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Immunologic Dysfunction Contributes to the Otitis Prone Condition

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Abstract

Acute Otitis Media (AOM) is a multifactorial disease occurring mostly in young children who are immunologically naïve to AOM pathogens. This review focuses on work from Rochester NY, USA over the past 12 years among young children who had AOM infections microbiologically-confirmed by tympanocentesis, so called “stringently-defined”. Among stringently-defined otitis prone children deficiencies in fundamental immune defense mechanisms have been identified that contribute to the propensity of young children to experience recurrent AOM. Dysfunction in innate immune responses that cause an immunopathological impact in the nasopharynx have been discovered including inadequate proinflammatory cytokine response and poor epithelial cell repair. Adaptive immunity defects in B cell function and immunologic memory resulting in low levels of antibody to otopathogen-specific antigens allows repeated infections. CD4+ and CD8+ T cell function and memory defects significantly contribute. The immune profile of an otitis prone child resembles that of a neonate through the first year of life. Immunologic deficits in otitis prone children cause them to be unusually vulnerable to viral upper respiratory infections and respond inadequately to routine pediatric vaccines.

Keywords

acute otitis media; otitis prone; innate immunity; adaptive immunity; *Streptococcus pneumoniae*; *Haemophilus influenzae*; *Moraxella catarrhalis*; antibody; CD4+ T cells; B cells; antigen presenting cells

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Acute otitis media (AOM) is extremely common, drives antibiotic use, emergence of antibiotic resistant bacteria and is costly. Temporary hearing loss is the most common complication; rarely there are intracranial complications.[1] The World Health Organization estimates that 51,000 deaths/year are attributable to AOM in children younger than 5 years old and that chronic otitis media (occurring in 65-330 million people) is the major cause of hearing loss in developing countries.[2,3] Each episode of AOM is typically followed by 4-12

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weeks of otitis media with effusion (OME) during which time the child has diminished hearing and this often leads to temporary delayed speech and language development and can be associated with permanent hearing loss.[4-6] In the US alone, the economic burden of otitis media exceeds $6 billion/year in medical treatment, surgical management, and loss of income for working parents.[7]

For several decades the underlying pathogenesis of AOM in children has been attributed to Eustachian tube dysfunction as most important.[8] Children demonstrate a diminished propensity to develop AOM over time and this change has long been thought due to the Eustachian tube anatomy of children transitioning to more “adultlike” by age three to five years old to account for the frequency of AOM infections being outgrown. Direct evidence for this explanation of otitis proneness (OP) is lacking in very young children since Eustachian tube functional testing has not been reported in children below age three years old.[9,10] Evidence supporting the role of Eustachian tube dysfunction derives mostly from studies in older children and adults[11] although persistent dysfunction occurs after children have outgrown their propensity for recurrent AOM[12] which argues against a causative link. OM has been described to be a heritable condition based on a twin study[13] and single nucleotide polymorphisms involving immune mediating cytokines have been described in OP children.[14-17] However, a clinical condition occurring as frequently as OP likely has other underlying causes.

Our work over the past decade brings forward an immunologic explanation for OP susceptibility and our work in Rochester, NY USA is the focus of this review. In our studies, clinically diagnosed children with AOM and confirmed by tympanocentesis were defined as stringently OP (sOP) if they experienced 3 separate AOM infections within a 6 month or 4 AOM infections within a 12 month time span. Children experiencing fewer AOM infections, confirmed by tympanocentesis, were defined as non-otitis prone (NOP). Other groups studying immunological characteristics of OP chldren have most often found deficiencies [8-21] but those studies did not restrict the definition of OP to cases where microbiologic confirmation occurred.[1] It is probable that a requirement for microbiologic proof of AOM refines the study population to allow clearer outcome differences during immunologic studies. Indeed, with a strict definition, we have identified multiple deficiencies in innate and adaptive immunity among young children who are sOP and introduced the term “prolonged neonatal-like immune profile (PNIP) because of striking similarities in immune responses seen in sOP children that resemble neonatal immunity.[22,23] Specifically, we observed that peripheral blood mononuclear cells (PBMCs) from sOP children between the ages of 6 and 12 months display a general skewing away from Th1 and Th17 immunity and toward Th2 and Treg dominance and a lack of enhanced inflammatory cytokine production by antigen presenting cells (APCs).[24,25] We found that the immune problems of sOP children were largely outgrown by age three years old coinciding with the epidemiologic observation of diminishing AOM in that age time frame. In previous studies, sOP children were not diagnosed by otolaryngologists. The clinical diagnoses were made by referring primary care physicians. Unfortunately, if 50% of the children entering those prior studies did not have recurrent AOM and were not authentic OP children then those previous studies may have missed the immune deficiencies we identified because their study populations were significantly contaminated by non-otitis prone (NOP) children. The innovation of our work

has been to apply stringent diagnostic criteria and microbiologic verification prospectively in a longitudinal study design that eliminates children who are misdiagnosed.

