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Higher serum levels of interleukin 10 occur at onset of acute otitis media caused by *Streptococcus pneumoniae* compared to *Haemophilus influenzae* and *Moraxella catarrhalis*

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Abstract

Objective—Acute otitis media (AOM) involves an inflammatory response to microbes in the middle ear that facilitates clearance of otopathogens. Clinically, *Streptococcus pneumoniae* (*Spn*) infections of the respiratory tract are characterized by greater inflammatory responses than nontypeable *Haemophilus influenzae* (*NTHi*) and *Moraxella catarrhalis* (*Mcat*). Interleukin 10 (IL-10) plays an important role in down-regulating the inflammatory response. We compared serum IL-10 levels in children before onset, at onset and after recovery from AOM caused by *Spn*, *NTHi*, and *Mcat*. We sought to determine if IL-10 could serve as a biomarker to distinguish AOM caused by *Spn* versus *NTHi* and *Mcat*.

Study Design—Prospective, longitudinal study in a primary care pediatric practice in Rochester, NY.

Methods—Participants were 54 children 6 to 30 months of age. Outcomes measured were serum IL-10 levels when healthy, at onset of AOM and after recovery from AOM.

Results—Serum IL-10 was elevated when children developed AOM ($p=0.013$) due to infections caused by *Spn* ($p=0.011$) but not AOM caused by *NTHi* or *Mcat*. Middle ear fluid levels of IL-10 mirrored those seen in serum but were 10-fold higher ($p=0.02$). Other effector cytokines in serum: IL-4, IFN- γ and TNF-alpha, did not show the same increases as IL-10 at onset of AOM.

Conclusion—Our study indicates that AOM caused by *Spn* elicits a significantly higher IL-10 response compared to *NTHi* and *Mcat* and may prove to be a biomarker of AOM infections by *Spn*.

Keywords

Interleukin 10; acute otitis media; *Streptococcus pneumoniae*; *Haemophilus influenzae*; *Moraxella catarrhalis*; cytokines

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DISCLOSURE OF CONFLICTS OF INTEREST

MEP and KL filed a patent in 2011 regarding IL-10 and other biomarkers of AOM and regarding biomarkers of *Spn* and *NTHi* infections.

Level of Evidence: 2b: Individual cohort study

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Introduction

Acute otitis media (AOM) is characterized by a local inflammation in the middle ear, nearly always in association with an upper respiratory viral infection (URI). The most common bacterial pathogens isolated from middle ear fluid (MEF) are *Streptococcus pneumoniae* (*Spn*) (25–50%), non-typeable *Haemophilus influenzae* (*NTHi*) (25–50%) and *Moraxella catarrhalis* (*Mcat*) (5–15%)¹. Genes encoding a wide array of cytokines are differentially regulated in cells in the blood, and in cells that migrate from blood to the middle ear at the onset of AOM². We have recently described variations in serum intercellular adhesion molecule 1 (sICAM-1) and S100A12 that correlated with the presence of *Spn* or *NTHi* in the middle ear, but not with *Mcat* or upper respiratory viral infection in children with AOM^{3,4}.

Interleukin 10 (IL-10) plays an important role in down-regulating the inflammatory response by inhibiting the release of immune mediators, phagocytosis, and the presentation of antigens to T-cells and macrophages^{5–7}. In our recent evaluation of the transcriptome of peripheral blood mononuclear cells (PBMCs) in children infected with *Spn* and *NTHi* at the onset of AOM², we found IL-10 was significantly up-regulated by *Spn* but not *NTHi*. The differential regulation of genes controlling cytokines, except for IL-10, were not significantly different when comparing *Spn* and *NTHi* infected children. The observation led us to hypothesize that the need to down-regulate the inflammatory response may be greater with *Spn* infections because *Spn* produces a greater inflammatory response compared to *NTHi* and *Mcat*.^{8,9}.

