were analyzed by RNA Seq followed by Qiagen Ingenuity Pathway Analysis. Results: Infected chinchillas were 100% positive for biofilm formation, ME effusion and bacterial culture by day 3 post-infection, and 80%, 60% and 40% positive for biofilm formation, ME effusion and microbiological culture, respectively, by day 17 post-infection. Approximately 30% of genes were differentially expressed between infected and uninfected groups on day 3 and 17 post-infection compared to the control respectively. About 27% of genes were differentially expressed on day 17 compared to day 3 in infected chinchillas. Pathway analysis indicated that the differentially expressed genes on day 3 and 17 were predominantly involved in hematological system development and function, immune cell proliferation and development, mucosal disease, inflammatory response, immune cell trafficking, cellular movement, and cell-to-cell signaling. Patterns of pathway activation and inhibition demonstrating consistency between day 3 and 17 were: activation of innate responses, recruitment/function of leukocytes, pro- and anti- inflammatory responses, and Toll-like receptor signaling; and inhibition of PPAR signaling, calcium signaling, GP6 signaling, LXR/RXR activation, and neuropathic pain signaling in dorsal horn neurons. Responses observed on day 17 that diverged from that on day 3 post-infection were: activation of adaptive immune responses such as Th1 and Th2 pathways, complement system, cAMP-mediated signaling, LXR/RXR activation, as well as inhibitions of inflammation, acute phase response signaling, LPS/IL-1 mediated inhibition of RXR function. Conclusion: Profiling of global gene expression in chinchilla ME induced by S. pneumoniae identified canonical pathways involved in controlling host defense at various stages of disease, suggesting their importance in OM pathophysiology. Further study is warranted to validate the relevance of identified pathways in governing the procession of infection/inflammation in clinical specimens.

Title: Quality of life in Swedish children receiving grommets – an analysis of pre- and postoperative results based on a national quality register

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Objective: Otitis media with effusion (OME) and recurrent otitis media (rAOM) are common childhood affections often treated with grommets. It is known that affected children have an affected quality of life (QoL), and various questionnaires have been used to evaluate this. Sweden has a national quality register for grommet insertions containing some quality of life questions that have hitherto never been analysed. Method: Data from 2010-2016 was extracted from the register and analysed with regards to QoL questions and physical findings. Results: Preoperative QoL data was available for 3835 children. Prior to surgery, parents of OME children were more likely to suspect that their child had a hearing loss (ORs 10.1 and 28.2 for suspecting a mild and severe hearing loss), but less likely to feel that the ear disease affected the child's general wellbeing compared to parents of rAOM children (ORs 0.54 and 0.33 for somewhat and much affected). When correlated to fulfilment of surgical criteria, OME children with a verified hearing loss scored worse on all questions compared to OME children without hearing loss, whereas parents of rAOM children with more than 3 verified AOM episodes were more likely to feel that their child's general wellbeing was affected compared to those with fewer AOM episodes. A significant improvement in individual scores was seen postoperatively ($p<0.001$). The degree of postoperative improvement in pure tone average correlated with the improvement in QoL ($p<0.001$) Conclusion: This is the first time the QoL aspect has been analysed in the Swedish register. Though the validity of the questions has not been proven, they provide valuable information. The relevance of surgical criteria in national guidelines is illustrated by their correlation with QoL, particularly for OME, and the postoperative improvement in QoL suggests parents find that their children benefit from surgery.

Title: Disparity In Allergy Testing Results Among Patients With Allergic Rhinitis And Eustachian Tube Dysfunction

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Objective: The reported incidence of allergy being related to Eustachian Tube Dysfunction (ETD) and/or Otitis Media with Effusion (OME) as determined by allergy testing ranges from 29% to 93% in children up to 18 years, and up to 35% among adults.[1] Most epidemiologic studies have shown that patients with OME have an increased prevalence of atopic conditions when compared with non-OME controls.[2] Current guidelines from otologists and allergists support the role of allergy in the development of OME.[5] Yet, recent International Consensus states "there is no convincing evidence that treating allergy has any effect on OME",[6] ignoring published data demonstrating that up to 89% of OME patients partially or completely resolve ETD symptoms after immunotherapy[7] or food elimination diets.[8] The lack of agreement on this relationship has led many physicians to discount the role that allergy might play in OME. We sought to discover if the disparity of the reported relationship of allergy to ETD might be due to the allergy testing method employed: skin prick tests (SPT) vs intradermal dilution testing (IDT). Method: There is no absolute way to evaluate allergy-testing techniques other than symptom resolution with allergy-directed therapy. This prospective study evaluated symptom improvement using symptom severity questionnaires completed before and after allergy immunotherapy (AIT) by 110 patients with chronic OME, asthma and chronic allergic rhinitis. Results: Positive SPT correlated with positive IDT at dilutions equal to or weaker than 1:12,500 w/v but not at 1:2,500 or 1:500. These stronger IDT dilutions identified 47 (43%) patients, with an additional 435 allergens who would not otherwise have been diagnosed. Even among SPT+/IDT+ patients, IDT detected 3.26 times more allergens per patient than did SPT (336 vs 103). After six months of AIT, 94.3% of patients reported average symptom severity improvements of 64%. The SPT-/ IDT+ group's responses to AIT were statistically similar to those reported by the SPT+ group. Conclusion: This data strongly supports our hypothesis: Positive IDT following negative SPT offer significant improvement in the ability to diagnosis AIT-responsive middle ear disease. IDT are clinically relevant, especially in patients with low sensitivity. Skin prick tests alone failed to identify 43% of OME individuals with allergic disease. There was no significant difference in AIT success or failure rates between SPT-/IDT+ and SPT+/IDT+ patients. Adding IDT tests following negative SPT more than tripled the number of detected allergens and doubled the number of people successfully treated for OME with immunotherapy. We believe that the discrepancy in allergy test results may account for why many physicians mistakenly discount the role that allergy plays as an etiology of OME. Importantly, OME patients found to be negative by SPT testing can never benefit from immunotherapy if they are incorrectly labeled as "non-allergic".

Title: Trans-Tympanic Delivery of Local Anesthetics for Pain in Acute Otitis Media

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Objective: Acute otitis media (AOM) commonly causes pain and distress in children. Up to 80% of children with AOM have mild to severe pain during the onset of the infection, of which about 40% have severe pain. Consequently, many AOM guidelines recommend the use of analgesics as an essential part of the treatment. The effectiveness of commercial ototopical products in AOM is also questionable. Nonetheless, local topical ain treatment remains desirable since side effects from systemic drug distribution would be avoided, the pain relief could be faster in onset, be more intense, and last longer than with oral analgesia. Therefore, we aim to develop a local drug delivery system to provide sustained pain relief in patients with AOM. Method: We hypothesized that the lack of efficient analgesic effects from ototopical drops was due to inability to penetrate the tympanic membrane (TM). The outermost layer in the TM, the stratum corneum, is impermeable to virtually all molecules except the small and moderately hydrophobic ones. Thus, we developed a formulation combining CPEs and known anesthetics to enhance drug flux into and across an intact TM, and achieve effective analgesia for AOM. The CPEs and anesthetics were delivered in a hydrogel-based formulation. It is an easy-to-apply liquid at room temperature, and gels quickly and firmly upon contacting the warm TM, holding the anesthetics and CPEs in place (i.e. on the TM). The sustained release and diffusion of drugs into the middle ear can thus be achieved by a single application of the formulation. Results: Successful drug delivery across intact tympanic membranes was demonstrated using the amino-amide local anesthetic in current clinical use, bupivacaine, and a highly potent site 1 sodium channel blocker anesthetic, tetrodotoxin, a very hydrophilic compound that blocks the same sodium channel as bupivacaine but at a different site, and has ultrapotent local anesthetic activity. Bupivacaine and TTX are known to strongly increase each other’s anesthetic effects when given in combination. The chemical permeation enhancers incorporated in the delivery system increased the permeability of the to the anesthetics considerably, resulting in high concentration of drug in the middle ear fluid. Conclusion: A local drug delivery system was developed to provide sustained pain relief from a single application in patients with AOM. A commonly used amino-amide anesthetic, bupivacaine, was successfully delivered across intact TMs, as was a highly potent site 1 sodium channel blocker anesthetic, TTX. The chemical permeation enhancers incorporated in the hydrogel system considerably increased the permeability of BUP and TTX across the TM.

Title: Treatment Outcome of Acute Otitis Media in Children Treated with the Clinical Practice Guidelines for Diagnosis and Management of Acute Otitis Media in Children in Japan

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Background: In 2018, Otologic Society of Japan, Japan Society for Pediatric Otorhinolaryngology, and Japan Society for Infectious Diseases in Otolaryngology updated "Clinical Guideline for Acute Otitis Media in Children in Japan." The first edition of this guideline was issued in 2006, and this latest edition is the 4th one. The most obvious difference from AAP Guideline might be the treatment options prepared for treatment failures caused by BLNAR (beta-lactamase non-producing ampicillin resistant Haemophilus influenzae). The options come from the high incidence of BLNAR and the unique health care system in Japan. The latest Guideline includes 17 clinical questions and recommendations. The recommendations are summarized to the treatment algorithms. The basic structure of the algorithm has been maintained since the first edition. Briefly, pediatric patients with acute otitis media are classified into three groups that is mild, moderate, or severe according to the scores for their ages, clinical symptoms and tympanic membrane (TM) findings. Each group has its own treatment course including not only antibiotic choice but also myringotomy. Objective: Our goal of this study is to clarify changes in bacteriology and disease severity after the guideline. Method: The changes in bacteriology and the disease severity was assessed when treated with the guideline in a representative hospital in Japan. The number of myringotomies was used as an indicator of disease severity. Results: Increase in susceptible strains of Streptococcus pneumoniae were observed during amoxicillin (AMPC) prescription were dominant. However, in the same period, decrease in susceptible strains of H. influenzae were also observed. Sever cases that requires myringotomies decreased maybe because guideline-recommended pneumococcal conjugate vaccine (PCV) has become available in Japan. Conclusion: The guideline in Japan has achieved good result in a part of bacteriology and disease severity of AOM. However, it also has some limitations, such as unexpected increase in the use of tosufloxacin (TFLX)-newly released fluoroquinolone antibiotic for children. While TFLX could increase susceptible strains of H. influenzae (BLNAS), a safety profile of fluoroquinolone is still controversial. This issue has remained to be solved.

Title: Acute Otitis Media in Mice with a Human Hematolymphoid System

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Objective: Otitis media (OM) is one of the largest pediatric health problems, and can have devastating impacts in developing countries. Much of the basic research done on OM pathogenesis, etiology and treatment options owes to animal models. In recent years, "humanized" mice have become a valuable tool with which to study the human immune system in an animal model setting. Here we describe and evaluate their use to model OM.
Method: OM was induced by inoculation of nontypeable Haemophilus influenzae (NTHi) into the middle ears (MEs) of NOD-Scid-IL2Rγ^{null} (NSG) mice that were previously transplanted with human hematopoietic CD34^{+} cells. Blood and ear tissue were collected at different times post OM induction. Human inflammatory cell mobilization was evaluated by flow cytometry and immunohistochemistry and RNA profile. The ME inflammatory response, leukocyte infiltration, tissue hyperplasia, bacterial clearance and OM recovery were compared to that in wild type (WT) BALB/c mice. Results: The ME inflammatory response, leukocyte infiltration, tissue hyperplasia, bacterial clearance and OM recovery profile were comparable in humanized NSG (huNSG) to WT. In the huNSGs, we observed the mobilization of human CD45^{+} immune cells into the ME cavity, suggesting that the human cells are participating in both ME inflammation and NTHi bacterial clearance. At 10 days, all MEs of WT and huNSGs with >30% engraftment were culture-negative, indicating normal recovery from OM. In contrast, animals with <30% engraftment were culture-positive, indicating failure to resolve OM. Hence, well-engrafted huNSG mice are able to clear bacterial ME infection within 10 days without significant sequelae. Utilizing the RT2 PCR profiler array (Qiagen), we observed ME expression of human-specific genes such as IL8, CCL2, HLA-A, IFNA1 and CXCL8. Conclusion: The ability to study human immune cells in an animal setting will allow us to address human-specific immunity in OM, while preserving the many advantages of an animal model. This preclinical model will have a number of potential uses in OM research, including using hematopoietic stem cells from OM patients with differing degrees of OM susceptibility to understand the role of human immune responses in proneness to this disease. Funding Source/Disclosures [Supported by NIH/NIDCD grants R03DC014801, DC000129 and the Veteran Affairs Administration Research Service.]

Title: Parental Perceptions and Management Strategies for Otitis Media In Greenland

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Introduction Otitis media (OM) in Greenland is a substantial problem and the country prevalence is among the highest in the world. However, little is known about how Greenlandic Inuit parents perceive and manage everyday life in families with children suffering from OM. We hypothesize that having a child with OM has dire consequences for the families that go beyond the advice and treatment offered in primary health care due to the nature of the disease. Objective The aim of this study was to investigate the perception of and management strategies for every-day life among Greenlandic Inuit parents with children suffering from OM. Methods We conducted a qualitative study based on semi-structured interviews and focus groups with parents to children with OM. The interviews took place in three different regions; the capital Nuuk (17,000 inhabitants), and two smaller towns (2,000-3,000 inhabitants) in West and East Greenland. Access to specialized health care, including Ear, Nose, and Throat specialists, differs among the regions, creating an underlying difference on the limitations of referral and thereby level of care. We conducted the data analysis using Systematic Text Condensation, a cross-case method. Results In total, 29 parents participated in the study. Although most parents perceived OM as a result of genetic or environmental dispositions, individual perceptions and cultural beliefs of causal associations between parental behavior and the occurrence of OM co-existed with the general understanding of medical explanation models for OM. This created a sense of guilt among the parents. Parents felt either in control of managing the disease of the child and used medically well-established strategies such as systematic ear mopping and antibiotics as prescribed. Others felt frustrated and considered contact to the health clinics as futile, thereby managing the disease by ‘waiting it out’. Emerging themes were shame and stigma related to the symptoms of OM in the local communities, which had led to social isolation as a consequence for several of the interviewed families with children suffering from OM. Conclusion Our results indicate that Greenlandic Inuit families are impacted by OM in a complex and severe manner. Guilt, shame and social isolation were predominant themes influencing the everyday life of the affected families. The parental perceptions of the disease and the management strategies go beyond the scope of the medical explanation and treatment models which poses a potential challenge for the parents’ experiences with the present treatment offer and indicate that the consequences of the disease reach beyond ear pain and fever. Due to the high prevalence of OM in Greenland the results of our study underline the need to develop a more holistic approach to prevention and treatment targeted children with OM and their families - both at the clinical level as well as a part of public health promotion at the community level.

Title: Acute Otitis Media and Pneumococcal Vaccination – An Observational Cross-sectional Study of Otitis Media among Vaccinated and Unvaccinated Children in Greenland

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Introduction: Streptococcus Pneumoniae (s. pneumoniae) is one of the main pathogens leading to otitis media (OM) and the leading pathogen to cause OM in children below six months of age. In 2010 the 13-valent pneumococcal conjugate vaccine (PCV13) was implemented in the Greenlandic children vaccination programme, but the effect of this change is not yet well documented. The objective of this study is to evaluate the effect of the implementation based on the number of episodes of acute otitis media (AOM). Methods: Data will be obtained from Greenlandic medical journals. The PCV13 vaccine is given in three doses, when the child is 3, 5 and 12 months old. We will include all children born from January 2015 to December 2016, thus eligible for the three doses of PCV13 including one year of follow-up time. Exclusion criteria will be uncertain vaccination status and predefined comorbidities. The children will be divided into two groups based on vaccination status defined as “Completed vaccination programme”: having received all three doses of PCV13 within the age of 15 months; and “Not vaccinated/uncompleted vaccination programme”: not having received all three doses of PCV13 within the age of 15 months and not fulfilling any exclusion criteria. Results: In total, we will include 1635 children. From the medical journals we register the following: gender, vaccination dates, the number of AOM-episodes, full pregnancy vs. premature birth, living in a town vs. a settlement and ceasarean section vs. vaginal birth. The potential absolute risk reduction and relative risk reduction will be calculated. Conclusion: Will be presented at the meeting according to the results.

Title: Determining factors for tympanostomy tube insertion in a country with a high insertion rate

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Introduction: Tympanostomy tube insertion is the procedure most often performed on children. Although most western countries have a high-level health system, the tympanostomy tube insertion rate differs among these countries. Objective: To investigate the determining factors for having tympanostomy tubes inserted in children in a western country with a high insertion rate. Methods: Informations were drawn from the Danish National Birth Cohort. This informations were interviews from more than 80,000 mothers, recorded prospectively from the first trimester and throughout the pregnancy until the child reached the age of 10 years and included information on OM, prenatal exposures, daycare attendance, breastfeeding and everyday life. Informations were also drawn from the Danish Health registries and the Danish Population Education registry. Results: In this material regarding 85,565 children, 25,331 of the children had one or more tympanostomy tubes inserted. In the children who were treated with tube insertion, 66% had experienced at least one episode of otitis media compared to 31% of those children not having a tube inserted. In the children who had experienced more than three episodes of otitis media, 75% had a tympanostomy tube inserted. A total of 24 variables were assessed in a prediction model to detect the risk of receiving treatment with tube insertion. Using a Random forrest function, a prediction model was maked with an overall error rate of 26.54% Conclusion: The variables associated with tube insertion were the same as those variables associated with the child experiencing otitis media. However, it was not possible to predict which children were going to have a tube inserted. The prediction model could not detect the children not going to have a tube inserted, and there was no difference between children with more than 3 episodes of otitis media with had a tube inserted and those not having a tube inserted.

Title: C-Reactive Protein and Non-Typeable Haemophilus influenzae

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Introduction: Lipopolysaccharide (LPS) on the Non-typeable Haemophilus influenzae (NTHi) surface plays an important role in interaction of the bacterium with the host and a component of the molecule, phosphocholine (PCho), can affect survival and the ability to cause disease, including otitis media (OM). PCho can bind to Platelet Activating Factor receptor (PAFr) to promote adhesion and evasion of immune responses. C-reactive protein (CRP), an acute phase response molecule whose concentration can rise dramatically in response to inflammatory signals, binds PCho and can initiate bacterial killing. PCho expressed on NTHi can therefore influence the immune response elicited against these bacteria. Objective: Study the interaction of CRP with NTHi to understand its role in bacterial survival. Method: NTHi can efficiently infect the Junbo mouse, a characterised model of chronic and acute OM. Serum and middle ear fluid (MEF) were collected from Junbo and wild type (WT) mice inoculated with variant NTHi strains predominantly expressing PCho, not expressing PCho, or unable to express PCho (lic1 mutation). Infection was assessed by bacterial counts and immunoblotting and samples collected from mice at 3 or 7 days post-inoculation. CRP concentrations in MEF and serum samples from mice were examined by ELISA. Binding of CRP to different NTHi strains and their PCho expressing variants and the biological consequences of this interaction were investigated. Results: CRP was detected at similar levels in MEF samples from Junbo mice infected with each WT NTHi strain and PCho-expressing variant tested; the only notable difference being for the lic1 mutant of strain 375. Higher but comparable levels of CRP were detected in the serum from both Junbo and WT mice infected with each NTHi strain. Control Junbo mice inoculated with PBS showed a baseline level of CRP in MEF that was significantly higher than that observed in mice inoculated with NTHi strains, suggesting that CRP may be being bound to infecting bacteria in vivo. Immunoblotting confirmed the selection of PCho expressing NTHi variants following infection in the Junbo mouse but lic1 mutants of NTHi strains that do not express PCho showed no significant difference in middle ear infection levels compared to WT strains. Conclusion: PCho expressed on NTHi LPS and its interaction with CRP is one aspect of host-microbial interaction that can contribute to NTHi survival in the normal human host and infection of the middle ear in a surrogate mouse model of OM. Many factors including host cell signalling and the presence of a plethora of other immune molecules can influence this interaction. The interaction of CRP with NTHi is currently under investigation to help advance our understanding of its role in the complex biological processes that influence bacterial killing and the onset and progression of OM caused by NTHi.

Title: NTHi Phosphocholine Induces Immune Tolerance in the Bulla Fluid of the Junbo Mouse Model of Acute Otitis Media.