The significance of our investigations extends beyond application to OP children. We have shown that sOP children are prone to more frequent clinically-diagnosed, (a subset confirmed by virus-specific detection) viral upper respiratory infections[26] and 23% of sOP children respond with sub-protective antibody levels after routine pediatric vaccinations.[22,27]

In this review we describe the accumulated results from 2006-2018, of studies involving 796 children, from whom we have prospectively collected about 20,000 samples that included blood, peripheral blood mononuclear cells (PBMCs), nasal swabs, nasal washes and/or oropharyngeal swabs during 3837 healthy planned visits at ages 6, 9, 12, 15, 18, 24, and 30-36 months of age and 1276 AOM visits, all before child age 36 months. 75 (~10%) children met the sOP definition. Children that did not meet the sOP criteria we classify as NOP. There are many factors that may contribute to children with recurrent AOM[28] but our work suggests that primary among those factors are immunologic deficiencies described here (Table 1 summarizes the findings).

MUCOSAL NASOPHARYNGEAL COMPARTMENT (Figure 1)

Innate Responses

Neutrophils—Neutrophil activation and bacterial ingestion represent part of the innate immune response. In a study of NP samples, we found that the neutrophil count increased roughly 600-fold during AOM in sOP and NOP children.[29] We further conducted studies of neutrophil recruitment and inflammation in the NP in children during health, viral URI and AOM in the presence or absence of NP colonization by *Streptococcus pneumoniae* (*Spn*).[29] We found no evidence of dysfunctional neutrophil recruitment or bactericidal ROS production by Neutrophils.[30] We compared difference of gene expression of inflammatory effectors from neutrophils of sOP and NOP children during AOM. Real-Time PCR (RT-PCR) was used to assess gene expression of inflammatory effectors. We found that sOP children had decreased mRNA expression of cytokine IL-1β from neutrophils.[29]

Epithelial and cytokines—We studied the role of epithelial and innate mucosal responses to NP colonization in our sOP child population during a viral URI that proceeded to cause AOM due to *Spn*.[30] We found significantly lower epidermal growth factor, epidermal growth factor receptor and angiogenin concentrations in the NP of sOP compared with NOP children suggesting lower capacity for epithelial repair in sOP children. We also found significantly lower pro inflammatory neutrophil chemoattractants such as MIP-1β, IL-8 and CXCL5 (ENA-78 in the NP of sOP children at onset of AOM. These findings of lower epitheliall repair and impaired proinflammatory response provide other mechanisms as to why sOP children succumb to repeated AOM events.

In a separate study, we investigated whether compartmental responses differed by comparing the cytokine levels in NP, MEF and peripheral blood of sOP and NOP children during an AOM event caused by *Spn*.[31] Nasal washes from sOP children contained significantly higher levels of proinflammatory cytokine IL-2 but lower levels of IL-7 and insulin-like growth

factor-4 (IGFBP-4), both involved in proliferation and T-cell homeostasis, but similar expression levels of IFN-γ and IL-17a, both of which are normally protective responses to an infection from innate and adaptive cells. We compared the RNA expression of IFN-γ, IL-2 and IL-17a from the MEF. We found significantly heightened transcription of IL-2 in sOP children and no significant difference in IFN-γ or IL-17a transcript levels. However, plasma IFN-γ and IL-2 levels were significantly lower in sOP children while IL-17a was too low to measure in both cohorts. These results show that the middle ear cytokine response mirrored those of the nasal mucosa versus the peripheral blood, suggesting that proximal mucosal sites may better predict the quality of the middle ear response than peripheral blood. Our results also highlight the differences between local and systemic immune responses that could co-ordinate anti-bacterial immune responses in young children. In addition, this data further highlights that the sOP child has immune deficits that fail to increase recall responses after additional exposure to otopathogens upon additional infections.

In another study NOP children had significantly higher levels of IL-6, IL-10, INF-γ, TNF-α, IL-1β, MCP-1, RANTES, IL-2 and IL-17 during viral URIs versus AOMs following the URIs, when compared to sOP children.[26] In that same study, we analyzed the relationship between the NP cytokine/chemokine level and sOP rate using a logistic regression analysis, generalized additive model, we found that sOP children had significantly lower nasal pro-inflammatory levels of IL-6, IL-10, TNF-α and RANTES than NOP children during viral URIs.

Taken together, sOP children have more frequent viral URIs than NOP children, due to deficient antiviral nasopharyngeal pro-inflammatory cytokine and chemokine responses, and this facilitates the development of AOMs. Our findings of lower epidermal repair processes and lower inflammatory response in the nasal mucosa of sOP versus NOP children provide another possible mechanism which might contribute to the predisposition of the sOP child to repeated AOM events.

We studied mucosal antibodies levels in the NP to *Spn*. Specifically, we investigated mucosal IgG and IgA levels in the NP and MEF from children to three *Spn* antigens (PhtD, PcpA and Ply).[32] We showed that higher naturally acquired mucosal antibody levels to these antigens was associated with reduced AOM caused by *Spn*. We then sought to correlate the mucosal antibody levels in sOP children to those same pneumococcal proteins with *Spn* NP colonization and the occurrence of AOM.[33] We found that sOP children had significantly higher colonization frequency by *Spn* ($p < 0.0001$) and significantly lower IgG and IgA levels to all 3 *Spn* proteins compared with NOP children except IgG to Ply D1. *Spn* colonization in NOP children led to 2-fold to 5-fold increase in mucosal IgG and IgA levels to all 3 proteins, whereas *Spn* colonization in sOP children generally failed to elicit antibody responses. Taken together, these data on mucosal antibody supports our hypothesis that sOP children have an immunological defect in responding to natural immunization by NP colonization and AOM.