In this paper we sought to determine if IL-10 levels in serum might serve as a biomarker for AOM caused by *Spn* and differentiate such infections from those caused by *NTHi* or *Mcat*. We describe our assessment of IL-10 concentrations compared to IL-4, IFN- γ and TNF- α concentrations in serum of young children with AOM to determine (i) if IL-10 serum levels are elevated to greater levels when AOM is caused by *Spn*, the otopathogen that produces the greatest inflammatory response in the middle ear; (ii) if recovery from AOM is associated with a return of IL-10 levels to pre-infection levels in children infected with *Spn*; (iii) whether otitis prone children (who have impaired immune responses to otopathogen colonization and infection^{10–13} and other predisposing factors, such as presence of young siblings and out-of-home daycare) show similar or divergent patterns of IL-10 response as children with infrequent AOM; and (iv) levels of IL-10 in middle ear fluid of children with AOM caused by *Spn* and *NTHi* mirror the response seen in blood.

Methods

Subjects and sample collection

The 54 subjects were selected as a convenience sample from children 6 to 30 months of age who participated in an ongoing prospective study funded by National Institute of Deafness and Communication Disorders. The sample collection from children was approved by the IRB at Rochester General Hospital. At 6, 9, 12, 15, 18, 24, and 30 months of age the children had serum collected; these samples allowed assessment of levels of cytokines when children were healthy, prior to onset of AOM infection and at times when they had a viral URI without concurrent AOM. Whenever a child developed AOM, serum was obtained and a tympanocentesis performed to determine the etiology of AOM. The general results of tympanocentesis cultures from the overall study population has been previously published.¹ Three weeks after an AOM episode, children returned for a convalescent blood sample.

The inclusion and exclusion criteria for study participation have been previously described.¹ AOM was diagnosed using pneumatic otoscopy by validated otoscopists¹⁴ according to the recommendations by the AAP¹⁵ but with a requirement of a bulging tympanic membrane.

Tympanocentesis was done to confirm bacterial AOM caused by *Spn*, *NTHi* or *Mcat*. The children were all treated with antibiotics directed to eradicate the otopathogens and when the convalescent blood sample was taken, all children were deemed to have recovered because they had no symptoms or signs of AOM although persistent middle ear effusion was considered normal. If the child experienced AOM three times within 6 months or 4 times within 12 months then they were classified as otitis prone. Children with immunodeficiency or chronic disease, or other infectious diseases were excluded from the study.

Clinical viral upper respiratory infection (URI) symptoms were assessed by a physician investigator. As described by Kalu *et al*¹⁶, we defined a viral URI clinically by the occurrence of acute onset of rhinorrhea, cough, decreased appetite, and malaise with or without fever. The general results of viral cultures in the study population have been previously published.¹⁷ All children in the study had a concurrent viral URI at onset of AOM.

Serum

Four milliliters of heparinized peripheral venous blood were collected from each child during every visit. The samples were centrifuged at 2,000 RPM for 10 minutes at room temperature. Peripheral blood mononuclear cells (PBMC) were isolated and serum was prepared and stored at -80°C until it was assayed.

MEF

MEF was collected from all children by tympanocentesis as previously described.¹ Samples were processed within 3 hours of collection. Trizol (Sigma) was added to stabilize an aliquot of the MEF samples for RNA and DNA detection.

Bacteriology—Bacterial cultures were processed as previously described.¹

Virology—Viral identification was done by a one-step RT-PCR reaction for RSV-A, RSV-B, Influenza-A, Influenza-B, and PIV-3 (Primerdesign, UK) using an iCycler (Biorad, CA) as previously described.¹⁷

MAP assay

IL-10, IL-4, TNF-alpha and IFN- γ levels in the serum were analyzed simultaneously by using a Bio-Plex Pro Assay (Bio-Rad Laboratories, Hercules, California, USA) which is a specific sandwich immunoassay formatted on magnetic beads. Capture antibodies directed against the cytokines were covalently coupled to beads. Coupled beads reacted with the sample containing the corresponding cytokine. After a series of washes to remove unbound protein, a biotinylated detection antibody was added to create a sandwich complex. The final detection complex was formed with the addition of streptavidin-phycoerythrin (SA-PE) conjugate. The protocol was performed according to the instruction of the manufacturer. Briefly, filter plates were wetted with 100 μl of assay buffer, and then 50 μl of 1x beads was added to the assay plate. After washing 2 times with 100 μl wash buffer, 50 μl samples diluted in 1:4 in buffer were added and incubated in the dark at RT with shaking at 300 RPM for 30 min. After washing 3 times with 100 μl wash buffer, 25 μl of detection antibody was added, incubated, and washed as above. Then 50 μl of streptavidin-PE was added and plates incubated for 10 min. After washing, the beads were resuspended in 125 μl of assay buffer and read with a Luminex instrument. The cytokine concentration was calculated based on a standard curve. MEF samples were analyzed in the same manner but only for IL-10 levels.