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Introduction: Phosphocholine is a minor component of the lipopolysaccharide (LPS) expressed on the surface of Nontypeable Haemophilus influenza (NTHi) but bacterial variants expressing it are more efficient in causing infection. The phosphocholine on the LPS is known to play an important role in host interactions and helps bacteria evade the immune response against them through evasion of antibody binding and modulation of cytokine expression. NTHi is a major OM pathogen but the mechanisms(s) behind the immune tolerance required for sustained and chronic infection are not clear. Objective: The objective of the current study was to elucidate the T-reg mediated immune tolerance induced by phosphocholine expressed on the surface of NTHi. Method: Phosphocholine expressing variants of NTHi strains were assayed for their effect on T-reg cell induction in-vivo using the Junbo mouse, a well-characterised chronic and acute otitis media infection model, and in-vitro using mouse bone marrow derived dendritic cell (DC) culture followed by a T-cell polarisation assay. Multicolour flow-cytometry and intracellular staining were used to characterise the immune cell population. Results: The cellular immune response in the bulla fluid of the Junbo mouse infected with NTHi expressing phosphocholine showed a rise in the T-reg (CD4+CD25+FoxP3+) cell population; this rise was not observed upon infection with NTHi variants (NTHilic1 – knockout and NTHiPCho-ve – natural non-expressing variant) deficient in phosphocholine on their LPS. In vitro stimulation of mouse bone marrow derived dendritic cells (DC) by NTHi strains showed variable levels of CD86 and CD80 expression that was dependent on NTHi phosphocholine levels. The DC's stimulated with phosphocholine deficient NTHi variants significantly reduced T-reg cell polarization compared to phosphocholine expressing NTHi. Conclusion: These observations indicate a novel mechanism used by NTHi to induce immune tolerance through T-reg mediated immune suppression, which could play an important role in long term and recurrent acute otitis media. Further characterisation of cytokine/chemokine signalling is on-going to provide a more detailed insight into the signalling cross talk induced by NTHi phosphocholine; this will help our understanding of the molecular mechanism(s) involved.

Title: Non-typeable Haemophilus influenzae adhesins HMW and Hia differentially bind human airway sialic acid glycans

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Non-typeable Haemophilus influenzae (NTHi) is one of the most common causes of OM in all forms. There is currently no vaccine against NTHi. The adhesins HMW (isoforms HMW1 and HMW2) and Hia are expressed by NTHi and facilitate attachment to the human airway epithelium. Strains express either HMW1 and/or 2 (~75% of strains) or Hia (~25% of strains). Expression of both HMW and Hia is phase-variable: changes in the length of simple-sequence repeats located in the encoding genes promoter regions results in changes in expression levels of these adhesins. HMW1 has been previously demonstrated to bind a 2-3 sialyl-lactosamine, located predominantly in the upper respiratory tract, and HMW2 binds a 2-6 sialyl-lactosamine, which is found in both the upper and lower human airway, and is a receptor for other human pathogens, such as Influenzae A virus. However, the target for Hia is currently unknown. We hypothesized that host glycans also act as a receptor for Hia-mediated adherence to host cells. We examined the glycan-binding ability of Hia using glycan arrays and Surface Plasmon Resonance (SPR). Glycan array and SPR results show that Hia binds preferentially to sialyated glycans, with highest binding of both proteins to a 2-6 sialyl-lactosamine, the same recptor as HMW2.Our analysis of HMW1, HMW2, and Hia, shows that both HMW2 and Hia, but not HMW1, preferentially bind to the human form of sialic acid, N-acetylneuraminic acid (Neu5Ac; the only form expressed in humans) over N-glycolylneuraminic acid (Neu5Gc). HMW1 shows no preference for Neu5Ac over Neu5Gc. Humans are one of the few mammals unable to produce Neu5Gc; a preference for structures containing Neu5Ac over Neu5Gc for both HMW2 and Hia demonstrates that unrelated NTHi adhesins have evolved to bind human specific glycan structures with highest avidity. Both HMW1/2 and Hia are currently being investigated as vaccine candidates; a combined vaccine approach would provide 100% strain coverage. Blocking host colonization by NTHi may result in lowered carriage rates, and decrease the incidence of NTHi disease. Knowledge of the host structures bound by these key adhesins will aid in the development of a vaccine against NTHi.

Title: Cationic Nanoparticle Enhanced trans-Tympanic Membrane Drug Delivery for Noninvasive Treatment of Otitis Media

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Introduction: Otitis media (OM) is typically treated with systemic oral antimicrobial agents, which often cause therapeutic resistance/side-effects and many children with chronic OM undergo surgery, as no alternative non-invasive therapies exist. The impermeability of TM, driven by tight junctions on the epithelial layer has limited the topical delivery efficacy of therapeutics to the ME cavity. Increasing the therapeutic flux through TM is critical for success of topical treatment. We report the potential of cationic nanoparticle (CNP)-based drug carriers for local non-invasive trans-TM drug delivery of drugs. Objective:We hypothesize that CNPs applied topically in outer-ear can effectively and rapidly translocate therapeutics into the ME cavity through the TM via enhanced diffusion. We synthesized and characterized antibiotic and steroid loaded CNP formulations for acute and chronic OM and tested the delivery efficacy enhancements of these formulations through the TM using an ex vivo chinchilla model. Method:To examine the role of NP charge and size in enhancing Fick's diffusion across TM, we synthesized Ag_2S quantum dots (QD) (~8nm, charge -26mV) and Gd_2O_3 NPs doped with 1% Ne (~100nm, charge +42mV). These NPs luminesce at 1000–1300nm with 808nm excitation, thus allowing imaging based detection. 200ul NPs aqueous suspension was applied to the external auditory canal (EAC) of excised chinchilla auditory bullae maintained with PBS buffer in ME and was sampled at varying time-intervals to detect diffusion of NPs. The diffusion of NPs was quantitatively verified by ICP-MS for Gd and Ag. The ability of ~100nm CNPs to diffuse through intact TM and deliver drugs was tested with biocompatible drug delivery systems based on cationic DOTAP(1,2-dioleoyl-3-trimethylammonium-propane) liposomes, with both hydrophobic (Dexamethasone, 2mg/ml) and hydrophilic (Ceftriaxone (CFX), 6.5mg/ml) cargoes and quantified by HPLC and LC-MS assays. The antibacterial efficacy of antibiotic loaded NPs was also tested with standard bacterial killing assays. Results:The TM maintained a tight barrier with 0.05% of -ve-charge Ag_2S QD diffusing to ME cavity, whereas intriguingly ~5% or 100X higher delivery of much larger but +ve charged Gd_2O_3 particles was detectable by imaging within 15min of application and confirmed by ICP-MS. For both the steroid and antibiotic cargoes, transport of drugs to ME was detected within 15min of application compared to minimal or undetected levels in ME for equivalent free drug application. CFX-Liposomes delivered 32.5ug/ml in ME in 15min far exceeding the MIC (~1ug/ml) for SP and NTHi strains. The CFX loaded liposomes maintained antibacterial activity equivalent to free drug, when tested on NTHi bacterial cultures. Conclusion:Local non-invasive trans-TM delivery of antibiotics and steroids is enhanced by CNPs formulations and non-invasive delivery of therapeutic levels of antimicrobials is feasible with a topical ear-drop type application.

Title: Development and Characterization of Middle Ear Cell Lines for the Study of Otitis Media

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Objective: In vitro cell culture systems are essential for studying the cell and molecular biology of the middle ear (ME) and the pathogenesis of otitis media (OM). Currently, the single human ME epithelial cell line available (HMEEC-1) was established from an adult cochlear implant (CI) patient from whom the phenotypic and genotypic characteristics are unknown. Significant differences exist in innate immunity and ME effusion composition of adults and children. Further, sex and race/ethnicity are risk factors for OM, however studies have not been performed to assess the contribution of intrinsic molecular properties of ME epithelial cells to OM proneness. The objective of this study was to develop ME epithelial cell lines from adult and pediatric CI patients and patients with recurrent OM (ROM) and OM with effusion (OME) of different sex and race to provide tools for comparative analyses of molecular expression and cytokine-responsive signaling relevant to the pathophysiology of OM. Method: 2mm pinch biopsies were obtained from CI, ROM and OME patients. Blood was obtained for genetic analyses. Effusions and audiometric data were obtained from OM patients. Primary cells established from biopsy were immortalized via lentiviral infection. Cell lines were characterized by karyotype analyses and soft agar assay. Cytokine and mucin expression profile were assessed via qPCR in cells stimulated with pro-inflammatory cytokines and lysate from bacteria involved in OM. Results: Primary and immortalized cell lines were developed. Soft agar assay demonstrated normal non-tumorigenic phenotype. Preliminary analyses demonstrated differences in basal mucin gene expression between cell lines with further analyses ongoing. Conclusion: The cell lines developed herein represent the first human ME epithelial cell lines from children with clinical and histopathological confirmation of OME and ROM and the first to permit comparison of intrinsic epithelial cell-based properties across patients of varying disease severity and differing sex and race. These analyses promise to inform our understanding of not only OM, but other conditions impacting the ME and respiratory mucosa in children, and impact interpretation of research findings and their applicability across sex and races with important implications for development of preventative and therapeutic interventions.

Title: Identification and Serotyping of Streptococcus pneumoniae in the Upper Respiratory Tract of Otitis Media Patients in the Greater Milwaukee Area of Wisconsin

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Objective: Otitis media (OM) is one of the most common childhood diseases. It is the leading cause of pediatrician visits and antibiotic prescription for children and a significant burden to public health care. Streptococcus pneumoniae (Spn) is one of the primary bacterial pathogens of OM. Pneumococcal conjugated vaccines were introduced into childhood vaccination schedules in the USA in 2000, however immunization attempts have been thwarted by replacement of prevalent pneumococcal serotypes by those not included in the vaccines. Identification of the prevalent pneumococcal serotypes in the OM population will provide information crucial to understanding the epidemiology of OM and development of improved pneumococcal vaccines. Methods: A single adenoid swab was collected from each OM patient during tympanostomy tube placement surgery. DNA was extracted from swabs and pneumococcal standard serotype strains. Spn-positive specimens were identified by PCR amplification of pneumococcal 16sRNA and lytA genes, or lytA and cpsA genes. Pneumococcal serotypes in Spn-positive specimens were identified by sequential multiplex PCR using specific primers for genes encoding capsular pneumococcal serogroup/types. Results: Overall, 41.5% of specimens/cases were pneumococcal-positive. Among the positive cases, at least 14 serotypes were identified. Serotypes included in vaccines, specifically serotypes 4, 5, 6A/B, 19F and 23F, were observed in 58.8% of pneumococcal-positive cases. Serotypes uncovered by vaccines, specifically serotypes 9NL, 10F/33C, 15A/B/C/F, 22A/F, 23B, 24F/A/B, 35A/C/42 and 35B, were observed in 47.1% of pneumococcal-positive cases; none was more predominant than the others. Multiple serotypes were observed in 35.3% of pneumococcal-positive cases. Conclusions: Although pneumococcal serotypes uncovered by vaccines were detected in OM patients, vaccine-covered serotypes comprised the predominant serotypes in the upper respiratory tract of OM patients in the Greater Milwaukee Area of Wisconsin. Colonization with multiple pneumococcal serotypes was also common. Further investigation is warranted to assess pneumococcal colonization in a larger population of OM patients relative to healthy control counterparts to aid in evaluating the efficacy of vaccination.

Title: Antibiotic Modulation of Gel-Forming Mucins in Experimental Otitis Media

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Objective: Specific pathogen influence on mucin expression and development of chronic otitis media with effusion (OME) remains an area of significant knowledge deficit. This study is to assess the relationship between gel-forming mucin (GFM) expression, specific pathogens, middle ear mucosal (MEM) changes, biofilm formation and antibiotic utilization. Method: Mixed gender chinchillas were inoculated with nontypeable Haemophilus influenzae (NTHi) strain 86028NP or Streptococcus pneumoniae (SP) strain TIGR4 via transbulla injection. Antibiotics was administered on day 3 to day 5 post inoculation. Mucin expression were measured by quantitative PCR on day 6, day 10 and day 17. Gross biofilm formation was recorded at the end of time points. Inflammatory cell infiltration and middle ear mucosal thickness were histologically measured. Results: SP infection resulted in higher mortality rate, incidence of biofilm formation and middle ear effusion compared with NTHi infection. However, NTHi persisted in the middle ear longer than SP with no substantive bacterial clearance detected on day 10 in any NTHi-infected chinchillas compared with 100% clearance on day 10 for half of the SP-infected chinchillas. The infection increased middle ear mucosal thickening and inflammatory cell infiltration. NTHi upregulated the mucin transcripts, Muc5AC, Muc5B, and Muc19 on day 10 compared to controls ($p = 0.0004$, 0.003, and 0.002, respectively). With SP infection, GFMs were upregulated, however, not at statistically significant level. In both NTHi and SP infections, the degree of GFM upregulation has a direct relationship to increased MEM hypertrophy, inflammatory cell infiltration and the presence of biofilm. Antibiotic treatment reduced the incidence of middle ear effusion and biofilm formation, limited the MEM changes and reversed the GFM upregulation. In NTHi infection, the rate of returning to baseline level of GFMs in treated chinchillas was quicker than those without antibiotic treatment.
Conclusion: Mucin gene expression in an animal OM model is upregulated in conjunction with MEM hypertrophy and biofilm formation. This upregulation is less robust and more quickly ameliorated with appropriate antibiotic therapy in comparison to the animals not treated. These findings contribute to the understanding of pathogen specific influences on mucin expression during the development of OM and provide new data which may have implications in how clinicians approach the treatment of OM.

Title: Bacterial Reservoirs in Otitis-prone Children are Associated with Repeat Surgery Outcomes: A Cohort Study

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Introduction: Recurrent acute otitis media (rAOM) remains a common and challenging paediatric disease. Bacteria associated with rAOM, predominantly non-typeable Haemophilius influenzae (NTHi), are highly immune-evasive and refractory to antibiotics. Children with rAOM (otitis-prone) usually require ventilation tube insertion (VTI) surgery and/or other otorhinolaryngology surgery; and repeat operations are common. Identifying children that may require repeat surgery is important for clinical management. Objective: To identify risk factors associated with follow up otorhinolaryngology surgery and repeat VTI in children undergoing VTI surgery for rAOM. Method: For this cohort study, 186 children with a history of rAOM undergoing VTI surgery were recruited between November 2007 and May 2009. Middle ear effusion (MEE) samples were collected from 148 children during VTI surgery. Follow up clinical information was collected retrospectively in 2015 from 142 children. Demographic risk factors (age at the time of VTI surgery, gender, recent antibiotic usage, median number of AOM episodes, and day-care attendance) were assessed against follow up surgery requirements. Microbiological risk factors (viral and bacterial detection by PCR) were calculated against repeat surgery for the subset of followed-up children where MEE samples were available (116/142). Results: Children were grouped according to requirements for follow up otorhinolaryngology surgery (no repeat surgery; surgery -ve: n=52, and repeat surgery; surgery +ve: n=90). Age, gender, antibiotic usage, day-care attendance and number of AOM episodes were similar between groups ($p \geq 0.073$). Presence of a common respiratory virus was associated with follow up otorhinolaryngology surgery (76% in surgery+ve versus 58.5% in surgery-ve; $p=0.05$). PCR detection of bacterial otopathogen in MEE at the time of surgery was associated with follow up otorhinolaryngology surgery (64.4% in surgery +ve versus 39% in surgery -ve; $p=0.009$), and specifically detection of NTHi (52.1% in surgery +ve versus 29.3% in surgery -ve; $p=0.019$). Of the 90 children who had follow up otorhinolaryngology surgery, 72.2% had repeat VTI. PCR detection of otopathogen in the MEE at the time of VTI was more common children who went on to have repeat VTI than those who did not (67.3% in VTI +ve versus 44.1% in VTI -ve; $p=0.005$). More specifically, NTHi detection in MEE at the time of VTI was associated with repeat VTI (52.7% in VTI +ve versus 35.6% in VTI -ve; $p=0.044$). Conclusion: Detection of bacterial otopathogens in the middle ear at the time of VTI is a strong indicator of children at-risk of repeat surgery. NTHi was the dominant otopathogen

detected in the middle ear, indicating that NTHi-targeted treatment strategies are likely to reduce repeat otorhinolaryngology surgery in children with rAOM.

Title: Prevention of non-typeable Haemophilus influenzae colonisation and otitis media in mice by microbial interference with a closely-related commensal species

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Introduction: Nontypeable Haemophilus influenzae (NTHi) is a major otitis media (OM) pathogen, and colonisation of the nasopharynx with NTHi is a prerequisite to development of OM. Therapies that prevent NTHi colonisation may therefore prevent OM and reduce the global OM burden. Microbial interference describes the use of commensal bacterial species to compete with pathogens for binding sites, nutrients and space in order to beneficially alter the host microflora. This strategy has been shown to prevent pneumococcal OM in children (using a nasal α-Streptococci probiotic spray), to prevent meningococcal carriage in humans and experimental meningococcal meningitis in mice (using intranasal delivery of the closely related commensal Neisseria lactamica). We have previously shown that pre-treatment of human respiratory epithelium with Haemophilus haemolyticus, a closely related commensal of NTHi, can prevent NTHi colonisation and infection of the epithelial cells. We have now assessed whether the murine equivalent of H. haemolyticus (Muribacter muris) can prevent NTHi colonisation and OM using a murine NTHi OM ascension model. Objective: To determine whether intranasal pre-treatment of mice with M. muris can prevent NTHi colonisation and development of NTHi OM. Method: BALB/c mice were intranasally treated with either 5x10^7 colony-forming units (CFU) of M. muris (n=12) or saline (n=15) on Day 0. On Day 1, mice were challenged with 1x10^4.5 plaque-forming units of Influenza A virus (strain MEM, H1N3) followed by a Day 3 intranasal challenge with 5x10^7 CFU of NTHi (spectinomycin resistant strain R2866Specr). Mice were monitored daily and scored clinically. Nasal washes and middle ear bullae were collected on Day 6. Homogenised ear tissue and nasal washes were plated onto selective media for viable bacterial counts. Results: Pre-treatment of mice with M. muris reduced NTHi nasopharyngeal colonisation density from a median 4.32 Log10CFU/mL in controls to 2.20 Log10CFU/mL (p=0.0002). Twenty five percent of mice pre-treated with M. muris developed NTHi OM (3/12) compared with 53% of mice with no pre-treatment (8/15); p=0.24. Of the mice that had NTHi detected in their middle ear, the density of NTHi was lower in those pretreated with M. muris compared with controls (median 2.20 Log10CFU/mL versus 4.34 Log10CFU/mL; p=0.133. Mice that were pre-treated with M. muris also had fewer symptoms of disease compared with controls, with less weight loss (10.4% versus 13.8%; p=0.0006) and lower clinical scores (5.54 vs 7.22; p<0.00001) compared with controls. Conclusion: We have demonstrated that microbial interference can reduce NTHi colonisation

and development of OM. Pre-treatment with the commensal also protected mice from the clinical symptoms of disease, potentially lessening OM severity. Human colonisation studies on microbial interference of NTHi are warranted in attempt to provide an effective therapy to reduce the burden of OM.

Title: Methods of Investigating Bacterial DNA Load in Middle Ear Fluids

Authors: P. Webster[2], B. Osterbauer[1], M. Gunawardana[2], A. Castonguay[2], R. Knight[3], M. Baum[2], B. Taylor[3], A. Müller[4], J. Koempel[1]

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Introduction: Globally, more than 700 million cases of otitis media are diagnosed each year, with 50% of affected children being under five years of age. Monitoring of predominant bacterial pathogens is important to inform new treatment strategies, vaccine development and to monitor the impact of vaccine implementation. Most previous approaches to investigating the microbiome of the middle ear have relied on culture methods. Only recently have molecular analyses been employed to detect microorganisms in fluids from the middle ears of otitis media patients. Objective: To develop robust methods for: quantifying bacterial DNA and identifying the bacterial species found in middle ear fluids (MEF); identifying virulence genes and antibiotic resistance genes in isolated bacterial DNA from MEF. Methods: MEF, collected via a Lukens tube, was removed from patients undergoing ear tube placement as a treatment for recurrent or chronic otitis media. DNA was extracted and purified using a BiOstic® Bacteremia DNA Isolation Kit. Isolated DNA was screened for the presence of virulence and antibiotic resistance genes using a Microbial DNA qPCR array (BAID-1901Z, Qiagen, Valencia, CA). Results: The molecular methods used for MEF analysis will be described in detail. Conclusion: After researching and testing several methods available, the final methods employed accurately quantified bacterial DNA as well as capably identify virulence and antibiotic resistance genes within the isolated bacterial DNA. These methods are reproducible and will be used going forward in future studies investigating these qualities in bacteria isolated from middle ear fluids.