SYSTEMIC BLOOD COMPARTMENT (Figure 2)

Professional antigen presenting cells (APCs)

APCs bridge the innate immune system to the adaptive immune system by facilitating presentation of antigens to B cells and T cells. Therefore we sought to determine whether there might be defects in numbers, phenotype and/or function of APCs in the peripheral blood of sOP infants.[25] APC phenotypic counts, MHC II expression and intracellular cytokine levels were determined in response to TLR 7/8 stimulation using R848. Innate immune mRNA expression was measured using RT-PCR and cytokines were measured using Luminex technology. We found significantly higher numbers of monocytes and conventional DCs but not plasmacytoid DCs in sOP children even when healthy compared to NOP age-matched infants. The presence of increased numbers of monocytes and cDCs in the blood we hypothesized was consistent with the existence of a heightened pro-inflammatory state in sOP children between recurrent AOMs. However, we found that sOP and NOP infants produce similar levels of innate associated cytokines upon PBMC stimulation with a TLR agonist (TLR7/8). Baseline cytokine/chemokine levels, as well as expression levels of TLRs and intracellular signaling molecules from PBMCs in response to TLR stimulation were similar among sOP and NOP children, suggesting that sOP APC function might not be a major contributor to sOP immune hyporesponsiveness.

Adaptive Responses

Antibody—The three major otopathogens causing recurrent OM in our study children have been *Spn*, nontypeable *H. influenzae* (NT*Hi*) and *Moraxella catarrhalis* (*Mcat*). We have shown that nasopharyngeal (NP) colonization is not only a necessary first step toward infection, it also is a natural immunizing event. [34,35] We had hypothesized that sOP children would have reduced levels of antibodies to the principal otopathogens resulting in there being less adaptive immunity to control the growth and eventual spread of the bacterium from the NP to the middle ear (ME). To test that hypothesis, we determined serum IgG titers against *Spn* proteins PhtD, PhtE, LytB, PcpA, PlyD1 rather than serotype-specific capsule polysaccharides.[34] We found that sOP children had significantly lower IgG titers to PhtD, PhtE, LytB, PlyD1 than NOP children at healthy visits with asymptomatic NP colonization and at onset of AOM, supporting our hypothesis.

In a similar study, we investigated antibody levels to NT*Hi* protein antigens (P6, D, OMP26). Antibody levels to the three antigens measured longitudinally during NP colonization between age 6 and 24 months showed <2-fold increases over time in sOP children compared to >4-fold increases in NOP children.[35] Similar to our findings for protein vaccine candidates of *Spn* and NT*Hi*, sOP children displayed a later and a significantly lower peak of serum IgG antibody rise than NOP children for *Mcat* protein antigens (OppA, OMP CD, Hag5-9) during NP colonization of *Mcat*.[36] However, at time of AOM caused by *Mcat*, only serum IgG antibodies to OppA or Hag5-9 were significantly higher for NOP compared to sOP children.

Although these studies do not inform regarding protective levels of antibody, the fact that sOP children have significantly lower adaptive antibody levels to *Spn*, NT*Hi* and *Mcat*

antigens from infections by these otopathogens support the finding as to why they also have significantly higher colonization frequency by *Spn*, NT*Hi* and *Mcat* than NOP children.[33]

To extend the findings regarding antibody hypo-responsiveness[37-39] we sought to determine whether sOP children would also have lower antibody responses to pediatric vaccine immunizations. We analyzed sera collected from sOP and age-matched NOP children age 6–24 months for IgG concentrations to the DTaP antigens (diphtheria toxoid (DT), tetanus toxoid (TT), pertussis toxoid (PT), filamentous hemagglutinin (FHA), and pertactin (PRN)), polio, hepatitis B, *H. influenzae* type b capsule polyribosyl-ribitol-phosphate (PRP) and *Spn* capsular polysaccharide conjugate vaccine. sOP children were significantly more likely to have non-protective responses against DT, TT, hepatitis B, polio 3, and *Spn* 23F, but not polio 1 and 2, PRP, or *Spn* 6B and 14.[22] Lower putative protective responses to PT, FHA, PRN pertussis antigens were also observed. A high percentage of these sOP children had non-protective antibody titers to the pediatric vaccines tested and sub-protective levels persisted until 24 months of age in many sOP children despite routine vaccination boosters. Currently these vulnerable sOP children do not show evidence of higher rates of vaccine-preventable infections. However, we speculated that they are protected by herd immunity and in the United States and other countries where parent refusal of vaccines has increased or immunizations are limited, herd immunity may become threatened.