Statistical Analysis

Each subject in this study had cytokines measured under more than one clinical condition, e.g. at onset of AOM, during a well visit, in a comparison between otitis prone and non-otitis prone (Table 1). Therefore the observations were related (perhaps correlated) and could not be treated as if they were independent, precluding parametric tests such as t-tests. As recommended in this situation¹⁸ we used generalized estimating equations (GEE), which most resemble linear regression modeling, for the main analysis to calculate probability (p) values. The two largest values (> 20) were imputed to be the highest value less than 20 (= 5.5), after which approximate normality held. The *Spn*, *NTHi* and *Mcat* comparisons were fully paired by subject, while each remaining comparison contained multiple measurements from at least some subjects. Analysis of the relationship between serum and middle ear fluid levels of IL-10 were calculated using a Mann-Whitney test. For all tests $p < 0.05$ was considered significant.

Results

Serum IL-10 levels are significantly elevated in children with AOM

54 children and 336 samples were included in the study for serum IL-10 testing simultaneously with 3 other cytokines (IL-4, TNF- α and IFN- γ) in one or more of the analyses (Table 1). To determine levels when children were healthy compared to at onset of AOM, 47 samples from 44 healthy non-otitis prone children were compared with 57 samples from 45 non-otitis prone children with AOM due to *Spn*, *NTHi* or *Mcat*. IL-10 was detectable in all the samples. The level of IL-10 in children with AOM was significantly ($p = 0.013$) higher than the level in children prior to AOM (Figure 1). In contrast, IL-4 could not be detected in 91% of samples, IFN- γ could not be detected in 74% of the samples and TNF- α could not be detected in 33% of the samples. Although TNF- α was detected in 67% of the samples, the levels were not significantly different at onset of AOM compared to healthy children (31.1 pg/ml, and 28.7 pg/ml, respectively). There was no correlation among concentrations of IL-10 and IL-4, TNF- α or IFN- γ (data not shown). Since IL-10 was the only cytokine significantly different in the serum of AOM patients among the cytokines surveyed, our subsequent analysis focused on IL-10.

Serum IL-10 levels are significantly increased in children with AOM due to *Streptococcus pneumoniae*

We had paired pre-infection and onset of infection samples from 21 non-otitis prone children who had AOM caused by *Spn*. We found a significant increase ($p = 0.011$) in serum IL-10 levels from prior to infection compared to onset of infection (Fig. 1).

Serum IL-10 levels do not significantly change in children with AOM due to *NTHi* or *Mcat*

We had paired pre-infection and onset of infection samples from 16 non-otitis prone children who had AOM caused by *NTHi* and 8 children with AOM caused by *Mcat*. We found no significant increase in IL-10 levels at onset of infection compared to samples taken pre-infection (Figure 1).

Serum IL-10 levels return to pre-infection concentrations after recovery from infection

The serum level of IL-10 in 24 non-otitis prone children prior to AOM returned to pre-infection levels when measured 3 weeks after AOM onset in 25 children (Figure 2).

Concurrent upper respiratory virus impact on IL-10 levels

All the children in the study had a concurrent clinical viral URI at onset of AOM. Therefore the difference in levels of IL-10 between *Spn* versus *NTHi* and *Mcat* were likely to be

attributable to the bacteria causing AOM. From 11 of 44 tested NP samples non-otitis prone children with AOM, we detected a virus (5 cases of *respiratory syncytial*, 3 of *influenza A*, 1 of *influenza B* and 2 of *parainfluenza* virus), too few positive samples to make distinctions among viral species. Our methods did not detect other respiratory viruses including rhinovirus, metapneumovirus or bocavirus.

No significant difference of serum IL-10 levels in OP and NOP children

We compared IL-10 levels in 7 otitis prone children with 44 non-otitis prone children at the time of onset of AOM. There was an insignificantly lower level of IL-10 among otitis prone children ($p=0.35$) (Fig. 2).