Title: Bacterial Virulence and Antibiotic Resistance in the Southern California otitis media population

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Introduction: Otitis media, or middle ear infection, affects more than 80% of all children (most before their third birthday) and demands more than $5 billion in healthcare costs each year. Treatment failure in children may be caused by a number of factors including antibiotic-resistant bacteria. Unfortunately, the prevalence of antibiotic-resistant bacteria typically causative of otitis media is increasing and has become a major concern for effective treatment. Previous studies have reported antibiotic resistance or reduced sensitivity of bacterial pathogens in middle ear fluids of up to 67%. This study will begin to identify the pathogenesis and related virulence factors of children with acute and chronic otitis media in the unique environment and multicultural population of Southern California. Objective: We aim to investigate the virulence and antibiotic resistance genes of bacterial DNA from middle ear fluid (MEF) in the diverse population of Greater Los Angeles. Methods: MEF, collected via a Lukens tube, was removed from patients undergoing ear tube placement as a treatment for recurrent or chronic otitis media. DNA was extracted and purified using a BiOstic® Bacteremia DNA Isolation Kit. Isolated DNA was screened for the presence of virulence and antibiotic resistance genes using a Microbial DNA qPCR array (BAID-1901Z, Qiagen, Valencia, CA). Results: A total of 145 middle ear fluid specimens were collected over a two-year period. The patients represented a diverse demographic of Hispanic (47.5%), Caucasian (24.8%), African American (6.3%), Asian (7.6%), and unidentified specimens (13.8%). Our results of bacterial resistance will be compared with previous published results. Conclusion: The results suggested a variety of resistance genes in the otitis media population of the Greater Los Angeles area.

Title: Bacterial DNA from Middle Ear Effusions of Subjects in Southern California

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Introduction: As the second most common infectious disease in children, acute otitis media accounts for more than 20 million antibiotic prescriptions per year and is the most common reason for antibiotic use in the pediatric population. The annual number of cases of otitis media in all segments of the population is estimated to be approximately 2.2 million (chronic otitis media with effusion) to 5 million (acute otitis media). Primary bacterial pathogens include Streptococcus pneumonia, Haemophilus influenza, and Moraxella catarrhalis, but the prevalence of these bacteria is variable. Previous studies on the distribution of otitis media pathogens in the USA have been performed on children from communities in temperate zones. The Los Angeles Metropolitan area, however, has a dry subtropical climate with dry summers, a winter rainy season, and modest transitions in temperature. Our unique multicultural population is not exposed to wild variations in seasonal weather and temperature extremes characteristic of other parts of the United States. However, the incidence of otitis media appears to occur at the same level as in these other areas, and during the same winter period as the rest of the country. Objective: We aim to screen for otitis media pathogens in the diverse population of Greater Los Angeles. Methods: Middle ear fluid, collected via a Lukens tube, was removed from patients undergoing ear tube placement as a treatment for recurrent acute or chronic otitis media. DNA was extracted and purified using a BiOstic® Bacteremia DNA Isolation Kit. Isolated DNA mass and DNA purity was measured using a UV 230/260/280 assay in a multiwell plate reader (Spectramax Plus, Molecular Devices, Sunnydale, CA). Bacteria load was measured by quantitative polymerase chain reaction (qPCR) analysis using a phylogenically general (16s rRNA) TaqMan probe. Results: A total of 145 middle ear fluid specimens were collected over a two-year period. The patients represented a diverse demographic of Hispanic (47.5%), Caucasian (24.8%), African American (6.3%), Asian (7.6%), and unidentified specimens (13.8%). The predominant bacterial families identified were the Proteobacteria and Firmicutes. However, the analysis revealed members of the Actinobacteria, Bacteriodetes, Cyanobacteria, Fusiobacteria, and small numbers of other families. The common otitis media pathogens, non-typeable Haemophilus influenzae and Moraxella catarrhalis belong to the Proteobacteria family, and Staphylococcus pneumoniae is a member of the Firmicutes. The distributions of bacteria within the patient population varied as a result of prior antibiotic exposure. Conclusion: The results suggest the common otitis media pathogens identified in other studies are a common cause of otitis media in the Greater Los Angeles area despite the different climate. However, other bacterial species appear to also be present in the population.

Title: Synergy between Chemical Permeation Enhancers and Drug Permeation across the Tympanic Membrane

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Objective: Ototopical drug delivery presents a promising alternative to oral therapeutics for drug administration to the middle ear. Localized delivery of therapeutics across the intact tympanic membrane (TM) and directly to the middle ear could minimize adverse systemic effects, improve patient adherence with therapy (due to reduced side effects and obviation of the need for extended treatment of often uncooperative toddlers), and therefore possibly achieve better therapeutic outcomes. However, non-invasive trans-tympanic delivery has seldom been explored until recently due to the impermeability of the TM. We have previously demonstrated that OM can be treated by the trans-tympanic delivery of ciprofloxacin (Cip) enabled by a combination of CPEs. However, the benefits of combinations of CPEs remains to be demonstrated formally, specifically whether their effects are truly synergistic, or simply additive. Synergistic interactions hold the potential to reduce the amount of CPEs needed to achieve a given effect, thus potentially also reducing toxicity. Method: We used formal pharmacological approaches, to establish whether the CPE interactions noted here are synergistic and also whether CPE combinations could be used to increase the peak effects that could be achieved. We investigate potentially synergistic effects among three CPEs delivered in a polymer matrix in enhancing trans-tympanic permeation using isobole analysis and combination indices. Sodium dodecyl sulfate (SDS), a surfactant, and limonene (LIM), a terpene, were chosen because they are both CPEs approved by the FDA for topical use. The clinically-used local anesthetic bupivacaine hydrochloride (BUP) was studied because it may reduce pain associated with OM. The effect of SDS, LIM, BUP, and their combinations on permeation enhancement was elucidated by measuring their effect on the permeability of Cip across the TMs of healthy chinchillas. Cip was selected because it is FDA-approved to be administered locally to the middle ear for the treatment of OM. Cip and the CPEs were delivered from a hydrogel reported previously, poloxamer 407-polybutylphosphoester (P407-PBP) . The hydrogel-based formulation is an easy-to-apply liquid at room temperature, and gels quickly and firmly upon contacting the warm TM, holding the antibiotic and CPEs in place (i.e. on the TM) throughout the permeability measurements. Results: We have formally demonstrated that CPEs have synergistic effects on drug flux across the TM, and that combination of CPEs can increase the maximal flux beyond what could be achieved by any concentration of a single CPE. We postulate that similar phenomena would be observed in skin, which is structurally similar, and in other settings where CPEs have been shown to be effective. Conclusion: Combinations of CPEs greatly enhanced the maximum drug flux achievable over that achieved by individual CPEs.

Title: Effects of Early Childhood Otitis Media and Ventilation Tubes on Psychosocial Wellbeing – a Prospective Cohort Study within The Danish National Birth Cohort

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Objective: Otitis Media (OM) is one of the most common infections among children in developed countries and may result in temporary conductive hearing loss (HL) if accompanied by middle ear effusion (MEE). Ventilation tube insertion (VTI) is recommended as treatment for recurrent acute OM or chronic MEE with HL. HL may lead to impaired development of psychosocial skills. However, evidence for the developmental consequences of OM and the effect of VTI is inconsistent. The objectives of this study were to investigate 1) whether OM in early childhood is associated with long-term consequences on psychosocial development and 2) if VTI prevents the possible negative consequences of OM. Method: This study examined prospectively collected data from over 50,000 children registered in the Danish National Birth Cohort (DNBC). Information about previous OM-episodes and VTI was obtained through systematic follow-up interviews at seven years, and The Strength and Difficulties Questionnaire (SDQ) containing questions about psychological wellbeing was completed. Five groups were defined based on OM-exposure and the presence of VTI. Comparison of mean SDQ-scores for the five exposure groups was conducted. Means were adjusted for à priori defined confounding factors, and baseline characteristics were analysed. Results: Data from 52,877 children in the DNBC showed an association between OM and increased SDQ-scores. VTI was associated with poorer SDQ-scores for boys, and only a slight beneficial effect on the girls' outcome. The groups differed in their baseline characteristics in e.g. maternal education, socio-economic status, breastfeeding, and prematurity. Conclusion: : Significant associations between parent-reported OM in early childhood and later psychosocial health difficulties were found. VTI was not found to be a resolving intervention for the observed negative behavioural and emotional consequences of OM.

Title: Single-Cell Transcriptomics Illuminate the Cellular and Molecular Landscape of the Normal and Infected Middle Ear

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Objective: During otitis media (OM) the expression of thousands of genes is regulated in the middle ear (ME). However, much less is known about the transcriptional contributions of individual cell types. Hence, we performed single-cell RNA-Seq of the mouse ME before and after induction of OM by ME inoculation with NTHi. Method: The 10X Chromium System was used to generate single-cell transcriptomes of more than 25,000 ME cells. PCA-generated cell clusters were classified based on marker genes, and expression of genes known to be important in innate immunity and in OM pathogenesis and recovery was assessed for each cell type. Results: In the normal murine ME, Seurat analysis of gene expression by 6,370 cells from three independent samples resulted in 17 PCA clusters. This included five epithelial cell clusters with gene expression profiles suggesting secretory, non-secretory, ciliated, intermediate and basal cell phenotypes. Three different stromal cell populations included one preferentially expressing epithelial and stromal cell growth regulators, one expressing ECM and bactericidal genes, and one expressing osteoblast genes. Other cell types included vascular and lymphatic endothelial cells, pericytes, M2 macrophages, dendritic cells, monocytes, NK-cells, different T-cell subtypes, B-cells, Type 2 innate immune lymphoid cells and melanocytes. 505 innate immune genes were differentially expressed across cell types. While all cells expressed unique innate immune profiles, macrophages, dendritic cells and monocytes expressing the highest numbers. Genes previously associated with OM were also differentially distributed across cell types, but again macrophages expressed more than did other cell types. At various time points after ME infection with NTHi, in addition to the above cell types, large numbers of neutrophils and a small number of RBCs were identified, and leukocytes represented a much higher proportion of recovered cells. Again, each cell type expressed a unique profile of immune genes. Conclusion: ME cell types known from morphological studies were found to display considerable molecular heterogeneity, and gene expression identified previously unrecognized cell types. Genes defining differences in naïve ME cells offer new insights into ME homeostasis. Differences in transcriptional responses to infection identify unexpected cell contributions to the pathogenesis and resolution of OM. For example, the only ME cells expressing beta-defensin are cytokeratin 14-positive basal epithelial cells, with no expression in surface epithelial cells. The growth suppressor gene ecrg4, often expressed in epithelia in other tissues, was expressed by stromal cells. While the cytokine Il1bwas highly expressed by leukocytes during OM, Il6 mRNA was produced only by stromal and endothelial cells. Supported by grants DC000129; DC012595 and DC014801 from the NIH/NIDCD and the Veterans Administration.

Title: Active Transport Across the Intact Tympanic Membrane is Mediated by Transcytosis

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Local delivery of antibiotics has been found to be effective in treating OM, but requires surgically breaching the tympanic membrane (TM). We previously discovered a family of peptides expressed on bacteriophage that are actively transported across the intact tympanic membrane (TM), providing a potential mechanism for noninvasive drug delivery. However, the mechanism by which this transport occurs is not known. We therefore analyzed these peptides to identify features associated with transport. Common peptide features included a central lysine residue, isoelectric point of 0.0 at physiologic pH and a hydrophobic C-terminus. Two conserved amino acid motifs were present in the peptides: SxDSTK and PxxP. 3-D structural analysis showed that peptides exhibiting the highest transport rates presented these motifs at the free end of the M-13 PIII N-terminus. Those in which the motifs were predicted to be closer to the phage pIII protein exhibited lower rates. These structural analyses strongly implicate the presentation of the conserved motifs, and especially SxDSTK, in determining peptide transport characteristics. Reasoning that the conserved motifs might point to the mechanism by which peptides transit the TM, we used the Motif Alignment and Search Tool (MAST) to search for proteins with related motifs. The highest predicted alignments were for exocyst complex component 1, which mediates docking of exocytic vesicles with fusion sites on the plasma membrane,and importin subunit alpha 6. The importin complex is involved in transport of protein across intracellular compartments and is capable of bidirectional transport. These identifications implicate transcytosis, by which cargoes are transported across polarized cells, as the transport mechanism. As transcytosis is initiated by endocytosis, we applied wortmannin, a potent inhibitor of receptor-mediated endocytosis, to the TM for 1 hour prior to applying a TM-transiting phage peptide. The inhibitor decreased phage recovery from the ME by >90%. Wortmannin is also know to inhibit other aspects of the endocytotic pathway, including vesicle fusion and perinuclear transport, and has been shown to inhibit both apical-to-basolateral and basolateral-apical transcytosis in polarized epithelial cells. The data strongly support transcytosis as the mechanism by which trans-TM peptides more actively across the membrane, initiated by binding to a cell surface receptor with transition into the endocytotic pathway. Supported by grants DC000129; DC012595 and DC014801 from the NIH/NIDCD and the Veterans Administration.

Title: Lack of the Hyaluronan Receptor CD44 Affects the Course of Bacterial Otitis Media and Reduces Leukocyte Recruitment to the Middle Ear

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OBJECTIVE: CD44 is a multifunctional molecule that plays major roles in both leukocyte recruitment and tissue proliferation. Since mucosal hyperplasia and leukocyte infiltration of the middle ear (ME) cavity are major features of otitis media (OM), we evaluated the role of CD44 in the pathophysiology and course of this disease in a mouse model of ME infection. METHODS: To assess the prevalence and distribution of CD44 in the ME before and during OM, the expression of Cd44 and genes related to its function was evaluated using gene arrays in wild-type mice, and in single-cell transcriptomes from normal and infected wild-type MEs. To evaluate the functional role of CD44 in OM, the MEs of mice genetically deficient in Cd44 were inoculated with non-typeable Haemophilus influenzae(NTHi). Histopathology and bacterial clearance were compared to those seen in wild-type controls. RESULTS: We observed strong up-regulation of CD44 and of genes related to its role in leukocyte extravasation into the ME, during the course of acute OM. Mice deficient in Cd44exhibited reduced early mucosal hyperplasia and leukocyte recruitment, followed by delayed resolution of infection and persistent inflammation. CONCLUSIONS: CD44 plays an important role in both pathogenesis and defense of the ME during OM. Targeted therapies based on CD44 could be useful adjuncts to the treatment of ME infections. Supported by grants DC000129; DC012595 and DC014801 from the NIH/NIDCD and the Veterans Administration.

Title: The impact of pneumococcal vaccine schedules on nasopharyngeal microbiology of Indigenous infants aged 12 months

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Introduction: Indigenous infants living in the remote north of Australia are at high risk of otitis media (OM). Participants were randomised (1:1:1) at 28-38 days of age to either Synflorix (PHiD-CV10) or Prevenar13 (PCV13) at 2, 4 and 6 months of age or an early start combination schedule of PHiD-CV10 at 1, 2 and 4 months followed by PCV13 at 6 months. Objective: To determine whether the combination schedule provides superior protection against vaccine type Streptococcus pneumoniae (Spn) compared to PHiD-CV10 alone, and superior protection against non-typeable Haemophilus influenzae (NTHi) compared to PCV13 alone, post primary schedule at 12 months of age. Method: Nasopharyngeal (Np) swabs, and Ear discharge (ED) swabs (if present), were collected at 12 months of age into STGGB, then transported, stored and cultured using standard methods. Antimicrobial resistance profiles for Spn, NTHi, Moraxella catarrhalis (Mc) and Staphylococcus aureus (Sa) were determined, and Spn isolates were serotyped using the Quellung method. Results: 259 Np swabs from 261 enrolled infants were collected. From 212 swabs processed to date, 74%, 58%, 81% and 5% were positive for Spn, NTHi, Mc and Sa respectively. Of the cultured Spn, 11% were PCV13 vaccine types (VT), predominantly 19A (4%) and 19F(3%). 37 ED swabs were collected from 28 infants in the cohort. Of the 32 ED swabs cultured to date, 16%, 52%, 3% and 19% were culture-positive for Spn, NTHi, Mc and Sa respectively. One child had a VT-Spn-positive ED. There were no statistically significant differences in VT-Spn or NTHi in the NP or ED by vaccine group, when comparing PCV13 with the combination group for NTHi (Np: 36/71 (50%) v 43/68 (74.1%), p=0.171; ED: 6/12 (50%) v 1/6 (16.7%), p=0.173) and comparing PHiD-CV10 with the combination group for VT-Spn (Np: 10/73 (13.7%) v 10/68 (14.7%), p=1.000; ED: 0/11 (0%) v 1/9 (11.1%), p=0.450) Conclusion: These preliminary data suggest that the use of a combined primary series of vaccination with PHiD-CV10 at 1, 2, and 4 months followed by PCV13 at 6 months of age compared to a primary series with either PHiD-CV10 or PCV13 did not result in reduced NP carriage with NTHi or Spn. These results should be cautiously interpreted due to the small number of swabs available for analysis.

Title: Otitis Media Hospitalisation in the Northern Territory of Australia: A Decade of Observation (2006-2015)

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Objective: In the Northern Territory (NT) of Australia rates of childhood pneumonia (22% hospitalised in first year), otitis media (OM; 90% at age 18 months) and chronic suppurative lung disease (CSLD; 1 in 68 in Central Australia) are among the world's highest. Streptococcus pneumoniae and Haemophilus influenzae are leading aetiological pathogens. We describe childhood OM coded hospitalisations in the NT over a 10-year period. Method: A historical cohort study of NT Indigenous infants born between 1st Jan 2006 – 31st Dec 2015 and followed until age 12 months. Three pneumococcal conjugate vaccines were used in the NT over this period: PCV7 (2006 – 2009); PCV10 (2009 – 2011); and PCV13 (2011 – 2015). Otitis media coded hospitalisations (ICD-10AM H65-H67.8 and H72-H72.9) were identified from the NT Government Hospital Inpatient Activity dataset (ICD-10AM codes: Z38-Z38.8). OM hospitalisation rates (episodes per 100 child-years) were calculated overall, annually, and by PCV era. Results: The 14,594 Indigenous children born during the study period presented for a total of 13,307 subsequent hospitalisation episodes in their first year of life. Fifty one percent (n=7490) were male, 70% (n=10,175) lived in remote communities and 32% (n=4675) were from the Central desert region. Of the 13,307 episodes, 957 (7%) were coded episodes of OM for 825 infants (6%); 79 (0.6%) had two episodes and 20 (0.15%) had three or more episodes. On 650 occasions (4% overall, 68% of OM episodes) OM was a leading diagnosis (coded 1st, 2nd or 3rd). The frequency of first OM coded hospitalisation peaked at 5 months and remained stable until 12 months. By region, the proportions of children with an OM coded hospitalisation were: remote Central Australia (10%), urban Central Australia (5.5%), remote Top End (5.5%), urban Top End (1.5%). There was no significant trend over calendar time. Over 80% of infants received three or more timely PCV doses before age 12 months and this was consistent over time. Compared to the PCV7 era (7.8 episodes per 100 child-years), OM rates were marginally yet significantly lower in both the PCV10 (6.5 episodes per 100 child-years; IRR 0.84, 95%CI 0.70 to 0.99) and PCV13 eras (6.5 episodes per 100 child-years; IRR 0.83, 95%CI 0.72 to 0.96). These differences were no longer significant when analysis was confined to episodes where OM was a leading diagnosis. Conclusion: One in 20 Indigenous infants in the NT of Australia were hospitalised and coded with a diagnosis of OM annually. These rates remained largely unchanged though the decade 2006-2015. While the majority (70%) of OM coded episodes were leading diagnoses, further investigation of comorbidities is necessary to clarify the clinical significance of the OM. Lower OM coded hospitalisation rates during eras of PCV10 and PCV13 vaccination, compared to the PCV7 era, might suggest a vaccine effect.

