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Association between Cytokine Gene Polymorphisms and Risk for Upper Respiratory Tract Infection and Acute Otitis Media

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Abstract

Background—We previously reported an association between tumor necrosis factor alpha (TNFα)$^{-308}$ and interleukin 6 (IL-6)$^{-174}$ polymorphisms and otitis susceptibility by history. Acute otitis media (AOM) occurs most commonly as a complication of upper respiratory tract infection (URI); it is not clear why some children develop AOM after URI and others do not. Our objective was to prospectively evaluate the association of TNFα^{-308} and IL-6^{-174} polymorphisms with URI and AOM development after URI.

Design/Methods—Children 6–35 mos. were prospectively followed for occurrences of URI and AOM. Blood or buccal mucosa samples were collected for DNA extraction to determine cytokine genotypes. Active and passive surveillance was used to capture all URI episodes during the one-year follow-up period in order to study the rate of AOM following URI. Data were analyzed using SAS and general estimating equations modeling.

Results—242 children were followed over 2689 patient months and had DNA genotyped; 1235 URI episodes occurred, 392 (32%) were complicated by AOM. Children who had IL-6^{-174} polymorphism had a higher susceptibility to URI during the study period (IDR:1.24) and were more likely to meet established otitis susceptibility criteria ($p<0.01$). Presence of TNFα^{-308} polymorphism was associated with increased risk for AOM following an episode of URI (OR: 1.43).

Conclusions—TNFα^{-308} and IL-6^{-174} genotypes are associated with increased risk for symptomatic URI and AOM following URI. Future studies may be designed to carefully look at the interaction of these genetic polymorphisms with modifiable environmental risk factors.

Keywords

Otitis Media; Cytokine Genetic Polymorphisms; Upper Respiratory Tract Infection

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INTRODUCTION

Cytokines are proteins that mediate and regulate a vast array of biological events; they function in a complex network in which production of one cytokine will influence production of several others. TNFα, IL-1β and IL-6 are well defined as acute-phase proinflammatory cytokines in a variety of disease states including the host inflammatory response to infection. In the pathogenesis of inflammation in the respiratory tract, proinflammatory cytokines play a major role as regulators of proliferation, chemotaxis, and activation of inflammatory cells (1). These cytokines are actively induced in nasal secretions of children during viral upper respiratory tract infection (URI), suggesting that these cytokines participate in regulation of virus-induced inflammation and/or recovery from the infection (2). In influenza, for example, duration of virus shedding is associated with levels of IL-6 in nasal secretions (3); and the levels of IL-6 and IL-8 are associated with increased local and systemic symptoms. In adenovirus infection in children, high serum IL-6 and TNFα are associated with increased disease severity (4). It is likely that high levels of cytokines in nasal secretions during viral URI are associated with enhanced degree of inflammation and the development of AOM as a complication of the URI.

The genetic control of proinflammatory cytokine production has been widely explored. Among many reported polymorphisms in the TNFα promoter gene, the G/A polymorphism at −308 has been shown to correlate with 20%–40% increase in TNFα production (5–7) and most significantly associated with susceptibility to infections (5,8,8–11). This polymorphism is correlated with susceptibility to or with poor outcome after cerebral malaria (10), septic shock (11), periodontitis(9), liver cirrhosis in hepatitis C infection (12), and meningococcal disease (13).

We previously studied the association between three proinflammatory cytokine gene polymorphisms (TNFα^{-308}, IL-1β^{+3953}, and IL-6^{-174}) and history of otitis susceptibility in children (14); we found that TNFα^{-308} and IL-6^{-174} polymorphisms (but not IL-1β^{+3953}) are significantly associated with otitis susceptibility. Although AOM occurs most commonly as a complication of URI, our retrospective study did not factor in the relationship between number of URI episodes and the risk of AOM developing after an individual URI episode. In the present study, we prospectively followed children from 6–35 months for up to one year for the occurrence of AOM after URI to determine the relationship between TNFα^{-308}and IL-6^{-174} polymorphisms and risk for AOM complicating URI.