Concurrence of serum and MEF IL-10 patterns

In children with AOM caused by *Spn* or *NTHi*, serum and MEF levels of IL-10 were compared. The mean IL-10 level in MEF for 4 samples taken from non-otitis prone children that had *Spn* in the MEF was 314 pg/mL (S.E. = 195 pg/mL) whereas the serum level was 4.8 pg/mL (S.E.= 0.9 pg/mL). ($p=0.02$) The mean IL-10 level in MEF for 4 samples taken from non-otitis prone children that had *NTHi* in the MEF was 178 pg/mL (S.E. = 95 pg/mL) whereas the serum level was 3.5 pg/mL (S.E.= 0.7 pg/mL). ($p=0.02$) (Fig. 3).

Discussion

In this assessment of IL-10 concentrations compared to IL-4, IFN- γ and TNF- α concentrations in serum of young children with AOM we provide data to support several conclusions. (1) IL-10 but not IL-4, IFN- γ or TNF- α serum levels are elevated at onset of AOM. (2) The elevation of IL-10 in serum at onset of AOM is statistically associated with infections caused by *Spn*, the otopathogen that produces the greatest inflammatory response in the middle ear. Similar elevations are not seen in infections caused by *NTHi* or *Mcat*. (3) Recovery from AOM caused by *Spn* is associated with a return of IL-10 levels to pre-infection levels. (4) Children prone to recurrent AOM infections show similar patterns of IL-10 response as children with infrequent AOM. (5) Levels of IL-10 in middle ear fluid of children with AOM caused by *Spn* and *NTHi* mirror the response seen in blood.

Differences in elevation of IL-10 in children at onset of AOM caused by *Spn* infection are consistent with the known role of IL-10 in dampening the innate and adaptive immune response so as to avoid inflammatory damage to tissues. *Spn* is more invasive and causes systemic infection unlike *NTHi* and *Mcat*. *Spn* is known to cause more severe symptoms and signs of inflammation than other otopathogens in AOM. Van der Poll et al ¹⁹ found that administration of *Spn* intranasally in mice caused increased production of IL-10 in the lungs during pneumococcal pneumonia. IL-10 levels reached 25.8 ± 3.0 ng/ml in the mice compared to pre-infection levels of 1.2 ± 0.2 ng/ml. Concentrations of IL-10 in middle ear effusions and plasma of adults with otitis media showed a strong correlation with the duration of effusion²⁰.

The role of increased IL-10 in bacterial infection is sometimes complex, varying with the pathogen, the host and the interaction with other anti-inflammatory and pro-inflammatory cytokines. Lee et al ²¹ found that IL-10 injected into the middle ear cleft of mice inhibited AOM induced by lipopolysaccharide (LPS) by reducing the mucosal and sub-mucosal infiltration of inflammatory cells. Oral et al ²² described that IL-10 could inhibit the CD28 signaling pathway and protect the middle ear mucosa from damage by limiting the activation of T-cells and natural killer cells and the general inflammation response. Diab et al ²³ demonstrated elevated IL-10 in *Haemophilus influenzae* b-infected rat brains at 8 hour, but at 18h of infection IL-10 mRNA expression declined to levels below that of uninfected

control rats whereas in *Spn* inoculated rats, IL-10 mRNA induction was elevated at both 8h and 18h of infection.

Emonts et al ²⁴ and Ilia et al ²⁵ showed that children with lower IL-10 levels due to an IL-10-1082 A/A polymorphism were less susceptible to recurrent episodes of AOM, leading to the notion that higher IL-10 levels signify a greater likelihood for chronic and AOM prone conditions. Our results do not support those observations since we found no significant difference in serum IL-10 levels in otitis prone and non-otitis prone children.

We did not assess the effect of viral infections on IL-10 levels at onset of infection because all of the children we studied had a concurrent viral URI clinically diagnosed and a limitation of our study is the absence of such an assessment and reliance on a clinical diagnosis for definition of viral URI. In a subset of children the presence of a virus was detected. It is known that the most common viruses to cause upper respiratory infections are *respiratory syncytial virus (RSV)* and *human rhinovirus (HRV)* ²⁶. Previous studies show that *RSV* and *HRV* are able to infect human immune cells and trigger them to release IL-10 inhibiting the host immune response and resulting in a longer and more severe respiratory infection.²⁷ Skovbjerg et al. ²⁸ found that IL-10, TNF-alpha and IL-8 were unrelated to the presence or absence of viruses. Another limitation of our study is the small number of subjects used to compare levels of IL-10 in serum versus middle ear fluid.