Title: Respiratory Pathogens are among the Dominant Taxa in Nasopharyngeal Microbiota of One-month Old Indigenous Australian Infants

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Objective: Nasopharyngeal (NP) pathogen carriage is a known otitis media (OM) risk factor, whereas dominance of the NP microbiota by Corynebacterium sp. and Dolosigranulum pigrum is potentially protective against OM. No studies have examined NP microbiota in Indigenous Australian children; a population at high-risk of early-onset, persistent and severe OM. The aim of this study was to compare the NP microbiota (including analysis of potentially protective commensal taxa) in infants with or without early-onset OM. Method: NP swabs were collected from Indigenous Australian infants aged 28-38 days. OM (any acute otitis media or otitis media with effusion) was diagnosed by research nurses using tympanometry. Swabs were cultured using standard methods. Total and species-specific bacterial loads were determined by qPCR. Sequencing of the 16S rRNA gene V4 region was done with kitome exclusion included in the analytic pipeline. Results: 111 infant NP swabs were included in the analysis. OM was diagnosed in 33% infants. Total bacterial load was similar in infants with or without OM (Wilcoxon p=0.2). After kitome exclusion, swabs from 87 infants had sufficient reads for microbiota analysis; 31% of these infants had OM. Swabs from infants with and without OM had similar alpha (Wilcoxon p>0.9) and beta diversity (PERMANOVA p=0.16). Thus, bacterial density, community structure and overall NP microbiota composition were not different in children with or without early-onset OM. The microbiota of 86/87 swabs clustered into six profiles which all included combinations of pathogenic and commensal taxa. Streptococcus pneumoniae (Spn), Haemophilus influenzae (Hi), Moraxella catarrhalis (Mc), and Staphylococcus aureus (Sa) were detected by PCR and/or culture in 50%, 48%, 48% and 49% of swabs, respectively, with 89% of infants colonised by at least one of these species. Co-colonisation by multiple pathogens was detected in 31% of infants with 5% positive for Spn and Hi; 11% positive for Spn, Hi and Mc; 6% positive for Spn, Hi and Sa; and 9% positive for Spn, Hi, Mc and Sa. Pathogen carriage rates (either alone or in combination) were similar in infants with and without OM (all chi-square adj-p>0.2). Analysis of potentially protective taxa showed lower D. pigrum relative abundance in infants with OM (median 0.05%, IQR 0-3%) compared to those with no OM (median 4%, IQR 0.2-9%; Wilcoxon p=0.02), whereas Corynebacterium relative abundance was similar in both groups (median 46% and 44%, respectively; Wilcoxon p=0.8). Conclusion: Pathogens were among the dominant taxa in most NP swabs from this cohort of one-month old infants with or without OM. Commensal taxa were also present in all swabs, but at lower relative abundance than studies from other populations. Development of microbiota modifying agents (e.g. probiotics) for preventing pathogen colonisation and subsequent OM in high-risk populations will require formulations suitable for use during the neonatal period.

Title: Acute otitis media in infants younger than two months of age: epidemiologic and microbiologic characteristics in the era of pneumococcal conjugate vaccines

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Objectives: To evaluate the epidemiology, microbiology, Streptococcus pneumoniae (SP) serotypes distribution and serious bacterial infections (SBIs) occurrence in infants <2 months of age with tympanocentesis-documented acute otitis media (AOM), before and after the introduction of pneumococcal conjugate vaccines (PCVs). Methods: The medical records of all hospitalized infants with AOM who underwent tympanocentesis during 2005-2014 were reviewed. Results: Of the 182 infants with AOM, 92 were diagnosed during 2005-2009 (prevaccine period) and 90 during 2010-2014 (postvaccine period). No changes were recorded in the number of AOM cases requiring tympanocentesis during 2005-2009 vs. 2010-2014 (92/12,619 patients, 0.007% vs. 90/13,563, 0.006%, P=0.57; the number of cases requiring tympanocentesis reached their lowest during 2013-3014. SP and nontypeable H. influenzae (NTHI) were isolated in 46/92 (50%) and 37/92 (40.2%) patients during 2005-2009 and decreased to 27/90 (30%) and 21/90 (23.3%). respectively, during 2010-2014 (P=0.006 and P=0.001). The number of culture-negative patients increased from 18/92 (19.6%) during 2005-2009 to 32/90 (35.6%) during 2010-2014 (P=0.02). There were only 6 (3.3%) patients <2 weeks of age. The most common SP vaccine serotypes isolated during 2005-2009 were 5, 3, 1, 19F and 14 (15.2%, 13.0%, 10.9%, 6.5%, and 4.3%, respectively) and 3, 5, 1, 14 and 19A (22.2%, 11.1%, 7.4%, 7.4%, and 7.4%, respectively) during 2010-2014. No changes were recorded in the percentages of vaccine serotypes (58.7% vs. 63%, P=0.7) and non-vaccine serotypes (41.3% vs. 37%, P=0.7) between 2005-2009 and 2010-2014. SBIs were recorded in 23/182 (12.64%) patients and urinary tract infections represented 19/23 (82.61%) of them (Escherichia coli isolated in 12, 63.2%). Conclusions: The overall number of AOM cases and of SP and NTHI-AOM decreased while culture-negative-AOM increased following the introduction of PCVs. SBIs associated with AOM were frequent and were represented mostly by urinary tract infections caused by pathogens unrelated to the etiologic agents of AOM.

Title: Regulation of innate immunity and inflammation and new therapeutic targets in otitis media

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Objective: Uncontrolled innate immunity and inflammation is a hallmark of otitis media (OM). Although appropriate innate immune and inflammatory response is critical for eradicating pathogens, dysregulated host immune response often results in middle ear immunopathology and hearing loss. Thus, inflammation and innate immune response must be tightly controlled. However, the underlying mechanisms remain largely unknown. There is currently an urgent need for understanding the molecular mechanisms underlying tight regulation of inflammation and innate immune response in OM pathogenesis and identifying novel therapeutic targets. Among various mechanisms underlying tight regulation of inflammation and innate immunity, both positive and negative regulators are thought to play a critical role in controlling dysregulated host immune response. Method: We used multiple experimental approaches including pharmacological, biochemical, immunological and genetic approaches. Results: Toll-like receptor (TLR)-dependent IRAK-TRAF-MAPK ERK, p38, NF-kB and phosphodiesterase 4 (PDE4) have been identified as the positive signalling pathways for mediating innate immune and inflammatory responses induced by nontypeable Haemophilus influenzae (NTHi), a major bacterial pathogen for OM. In contrast, deubiquitinase CYLD, interleukin-1 receptor-associated kinase M (IRAK-M), and mitogen-activated protein kinase (MAPK) phosphatase-1(MKP-1) have been demonstrated to act as negative regulators. Furthermore, the therapeutic potential for targeting the identified pathways has also been explored. Importantly, non-invasive ototopical delivery of the pharmacologic agents which either suppress the positive pathways or up-regulate the negative pathways have been shown to be a promising therapeutic strategy for the management of OM. Conclusion: Together, these studies bring new insights into our understanding on tight regulation of inflammation and innate immune response and may help identify novel therapeutic targets for treating otitis media.

Title: Otopathogenic Pseudomonas aeruginosa Induces Otitis Media and Inner Ear Damage in an Experimental Mouse Model

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Introduction: Otitis media (OM) is the most common middle ear disease especially affecting children. Although the pathogenesis of OM caused by most common pathogens namely, Streptococcus pneumoniae, Non-typeable Haemophilus influenzae (NTHi) and Moraxella catarrhalis is well characterized, the molecular mechanisms underlying middle ear infection induced by microbes such as Pseudomonas aeruginosa are not known. This lack of understanding about pathogenetic events can be attributed to the lack of a good animal model. Objective: The aim of this study was to establish a mouse model of P. aeruginosa induced OM and characterize the middle ear inflammatory responses as well their impact on inner ear. Methods: Otopathogenic P. aeruginosa was inoculated into the middle ears of mice through transtympanic administration. Animals that received phosphate buffered saline (PBS) as well as naïve animals served as the control groups. Mouse middle ears were harvested at different post-infection time periods to determine bacterial load, recruitment of inflammatory cells, cytokine production and fluid accumulation. Infected animals were also subjected to auditory brainstem recordings (ABRs) to determine hearing thresholds. Inner ears harvested from infected mice were subjected to confocal microscopy and scanning electron microscopy to evaluate the effects of middle ear infection on inner ear. Results: We observed that otopathogenic P. aeruginosa can successfully colonize the middle ear of normal mice and induce characteristic features of OM without any surgical manipulation. We also observed that P. aeruginosa middle ear infection damages sensory cells in the inner ear leading to hearing loss in our mouse model. Conclusions: The results of our study suggests that otopathogenic P. aeruginosa can induce OM in a normal mouse ear. The availability of P. aeruginosa induced OM mouse model established in this study will open up avenues to understand the molecular mechanisms involved in the pathogenesis of disease as well as to develop novel treatment modalities.

Title: Acute Otitis Media Diagnosis and Management Guidelines: How Can We Explain Worldwide Differences?

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Objective: In order to reduce acute otitis media (AOM) burden and limit unnecessary antibiotic use, guidelines and consensus statements have been published in many countries over the past decades. We review the differences between AOM guidelines worldwide and attempt to track the origins of these dissimilarities Method: The key-words: 'acute otitis media' AND 'children' AND ['treatment' or 'management'] AND ['guideline' or 'consensus'] were used in various electronic databases between 1/1/1989 through 9/30/2018. Overall, 99 sources from 64 countries were retrieved. According to the United Nations Annex definition, 60 were from 26 developed countries, and 39 were from 38 developing countries. We performed a qualitative review for the following key points: diagnosis, treatment decision algorithm, antibiotic treatment options, role of complementary treatment, treatment failure, salvage and preventive strategies. Results: Tympanic membrane bulging, opacity and presence of middle ear fluid are essential for diagnosis, as marginal/uncertain cases are not accepted. Guidelines from developed countries offer the use of pneumatic otoscopy and tympanometry to aid diagnosis. Withholding antibiotic therapy and a 'watchful waiting' (WW) approach in mild-moderate cases are preferred in settings where follow-up visits are both possible and attainable, mostly in developed countries. While amoxicillin is mostly accepted as the 1st-line antibiotic therapy, options for 2nd- and 3rd-line antibiotics vary, according to local bacteriology and antimicrobial susceptibility data and costs. Other treatments, such as complementary and alternative medicine, steroids or anti-histamines, are either rejected or ignored. Reduction of known risk factors and call for vaccinations (influenza, pneumococcal conjugate vaccines) are encouraged mostly in developed countries, where such immunizations have been implemented in National Immunization Programs. Conclusion: Despite regional differences, AOM guidelines worldwide share common grounds on various matters concerning diagnosis and management: diagnosis based on TM findings using otoscopy and/or pneumatic otoscopy in conjunction with tympanometry, WW approach in appropriate cases, oral analgesic treatment using ibuprofen/paracetamol, reduction of risk factors and preventive measures to reduce AOM burden.

Title: The Yield of Respiratory Viruses Detection Testing is Age-Dependent in Children with Uncomplicated Acute Otitis Media

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Introduction: Acute otitis media (AOM) pathophysiology presumes bacterial super-infection complicating an anteceding nasopharyngeal viral infection that ascends to the middle ear. Studies of nasopharyngeal secretions serve as reliable surrogate to evaluate the contribution of viruses to AOM and upper/lower respiratory tract infections (URIs/LRIs). Objectives: We sought to study the results of NP respiratory viral panel tests obtained from children with uncomplicated AOM hospitalized during the URI/LRI seasons in 6 consecutive calendrical years which had seen a shift from traditional antigen detection assays to a PCR-based multiplex assay. A secondary objective was to study the yield of these tests in relation to children's age, in order to identify which age group would benefit the most at the greatest cost effectiveness Methods: We identified children aged 0-6 years admitted to our Pediatrics department in a university-affiliated, secondary hospital with uncomplicated AOM and concurrent URI/LRI during October-April between 2012-2017, when viral studies are performed. Viral studies were performed either using antigen detection tests, for respiratory syncytial virus (RSV) and influenza A/B (2012-2016) and for various common respiratory viruses, utilizing multiplex polymerase chain reaction assays (2017). Results: 249 children with a median age of 15 months (IQR 7-22) were included. There were 155 (62%) males. In 88 (35%) cases, viral studies were positive, most of them in children <24 months (78, 89%). RSV was positive in 52 (59%) cases, followed by influenza A, 11 (13%) and B, 5 (6%). RSV co-infection was documented in 10 (11%) cases. Children ≤12 months were the age group with most positive test results (43, 49%) (p=0.004), and had statistically significant more positive RSV results (31, 60%) when compared with older children (p=0.038). There were no statistically significant differences in the positive viral results between those who were treated with antibiotics before admission and those who were not (p=0.318). Conclusion: The yield of nasopharyngeal viral studies in uncomplicated AOM is limited to children <24 months, with the greatest benefit in infants aged ≤12 months.

Title: Do Affordable Bone Conduction Headphones Help Children with Glue Ear to Hear Speech more easily? What do Children Think about Wearing these Headsets?

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Objective: To assess the effect of using a BC headset paired with a remote microphone on speech recognition thresholds of children with Otitis Media with effusion (OME) during the watchful waiting period of their usual NHS (National Health Service) care. To assess what children thought of the bone conduction headset in everyday settings and whether they used a BC headset at home during a 6 month period, during their usual NHS care, often waiting for a grommet operation. Method: 20 children aged 3-6 years with bilateral OME, flat tympanograms and hearing worse than 20 dB HL averaged over 3 frequencies, were studied. Speech recognition thresholds (SRT's) were measured in a quiet room and with 65dB(A) speech-shaped background noise, with and without the headset. 10 children were given the BC headset to take home for 6 months, and 10 children were given listening games to play on a phone/ ipad/ tablet for 6 months. Families completed questionnaires at the start and end of the 6 month period, including a wearability/ usability questionnaire for families who had been using the headset. Results: There was a significant effect of using the headset: The median SRT in quiet (N=17) was 50 dB(A) (range: 34-68) without the headset and 32 dB(A) (range: 18-44) with the headset, and this difference was statistically significant (T=0, $p<0.001$). The SRT in noise (N=19) was 68 dB(A) (range: 59-70) without the headset and 54 dB(A) (range: 41-60) with the headset, and the difference was statistically significant (T=0, $p<0.001$). Questionnaires indicated the BC headsets were acceptable to children. Conclusion: Use of a BC headset paired with a remote microphone significantly improved speech recognition in quiet and in noise for children with OME. The BC headsets were acceptable to children, who wore them in a wide variety of settings. 9 of the families asked to keep the BC headset at the end of the study. Perhaps this type of affordable bone conduction headset, on the market for listening to music and mobile phones, be used during the watchful waiting period to support a child's speech, language, listening skills, auditory processing, social communication, behaviour, quality of life and access to education, while they wait for a grommet operation or resolution of their glue ear.

Title: Sequential Episodes of Experimental Otitis Media Promote Pathogenic Lifestyles Through Microevolution of Nontypeable Haemophilus influenzae

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Introduction: Nontypeable Haemophilus influenzae (NTHI) is a causative agent of both chronic and recurrent otitis media (OM). During chronic infection, bacterial adaptation promotes fitness and facilitates long term survival within the host. Objective: We used a preclinical chinchilla model of OM to determine the potential role for microevolution in the adaptation of NTHI during sequential episodes of the disease. Methods: OM was introduced into three chinchillas. Seven days following inoculation, NTHI were recovered from middle ear effusions and mucosal-associated biofilm. These two bacterial populations were used to infect each of three naïve chinchillas. After a 7-day infection, bacteria were again recovered and used to infect a third and a subsequent fourth cohort. Results: Between the first and the last cohorts we observed a significant increase in biofilm development and in the bacteria recovered from biofilms during the first week of infection, suggesting in vivo strain adaptation by passage through prior animals. We also observed a significant increase in the number of intracellular bacterial communities (IBCs) of NTHI within middle ear mucosae, as well as increased fibrosis and inflammation. The genomes of the bacteria collected from the fourth cohort were sequenced and, compared to the parent strain, mutations were identified in 14 genes. These mutations were categorized into six classes based on mutation type and the locus where the mutation occurred: transcriptional phase variation, translational phase variation, intragenic single nucleotide polymorphisms (SNPs), intragenic SNPs, promoter region SNPs, or deletions. Moreover, in three genes multiple separate mutations were observed in a single locus. Confirmatory sequencing showed a subset of these mutations arose within the first 7 days of infection. Conclusions: These data provide the first demonstration of microevolution during experimental OM, with mutations arising along with increased bacterial growth, more severe markers of disease and greater biofilm and IBC formation over time. A deeper understanding of microevolution of NTHI during disease will give insight into how IBCs develop and the bacteria survive for protracted periods of time within the host. Understanding how strains of NTHI enter this chronic lifestyle will ultimately reveal targets for therapeutic modalities in the treatment of OM.

Title: Diverse Paths to Serum Resistance in Nontypeable Haemophilus influenzae Isolated from Children with Otitis Media

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Introduction: Nontypeable Haemophilus influenzae (NTHi) is a human-restricted opportunistic pathogen that causes considerable morbidity, particularly in otitis media (OM) infections. Like many bacteria, NTHi is highly diverse, including variation among isolates' serum resistance (SR) levels. Although several SR mechanisms have been identified in model NTHi strains (including phase-variable genes), how these mechanisms are distributed across NTHi and what role SR plays in OM infections has not been rigorously assessed, despite the known presence of complement in the middle ear. Objective: Using a well-curated collection of 212 clinical NTHi strains isolated longitudinally from healthy or otitis media prone-children, we set out to: 1) survey SR across the collection to test for correlations with NTHi clonal type and subject health status; 2) identify loci and alleles responsible for variability in SR; and 3) measure the response to selection for SR among diverse serum sensitive-strains. Methods: 1) We implemented a novel high-throughput assay to quantify SR across our collection and applied multivariate regression analysis to test any associations with clinical metadata. 2) We collected whole genome assemblies for each strain for the performance of genome-wide associations studies to identify SR genes within and among NTHi lineages. 3) We subjected phylogenetically diverse sensitive-strains to serial serum selection, and used genomic sequencing to identify differences between sensitive parental strains and newly evolved SR strains. Results: As expected, we found broad phenotypic variation among strains collected from both cohorts of children. By using selectively compromised serum, we discovered that diverse sensitive-strains were differentially susceptible to the distinct branches of complement. Testing for associations between NTHi SR, clinical provenance, and bacterial genomic variation are ongoing; preliminary results suggest rapid switches in the SR phenotype, even within closely related strains infecting the same subject and only sometimes explainable by switching of known phase-variable genes. Remarkably, after serial serum exposure, diverse sensitive strains (n=18) rapidly increased in SR by ~400-fold on average, despite their phylogenetic diversity and distinct pathway sensitivity profiles. Genomic analyses to identify the causative mutations is ongoing. Conclusion: These results underscore the rapid and dynamic changes within NTHi strains during carriage and disease; they indicate fluctuating selective pressures acting on the SR trait. This indicates unknown fitness costs associated with SR-associated surface structures (particularly lipooligosaccharide moieties). Our new

bacterial genomic and phenotypic resource will elucidate the ‘genetic architecture’ of the highly variable SR phenotype across a large diverse collection of clinical strains, which may inform translational endeavors, particularly in NTHi vaccine development.

Title: Nasal microbiota profiles and chronic otitis media with effusion

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Objective: To determine the relationship between nasal microbial composition and risk of chronic otitis media with effusion (COME) in preschool children. Method: A case-control study of 178 preschool children aged 3 and 4 years was conducted. The cases were undergoing placement of tympanostomy tubes for COME. The controls were healthy children from primary care practices. Nasal swabs were collected and a structured questionnaire was administered. The microbial community of the anterior nares was assessed by amplifying the V1-3 region of the 16S rRNA gene and sequenced on the Illumina MiSeq platform. Results: Children with COME had a lower Shannon diversity index than healthy controls (1.62 [.80] versus 1.88 [.84], respectively; P=.046). The nasal microbiota of cases and controls differed in composition using Bray-Curtis dissimilarity (p=.002). Children with COME had a higher abundance of otopathogens and lower abundance of commensals including alpha haemolytic Streptococci and Lactococcus. However, the proportion of controls in which any reads of otopathogens were detected did not differ from the proportion of cases (P≥.05). Cluster analysis revealed 4 distinct nasal microbial profiles. Profiles that were Corynebacterium-dominated (aOR 4.18 [95%CI, 1.68-10.39], Streptococcus-dominated (aOR 3.12 [95%CI, 1.08-9.06], or Moraxella-dominated (aOR 4.70 [95%CI, 1.73-12.80] were associated with COME, compared to a more mixed microbial profile when controlling for age, ethnicity, and recent antibiotics use. Conclusion: Children with COME have a less diverse nasal microbial composition with a higher abundance of pathogens, compared to healthy children who have a more mixed bacterial profile with a higher abundance of commensals. The presence of otopathogens in the nasal passages of controls may indicate that they are respiratory pathobionts, remaining asymptomatic until activated into overgrowth and dispersion by stimuli such as viral upper respiratory infection. Further research is required to determine how nasal microbial composition may be involved in the pathogenesis or maintenance of COME, and whether modification of the nasal microbiota is an effective prophylactic or treatment for children at risk of COME.