Memory B cells—Knowing that a high percentage of sOP children develop lower levels of antibody to otopathogenproteins during natural exposure via the mucosal route, we sought to determine the percentages of memory B cells to *Spn* antigens compared to NOP children.[40] We found that sOP children had significantly lower frequencies of antigen-specific memory B cells against 3 *Spn* protein antigens (PhtD, PhtE, and Ply). Additionally, these frequencies correlated positively with serum IgG levels to the same antigens. Since sOP children failed to develop protective antibody levels to standard pediatric vaccines when given parenterally, we examined memory B cell responses to the DTaP vaccine antigens (DT, TT, and PT) in healthy sOP and NOP children.[41] We found the frequency of total memory $CD19^+$ $CD27^+$ B cells was significantly lower in sOP children. Further, sOP children had significantly fewer memory B cells specific for DT, TT, and PT, and antigen-specific B cell frequencies correlated with serum IgG titers as in the earlier study.

We further analyzed specific aspects of the B-cells in sOP children. We found fewer switched memory B-cells as measured by CD19, CD27, IgG and IgM surface markers.[42] The B-cells of sOP children also showed reduced levels of expression of co-stimulatory molecules and TNF family receptors: transmembrane activator and calcium modulating cyclophilin-ligand interactor (TACI), together with B cell maturation antigen (BCMA) and B cell activating factor receptor (BAFFR).

Overall, our data indicate that sOP children have reduced memory B-cells to otopathogen antigens (and vaccines), reduced switched memory B-cells with IgM and IgG receptors and poor expression of TNF family receptors compared to NOP children which may lead to failure in generating *Spn* and NT*Hi* specific or vaccine antigen specific antibody generation.

CD4 T cells—We hypothesized that alterations in CD4 T-cell subsets in sOP children might also contribute to immune hyporesponsiveness, as compared to NOP children. Using 6 *Spn* and 3 NT*Hi* protein antigens, we enumerated *Spn*- and NT*Hi*- specific functional CD4 T-helper memory cell subsets in the peripheral blood of cohorts of sOP and NOP children with AOM or NP colonized with either *Spn* or NT*Hi*.[43] We found significantly reduced percentages of functional $CD45RA^{Low}$ memory CD4 T cells producing cytokines (IFN-γ, IL-2, IL-4 and IL-17A) in sOP children following AOM and NP colonization with either *Spn* or NT*Hi*. Thus we showed that sOP children also have a diminished ability to generate memory T cell responses after NP colonization and AOM. These data had particular importance with regard to IL-17A since data from mouse NP colonization models suggest that protection against *Spn* carriage is dependent on IL-17 expressing CD4 T cells by a mechanism involving IL-17A increasing *Spn* killing by neutrophils.[44,45] However, the percentage of functional memory CD4 T-cells was similar for sOP and NOP when stimulated with SEB ruling out an intrinsic defect in CD4 T-cells of sOP children.[43]

Peripheral blood mononuclear cell (PBMC) cytokine production and T cell skewing—Proinflammatory cytokines are known to be critical for protective immunity against *Spn* infection. Therefore, we compared extracellular cytokine levels from PBMCs of sOP and NOP children in response to *Spn* stimulation.[24] sOP children produced significantly lower levels of IFN-γ, IL-17A, IL-21 and IL-23 than NOP children. RNA analysis of *Spn* stimulated PBMCs also showed significant lower expression levels of IFN-γ, IL-2, IL-13, IL-17A, IL-23 and TGF-β. Furthermore, Th1 and Th17 fate-determining transcription factors T-box transcription factor 21 (TBX21) and RAR-related orphan receptor C (RORC) showed significantly lower expression levelsin samples from sOP children compared with NOP children while Foxp3 (Treg cell) expression was significantly higher from sOP children.

In a subsequent study, we heat killed *Spn* stimulation for in vitro stimulation of T cells.[24] We found significantly diminished memory CD4 ($CD69^{+}CD45RA^{-}$) T-cells producing IL-2, TNF-α and IL-17 in sOP children. Addition of exogenous Th17-promoting cytokines (IL- 6, −1β, −23 and TGF-β) restored CD4+ Th17 cell function in cells from sOP children to levels measured in NOP children. We also observed that both CD4 naïve ($CD69^{+}CD45RA^{+}$) and memory populations were significantly less activated in sOP children. In additional unpublished experiments we stimulated T-cells with TCR stimulating reagents mimicking DC activation (anti-CD3/CD28 Dynabeads), and found sOP children (age 6-9 months) had significantly impaired responses as measured by CD69 expression. Taken together our results indicate that sOP children generate fewer memory T cells and their T cells have deficiencies in their T cell receptors or T cell signaling after antigen priming.

Viral URI co-pathogenesis with otopathogens

A risk factor known to be associated with AOM is a preceding or concurrent viral upper respiratory tract infection (URI).[46] In our studies, at onset of AOM, 93% of the children had clinical signs of a viral URI.[47] We examined the differential impact of respiratory syncytial virus (RSV) and parainfluenza virus (PIV) URIs on the frequency of AOM caused by *Spn* and NTHi in sOP and NOP children as a potential mechanism to explain increased

susceptibility to AOM.[48] RSV was substantially more likely to contribute to AOM in sOP than in NOP children, and additionally sOP children were significantly more likely to be infected by RSV. This corresponded with significantly lower serum antibody titers against RSV in sOP children. PIV infections did not differentially affect AOM events in sOP and NOP children. To investigate the interface of a diminished neutralizing antibody response to virus and the correlative heightened viral replication we detected with neutrophil phagocytic function during AOM, an *ex vivo* phagocytic assay was developed. RSV impaired the phagocytic activity of neutrophils isolated from infected children significantly more than PIV. These data suggested that a failure to neutralize RSV could disrupt the capacity of neutrophils to engulf the bacterial otopathogen, facilitating the development of AOM. Taken together, the data show that lower innate and adaptive immune responses to RSV in sOP children allowed for viral interference with innate antibacterial immune responses, thus contributing to increased frequency of AOMs.