Previous studies showed variable detection of IFN- γ , IL-4 and TNF-alpha in children with AOM. Kele ²⁹ reported IFN- γ levels were significantly lower and IL-4 levels significantly higher in plasma samples from AOM patients compared to controls. Johnston et al ³⁰ found no significant difference in plasma IL-4 but significantly elevated levels IFN- γ comparing AOM cases to controls in older children (mean = 4.5 years old). The difference in results from those studies compared to ours may be due to age of the child, certainty of AOM diagnosis (since those studies did not confirm AOM with tympanocentesis) and methods applied. With xMAP technology, we found IFN- γ and IL-4 were largely undetectable whereas TNF-alpha was detected in 67% of serum samples but no difference was found between the AOM and healthy state. Willett et al ³¹ detected TNF-alpha by ELISA in 40% of 65 middle ear effusions from 41 children with chronic otitis media with effusion; the mean concentration was 1.24 +/- 3.1 pg/mg. Skotnicka et al ³² detected TNF-alpha in 47.4% of middle ear effusions tested.

Conclusion

In conclusion, at onset of AOM a significant rise in serum IL-10 levels occurs when the infection is caused by *Spn* but not *NTHi* or *Mcat*. When children recover from AOM caused by *Spn*, IL-10 serum levels return to pre-infection values. This result suggests that IL-10 may be a useful biomarker to assist in diagnosis of AOM caused by *Spn* in young children (6–30 months of age). Serum IL-6 may be a similar cytokine biomarker for AOM caused by *Spn* ³³. The relatively high production of IL-10 at onset of AOM caused by *Spn* (Gram positive infections) but not by *NTHi* and *Mcat* (Gram negative pathogens) is consistent with the clinical observation of a greater inflammatory response that requires dampening in association with *Spn* infection compared to *NTHi* and *Mcat* infection.