Title: Otitis Media App: Using the Grading Recommendations and Assessment and Development and Evaluation (GRADE) Approach for a Smartphone App Guideline in the Prevention and Management of Otitis Media among Australian Indigenous Children.

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Introduction: Approximately 10% of remote Aboriginal and Torres Strait Islander (Indigenous) children younger than 3 years of age have healthy ears. Indigenous Health services across the country struggle to deliver diagnostic and management services and are challenged by high staff turnover. User-friendly mobile phone health care apps with evidence-based information that is suitable and accessible for all potential users are needed. Objective: An OMapp may assist in improving diagnosis and management of ear disease and conductive hearing loss in Indigenous children with a high disease burden. Methods: In 2010, The Recommendations for Clinical Care Guidelines on the Management of Otitis Media in Aboriginal and Torres Strait Islander Populations was published. In 2016, the National Health and Medical Research Council Centre of Research Excellence for Indigenous Children's Health Ears (CRE_ICHEAR) appointed a Technical Advisory Group to update this guideline following the GRADE approach. In 2018, the guideline was incorporated into an OMapp. Results: (a) Key Features of the OMapp: The OMapp is a multi-platform app available free of charge with downloadable content for off-line use. It has four basic windows: (i) Clinical (Diagnosis and Management): diagnostic, prevention and treatment algorithms for all types of OM; (ii) Communication tools: audio recordings in multiple Aboriginal languages to assist caregiver understanding of their child's ear health and hearing needs; (iii) Educational resources: for professionals, families and children including videos of pneumatic otoscopy, audiograms and tympanograms, hearing loss simulations, and cartoons to explain the ear and hearing health service pathways; and (iv) Guidelines: evidence summaries for all strategies and recommendations for prevention and treatment with links to GRADEpro Summary of Findings tables, strength of recommendations,

quality, effect size and a simple Population Intervention Comparison Outcome Time (PICOT) statement for each intervention and for multiple outcomes. (b) Pros and cons: OMapp is fully downloadable for access offline. The user-friendly windows and audio-visual features are intended to suit a diversity of users. Translation into Indigenous languages will enhance comprehension and adherence to recommended strategies for OM and hearing loss prevention and treatment. Updates necessarily demand internet connection, which is not universal across Australia. Conclusion: The potential impact of the OMapp in reducing the burden of OM among Australian Indigenous children should be rigorously evaluated. Future capability for maintaining the OMapp evidence base in real-time will be an ongoing challenge.

Title: Adjunctive Treatment with Oral Cotrimoxazole Improves Chronic Suppurative Otitis Media in Australian Aboriginal Children: Results from a Randomised Controlled Trial

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Introduction: The prevalence of chronic suppurative otitis media (CSOM) among children living in remote Australian Aboriginal communities remains high. Clinical failure (unable to achieve dry ears) can affect over 70% of children even when using recommended treatment. The role of adjunctive therapies (in addition to cleaning and topical antibiotics) is unknown. Objective: To determine whether twice daily adjunctive treatment with: A) antiseptic ear wash (>= 20mls povidone-iodine 0.5% solution syringed into the ear canal) or no ear wash; or B) oral antibiotics (cotrimoxazole 4mg/kg per dose of trimethoprim component) or placebo (in addition to standard treatment-twice daily ear cleaning and ciprofloxacin drops) reduced the proportion of children with discharging perforations after 16 weeks of treatment. Methods: The Indigenous Healthy EARs using BEtadine, Tissues and Antibiotics (IHEARBETA) study was a parallel group, factorial design, randomised (allocation concealed), assessor blinded, controlled clinical trial (ACTRN12614000234617). Children aged 6 months to 17 years with a clinical diagnosis of CSOM were eligible to participate. Clinical and microbiological assessments were completed at 16 weeks and 12 months. The primary outcome was clinical failure (presence of any ear discharge) after 16 weeks of treatment. A Chi-squared test and a binomial regression with a logarithmic link were used to estimate the main effects of the two interventions with adjustment for the other intervention and stratification variables. Results: Of the 1732 children screened in 28 communities, 355 (20%) had discharging perforations and 280 (16%) were randomised. Mean age at randomisation was 7 years. Across all groups, 50% of children in the study had dry ears after 16 weeks of treatment. Clinical failure occurred in 66 (49.3%) in the betadine group versus 69 (50.7%) in the no betadine group (adjusted risk difference (RD)= -0.5% (-12,11), p= 0.88) and 56 (41.8%) in the cotrimoxazole group versus 79 (58.1%) in the placebo group (adjusted RD=-16% (-28,-4), p=0.015). Conclusion: These results confirm that adjunctive treatment with oral cotrimoxazole (but not dilute povidine-iodine washes) improves the short term outcomes for children with CSOM. This is an important addition to the treatment options available to children with CSOM. The microbial data and 12 month clinical outcomes will be available in 2019. This will tell us more about the mechanism of action and whether the benefits persist long term.

Title: Hearing Outcomes at 1 to 3 Years of Age for Aboriginal Children Living in Remote Communities Allocated to Combination Schedules of Pneumococcal Conjugate Vaccines

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Aboriginal children living in remote communities of the Northern Territory in are at high risk of early and persistent otitis media, which causes disabling hearing loss and social disadvantage. Objective: To measure conductive hearing loss (CHL) in young Aboriginal children at high risk of otitis media. Methods: Aboriginal infants 28 to 38 days of age were allocated (1:1:1) to either Prevenar13™ (P, PCV13) at 2-4-6 months of age (_PPP), Synflorix™ (S, PHiD-CV10) at 2-4-6 months (_SSS), or an investigational schedule of Synflorix at 1-2-4 months plus Prevenar13 at 6 months (SSSP). At 12 months consented infants were randomised to S or P. Outcomes were measured at 1, 2, 4, 6, 7, 12, 18, 24, 30, 36 months of age. Hearing (dB, 3 frequency average) was assessed by an audiologist in a sound-proof booth at 12, 18, 24, 30 and 36 months. Results: 425 infants were randomised to _PPP(143), _SSS(141), or SSSP(141). 261 were randomsied to S or P at 12 months of age. 167 hearing assessments are included in this analysis. Investigators remain blinded. Overall, 17% showed no detectable CHL(<16dB), 64% mild CHL (16 to 30 dB), and 19% moderate CHL (31 to 45 dB). Of 61 assessments in children < 30 months of age, these figures were 0%, 77%, and 21%. Of 106 assessments in children > 30 months, these figures were 26%, 56% and 18%. Of 122 cases of any OM and where a hearing assessment was also made, 82 (67%) had mild CHL, 29 (24%) moderate CHL and 11 (9%) had no detectable CHL. Conclusion: Among Aboriginal infants in remote communities, almost all children (90%) with OM have mild or moderate CHL. Differences in infant vaccine schedules may not impact on this outcome. No child less than 30 months of age had normal hearing. Any child with a clinical diagnosis of any OM requires appropriate medical treatment, hearing assistance, and strategies for language stimulation and support with communication. As AOM is asymptomatic, primary health care services must ensure that every ear of every child is assessed at every opportunity. Prevention remains a huge challenge. Social determinants such as household

crowding are major predictors of early onset OM and a childhood of chronic conductive hearing loss.

Title: Baseline and end of therapy hearing in Aboriginal children with chronic supporative otitis media receiving standard topical treatment plus adjunct therapies: a factorial randomised control trial

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Introduction: Hearing is significantly affected by chronic suppurative otitis media (CSOM). This remains high among Australia Aboriginal children in remote communities, with impacts on learning and education outcomes. A factorial design randomised controlled trial was conducted to evaluate adjunct treatment with twice daily antiseptic ear washes (betadine) or no wash, and oral cotrimoxazole ([4mg/kg trimethoprim] BD) or placebo equivalent. The intervention period was 16 weeks. All children also received current standard treatment (twice daily ear cleaning and ciprofloxacin drops). Twenty-eight urban and remote communities in the Northern Territory participated between 2015-2018. Objective: To determine if adjunct betadine ear washes or oral cotrimoxazole improve hearing outcomes at the12 month follow up. Method:Children with CSOM aged 6 months to 17 years of age were eligible. CSOM was defined as discharge through a perforation for >6 weeks in the worst ear. Routine audiological assessments were included if undertaken within a 2-year period before randomisation (pre-intervention) or 6 to 18 months (post-intervention). Hearing level was defined as the pure tone 4 frequency average hearing (dB) at 500, 1000, 2000 and 4000 Hz in the better hearing and worse hearing ears. Grades of hearing loss were defined for the worse ear as normal (<16dB), mild (16 to 30dB), moderate (31 to 60dB) and severe (61 to 80dB). Mean hearing level differences were estimated using analysis of variance models Results: Of the 280 children with CSOM, an audiogram was performed at baseline in 165 children and at ~12 months after randomisation in 129 children; 89 children had both baseline and 12-month audiograms. The mean hearing level in the worse hearing ear was around 33dB both before and after the intervention period. 3% of children had normal hearing in their worse ear before the intervention period. Comparisons of post-intervention hearing levels in the worse ear were: i) betadine 32.3dB (SD 10.28, n=65) versus no betadine 33.31dB (SD 9.75, n=64); mean difference -1 dB (95% Confidence Interval (CI) -4.5 to 2.5); and ii) cotrimoxazole 32.4dB (SD 9.51, n=61) versus placebo 33.16dB (SD 10.46, n=68); mean difference of -0.76 (95%CI -4.3 to 2.7). There were no differences between the unadjusted and adjusted estimates. Conclusion: Our analysis of children at ~12 months follow-up shows the very high level of persistent hearing loss in children at baseline. There was no difference in hearing levels between adjunct betadine washes versus no wash, or between adjunct oral cotrimoxazole versus placebo. The average hearing loss was above 30dB which WHO states is disabling hearing loss in children. Further studies are needed to determine which medical treatments optimise the hearing in children

with CSOM. Strategies for prevention are also needed. In this high risk population, the environmental causes of persistent and severe OM must be addressed.

Title: Immunogenicity of a Combination 4-dose Primary Course Schedule of 10-valent Pneumococcal Haemophilus influenzae Protein D conjugate Vaccine, Synflorix (PHiD-CV10) at 1-2-4 Months Plus Prevenar13 (PCV13) at 6 Months, Compared to each Vaccine Alone at 2-4-6 Months: a Randomised Controlled Trial

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Aboriginal children living in remote communities are at high risk of early and persistent otitis media, which causes disabling hearing loss and social disadvantage. Objective: To assess the superiority of an early combination schedule of two pneumococcal conjugate vaccine (PCV) formulations in providing earlier, broadened, higher immune protection against pneumococcus and non-typeable Haemophilus influenzae (NTHi). Method: Aboriginal infants 28 to 38 days of age were allocated (1:1:1) to either Prevenar13™ (P, PCV13) at 2-4-6 months of age (_PPP), Synflorix™ (S, PHiD-CV10) at 2-4-6 months (_SSS), or an investigational schedule of Synflorix at 1-2-4 months plus Prevenar13 at 6 months (SSSP). Primary outcomes were immunogenicity at 7 months of age for pneumococcal serotypes 3, 6A, and 19A and protein D (GMC ratios and Difference in proportion of infants with IgG above thresholds 0·35 µg/ml or 100 EL.U/mL, respectively). Results: 425 infants were randomised to _PPP(143), _SSS(141) or SSSP(141). Primary outcomes (Figure) were: 1. Compared to _SSS, the SSSP group had superior immunogenicity for serotypes 3, 6A, and 19A (Differences 61%, 32%, and 18%, respectively, $p<0.001$ for each) 2. Compared to _PPP, the SSSP group had superior immunogenicity for protein D (Difference 57%, $p<0.001$) Secondary outcomes confirm overall benefits of the SSSP schedule at 2 and 4 months of age, and no harm of SSSP compared to _PPP.
Conclusion: The 4-dose early combination schedule of Synflorix plus Prevenar13 (SSSP) provided safe, earlier, higher and broader overall immune protection throughout the first 7 months of life compared to standard 3-dose single vaccine schedules (_SSS or _PPP).

Title: Epidemiological Data on Hearing Impairment among Greenlandic Adolescents: Item Development and Findings from the Health Behaviour in School-aged Children Study 2018

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Objectives – Ear-infections is the leading cause of hearing impairment among children worldwide and a major public health problem in many indigenous populations. Studies have shown that early onset of impaired hearing compromise communication skills, academic performance, psychosocial behavior and emotional development, but studies are based on clinical examinations and not from representative studies of populations as studies on self-reported hearing impairment are scarce. The purpose of the present study was therefore two-sided; first to follow modern psychometric methods to develop an item bank for collection of data on hearing impairment among Greenlandic adolescents, and secondly to report data on the child-reports on hearing impairment from a national survey. Methods – Data was part of the 2018 survey including 2.273 students corresponding to 47,6% of all Greenlandic schoolchildren in the age range from 10 to 17 years. The analyses performed aimed to describe the data characteristics and the frequency of self-reported hearing impairment among Greenlandic adolescents. Descriptive statistics are presented for hearing impairment, and binary logistic regression illustrate the strengths of association of school-related (risk) factors and poor self-rated health on hearing impairment. Results – An average of 4% reported to have pain in the ear almost every day, and almost 10% reported ear pain at least weekly. 3% reported to have inflammation from the ear at least weekly. 5% reported to have such impaired hearing that they were not at all able to follow what happened in school. 56% reported that their hearing impairment had lasted for less than 3 months, and 30% reported to have had hearing impairment for more than 1 year. 13% reported to have had them for more than 10 years. Logistic regression showed that girls had significantly higher odds of low self-rated health and poor school environment when experiencing impaired hearing. For boys tendencies were different and no associations were statistically significant except experiencing an academic achievement below average. Conclusion – The study confirms clinical knowledge and case stories that there are large proportions of Greenlandic adolescents, who have impaired hearing. The updated development of items collecting data among children and adolescents for practical use in epidemiological studies are now available in Danish, Greenlandic and English. Longitudinal studies will examine causal associations between hearing impairment and other risk factors as well as social and health outcomes, to prevent this widespread detrimental impairment. The developed items on perceived hearing impairment will support future studies in finding unrecognized hearing impairment among

children and adolescents. Furthermore, we hope these surveys will be beneficial to children with hearing impairment attending school.

Title: Changes of Osteoclasts in ossicles of mouse due to injection of lipopolysaccharide

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Objective: Repeated otitis media to cause tympanic membrane perforation and ossicular bone erosions, resulting in conductive hearing loss. However, there are few reports on the mechanism of auditory ossicular lesions. We believe that elucidation leads to prevention and treatment. The aim of this study is that the number of osteoclasts Induced by LPS increase in ossicles and then ossicles are eroded and or fixed. Method:Lipopolysaccharide (LPS) was injected in the right ear of the mouse and saline was administered to the left ear. We compared the induced amount of osteoclasts in the ossicles on both sides stained by tartaric acid resistant acidic phosphatase, a marker of osteoclasts at one week after injections. Results: The number of osteoclasts increased significantly in three auditory ossicles respectively in LPS-administered ears compared to control ears ($p<0.05$). Conclusion:In the otitis media animal model, osteoclasts involved in ossicular lesions. Therapeutic drugs such as osteoporosis drugs have been developed and can reduce osteoclasts in ossicular lesions of otitis media and can prevent conductive hearing loss.

Title: Biofilm Distribution on Ventilating Tubes: An in vivo Study

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Introduction: Tympanostomy tube (TT) insertion is a common procedure in children with chronic otitis media with effusion. Post-TT otorrhea (PTTO) may eventually lead to TT obstruction and dysfunction in 7-13% and 7% of cases, respectively. It has been reported that bacterial biofilms have been involved in chronic PTTO, and more specifically, Pseudomonas aeruginosa. However, the sites on TTs which are prone to harbor biofilms were studies in in vitro conditions, but not in TTs which were removed from children. Methods: This in vivo study included 30 TTs removed from children. Of them, 65% were removed due to retained TT (average time from initial insertion: 17±8 months), 35% were removed due to persistent otorrhea and 20% due to TT obstruction. Of them, 28 TTs were collar-buttons and 2 were T-tubes. All TTs were processed by appropriate fixation with formaldehyde and staining using propidium iodide, and the presence and topographic distribution of biofilms on the TTs were evaluated by using scanning electron microscope, confocal scanning microscope and stereo-microscope. Results: Bacterial biofilms were more likely to be formed on the perpendicular junction and in the internal lumen of the TTs in most cases. When TTs were sliced into medial and lateral parts, there were more biofilm colonies on the medial part, facing the middle ear mucosa. In children with PTTO, considerable biofilm adhesions were noted on the entire TTs surface. Conclusion: Analysis of topographic distribution shows biofilm adhesion in specific TT areas: perpendicular junctions and the internal lumen of the TT. These "prone zones" can be the future target areas for changes in TT geometry or can be specifically coated with anti-biofilm materials to decrease biofilm adherence.

Title: Efficacy of Transtympanic Ciprofloxacin and Gemifloxacin Gel formulation against Streptococcus Pneumoniae and Nontypeable Haemophilus influenza Experimental Otitis Media in a Chinchilla Model

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Introduction: The treatment of AOM often requires prolonged and subsequent systemic exposure to antibiotics especially in young children. Lack of adherence has been associated with treatment failure or early relapse. Subsequent and recurrent exposure to antibiotics is partially responsible for emergence of drug-resistant bacteria. Trans tympanic drug delivery of antibiotics directly to middle ear (ME) has the potential to provide local bioavailability sufficient to achieve sterilization while minimizing systemic exposure. Objectives: To evaluate pharmacokinetics (PK), middle ear fluid (MEF)/ nasopharyngeal (NP) and plasma concentrations of ciprofloxacin and gemifloxacin trans tympanic gel formulation against Streptococcus Pneumoniae (S. pneumoniae) and Nontypeable Haemophilus influenza (NTHi).Efficacy of ciprofloxacin and gemifloxacin trans tympanic gel formulation against S. pneumoniae and NTHi in chinchilla model of experimental otitis media (EOM) as compared to untreated control animals.Determine the effect of trans tympanic treatment on the NP colonization in the chinchilla EOM model as compared to untreated animals. Methods: S. pneumoniae and NTHi with selected antimicrobial susceptibility patterns, were inoculated initially into the nasopharynx and 3 days later directly into the chinchilla middle ear bulla. The animals were monitored by tympanometry and otoscopy for development of disease. Plasma, NP and MEF were collected for trans tympanic gemifloxacin/ ciprofloxacin PK studies at baseline, during and after treatment. NP and MEF quantitative cultures were performed subsequently to determine efficacy and changes in NP colonization during and after treatment as compared to controls. Results: In chinchilla model of EOM, one application of gemifloxacin-containing gel (1mg/ml) abutting intact inflamed tympanic membrane sterilized MEF in >92% (n=13/14) of animals challenged with S. pneumoniae with MIC ≤ 0.02µg/ml compared to untreated ears in which only 25%(n=1/5) cleared infection (p-value < .005). Likewise in NTHi group (MIC < 0.001) MEF was sterilized in 100% (12/12) animals compared to 0% (0/8) of animals either not treated or treated with gel only (p-value <.001). There was no evidence of systemic absorption of gemifloxacin in the chinchilla plasma at 24 hours and 1 week after application as measured by high-performance liquid chromatography. Based upon NP quantitative culture results we did not find any effect on the NP colonization in treated animals as compared to controls. Results with ciprofloxacin gel were similar. Conclusions: Trans tympanic gel formulation achieved high antibiotic flux across the tympanic membrane and cured S. pneumoniae and NTHi EOM successfully without causing any tissue toxicity. This delivery mechanism seems highly promising alternative to oral antibiotics for treatment of OM. The localized antibiotic delivery directly to ME enhances local bioavailability while minimizing systemic antibiotic exposure.