Correlation between sOP and Neonatal immunity

The immune system of sOP children clearly differs in many ways from that of NOP children. A terminology of “prolonged neonatal-like immune profile” was proposed to provide context to the overarching theme of the immune differences in sOP vs. NOP children.[22,23,27,42] The neonatal immune system is required to handle the abrupt transition to multiple, simultaneous antigenic stimulations from microbial colonization, exposure to food antigens, etc., so the responses must be muted through mechanisms of immune suppression. Thus the neonatal immune response tends to be anti-inflammatory rather than pro-inflammatory, resulting in predominance of anti-inflammatory innate cytokines. In neonates responses to TLR signals are dampened[49]. Newborn APCs are relatively lower in number with low basal levels of MHC-II surface expression and a decreased ability to produce cytokines.[39,50,51] Neonatal CD4+ T cells show preferential differentiation of Th2 cells over Th1 cells and higher numbers of T reg cells.[39,52,53] Defects in plasma cell differentiation occur due to limited T cell help[54]. Neonates produce lower levels of antibody following antigenic stimulation[39]. The ability of B cells to proliferate and differentiate into plasma cells and memory cells influences the levels of antibody and recall responses on antigen re-exposure, respectively. Neonatal B cells have decreased TNF family receptors[55]. TNF super family members expressed by B cells include BAFF and APRIL and 3 receptors: BAFFR, BCMA and TACi. Preterm newborn neonatal B cells show reduced proliferation in response to BAFF and anti-IgM and express less TACI, BCMA and BAFFR than adult B cells.[55,56] Taken together, these multiple features of neonatal immunity that have been identified in the sOP child provide the foundation for the proposed prolonged neonatal-like immune profile moniker.

Another conceptualization of the sOP child immune system might focus on immunosuppression and a chronic hyper-inflammatory state in the nasopharynx due to recurrent viral URI and otopathogens colonization. Early in life, the NP of children is colonized with multiple bacterial species, including potential otopathogens that occupy the nasal epithelial surface. NP colonization is the initial, necessary step in the pathogenesis of AOM. During viral URI, the major bacterial otopathogens can disseminate to the middle ear. Despite similar clinical care and demographic factors, we have shown that sOP children are

more susceptible to bacterial AOM during viral URI than NOP children.[26] This might be viewed as a failure of commensal-immune equilibrium. Both human and mouse studies have demonstrated that colonization is an immunizing event, generating both B and T cell memory to the colonizing bacteria. Accumulating data suggests adaptive immunity to NP colonizers requires boosting from successive encounters to reach a threshold of protection.[57] This may indeed be the reason for reduced colonization rates in healthy adults compared to children. Th1 and Th17 memory cells that promote neutrophilia are protective against otopathogens, with an additional role played by antibodies raised against capsular and protein antigens.[58-62] When high levels of anti-capsular antibodies are generated via vaccination (as is the case with pneumococcal conjugate vaccine), the B cell response alone is sufficient to prevent future colonization with the immunizing serotypes.[63] Cross-serotype protection is the ideal immune response and has mostly been associated with T cell memory.[59,61] Overall, these data suggest both B and T cell responses are important for protection, and development of immune memory over successive childhood infections plays an important role in future protection from both colonization and subsequent AOM infections.

In addition, the epithelial surface is the physical surface which bacteria utilize to colonize the NP, often forming biofilms to occupy a niche in this space. Epithelial cells provide the physical barrier, via tight and adherens junctions, that prevents otopathogens colonization. In addition, epithelial-derived cytokines and chemokines are central initiators and modulators of the local innate immune response[64]. Therefore, in addition to pathogen-specific memory T and B cells, epithelial barrier and immune function are likely a critical factor in determining the establishment and density of NP bacterial colonization, a necessary step in AOM pathogenesis. Taken together, observations from sOP children demonstrate an immunosuppressed state with its downstream effects on adaptive immune responses/immune memory and inadequate innate immune responses in the NP along with poor NP epithelial cell repair.

Conclusions

All infants are born immunologically naïve to respiratory viruses and potential bacterial otopathogens. All have Eustachian tube anatomy that favors reflux of nasopharyngeal secretions containing viruses and bacteria into the middle ear to cause AOM. Environmental risk to experience viral URI and NP colonization by potential bacterial otopathogens can be delayed by avoidance of exposure to others who harbor and can transmit the organisms. However, infants and young children cannot avoid their parents or siblings and interactions with the wider family group and others in society eventually occurs. AOM infection risk may be mitigated somewhat by avoidance of day care and pacifier use and providing breast feeding.[65] Nevertheless, when children are exposed to respiratory viruses leading to occasional or recurrent viral URI, a subset consequently experience AOM and about 10% of children experience recurrent AOM if stringently diagnosed. These children account for approximately half of all AOM cases observed, suggesting a high impact for improved understanding of the immune mechanisms contributing to cause susceptibility in this population of children. Infection history of a child early in life is known to have profound influences on later immune development[66]. We recently found that.sOP children experience NP colonization by Spn, NTHi, and Mcat at a substantially greater rate and at earlier ages

than NOP children (unpublished). However, the immune responses against these pathogens is reduced at 6 months of age before onset of AOM, with lower bacterial otopathogens-specific antibody titers and weaker responses by T and B cells upon stimulation with bacterial antigens *in vitro,* Thus, the events that predispose children to experience recurrent AOM may occur earlier than we have studied them. Microbiome modulation of the immune response commencing shortly after birth is currently under investigation by our group.