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References

1. Casey JR, Adlowitz DG, Pichichero ME. New Patterns in the otopathogens causing acute otitis media six to eight years after introduction of pneumococcal conjugate vaccine. *Pediatr Infect Dis J*. 2010; 29(4):304–9. [PubMed: 19935445]
2. Liu K, Chen LL, Pichichero ME. Transcriptome signature in young children with acute otitis media due to *Streptococcus pneumoniae*. *Microbes Infect*. 2012 Jan 23. Epub ahead of print.
3. Liu K, Casey JR, Pichichero ME. Serum Intercellular Adhesion Molecule 1 Variations in Young Children with Acute Otitis Media. *Clinical and Vaccine Immunology*. 2010; 17(12):1909–1916. [PubMed: 20926702]
4. Liu K, Pichichero ME. Clinical Significance of serum S100A12 in acute otitis media in young children. *Pediatr Infect Dis J*. 2012; 31(3):e56–58. [PubMed: 22198825]
5. Sabat R, Grutz G, Warszawska K, Kirsch S, Witte E, Wolk K, Geginat J. Biology of interleukin-10. *Cytokine and Growth Factor Reviews*. 2010; 21:331–344. [PubMed: 21115385]
6. Skovbjerg S, Martner A, Hynsjo L, Hessle C, Olsen I, Dewhirst FE, Tham W, Wold AE. Gram-Positive and Gram-Negative bacteria induce different patterns of cytokine production in human mononuclear cells irrespective of taxonomic relatedness. *Journal of Interferon and Cytokine Research*. 2010; 30(1):23–32. [PubMed: 20028205]
7. Skotnicka B, Hassmann E. Proinflammatory and immunoregulatory cytokines in the middle ear effusions. *International Journal of Pediatric Otorhinolaryngology*. 2008; 72:13–17. [PubMed: 17976828]
8. Palma AAI, Herva E, Savolainen H, Karma P, Makela PH, Kilpi TM. Association of Clinical Signs and Symptoms with Bacterial Findings in Acute Otitis Media. *Clin Infect Dis*. 2004; 38:234–242. [PubMed: 14699456]
9. Rodriguez WJ, Schwartz RH. *Streptococcus pneumoniae* causes otitis media with higher fever and more redness of tympanic membranes than *Haemophilus influenzae* or *Moraxella catarrhalis*. *Pediatr Infect Dis J*. 1999; 18:942–944. [PubMed: 10530598]
10. Kaur R, Casey JR, Pichichero ME. Serum antibody response to five *Streptococcus pneumoniae* proteins during acute otitis media in otitis-prone and non-otitis-prone children. *Pediatr Infect Dis J*. 2011; (8):645–650. [PubMed: 21487325]
11. Sharma SK, Casey JR, Pichichero ME. Reduced Memory CD4⁺T-Cell Generation in the Circulation of Young Children May Contribute to the Otitis-Prone Condition. *J Infect Dis*. 2011; 204:645–653. [PubMed: 21791667]
12. Khan MN, Kaur R, Pichichero ME. Bactericidal antibody response against P6, protein D and OMP26 of nontypeable *Haemophilus influenzae* after acute otitis media in otitis-prone children. *FEMS Immunol Med Microbiol*. 2012; 65(3):439–447. [PubMed: 22463053]
13. Sharma SK, Casey JR, Pichichero ME. Reduced serum IgG responses to pneumococcal antigens in otitis-prone children may be due to poor memory B-cell generation. *J Infect Dis*. 2012; 205(8): 1225–1229. [PubMed: 22383675]
14. Kaleida P, Stool SE. Assessment of otoscopists' accuracy regarding middle-ear effusion. *Am J Dis Child*. 1992; 146:433–435. [PubMed: 1558077]
15. Subcommittee on Management of Acute Otitis Media. Diagnosis and Management of Acute Otitis Media. *Pediatrics*. 2004; 113:1451–1465. [PubMed: 15121972]
16. Kalu SU, Ataya RS, McCormick DP, Patel JA, Revai K, Chonmaitree T. Clinical spectrum of acute otitis media complicating upper respiratory tract viral infection. *Pediatr Infect Dis J*. 2011; 30(2):95–99. [PubMed: 20711085]
17. Friedel V, Chang A, Wills J, Vargas R, Xu Q, Pichichero ME. Impact of Respiratory Viral Infections on Alpha Hemolytic Streptococci and Otopathogens in the Nasopharynx of Young Children. *Pediatr Infect Dis J*. 2012 In Press.
18. Hanley JA, Negassa B, Edwardes MD, Forrester JE. Statistical Analysis of Correlated Data Using Generalized Estimating Equations: An Orientation. *Am J Epidemiol*. 2003; 157:364–375. [PubMed: 12578807]
19. Van der Poll T, Marchant A, Keogh CV, Goldman M. Interleukin-10 Impairs Host Defense in Murine Pneumococcal Pneumonia. *J Infect Dis*. 1996; 174:994–1000. [PubMed: 8896500]