Title: In vitro Biofilm Formation Distinguishes Strains of Streptococcus pneumoniae that Persist in the Nasopharynx from Non-persistent Strains in Children with Acute Otitis Media

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Objective: Persistent nasopharyngeal (NP) carriage of Streptococcus pneumoniae is associated with recurrence of acute otitis media (AOM) and prolonged middle ear effusion. Biofilm is found frequently in the middle ear in those with chronic otitis media, but the role of biofilm formation in persistent carriage remains uncertain. Our aim was to study whether in vitro biofilm formation distinguishes between pneumococcal strains that persist in the NP compared to those that do not. We also studied the genomic differences between persistent and non-persistent strains. Method: Clinical isolates were obtained from the NP of children (6-35 months) with AOM enrolled in a study of antimicrobial treatment vs. placebo. Children were followed for at least 2 months, and NP cultures were taken at follow-up visits. Persistent carriage was defined as detection of the same serotype of S. pneumoniae from the NP for ≥45 days. Biofilm formation was assessed by measuring optical density (OD) values in microtiter plates after crystal violet staining. The whole genome sequencing data was generated using the Illumina HiSeq platform. We performed comparative genomic analysis of 63 persistent and 16 non-persistent strains using SEED, BLAST, and BRIG. Results: Persistent carriage was detected in 18% (31/177) of children. In vitro biofilm formation was significantly greater among persistent strains of S. pneumoniae compared with serotype matched, non-persistent strains [mean OD 0.367 (SD 0.109) vs. 0.292 (SD 0.066); P=0.01]. Overall bacterial growth did not differ between persistent and non-persistent strains [mean growth 0.656 (SD 0.163) vs. 0.609 (SD 0.157); P=0.38]. Repeat isolates from the same child demonstrated comparable in vitro results, suggesting that persistent strains were already better biofilm producers at the time of initial colonization. Comparative genomic analysis indicated a great homogeneity among the subsequent strains from the same child. Genes coding for cell wall surface anchor family proteins, phage replication proteins, diaminobutyrate-pyruvate transaminase, and histidinol-phospate aminotransferase appeared to be more common among persistent strains as compared with non-persistent strains, but the results were not consistent between different serotypes. Conclusion: In vitro biofilm formation distinguishes persistent strains of S. pneumoniae from those that are cleared from the NP. Further studies are needed to identify genes that contribute to the persistence of S. pneumoniae in the NP.

Title: Immunologic dysfunction in the otitis prone child

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Objective. Acute Otitis Media (AOM) is a multifactorial disease influenced by the immunological response occurring mostly in young children who are immunologically naïve to AOM pathogens. We sought to identify deficiencies in fundamental immune defense mechanisms in otitis prone children during a 10-year prospective study Methods. Nasopharyngeal and blood samples were prospectively collected children at 6-36 months of age (6, 9, 12, 15, 18, 24 and 30-36 months old) and at every AOM episode. The diagnosis of AOM was made by validated otoscopists and confirmed by tympanocentesis. Stringently-defined otitis prone (sOP) classification was made if a child had 3 AOMs within 6 months or 4 AOMs within a year. Cytokines /chemokines were quantitated with molecular methods. B cells, T cells and antigen presenting cells (APCs) and immunology signaling pathways were identified, characterized and quantitated by flow cytometry, Elispot and molecular methods,. Results. We identified dysfunction in innate responses that cause an immunopathological impact in the nasopharynx resulting in pathogenesis. These include inadequate expression of TLRs, proinflammatory cytokine secretion, epithelial cell repair and defects in professional APCs. Adaptive immunity defects in B cell function and immunologic memory were identified resulting in low levels of antibody to major otopathogen-specific protein antigens. CD4+ and CD8+ T cell function and memory defects were identified. We sought a mechanistic explanation in B cell dysfunction by examination of TNF family receptors (TNFRs) TACI, BCMA, and BAFFR receptor expression and found significantly lower BAFFR and TACI expression; significantly lower proliferation of B-cells stimulated with exogenous BAFF; and diminished expression of co-stimulatory receptors B7–1 and B7–2 among sOP children. We sought a solution for T cell dysfunction and found addition of exogenous Th17-promoting cytokines restored Th17 function in cells from sOP children. We found that sOP children are unusually vulnerable to other respiratory infections: viral URI, sinusitis, lobar pneumonia and influenza suggesting their broad immunologic defects are associated with susceptibility to other infections. Conclusions. In the first years of life when the diagnosis of otitis proneness is stringently applied by requiring microbiologic confirmation of authentic repeated AOMs, broad immunologic deficits in innate and adaptive immunity can be identified and present a new opportunity for therapeutic intervention.

Title: Acute Otitis Media Treatment Guidelines in the Pediatric Emergency Department: How Adherent Are We?

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Objective: To quantify the rates of appropriate and inappropriate treatment of uncomplicated AOM visits. Also, we studied the pre-AOM visit and discharge antibiotic prescription rates, the type and duration of antibiotics prescribed for AOM, and the total time spent with antibiotics in each uncomplicated AOM episode. Method: Records of children 6-36 months of age with AOM visiting a university-affiliated pediatric emergency department (PED) in central Israel between 2014 and 2016 were retrieved and reviewed for the treatment given: watchful waiting (WW) vs. antibiotic treatment. If antibiotics were prescribed, the type and duration were recorded. Overall time spent with antibiotics was also calculated (before, during and after admission). We evaluated appropriate and inappropriate treatment rates of eligible AOM cases, in respect to the local guidelines, published in 2013, which encourage WW in most mild-moderate cases. Results:Out of 1493 AOM visits, 863 (57.8%) were boys, with a median age of 14.9 months (IQR, 9-19). The overall pre-visit antibiotic rate was 24.1%, but among those children who had been examined by a physician, this rate was 95.2%. Amoxicillin was the most common antibiotic, administered in 66.3% of the cases. Only 21 (5.8%) children had been treated with antibiotics for ≥7 days prior to their visit, and were considered as treatment failure. Antibiotic therapy upon discharge was recorded in 1394/1449 (96.2%) visits, again with amoxicillin as the most common antibiotic therapy, in 80.8% of the cases. Appropriateness of treatment (WW or antibiotics) could be analyzed in 1134 visits; 20.9% were considered as inappropriate. Of them, 98.3% were prescribed with the wrong antibiotic type and duration. The mean duration of the recommended antibiotic therapy upon discharge was 7.84±1.21 days (recommended by the guidelines: 10 days). Conclusion: Adherence rate to the local guidelines of diagnosis and treatment recommendations for uncomplicated AOM was overall very high, as measured by whether appropriate treatment was given and the type and duration of antibiotics. The total time spent with antibiotics was shorter than recommended but was not associated with a high rate of complications.

Title: Development of a Primary Pediatric Middle Ear Epithelial Cell Model for the Study of MUC5B Regulation in Otitis Media

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Objectives: Chronic Otitis Media (OM) is characterized by the production of fluid in the middle ear often mucoid, with accumulation of MUC5B mucin. Current middle ear (ME) human cell models employ immortalized cell lines which are limited in their ability secrete mucin proteins. Our laboratory has developed a novel in vitro model using fibroblast reconditioned media with patient middle ear epithelial cells enabling the study of MUC5B protein in chronic OM development. Methods: Primary middle ear epithelial cells (pMEECs) were recovered brushing the middle ear cavity of 2 pediatric patients during cochlear implant placement at Children's National Medical Center with consent of the legal guardians and in accordance with the Institutional Review Board. Cells were cultured in conditional medium containing calf bovine serum, irradiated 3T3 NIH fibroblast secretions and 10μM Y-27632 dihydrochloride (Rho kinase inhibitor) to induce proliferation. Cells were then plated in transwells and differentiated at air-liquid interface (ALI) with BEBM supplemented with epithelial specific singlequots (Lonza). Pictures were taken; proteins were analyzed by western blot and mass spectrometry, and mRNA by quantitative PCR after 1 to 4 weeks of culture. Results: pMEECs required a mean of 20 days to proliferate in conditional medium. Bright field microscope pictures showed pMEECs cultured at ALI formed a tight epithelium until week 2 to week 4 depending on the patient, and had a differentiated airway epithelial phenotype. Keratins 5, 14 and 15 were detected by immunofluorescence and western blot to confirm epithelial cell differentiation. Importantly, MUC5B mRNA was expressed at all-time points in pMEECs for both patients. MUC5B protein was detected in pMEEC lysates for both patients by western blot. Interestingly, MUC5B was secreted only by one patient sample (detected by mass spectrometry), showing an increase in MUC5B peptide count (PC) overtime between day 1 (0 PC) and week 4 of differentiation (69 PC = 1.05% total PC). Conclusion: We successfully created a primary pediatric middle ear epithelial cell model potent to produce mucins for the study of MUC5B regulation in OM.

Title: Development of a Handheld Optical Coherence Tomography Device with an Otoscopy Probe for In-office Non-invasive Imaging and Characterization of Middle Ear Effusions

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Introduction: A major diagnostic challenge for pediatricians, primary care providers, and otolaryngologists is the determination and characterization of middle ear effusions (MEEs) associated with otitis media (OM). While standard and pneumatic otoscopy have been historical methods for making this assessment, they rely on the subjective interpretation of the surface view of the eardrum. Consequently, ear infections are the leading cause for antibiotic prescription and over-prescription, as well as surgery in children, in the U.S. Development of new in-office diagnostic tools and methods to objectively determine the presence of MEE could significantly improve existing treatment protocols and judicious use of interventions. Optical coherence tomography (OCT), a promising high-resolution imaging modality that performs near-infrared optical ranging into tissues in a manner analogous to ultrasound imaging, is demonstrated for imaging the human middle ear and its contents. Traditional OCT systems, such as those used in ophthalmology, are often bulky desktop instruments and utility of OCT for in-office otological use necessitates a more portable form-factor. Objective: Develop and clinically test the ability of a handheld M-mode (motion-mode) OCT device with an otoscopy probe to collect human middle ear images for interpretation via blinded reader quiz, compared to that of a cart-based traditional 2-D OCT device with an otoscopy probe. Methods: Several iterations of portable clinical OCT devices with an otoscopy probe were constructed and used at two U.S. tertiary care, freestanding hospitals to collect bilateral OCT images from 25 subjects presenting in the office setting with or without ear complaints and 65 subjects scheduled for tympanostomy tube surgery. Clinical histories and diagnoses including acute OM, recurrent OM, and chronic OM with effusion were recorded for each subject. Cross-sectional OCT images with micron-scale (5-15 μm) resolution and imaging depths up to 2 mm were obtained. Blinded reader quizzes were conducted using the cart-based traditional 2-D OCT images, M-mode OCT images generated from the 2-D OCT data, and images collected by the M-mode OCT device. Readers from four groups of tiered medical expertise were trained and instructed to identify the presence of middle ear content, specifically MEEs. Results: In the traditional 2-D OCT reader quiz, detection of MEE had high accuracy (90.6%), sensitivity (90.9%), and specificity (90.2%). In the reader quiz for M-mode OCT images generated from the 2-D OCT data, detection of MEE had accuracy (87.5%), sensitivity (83.3%), and specificity (95.8%). The reader quiz of the images collected by the M-mode OCT device is ongoing. Conclusion: Use of M-mode OCT for in-office non-invasive imaging and characterization of MEE shows promise for improving physician assessments in diagnosing presence of MEE, but with a smaller, more in-office appropriate form factor than cart-based OCT systems.

Title: Micro RNAs Implication in the Progression of Middle Ear Infection

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Objective: Otitis Media (OM) affects millions of children every year and costs over $2 billion/year to the US healthcare system. OM is an infection of the middle ear mostly due to Non-typeable Haemophilus influenza (NTHi), and characterized by fluid build-up. During chronic OM, this fluid becomes mucoid as a result of the remodeling of the middle ear epithelium. Currently, there is no medication beneficial to chronic OM, leading to surgery to clear the middle ear. Micro RNAs (miRNAs) are small RNA sequences (<22 nucleotides) that can be carried in small vesicles (exosomes) for cell to cell communication, regulating gene expression. In this study we aimed at investigating the role of miRNAs in the progression of OM. Methods: Middle ear fluids (MEF) were collected during tympanostomy tube placement at Children's National with consent. A human middle ear cell line (HMEEC) was grown and exposed to NTHi lysates to simulate acute stages of OM. Exosomes from both MEF and cell secretions were isolated with Exoquick-TC, miRNAs purified with SeraMir kit and analyzed by Nanostring. HMEEC were transfected with miR-378 mimic or negative control and gene expression for MUC5B, MUC5AC and IL-8 was assayed by PCR, the transcriptome was analyzed by mRNA-seq technique performed by Children's National Genomics Core. miR-378 transfection efficiency was evaluated by PCR and flow cytometry to detect its presence inside of HMEEC. Results: miR-378 was the most upregulated miRNA in HMEEC secretions in response to NTHi lysates and one of the most abundant in MEF. Notably, miR-378 was previously shown to increase mucin expression in airway epithelial cells. Given these findings, we proceeded to test its effect on HMEEC. Cells were transfected with miR-378 using lipofectamine and the presence of miR-378 in cells was confirmed by PCR and flow cytometry. miR-378 exposure resulted in mRNA induction after 24hrs: mucins MUC5B 3.7-fold, MUC5AC 20-fold and IL-8 2-fold compared to negative control miRNA ($p<0.05$). As a potential mechanism of action, the preliminary mRNA-seq results showed that miR-378 induces Wnt-1 pathway (3-fold, $p<0.0001$) and the dowregulation of HIF1-α inhibitor (1.9-fold, $p<0.00001$) after 6hrs of incubation, pathways previously implicated in mucin regulation. Conclusion: This study shows the mucogenic and inflammatory effect of miR-378 on the middle ear epithelium. This work will provide the groundwork to find treatment strategies limiting the progression of OM targeting miRNAs.

Title: Surgical management of large-sized labyrinthine fistula caused by choleteatoma: a long term outcome and hearing changes

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Objective: To evaluate long-term hearing changes and recurrence after surgical management of cholesteatoma matrix overlying large-sized labyrinthine fistula (≥ 2 mm) Method: A retrospective review of medical records was carried out for 26 patients who underwent surgical resection. Among them, 25 patients underwent complete resection of the cholesteatoma matrix. A long-term recurrence and hearing changes were assessed with an investigation of associated complications. Results: For complete resection group, average bone conduction threshold was changed from 38.6 to 45.1 dB immediate postoperatively (p=0.073) with 5 cases (20%) with more than 10 dB loss although 3 of them already had more than 70 dB preoperative hearing level. Minimal hearing changes (46.5 dB, p=0.353) were observed thereafter over 46.8 months (15-87 months) without recurrent case. The risk of postoperative hearing loss was significantly increased as the size of fistulae increases (p=0.011), however, a definite cut-off size cannot be determined. Two cases (8%) with large fistulae (3.1 mm, 3.8 mm) experienced intraoperative perilymph leakage with resultant significant additional hearing loss in one case. In a case, cholesteatoma matrix was partially removed because of extension to oval window. Conclusion: Complete removal of cholesteatoma matrix could also be applied even for large-sized fistula (≥ 2mm) with successful long-term preservation and disease control. Intraoperative hearing loss could be minimized by considering preoperative hearing level and great cautions for cases with larger than 3 mm fistula.

Title: Impact of History of Otitis Media and Hearing Loss on Children's Academic Achievement: The U.S. Early Childhood Longitudinal Study–Kindergarten Class of 2010–11 (ECLS–K:2011)

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Objective: To describe associations between parental report of ear infections (otitis media history since birth) and difficulty hearing with measured hearing loss and reading achievement scores in early primary school. Method: ECLS–K:2011 children (n=18,170) were drawn from a national sample of public and private schools in 2010–11. Information on children's health, including medically-diagnosed ear infections (EIs) and hearing trouble (HT), was reported by parents; additional information was provided by teachers, schools, and daycare providers. Children's cognitive, socio-emotional, and physical development were directly assessed. Pure-tone audiometry was performed on a subsample of over 3500. Reading scale scores based on the full set of assessment items were calculated using item response theory (IRT) procedures. The IRT scale scores represent estimates of the number answered correctly if children had received all of the questions in a given content domain. Using national sampling weights, logistic regression models were statistically-adjusted for covariates. Results: Prevalence of EIs was reported for specific time intervals: birth to age 2 (45% of children), age 2 to kindergarten entry (72%), kindergarten year (30%), first grade (20%), second grade (17%) and third grade (15%) for participants without missing data (n=6,580). Prevalence estimates for each time interval using the full sample of children were similar. By third grade, 80% of children had at least one EI and 30% had one or more time periods with recurrent EIs (3 or more). Overall, 2.8% of children had reported HT (80% "a little" HT; 20% "moderate/worse" HT). Among those who had EIs, HT was reported for 6.2% in kindergarten, 7.4% in first grade, 7.2% in second grade, and 7.0% in third grade. Measured hearing thresholds were 5 to 7 dB higher (worse) on average at frequencies of 2, 4, and 8 kHz for children with reported HT. After controlling for children's age, sex, race/ethnicity, birth weight, complications at birth, recurrent EIs (3 or more) in any time interval before kindergarten entry was significantly associated with HT in kindergarten (odds ratio [OR]=3.6; 95% CI: 2.0-6.6), first (OR=4.9; 95% CI: 2.5-9.4), second (OR=5.4; 95% CI: 3.0-9.6), and third grade (OR=5.1; 95% CI: 2.7-9.8). Children with HT had lower reading scores in kindergarten (HT vs no HT, 0.26 vs 0.59), first grade (1.34 vs 1.74), second grade (1.97 vs 2.32), and third grade (2.38 vs 2.74), $p<0.0001$ for each grade. Conclusion: EIs are associated with reported HT and HT is associated with children's academic achievement; hence, EIs indirectly impact children's academic (reading) achievement test scores in early primary school.

Title: Office Insertion of Tympanostomy Tubes in Young Children: Lessons Learned over 10 Years

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Objective: To describe lessons learned by the author during the past 10 years of performing in-office insertion of tympanostomy tubes in young children, without general anesthesia. Despite worldwide controversy and limited uptake of this method, the author has found widespread acceptance among parents and families who wish to avoid the inconvenience and possible adverse neurocognitive effects of general anesthesia. Method: After informed consent and child premedication with 30 mg/kg acetaminophen 1 hour prior to the procedure, office insertion of Armstrong beveled fluoroplastic tubes is performed, without topical anesthesia, using the papoose board for protective stabilization and the binocular microscope for visualization. An assistant stabilizes the child's head and the parent(s) are in the room. Ofloxacin drops are applied after insertion and the child is permitted to feed immediately. Results: Over the past 10 years approximately 300 children have received in-office tympanostomy tubes, with 95% below age 2 years. The primary indication has been recurrent acute otitis media with persistent middle-ear fluid and otalgia, unacceptable antibiotic burden, or both; chronic middle-ear effusion with hearing loss was the second most common indication. Insertion times range from 3.5 to 12.0 minutes, with mean time (for both ears) over tha past year about 4.5 minutes. Successful insertion is achieved in more than 98% of children, with occasional failure cause by ear canal stenosis or obstructed visualization because of severe inflammation and oozing. Success if facilitated through advance planning, two-handed technique, using an experienced assistant, and proactive counseling of parents. There were no intraoperative complications or tube medializations. Four children had early extrusion of 1 tube that required reinsertion. Children recovered promptly without any reports of persistent traumatic reaction to the procedure. Conclusion: In-office insertion of tympanostomy tubes is an option that appeals to many parents and caregivers as an attractive alternative to general anesthesia. In the author's experience over 10 years the procedure is highly successful without complications. This procedure continues to remain in high demand in the author's clinical practice and appears to fill a significant need given concerns over the neurotoxicity of general anesthesia in young children based on USA FDA warnings.

Title: Single-Cell Transcriptomics of the Normal Human Middle Ear Mucosa

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Objective: The normal human ME mucosa has been extensively studied using histological and ultrastructural methods to identify the various cell types present. Immunochemistry has also been used to assess the expression of specific genes. Here we utilized single-cell RNA sequencing to identify ME mucosa cell types. Methods: We performed single-cell RNA-Seq on ME mucosa samples obtained from patients undergoing labyrinthectomy for removal of vestibular schwannomas. The 10X Chromium System generated single-cell transcriptomes of thousands of ME cells. PCA-generated cell clusters were classified based on marker genes, and the differential expression of genes across cell types was evaluated. Results were compared to our sample of murine mucosal cells from the normal ME. Results: In the normal human ME, PCA analysis using 10X CellRanger identified 11 cell clusters. The markers used to identify murine cell types only partially overlapped with those present in the human cells. In these cases, other marker genes were employed to identify cells. Five epithelial cell clusters made up ~75% of the cells. There was one cluster of stromal cells. An endothelial cell cluster contained primarily vascular, but some lymphatic, endothelial cells. One pericyte cluster was present, as was a small neuroendocrine cell cluster. A monocyte cluster contained mature and M2 macrophages well as dendritic cells. A lymphocyte cluster contained T, NK, B and type 2 lymphoid cells. A cluster of erythrocytes presumably reflected the surgical excision of the samples. No melanocytes were observed. As with marker genes, there were similarities and differences between gene expression by human and mouse cell types, including innate immune and OM-related genes. Conclusions: Single-cell transcriptomes identify a broad spectrum of cell types in the normal human ME mucosa. They provide cellular resolution information on differences in the expression of genes known to be important to OM pathogenesis and recovery at the initiation of OM. Supported by grants: DC000129; DC012595 and DC014801 from the NIH/NIDCD and the Veterans Administration.