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Highlights

- AOM cases, when microbiologically-confirmed, reveal a unique otitis prone cohort.
- Otitis proneness in children may result from multiple, transient immune dysfunction.
- Nasopharyngeal and systemic immune compartments may display dysfunction.
- Innate and adaptive immune systems may display dysfunction.
- The immune profile of young otitis prone children may resemble that of a neonate.

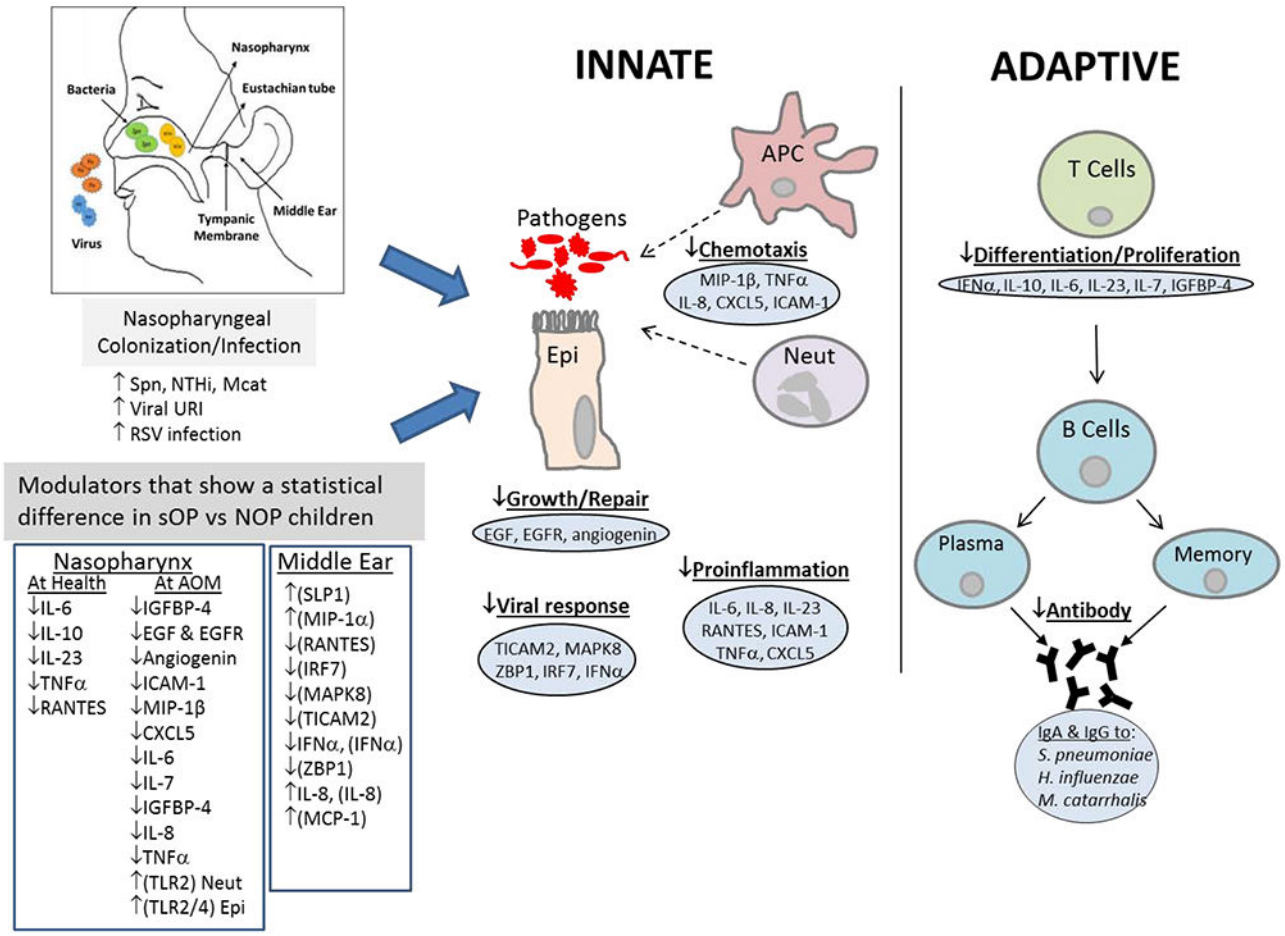

Figure 1. Nasopharynx and middle ear responses in sOP versus NOP children.

Relative levels that show a statistical difference between sOP versus NOP children are depicted with up or down arrows. Modulator measurements are of proteins except those in brackets are of mRNA.