20. ShouQin Z, Jie L, Hua L, QuanGeng Z, Yang W, DeMin H. Role of interleukin-10 and transforming growth factor beta 1 in otitis media with effusion. *Chinese Medical Journal*. 2009; 122 (18):2149–2154. [PubMed: 19781301]
21. Lee JH, Byun JA, Won Yeo S, Yong Soo Park J. Role of Interleukin-10 on Mouse Otitis Media Model Using Lipopolysaccharide. *Korean J Otorhinolaryngol-Head Neck Surg*. 2011; 54:43–7.
22. Oral HB, Kotenko SV, Yilmaz M, et al. Regulation of T Cells and cytokines by the interleukin-10(IL-10)-family cytokines, IL-19, IL-20, IL-22, IL-24, IL-26. *Eur J Immunol*. 2006; 36:380–388. [PubMed: 16365913]
23. Diab A, Zhu J, Lindquist L, Wretling B, Bakhiet M, Link H. *Haemophilus influenzae* and *Streptococcus pneumoniae* induce different intracerebral mRNA cytokine patterns during the course of experimental bacterial meningitis. *Clin Exp Immunol*. 1997; 109(2):233–41.
24. Emonts M, Veenhoven RHSP, Wiertsema JJ, et al. Genetic Polymorphisms in Immunoresponse Genes TNFA, IL6, IL10, and TLR4 Are Associated With Recurrent Acute Otitis Media. *Pediatrics*. 2007; 120:814–823. [PubMed: 17908769]
25. Ilia S, Goulielmos GN, Samonis G, Galanakis E. Host's Response in Otitis Media Understanding Genetic Susceptibility. *Pediatr Infect Dis J*. 2008; 27:929–933. [PubMed: 18724267]
26. Bulut Y, Guven M, Otlu B, et al. Acute Otitis Media and respiratory viruses. *Eur J Pediatr*. 2007; 166:223–228. [PubMed: 16967296]
27. Bartz H, Buning-Pfaue F, Turkel O, Schauer U. Respiratory syncytial virus induces prostaglandin E2, IL-10 and IL-11 generation in antigen presenting cells. *Clin Exp Immunol*. 2002; 129:438–445. [PubMed: 12197884]
28. Skovbjerg S, Martner A, Hynsjo L, et al. Gram-Positive and Gram-Negative Bacteria Induce Different Patterns of Cytokine Production in Human Mononuclear Cells Irrespective of Taxonomic Relatedness. *Journal of Interferon and Cytokine Research*. 2010; 30(1):23–32. [PubMed: 20028205]
29. Keles E, Yalcin S, Bulut V, Kaygusuz I, Karlidag T, Aplay HC. The role of allergy in the etiology of otitis media with effusion; immune system and cytokines. *Kulak Burun Bogaz Ihtis Derg*. 2004; 13(3–4):51–56. [PubMed: 16055981]
30. Johnston NAB, Diego A, Preciado D, Ondrey FG, Daly KA. Presence of otitis media with effusion and its risk factors affect serum Plasma cytokine profile in children. *International Journal of Pediatric Otorhinolaryngology*. 2008; 72(2):209–214. [PubMed: 18055023]
31. Willett DN, Rezaee RP, Billy JM, Tighe MB, DeMaria TF. Relationship of endotoxin to tumor necrosis factor-alpha and interleukin-1 beta in children with otitis media with effusion. *Ann Otol Rhinol Laryngol*. 1998; 107(1):28–33. [PubMed: 9439385]
32. Skotnicka B, Hassmann E. Cytokines in children with otitis media with effusion. *Eur Arch Otorhinolaryngol*. 2000; 257(6):323–6. [PubMed: 10993552]
33. Heikkinen T, Ghaffar F, Okorodudu O, Chonmaitree T. Serum interleukin-6 in bacterial and nonbacterial acute otitis media. *Pediatrics*. 1998; 102:296–299. [PubMed: 9685429]

Fig. 1.

Serum IL-10 levels in children are plotted as boxplots showing the median value, the 25th percentile (box), 75th percentile (bars) and outliers (dots) for each analysis. The vertical axis is the IL-10 measurements in picograms/milliliter.

Comparison at onset of AOM caused by *Spn* or *NTHi* or *Mcat* compared to the pre-infection healthy state. ($n=39$ and 45 , respectively) $p=0.013$.

Comparison in children at onset of AOM caused by *Spn* versus the pre-infection healthy state. ($n=15$ and 15 respectively) $p=0.011$.

Comparison in children at onset of AOM caused by *NTHi* versus the pre-infection healthy state. ($n=8$ and 8 , respectively) $p=0.29$

Comparison in children at onset of AOM caused by *Mcat* versus the pre-infection healthy state. ($n= 8$ and 8 , respectively) $p=0.85$

Fig. 2.

Serum IL-10 levels in children are plotted as boxplots showing the median value, the 25th percentile (box), 75th percentile (bars) and outliers (dots) for each analysis. The vertical axis is the IL-10 measurements in picograms/milliliter.

A IL-10 serum concentration prior to AOM infection compared to after recovery from infection ($n=24$ and 25 , respectively) $p=0.2$

IL-10 serum concentrations in otitis prone ($n=7$) and non-otitis prone ($n=44$) children. $P=0.8$

Fig. 3. IL-10 MEF and serum levels in children with AOM caused by *Spn* ($n=4$) or *NTHi* ($n=4$). MEF levels for IL-10 were significantly higher in MEF than serum for both *Spn* and *NTHi*, ($p=0.02$ for both).

Table 1

Subjects and Samples Tested for IL-10

	N Subjects	N Data
All	54	336
Spn+NTHi+Mcat AOM	44	47
Healthy	45	57
Spn AOM	21	21
Healthy	21	21
NTHi AOM	16	16
Healthy	16	16
M cat AOM	8	8
Healthy	8	8
Pre-infection	24	27
Convalescence	25	27
Otitis prone	7	7
Non-otitis prone	44	47
Virus and AOM	11	11
Non virus infected	33	35