Title: In Vitro Analysis Demonstrates that Peptides are Actively Transported Across the Human Tympanic Membrane

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Objective: Using phage display, we identified a family of peptides that can actively transport bacteriophage across the intact tympanic membrane (TM) of rats and other mammalian species. However, it was not clear whether the peptides require the phage for transport, or whether they can cross the TM independently. It was also not clear whether the peptides would mediate transport across the human TM. Methods: Using an in vitro assay consisting of a modified Ussing chamber into which a TM could be mounted between an upper and lower fluid chambers, we applied a trans-TM peptide linked to a DNA oligo template on the TM surface. After 2 hours, fluid was recovered from the lower chamber and qPCR was used to quantify the amount of peptide present. Controls were peptide lined to phage at the same molar concentration. In a second study, TM fragments from human patients undergoing TM reconstruction procedures were place in the device. Phage expressing trans-TM peptides were applied to the human TM and 2 hours later, fluid was collected from the lower chamber and phage was tittered to evaluate concentration present. Control human TMs were exposed to untargeted WT phage. Results: In the first experiment, the amount of DNA-tagged peptide recovered from the lower chamber was comparable to the amount of phage recovered. In the human TM experiments, recovery of trans-TM phage from the lower chamber was similar to that seen with the TMs of rat or other mammalian species used. Conclusions: The results indicate that transport of trans-TM peptides across the TM is not dependent on phage cargo. Moreover, transport is also independent of size since recovery amounts were similar between the peptides linked to DNA-oligo and peptide linked to a 1um phage particle. This would be consistent with a transcytotic transport mechanism as opposed to a transmembrane transporter or paracellular movement. Finally, active peptide transport across the TM occurs in the human TM ex vivo which is critical for any use of peptides in drug delivery. Supported by grants: DC000129; DC012595 and DC14801 from the NIH/NIDCD and the Veterans Administration.

Title: Otitis Media Without Macrophages

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Objective: Macrophages are thought to play an important role in otitis media (OM). These phagocytic cells, when classically activated, are important sources of pro-inflammatory mediators and chemotactic factors that attract other leukocyte classes. When alternatively activated into an M2 phenotype, they are anti-inflammatory and can play a major role in the resolution of inflammation. However, given the complex cellular landscape of the middle ear (ME) and OM it is difficult to separate the contributions of a single cell type. Method: To evaluate the role of macrophages in OM we induced OM in a mouse deficient in CCR2 ($CCR2^{-/-}$), the major receptor for chemoattraction in macrophages, by intrabullar injection of nontypeable Haemophilus influenzae(NTHi). Some of these mice were also treated intravenously with liposomes containing clodronate ($CCR2^{-/-}$/clodronate), which induces apoptosis in peripheral blood macrophages, at two day intervals before and after NTHi inoculation. Histological analysis and immunohistochemistry was used to evaluate the MEs of experimental and control (wild-type, inoculated with NTHi, WT; CCR2 KO saline liposome, $CCR2^{-/-}$/saline) mice for OM. Results: Mucosal hyperplasia and the number of leukocytes present in the ME were similar across all three groups of mice over the first few days of OM, although $CCR2^{-/-}$mice showed more initial mucosal growth. However, leukocyte infiltration was significantly greater at 7 days after inoculation in $CCR2^{-/-}$/clodronate mice. The number of macrophages in the MEs on day 2 after NTHi inoculation was less than 50% of that seen in WT animals in $CCR2^{-/-}$mice, and only 10% in $CCR2^{-/-}$/clodronate MEs, with only slight recovery of macrophages by day 3. On day 7, WT mice had only a few macrophages in the ME, while macrophages persisted in $CCR2^{-/-}$and $CCR2^{-/-}$/clodronate mice. Numbers of ME neutrophils in the ME, always much higher than the number of macrophages, were higher by more than two-fold in $CCR2^{-/-}$/clodronate mice comparted to WTs, and 8-fold higher at day 3. While they were absent at day 7 in WTs, they persisted in $CCR2^{-/-}$and $CCR2^{-/-}$/clodronate MEs. Finally, while WT mice cleared NTHi by day 7, some $CCR2^{-/-}$and $CCR2^{-/-}$/saline, and most $CCR2^{-/-}$/clodronate, MEs were culture-positive at that time. Conclusion: The results suggest that macrophages play a very significant role in OM, despite the fact that they are typically present in small numbers than neutrophils. They appear to inhibit neutrophil entry into the ME cavity, and to promote the resolution of ME infection and inflammation. Supported by grants DC000129; DC012595 and DC014801 from the NIH/NIDCD and the Veterans Administration.

Title: Delivery of Antibiotic Through the Intact Tympanic Membrane by Peptide-Mediated Active Transport

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Objective: While systemic antibiotics are not recommended for the treatment of uncomplicated acute otitis media (OM) in children over two years of age, they are useful in the treatment of younger children and of complicated forms of the disease. Local delivery has also been shown to be efficacious in treating complicated OM, but it requires surgically breaching the tympanic membrane. Using Phage display, we previously discovered a family of peptides that are actively transported across the intact tympanic membrane (TM), providing a potential mechanism for noninvasive ME delivery. Since these peptides can deliver bacteriophage that are 1 μm in length to the ME, we reasoned that they should also be able to deliver drugs. Method: As a first attempt at peptide-mediated drug delivery, single amoxicillin molecules were linked to a trans-TM peptide and applied to the rat TM in vivo after ME inoculation of nontypeable Haemophilus influenzae (NTHi). After 8 hours, the ME was opened and the bacteria were titered. Individual antibiotic molecules linked to peptide only modestly reduced NTHi-load in the ME, suggesting that larger drug packages would need to be delivered in order to be effective. To assess the impact of a substantial drug package, we used EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide/N-hydroxysuccinimide) chemistry to conjugate neomycin to the major coat protein of M-13 phage. Results: Using this technique, we were able to link several hundred neomycin molecules per phage particle to phage expressing one of two different trans-TM peptides. Application of either neomycin-phage to rat TM in vivo, after ME inoculation with NTHi, resulted in 80% reduction in NTHi in the ME after only 8 hours, when compared to control MEs receiving NTHi but no TM treatment. Additional control MEs, inoculated with NTHi and receiving antibiotic crosslinked to WT phage on the TM, showed no reduction in ME NTHi titer either. Conclusion: The results indicate that active transport of drug packages by trans-TM peptides can deliver potent concentrations of antibiotics to the ME for the treatment of OM. Supported by grants DC000129; DC012595 and DC014801 from the NIH/NIDCD and the Veterans Administration.

Title: SPINK5 Variants Confer Susceptibility to Autosomal Dominant Non-syndromic Otitis Media

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Introduction: Previously population-specific rare variants within genes A2ML1 and FUT2 were observed to confer risk for otitis media. These studies were performed by exome and Sanger sequencing, linkage and association analyses, microbiome sequencing and analyses, and middle-ear specific protein localization in wild-type mouse. Objective: To assess how SPINK5, which encodes an epithelial serine protease inhibitor, increase susceptibility to autosomal dominant non-syndromic otitis media. Methods: Exome sequencing on DNA from Filipino, European-American, and Finnish cohorts with otitis media. Microbiome analyses on oral, nasopharyngeal and middle ear samples. Expression studies on murine tympanic membrane and middle ear mucosa. Results: Genome-wide-significant linkage (LOD=4.5) for the rare SPINK5 variant c.1682A>G (p.Glu561Gly) in a genetically heterogeneous, intermarried, indigenous Filipino population. A second rare, missense SPINK5 variant c.802C>T (p.Arg268Cys) co-segregates with otitis media in a European-American family and was also observed in four additional European-American or Finnish probands with otitis media. Seven additional SPINK5 missense, stop or UTR variants were identified in multi-ethnic probands, including one that is absent in the gnomAD database. SPINK5 is expressed in tympanic membrane and middle ear mucosa. Carriage of a SPINK5 variant is associated with overall shifts in the microbiota of the oral cavity and nasopharynx. Additionally in SPINK5 variant carriers compared to wild-type individuals we identified relatively abundant bacterial taxa in the middle ears that are more similar to taxa identified in the outer ear or nasopharynx. Conclusion: These findings suggest that changes in the head and neck microbiota due to SPINK5 variants also play a role in otitis media susceptibility.

Title: Biological Networks Underlying Genetic Susceptibility to Upper and Lower Airway Conditions

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Introduction: A unified airway theory has been proposed, by which the upper and lower respiratory tracts act as a single functional unit. This theory is typically applied to allergic disease, in part due to the observation that patients with allergic rhinitis are more likely to have asthma, and vice versa. Additional support for this theory is that the middle ear mucosa, upper airway, and lower airway are all lined with ciliated epithelium, which is involved in the active transport of mucus and particulate matter. It stands to reason that these tissues may respond similarly to environmental insult. In addition, data from the Human Microbiome Project indicates that the microbiome of the upper and lower airway show remarkable similarities. We believe that this unified airway theory can also be applied to infections, with the host genetic background contributing to susceptibility to both upper and lower airway infections. Objective: The goal of this study is to investigate the unified airway theory with respect to infectious and non-allergic disease, through both literature search and analysis of our own RNA-Sequencing data. Methods: Literature searches were completed in PubMed for chronic rhinosinusitis with and without nasal polyps, otitis media, chronic bronchitis, pneumonia, and idiopathic pulmonary fibrosis. Genome-wide studies were included in these analyses (linkage, genome-wide association studies (GWAS), and whole exome sequencing). Candidate gene studies were not included. Linkage results were included if reported with LOD≥3.3. GWAS results were included using two thresholds, first with a standard threshold for genome-wide significance ($p<5x10^{-8}$), and also with a more lenient threshold ($p<2.5x10^{-6}$). Genes identified by exome sequencing were included if $p<2.5x10^{-6}$. NetworkAnalyzer software was used to perform GO-term enrichment analyses on the combined gene list (upper and lower airways) and for each condition separately. Results: Initial analyses suggest that there may be shared genetic susceptibility to both upper and lower airway infections. As expected, genes related to the immune response, including antigen processing and presentation, are enriched in both upper and lower airway infections. Other enrichments include extracellular matrix, cytoskeleton, cilia, and calcium ion binding. Enrichments specific to otitis media are actin cytoskeleton, carbohydrate metabolism, and sensory perception of sound. Conclusion: Further analyses will be completed to fully investigate the common and condition-specific pathways and networks. In addition, we are in the process of examining the intersection of RNA-Seq datasets from our samples, for infections of the ear, nose, and lung. Eventually, these results may provide insight into the pathogenesis of both upper and lower airway infections, including the role of the host genetic susceptibility. This information may also allow for preventative measures and improved treatments for these infections.

Title: Identification of Rare, Missense PLG Variants in Families with Otitis Media

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Introduction: The PLG gene encodes plasminogen which, when activated by proteolysis, is converted to plasmin and angiostatin. Plasmin degrades blood plasma proteins, including fibrin in clots, while angiostatin inhibits angiogenesis. Deficiency in plasminogen results in a build-up of fibrin-rich lesions on the mucous membranes of the body. Plasminogen deficiency is most frequently associated with ligneous conjunctivitis, in which the wood-like pseudomembranous lesions develop on the mucous membranes in the eyes. However, similar lesions have also been observed in the middle ear, gingiva, respiratory tract, and female reproductive tract. Previous studies that show association between PLG variants and plasminogen deficiency consider homozygotes to be affected and heterozygotes to be “healthy”. Interestingly plasminogen-deficient $Plg^{-/-}$ mice spontaneously develop chronic otitis media (OM). Previously a rare PLG variant c.112A>G (p.(Lys38Glu)) was identified as a genome-wide significant risk factor for OM. Objective: We aim to identify rare, coding PLG variants in families with OM. Method: DNA samples from affected individuals from 28 Minnesota and 214 Finnish families were submitted for exome sequencing. Coding variants within PLG were Sanger-sequenced in additional family members. PLG variants were checked for minor allele frequency (MAF) within the Finnish population in the genome Aggregation Database (gnomAD) and prediction of functional effect on protein based on multiple bioinformatics tools, namely CADD, MutationTaster, PolyPhen-2 and PROVEAN/SIFT. Results: From the families with exome data, three Minnesota and five Finnish families were positive for rare, damaging PLG variants. Four PLG variants were identified as heterozygous, namely: c.112A>G (p.(Lys38Glu)) in one Finnish and two Minnesota families; c.782G>A (p.(Arg261His)) in three Finnish families; c.1481C>T (p.(Ala494Val)) in one Finnish proband; and c.2045T>A (p.(Ile682Asn)) in one Minnesota family. Three out of four multi-affected families showed incomplete co-segregation of PLG variants with OM in an autosomal dominant pattern, suggesting intra-familial heterogeneity or the occurrence of phenocopies. Two of the identified variants, c.112A>G (p.(Lys38Glu)) and c.782G>A (p.(Arg261His)), were previously associated with autosomal recessive type I plasminogen deficiency, however no second coding PLG variant was identified in the same families. All four variants were predicted to be deleterious by majority of bioinformatics tools used, with CADD scores of 19.0-27.6. The four variants were rare (MAF<0.005) in all gnomAD populations, except for c.1481C>T (p.(Ala494Val)) which had a MAF=0.056 in the Ashkenazi Jewish population. Majority of our families with PLG variants had recurrent acute OM. Conclusion: We identified four PLG variants in eight families with

autosomal dominant OM, suggesting a novel association between heterozygous PLG variants and familial OM.

Title: The Relationship between ABO Variants and Otitis Media in Finnish Families

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Introduction: Previously an intronic variant rs781822643 within the ABO locus was identified as a genome-wide significant protective factor against otitis media. Earlier studies demonstrated an association between blood type and otitis media, such that type O is protective against otitis media with effusion, while type A increased risk for acute or secretory otitis media.
Objective: To determine if there was any association between ABO variants or blood types and otitis media. Method: DNA samples from 214 probands from Finnish families with recurrent acute otitis media (RAOM) and/or chronic otitis media with effusion (COME) were submitted for exome sequencing. Of these Finnish probands, 43 had blood types available from clinical records. Using the exome data, blood type was predicted for the rest of the Finnish cohort: (1) Heterozygous genotypes for a haplotype with three ABO variants [c.703G>A(p.Gly235Ser);c.796C>A(p.Leu266Met);c.803G>C(p.Gly268Ala)] conferred a B phenotype. (2) For ABO c.260insG (p.Val87_Thr88fs*), the wildtype genotype resulted in type O while homozygous genotype conferred the A phenotype. (3) Type AB was assigned if heterozygosity for the 3-variant haplotype and homozygosity for c.260insG co-occurred. (4) All other genotypes were assigned as type A. For the general Finnish population, the minor allele frequencies for variants c.260insG and c.703G>A were derived from the Finnish genotypes in the genome Aggregation Database (gnomAD). Fisher exact tests were performed when (a) comparing frequencies of ABO genotypes in the Finnish probands with otitis media vs. counts in gnomAD Finnish, and (b) within the Finnish family cohort, comparing occurrence of RAOM vs. COME according to ABO genotype/haplotype and predicted blood type. Age and sex were also included in the study as potential covariates in logistic regression analysis.
Results: Fifteen coding variants within the ABO gene were identified, of which the [c.703G>A(p.Gly235Ser);c.796C>A(p.Leu266Met);c.803G>C(p.Gly268Ala)] and c.260insG variants were the most significant expression quantitative trait loci for airway or mucosal tissue in the Genotype-Tissue Expression database. The proportions of predicted blood types within the Finnish family cohort was comparable to the general Finnish population according to national data. Female sex is protective against having both RAOM and COME (OR=0.51; p=0.02). No association between otitis media and genotypes for the ABO c.260insG and c.703G>A genotypes were identified. Type O was protective against RAOM (OR=0.33; p=0.04). On the other hand, type A was associated with increased risk for COME (OR=2.20; p=0.03). These findings remained significant even when adjusted for age and sex.
Conclusion: Within the Finnish family cohort, blood type O is protective against RAOM while type A increases risk for COME. This suggests that the association between the ABO locus and otitis media is specific to blood type, otitis media type and cohort.

Title: RGD: Resources for Translational Research in Otitis Media

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Introduction: RGD (https://rgd.mcw.edu) is a multispecies resource for translational research and comparative genomics. Most notably for researchers working with chinchilla as a model for otitis media (OM), RGD now contains all of the data and functionality of the previous Chinchilla Research and Resource Database, and more. Results: The full incorporation of chinchilla data into RGD provides chinchilla researchers with the advantage of being able to leverage a wealth of both human data and data associated with commonly used model organisms such as rat and mouse. RGD curators manually review the literature for information on the association of genes with diseases, pathways and phenotypes, as well as information on gene functions, processes and subcellular localization. The information is standardized and assigned to the appropriate genes for the species studied, then propagated to the orthologous genes in other species. This data is augmented by the output of a number of automated pipelines which regularly import a substantial volume of data from sources such as Mouse Genome Informatics for phenotype annotations based on mouse knockouts, and OMIM, ClinVar and the Comparative Toxicogenomics Database for human disease annotations. The consolidation of data from multiple sources, including the sharing of data across species, gives researchers easy access to a substantial amount of data even when their gene or genes of interest have not been studied in their organism of choice. To facilitate access to data and tools of interest to chinchilla researchers, RGD has added a new species-specific portal for chinchilla at https://rgd.mcw.edu/wg/chinchilla/. The Chinchilla Portal gives links directly to tools such as the OLGA Object List Generator and Analyzer for finding chinchilla genes that match specified criteria, and the PhenoMiner tool to query for quantitative phenotype data in chinchilla. For researchers interested in viewing a gene of interest in its genomic context, the portal provides a link to the chinchilla JBrowse genome browser. The portal also supplies direct links to data of interest to chinchilla researchers such as the ontology report page for otitis media which lists all of the genes associated with OM in chinchilla and other species, including human. Conclusion: The RGD website provides seamless access for a wide variety of data types across multiple species, including chinchilla, making RGD an invaluable resource for researchers interested in chinchilla specifically, and in otitis media and related diseases in general.

Title: Phase Variation of Nontypeable Haemophilus influenza Affects Mucosal Immune Responses in The Nasopharynx

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Objective: Nontypeable Haemophilus influenzae (NTHi) are considered the chief pathogens in both acute otitis media and otitis media with effusion (OME), and most strains cultured from middle ear effusions are identical with those found in the nasopharynx of patients with OME. NTHi has phosphorylcholine (ChoP) on the surface of lipooligosaccharides (LOS), and our previous study revealed that the sickness-period of otitis media with effusion caused by ChoP (+) NTHi was longer than that of ChoP (-) NTHi in clinical cases. In this study, we investigated the host immune responses to phase variation by using mouse models of nasopharyngeal clearance with clinically isolated NTHi strains. Method: Animals were inoculated with ChoP (+) and ChoP (-) NTHi strains into the nose, and then euthanized at 12 hours, day 1, 3, and 7 after inoculation. Nasopharyngeal washes (NW) were collected, and then used for viable bacterial counts and an estimation of various inflammatory cytokines by Bio-Plex® assay. Results: The numbers of ChoP (+) NTHi in NWs from 12 hours to day 7 were higher than of ChoP (-) NTHi, and the levels of interleukin-(IL-)1, IL-6, KC and tumor necrosis factor-increased in NWs in the both ChoP (+) and ChoP (-) NTHi strains, and the levels of these cytokines were significantly higher in ChoP (+) NTHi than those in ChoP (-) NTHi at 12 hours and day 1 after inoculation, however, the level of these cytokines was not different between ChoP (+) and ChoP (-) NTHi at day 3 and 7. Conclusion: ChoP expression of NTHi affected mucosal immune responses in nasopharynx, as well as adhesion on nasopharynx. ChoP expression of NTHi may be related to prolongation of the duration of OME by not only nasopharyngeal adherence but also mucosal inflammation.