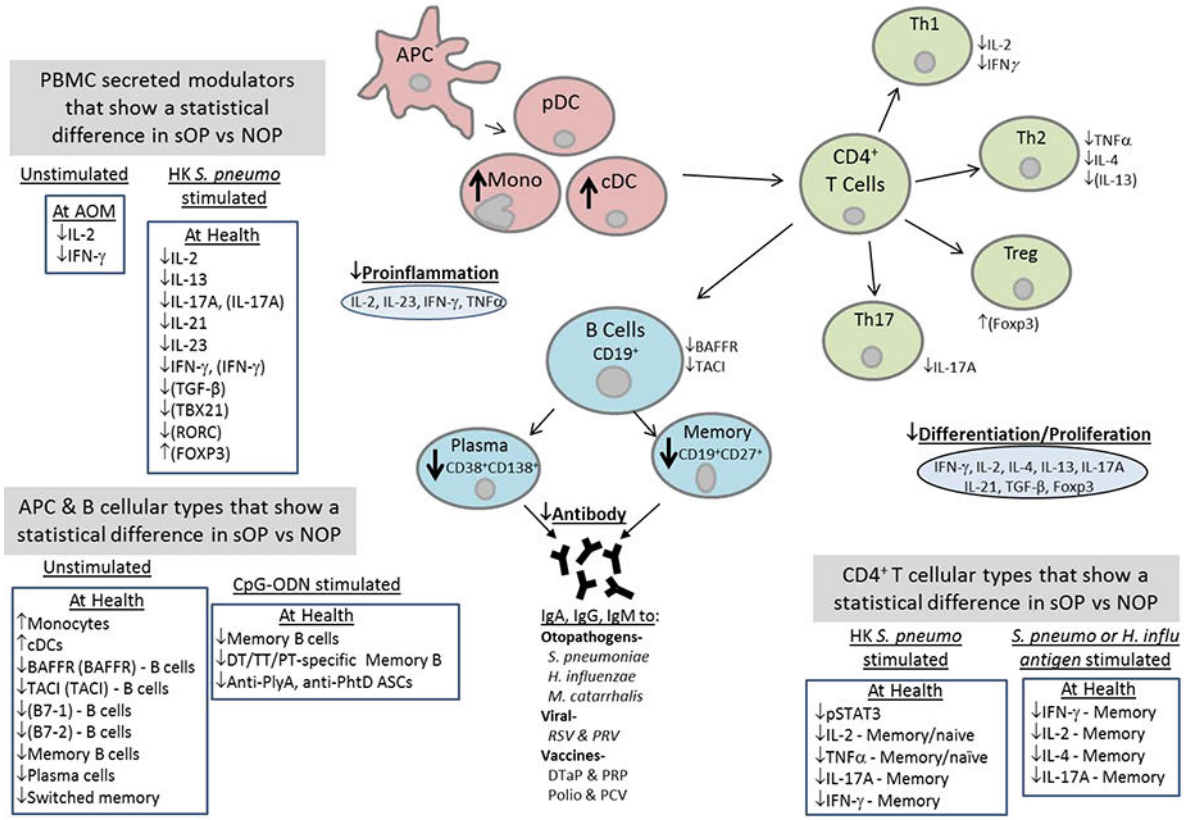

Figure 2. PBMC and serum immune responses in sOP versus NOP children.

Relative levels that show a statistical difference between sOP versus NOP children are depicted with up or down arrows. Modulator measurements are of proteins except those in brackets are of mRNA.

Table 1.

Immune Measures in sOP vs NOP Children

MUCOSAL (NP & MEF)			
	sOP:NOP	**Visit Type(s)**	**Ref**
Innate Responses			
Immune modulators-NP			
MIP-1β	↓	AOM	30
CXCL5	↓	AOM	30
IL-8	↓, NSD	AOM	26,30
IL-6	↓	AOM, URI@Healthy	24,26,30
EGF	↓	AOM	30
EGFR	↓	AOM	30
Angiogenin	↓	AOM	30
ICAM-1	↓	AOM	30
IL-7	↓	AOM	31
IGFBP-4	↓	AOM	31
IL-23	↓	Healthy	24
TNFα, IL-6, IL-10, RANTES,	↓	URI@Healthy	26
MCP-1	NSD	AOM	26,30
IL-2	↑	AOM	26,31
			26
IL-2	NSD	URI@Healthy	26
IL-17a	NSD	AOM, URI@Healthy	26,31
IFN-γ	NSD	AOM, URI@Healthy	26,31
IL-1β, IL-8, MIP-1α	NSD	URI@Healthy	26
TLR2/4 (RNA)	↑	AOM	30
TLR2 (RNA) (Epithelials & Neutropils)	↑	AOM	30
ICAM-1	↓	AOM	30
Immune modulators-MEF			
IL-2 (RNA)	↑	AOM	31
IL-8 (RNA)	↑	AOM	67
SLPI (RNA)	↑	AOM	67
MIP-1α (RNA)	↑	AOM	67
RANTES (RNA)	↓	AOM	67
IFNα1 (RNA)	↓	AOM	67
IRF7 (RNA)	↓	AOM	67
MAPK8 (RNA)	↓	AOM	67
TICAM2 (RNA)	↓	AOM	67

MUCOSAL (NP & MEF)			
	sOP:NOP	**Visit Type(s)**	**Ref**
ZBP1 (RNA)	↓	AOM	67
Adaptive Responses			
Antibodies			
IgG to *Spn* & *Mcat* Ags	↓	Healthy	33,68
IgA to *Spn* & *Mcat* Ags	↓	Healthy	33,68
Colonization			
Spn, NTHi, Mcat	↑	Healthy, URI@Healthy, AOM	26,33,68
Viral Infection			
RSV	↑	AOM	48
URIs	↑	Healthy	26
BLOOD			
	sOP:NOP	**Visit Type(s)**	**Ref**
Baseline Responses			
PBMC (unstimulated)			
IL-2	↓	AOM	31
IFN γ	↓	AOM	31
BAFF, APRIL	NSD	Healthy	42
APCs (unstimulated)			
Total Monocytes & cDCs	↑	Healthy	25
MHC II	↓	Healthy & AOM	69
PBMC (R848 stimulated)			
IL-1β, IL-6, IL-8, IL-10, IL-12, IFN-α, TNF-α, IFN-γ, CCL2, CCL4, CCL5, CXCL10	NSD	Healthy	25
APCs (R484 stimulated)			
Intracellular IL-12, TNF-α, IFN-α	NSD	Healthy	25
Innate & Adaptive Responses			
PBMC (HK-*Spn* stimulated)			
IL-2, IL-4, IL-6, IL-10, IL-13	NSD	Healthy	24
IFN-γ	↓	Healthy	24
IL-17A	↓	Healthy	24

MUCOSAL (NP & MEF)			
	sOP:NOP	**Visit Type(s)**	**Ref**
IL-21	↓	Healthy	24
IL-23	↓	Healthy	24
IL-2 (RNA)	↓	Healthy	24
IL-13 (RNA)	↓	Healthy	24
IL-17A (RNA)	↓	Healthy	24
IL-23 (RNA)	↓	Healthy	24
TGF-β (RNA)	↓	Healthy	24
IFN-γ (RNA)	↓	Healthy	24
TBX21 (RNA)	↓	Healthy	24
RORC (RNA)	↓	Healthy	24
FOXP3 (RNA)	↑	Healthy	24
Adaptive Responses			
Antibodies			
IgG to *Spn, NTHi, Mcat* Ags	↓	Healthy & AOM	34-36,40,43
Antibodies to pediatric vaccine Ags (DTaP, Hib, Polio, *Spn* PSs)	↓	Healthy	22,41,42
IgG to RSV	↓	Healthy	48
B cells			
Total B cells	NSD	Healthy	41
BAFFR, TACI	↓	Healthy	42
BCMA	NSD	Healthy	42
BAFFR, TACI, B7-1, B7-2 (RNAs)	↓	Healthy	42
BCMA, CD40L (RNAs)	NSD	Healthy	42
%Memory B cells	↓	Healthy	41,42
%Memory B cells response to DTaP	↓	Healthy	40,41
%Switched memory	↓	Healthy	41
%Plasma cells	↓	Healthy	42
Spn Ag-specific ASCs	↓	Healthy	42
$CD4^+$ T cells			
Total $CD4^+$ T-cells	NSD	Healthy	43
Naïve $CD4^+$ T-cells	NSD	Healthy	43
Memory $CD4^+$ T-cells	NSD	Healthy	43
$CD4^+$ T-cells (HK-*Spn* stimulated)			
pSTAT3	↓	Healthy	24

MUCOSAL (NP & MEF)			
	sOP:NOP	**Visit Type(s)**	**Ref**
pSTAT3 (+Th17 cytokines)	NSD	Healthy	24
%CD4$^+$ T-cells (HK-*Spn* stimulated)			
IFN γ	NSD	Healthy	24
IL-2	↓	Healthy	24
IL-17A	↓	Healthy	24
TNF-α	↓	Healthy	24
%Naive CD4$^+$ T-cells (HK-*Spn* stimulated)			
IL-2	↓	Healthy	24
TNF-α	↓	Healthy	24
%Memory CD4$^+$ T-cells (HK-*Spn* stimulated)			
IFN γ	↓	Healthy	24
IL-2	↓	Healthy	24
IL-17A	↓	Healthy	24
TNF-α	↓	Healthy	24
%Memory CD4$^+$ T-cells (*Spn* or *NTHi* antigen stimulated)			
IFN γ	↓	Healthy	43
IL-2	↓	Healthy	43
IL-4	↓	Healthy	43
IL-17A	↓	Healthy	43
%Memory CD4$^+$ T-cells (SEB stimulated)			
IFN γ, IL-2, IL-4, IL-17a	NSD	Healthy	43

NSD: No statistical difference detected

AOM: Child with acute otitis media (AOM).

Healthy: Child at a normal healthy visit checkup.

URI@Healthy: Child came in during a normal healthy visit checkup but was diagnosed with an upper respiratory infection (URI).

NP: Nasopharyngeal

MEF: Middle ear fluid

PBMC: Peripheral blood mononuclear cells

Spn: *S. pneumoniae*; HK-*Spn*: heat killed *Spn*; NT*Hi*: nontypeable *H. influenzae*; *Mcat*: *Moraxella catarrhalis*