Title: Effect of Middle Ear Inflammation on Progression of Middle ear Cholesteatoma

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Objective:Middle ear cholesteatoma is characterized by a mass lesion formed by keratinizing squamous epithelium, keratin debris with or without inflammatory reaction. It is known that some cytokines, including interleukin(IL)-17, tumor necrosis factor alpha (TNF-α),inducesabnormally excessive growth of the epidermal layer of the skin.In this study, we examined if the inflammatory responses in the middle ear affect progression of cholesteatoma according to classification and staging system in Japan. Method:We used 16 granulomas from the middle ear. When we performed tympanoplasty for 16 cholesteatoma cases, we excised samples surgically. Frozen sections were made for HE staining and immunohistochemistry for IL-17 and TNF-α. The number of IL-17 and TNF-α positive cells in immunostaining were classified into three grades, from 1 (low), 2 (moderate) to 3 (high).RNA wasalso extractedfrom the granulomas, and IL-6, IL-17A, IL-17F, TNF-α-mRNAexpressions inthe granulomas were investigated by real-time RT-PCR. We analyzed the relation of the level of these cytokine to classification and staging system in Japan. Results:The grades of IL-17 staining had positive correlation with the degree of progressof cholesteatoma, and the grade of TNF-α staining had negative correlation with the degree of progressof cholesteatoma. However, there was no statistical difference between the grade of staining and progression of cholesteatoma. In the relationship between the -ΔCT value of each target gene and the degree of progress cholesteatoma, In IL-6-mRNAexpression, statistically significant differences were observed at a significance level of 5% for Spearman's correlation coefficient. In addition, although there was no statistically significant difference in IL-17A, IL-17F, TNF-α-mRNAweak positive correlation was observed. Conclusion:In this study, IL-17 and TNF-α did not show the evidence to facilitate progression of cholesteatoma in the middle ear. Although IL-6 has been reported to be inflammatory cytokines, it is reported that IL-6 suppress the terminal differentiation of human keratinocytes. IL-6 may be related to inhibition of the expansion of cholesteatoma.

Title: Effect of Mucosal Adjuvant on Innate Lymphoid Cells 2 in Mucosal and Lymphoid Tissues.

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Objective:Acute otitis media (AOM) is one of the most common infectious diseases in children, andNontypeableHaemophilus influenzae(NTHi) is Gram-negative bacteria that are considered major pathogens of AOM and respiratory tract infections.Previously, we reported that intranasal immunization with outer membrane protein or P6 from NTHi and mucosal adjuvants can induce antigen-specific immune responses in systemic and upper airway, and it enhances NTHi clearance from the nose and middle ear. Mucosal adjuvants, such as cholera toxin, are important to elicit mucosal and systemic immune responses to bacterial antigens. In recent years, innate lymphoid cells (ILC), is known to participate in various mucosal immune responses. In this study, we investigate the effect of mucosal adjuvant via nasal route on innate lymphoid cells 2 (ILC2) in mucosal tissues, cervical lymph nodes and spleen. Method:BALB/c (male: 5-6 weeks old)mice were intranasally administered 1 mg cholera toxin(CT) once a week for 1 or 2 times. Control mice were administered phosphate-buffered saline alone. At 1 week after CT administration, these mice were sacrificed to collect nasal mucosa, lung, cervical lymph nodes and spleen. Mononuclear cells (MNCs) were isolated from these tissues by enzymatic digestion orphysical disassociation. ILC2 were concentrated by using the Mouse ILC2 Enrichment Kit. The collected ILC2 rich cells were stained with Pacific blue-labeled anti-CD 45 antibody, FITC-labeled anti Linear (+) antibody, PE-labeled CD 278 antibody, and flow cytometric analysis was performed to examine the proportion of ICL2 in ILC2 rich cells. Results:The percentage of ICL2 in the nasal mucosae increased according to the number of doses compared to the control, but no change was observed in the cervical lymph nodes and the spleen. In the lung tissues, the proportion of ICL2 was slightly increased in a single dose, but not in second dose compared to the control. Conclusion:CT has been used as a potent adjuvant for inducing mucosal immune responses. Our studyindicated that nasal administration of CT adjuvant may affect ICL2 population in the nasal mucosa, and ICL2 may be related to inducing mucosal immune responses to antigen.

Title: Beyond the Otopscope: Emerging Technologies for the Diagnosis of Otitis Media

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Objective: To present new experimental technologies for otitis media (OM) diagnosis which have already been tested in clinical settings in humans. Method: We searched the following MeSH terms: ["diagnosis"] AND [all forms of OM] AND ["human"] AND ["ear"] and ["tympanic membrane"] in papers published in various electronic databases between 1/1/2005-4/20/2018. Our search revealed innovative diagnostic technologies which rely on and take advantage of the physical properties of the tympanomastoid cavity components: tympanic membrane (TM) thickness, its translucency and compliance; middle ear fluid characteristics; biofilm presence; increased tissue metabolic activity in OM states and fluid presence in the mastoid cavity. All these parameters are taken into account to establish OM diagnosis in a more objective manner. Results: Initial search revealed 549 publications, which were then reduced to 47 publications reporting on 12 new technologies for OM diagnosis. We discuss the following technologies: spectral gradient acoustic reflectometry, digital otoscopy, TM image analysis, multi-color reflectance imaging, anti-confocal middle ear assessment, optical coherence tomography, quantative pneumatic otoscopy, trans-mastoid ultrasound, wideband measurements, TM thickness mapping, shortwave infra-red imaging and wideband acoustic transfer functions. Conclusion: New experimental technologies are gradually introduced to overcome the difficulties of standard otoscopy. Their main advantage is the objective evaluation of the TM morphology, determination the presence of middle ear fluid and evaluate its content, and thus they can potentially replace standard otoscopy. Because these technologies are still under investigation and are pending widespread clinical use, their implementation in the market depends on their success in clinical trials, as well as on their future cost.

Title: Aboriginal Australian Otitis Prone children have reduced natural antibody titres to NTHi vaccine candidate antigens associated with attachment and biofilm formation

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Introduction: Nontypeable Haemophilus influenzae (NTHi) is the most common bacterial otopathogen associated with otitis media (OM), persisting in biofilms on the middle ear mucosa of otitis-prone (OP) children. Australian Aboriginal children acquire NTHi in early infancy and have exceptionally high rates of NTHi OM. Protein-based vaccines specifically targeting NTHi disease are under development. These include antigens associated with both infection and persistence (rsPilA, ChimV4 and Protein D). We have previously shown that Australian Aboriginal OP children have lower Protein D serum IgG titres than their non-Aboriginal counterparts. Objective: To assess the natural serum antibody titres to NTHi vaccine candidate antigens rsPilA, ChimV4, OMP26 (and confirm Protein D titres) in children; focusing on high-risk OP cohorts including Australian Aboriginal children. Method: Serum was collected from Aboriginal OP children (n=77), non-Aboriginal OP children (n=70) and healthy non-Aboriginal controls (n=36). Naturally acquired antigen-specific serum IgG was measured using an in-house multiplex fluorescent bead immunoassay. Antibody titres were adjusted for age, and Geometric Mean Concentrations (GMCs) compared between cohorts using a univariate analysis model. Results: Australian Aboriginal OP children had lower serum IgG titres to rsPilA, ChimV4 and Protein D (GMC: 88.94, 429.66 and 194.81 AU/mL respectively) compared to non-Aboriginal OP children (GMC: 240.57, 1210.76 and 493.24 AU/mL respectively), and Healthy Controls (227.69, 872.18 and 509.28 AU/ml respectively) $p<0.05$. No significant differences between non-Aboriginal OP children and healthy children were observed. Serum IgG titres to OMP26 were similar between groups (GMC: Australian Aboriginal OP children 1060 AU/mL, non-Aboriginal OP children 1054 AU/mL and healthy children 820.2 AU/mL), $p>0.5$. Conclusion: Australian Aboriginal OP children had lower antibody titres to most major NTHi vaccine candidate antigens, suggesting a failure to develop antibodies in response to NTHi exposure. Similar anti-OMP26 IgG titres show that this deficiency does not exist to all NTHi antigens. Our data further demonstrate that both population and antigen-specific differences occur in response to infection with NTHi, and this may contribute to the increased susceptibility of Australian Aboriginal children to OM. Importantly, this vulnerable population may benefit from active immunisation with antigens associated with biofilm/adhesion and should be a major consideration for development of future vaccines.

Title: Parent Satisfaction, Adherence to Guideline and Symptom Relief in Children with Otitis Media undergoing Tympanostomy Tube insertion - a Prospective Multicentre Study

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Objective: This study investigates parent satisfaction, adherence to guideline, and symptom relief in children younger than 12 years undergoing tympanostomy tube (TT) insertion for otitis media (OM) using electronic patient-reported outcome (ePRO) data in private ear-nose & throat (ENT) practice settings. Methods: A total of 3.572 children aged 0-11 years and registered in the DØNHO database were included. Following parental consent to participate, the Danish National Tympanostomy tube Insertion Questionnaire's (DANTIQ) consisting of a pre-surgical questionnaire and follow-up questionnaires at 1, 3, 6, 9 and 12 months after surgery were e-mailed to the parents. The pre-operative questionnaire collected information on symptom duration, number of acute OM (AOM) episodes within one year before TT insertion and ear-related symptoms. The post-operative questionnaires collected information on symptom relief, number of AOM episodes and parental satisfaction. Criteria for adherence to guideline were 1) Symptom duration of three months or longer (COME), 2) three or more AOM episodes within six months and 3) four or more AOM episodes within 12 months (RAOM). Results: Pre- and post-operative questionnaires from 2,462 children were eligible for complete analysis. Before surgery, 89.8% of parents reported a symptom duration of three months or longer and/or recurrent AOM (RAOM) in accordance with the Danish National Guideline. Complete symptom regression was reported in more than half of the children post-operatively. For the rest, significant symptom relief was reported 1-12 months following TT insertion. Parent satisfaction rose from 94.6% to 97.2% throughout the observation period. Conclusions: We report a high degree of adherence to guidelines, a consistently high rate of symptom relief 1-12 months following TT insertion in Danish children below 12 years of age. Furthermore, parental satisfaction throughout the 12-month observation period was compelling.

Title: A Phase 1 Safety Study of Repeated Doses of Intranasal OP0201 Metered Dose Inhaler Compared to Placebo in Healthy Adults: A Potential Treatment for Otitis Media

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Introduction: OP0201 nasal aerosol is a novel, surfactant being developed to treat and prevent otitis media. OP0201 is a 20:1 fixed combination of dipalmitoylphosphatidylcholine (a phospholipid surfactant) and cholesteryl palmitate (a neutral phospholipid spreading agent) suspended in propellant. Intranasal OP0201 is intended to absorb to mucosal air-liquid interface and reduce Eustachian tube (ET) interfacial surface tension and in turn reduces ET passive opening pressure resulting in 'de-sticking' and restoring ET physiologic activity. ET dysfunction is an important underlying cause of otitis media. Objective: To evaluate safety and tolerability of 14 days of intranasal OP0201 (30 mg per day) compared to placebo in healthy adult volunteers. Method: Phase 1, randomized, double-blind, placebo-controlled, parallel-group, dose-escalation study in adults at 1 study center in the United States. Two dose cohorts are planned (N=15 per cohort). Within each cohort, subjects are randomized 4:1 to receive OP0201 or placebo (stratified by gender; male versus female). Eligible subjects are admitted to the study center and randomized. Subjects remain in resident at the study center until all Day 14 assessments are completed. A Day 21 follow-up visit is planned. Study treatment will be administered to the subjects by the study center staff three times a day for 14 days. Safety endpoints include adverse events (AEs), otoscopy, tympanometry, nasal and epipharynx endoscopy, olfactory test, pure tone hearing test, electrocardiogram, physical examination and clinical laboratory tests. Results: Fifteen subjects were randomized to Cohort A (30 mg per day); all completed the study as planned. Subjects were Caucasian (60%) or Black (40%), female (53.4%), with a mean age of 34 years (range 20-49). No severe or serious AEs occurred during the 14 days of treatment or during follow up. All AEs resolved without sequelae. Twelve mild or moderate AEs occurred; 3 reported as not related to blinded study treatment (pain upper right arm, somnolence, toothache) and 9 reported to be related to blinded study treatment (headache [5 reports in 4 subjects], nasopharynx dryness/irritation [3 reports in 3 subjects, all mild] and common cold [1 report]. All safety results were within normal limits or changes were rated as not clinically significant. After all subjects in Cohort A completed the Day 14 visit, a Safety Review Committee reviewed all the blinded safety data and determined the doses administered were safe and well tolerated, and recommended escalation to the next cohort (Cohort B 60 mg/day). Conclusion: In this Phase 1 study, OP0201 was found to be safe and well tolerated in adult volunteers. Future development studies are planned to evaluate OP0201 to treat acute and chronic otitis media in infants and children.Introduction: OP0201 nasal aerosol is a novel, surfactant being developed to treat and prevent otitis media. OP0201 is a 20:1 fixed combination of dipalmitoylphosphatidylcholine (a phospholipid surfactant) and cholesteryl

palmitate (a neutral phospholipid spreading agent) suspended in propellant. Intranasal OP0201 is intended to absorb to mucosal air-liquid interface and reduce Eustachian tube (ET) interfacial surface tension and in turn reduces ET passive opening pressure resulting in 'de-sticking' and restoring ET physiologic activity. ET dysfunction is an important underlying cause of otitis media. Objective: To evaluate safety and tolerability of 14 days of intranasal OP0201 (30 mg per day) compared to placebo in healthy adult volunteers. Method: Phase 1, randomized, double-blind, placebo-controlled, parallel-group, dose-escalation study in adults at 1 study center in the United States. Two dose cohorts are planned (N=15 per cohort). Within each cohort, subjects are randomized 4:1 to receive OP0201 or placebo (stratified by gender; male versus female). Eligible subjects are admitted to the study center and randomized. Subjects remain in resident at the study center until all Day 14 assessments are completed. A Day 21 follow-up visit is planned. Study treatment will be administered to the subjects by the study center staff three times a day for 14 days. Safety endpoints include adverse events (AEs), otoscopy, tympanometry, nasal and epipharynx endoscopy, olfactory test, pure tone hearing test, electrocardiogram, physical examination and clinical laboratory tests. Results: Fifteen subjects were randomized to Cohort A (30 mg per day); all completed the study as planned. Subjects were Caucasian (60%) or Black (40%), female (53.4%), with a mean age of 34 years (range 20-49). No severe or serious AEs occurred during the 14 days of treatment or during follow up. All AEs resolved without sequelae. Twelve mild or moderate AEs occurred; 3 reported as not related to blinded study treatment (pain upper right arm, somnolence, toothache) and 9 reported to be related to blinded study treatment (headache [5 reports in 4 subjects], nasopharynx dryness/irritation [3 reports in 3 subjects, all mild] and common cold [1 report]. All safety results were within normal limits or changes were rated as not clinically significant. After all subjects in Cohort A completed the Day 14 visit, a Safety Review Committee reviewed all the blinded safety data and determined the doses administered were safe and well tolerated, and recommended escalation to the next cohort (Cohort B 60 mg/day). Conclusion: In this Phase 1 study, OP0201 was found to be safe and well tolerated in adult volunteers. Future development studies are planned to evaluate OP0201 to treat acute and chronic otitis media in infants and children.

Title: Topical and Systemic Interventions for Chronic Suppurative Otitis Media: A Suite of Cochrane Reviews

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Introduction: Chronic suppurative otitis media (CSOM) is the leading cause of preventable, permanent hearing loss globally. Objective: To systematically review the effectiveness of the most common topical and systemic treatments for CSOM using seven key comparisons identified in a scoping review and global consultation process including patients, clinicians and researchers: i) topical antibiotics, ii) topical antibiotics with steroids, iii) systemic antibiotics, iv) topical versus systemic antibiotics, v) antiseptics, vi) topical antibiotics versus topical antiseptics and vii) aural toileting (ear cleaning). Method: The Cochrane ENT Information Specialist searched the Cochrane ENT Register; Central Register of Controlled Trials; Ovid Medline; Ovid Embase; CINAHL; Web of Science; Clinicaltrials.gov; ICTRP and additional sources for published and unpublished trials. The date of the search was 12 March 2018. The reviews included randomised controlled trials with at least a one-week follow-up with patients (adults and children) who had chronic ear discharge of unknown cause; or CSOM, where the ear discharge had continued for more than two weeks. We used the standard Cochrane methodological procedures. Our primary outcomes were: resolution of ear discharge or 'dry ear' (whether otoscopically confirmed or not), measured at between one week and up to two weeks; two weeks to up to four weeks; and after four weeks; health-related quality of life using a validated instrument; ear pain (otalgia) or discomfort or local irritation. Secondary outcomes included hearing; serious complications; suspected ototoxicity including sensorineural hearing loss; balance problems/dizziness/vertigo; tinnitus. The same methods and outcome measures are shared across the seven reviews allowing evidence for all treatment options to be compared for relative effectiveness and quality of evidence. Results: The searches retrieved a total of 2990 unique references. After title and abstract screening we assessed 212 full texts. 49 studies met the inclusion criteria (with some studies addressing multiple comparisons): 26 studies examined topical antibiotics +/- steroids, 16 studies examined systemic antibiotics, 5 studies examined topical antiseptics, 6 studies examined topical versus systemic antibiotics and 8 studies examined antibiotics versus antiseptics and 3 studies examined aural toilet for CSOM. Conclusion: The findings from these reviews will help inform global policy and practice in CSOM treatment and direct future research efforts in trials examining the effectiveness of topical and systemic treatments for CSOM. This suite of Cochrane reviews has highlighted

a lack of high-quality clinical trials for CSOM treatments, with poor reporting of adverse effects, limited duration of follow-up and few studies reported the impact of treatment on hearing outcomes.

Title: Immunization with Outer Membrane Vesicles from htrB Mutant Nontypeable Haemophilus influenzae Protects against Experimental Otitis Media in Chinchillas

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Introduction: We previously reported that immunization with nontypeable Haemophilus influenzae [NTHi] outer membrane vesicles [OMVs] protected chinchillas against experimental otitis media [Clin Vaccine Immunol 2017]. NTHi OMVs expressing native lipooligosaccharide would be unacceptable in human vaccines because of excessive reactogenicity. The NTHi htrB gene encodes an acyltransferase involved in lipid A biosynthesis and lipooligosaccharide from htrB mutant strains results in a markedly reduced inflammatory response. Objective: To construct prototype htrB mutant NTHi, to assess inflammation induced by htrB mutant OMVs in vitro, and to determine whether immunization with htrB mutant OMVs combined with NTHi HMW1/HMW2 adhesion proteins protects against experimental otitis media. Methods: htrB mutant NTHi were derived from prototype strains 5, 12, and 15 by insertional inactivation of the respective htrB genes with a spectinomycin resistance cassette. OMVs from parent/htrB mutant pairs were compared in vitro in cytokine release assays using human peripheral blood mononuclear cells [PBMC]. HMW1/HMW2 proteins were purified from each prototype strain using previously described methods [Clin Vaccine Immunol 2014]. Chinchillas were immunized with three monthly subcutaneous injections of purified OMVs combined with HMW1/HMW2 proteins with monophosphoryl lipid A [MPLA] used as adjuvant. Control animals were immunized with MPLA alone. One month after the last immunization, chinchillas were challenged by direct intrabullar inoculation with the homologous NTHi strain. Protection was assessed by comparing total number of animals infected in each immunized group versus the MPLA controls and by comparing relative bacterial densities in middle ear fluid [MEF] specimens from immunized animals versus controls. Results: In PBMC cytokine release assay, the htrB mutant OMVs induced 100- to 10,000-fold less TNFα and IL-1β than did the respective parent OMVs. Immunization with htrB mutant OMVs plus HMW1/HMW2 proteins provided nearly complete protection against culture-positive otitis media following homologous NTHi challenge: 8 of 8 strain 5 challenged animals, 13 of 16 strain 12 challenged animals, and 8 of 8 strain 15 challenged animals [$p < .001$]. All MPLA-immunized control animals developed moderately severe culture-positive otitis media with all three strains. In OMV-immunized animals that did develop culture-positive otitis, MEF bacterial concentrations were markedly lower than those of MPLA controls: geometric mean 1.7×10^2 vs 2.7×10^7 [$p < .001$]. Conclusions: These data confirm the importance of the htrB gene product in impacting the inflammation caused by NTHi LOS. They also show that inactivation of the htrB gene does not impair the ability of NTHI OMVs to function as potent immunogens and protective vaccines in the chinchilla model. Finally, they suggest htrB mutant OMVs hold significant potential as components of future NTHi vaccines.