METHODS

Study design and subjects

This was a prospective, longitudinal, cohort study which was designed to capture all symptomatic URI episodes that occurred in each child during a one-year follow up period in order to study the incidence and characteristics of OM complicating URI (15). The study was performed from January 2003 through March 2007 at the University of Texas Medical Branch (UTMB) at Galveston and was approved by the Institutional Review Board. Written informed consent was obtained from all subjects. Healthy children at the peak age incidence of OM (6 mos. to 3 yrs.) living in the city of Galveston were recruited from the primary care clinic at UTMB. Children with chronic medical problems or anatomical or physiological defect of the ear or nasopharynx were excluded.

At enrollment, a blood sample or buccal mucosa swab was collected for DNA analysis. During the follow-up period, parents were asked to notify the study office as soon as the child began to have URI symptoms (nasal congestion, rhinorrhea, cough, or sore throat, with our without fever). Children were seen by a study physician as soon as possible after the

URI onset and followed a few days later for AOM complications; parents were compensated for time and travel.

AOM was defined by acute onset of symptoms (fever, irritability, or earache), signs of tympanic membrane inflammation and presence of fluid as documented by pneumatic otoscopy and/or tympanometry. Asymptomatic children with tympanic membrane inflammation and fluid were also diagnosed with AOM. Children diagnosed with AOM were managed based on the standards of care (16).

Study personnel conducted 2 home visits at 2 and 3 weeks after URI onset. During these visits, the child's interval health history and tympanometry were obtained to document the presence of otitis media with effusion. If the tympanogram remained abnormal for 3 weeks after the onset of symptoms, the test was repeated every two weeks until returning to normal or the next URI episode. Parents were advised to bring the child for examination whenever they suspected the child to have any symptoms of AOM.

At each visit, information was collected on specific URI and AOM symptoms; tympanometry was performed and the child's ears were examined using pneumatic otoscopy by trained investigators (TC, KR, JP). AOM complicating URI was considered when AOM occurred within 28 days of the URI unless a new onset of URI occurred within this period.

In addition to the parent's self reports of URI, the study personnel called the parents twice monthly for any current URI symptoms and occurrence of other URI or AOM episodes missed since the last contact. An extensive review of medical records was performed at the completion of each child's study. UTMB is the sole provider of pediatric healthcare in Galveston; diseases diagnosed and treated in our children are likely to be within our medical records. URI and AOM episodes not seen by the study group but captured from parent's interviews or from medical records were recorded as "missed episodes".

Eligible children were classified as otitis susceptible if they had ≥4 OM episodes in one year, ≥ 3 in six months, ≥ 6 by age 6, or the first OM episode prior to age 6 months (14). Children who had two or fewer AOM by the time they turned 2 years old were classified as non-susceptible.

DNA Analysis

DNA was extracted from peripheral blood mononuclear cells or buccal epithelial cells of enrolled children. Polymerase chain reaction (PCR) was performed on the extracted DNA with the use of respective cytokine primer sets that spanned the single nucleotide polymorphism (SNP) sites. The resultant PCR products were digested with polymorphic site-specific enzymes: NCo1 for TNFα^{-308} and Hsp92II for IL-6^{-174}. All SNPs that were identified by PCR and restriction fragment length polymorphism were confirmed by DNA sequencing. The laboratory staff who conducted the polymorphism assays were blinded to the otitis susceptibility classification of the children. The study of SNPs yielded 3 genotypes for each cytokine: homozygous "normal" (low cytokine-producing) genotype, and homozygous and heterozygous polymorphic (high cytokine-producing) genotypes. Children were considered to be polymorphic if they were either homozygous or heterozygous for the polymorphism.

Statistical Analysis

Odds ratios and p-values were calculated using SAS® (SAS Institute Inc, Cary, NC) statistical software. Chi-square analysis to compare polymorphisms vs AOM Susceptibility was performed using STATA 9.0 © (Stata corporation, College Station, TX) statistical software. To model the number of URIs count data and determine incidence density ratios

(IDR) we used Poisson regression to model the number of URIs as a function of polymorphism status and adjusted for breastfeeding (BF), daycare (DC) attendance, cigarette smoke exposure (CSE). The log of the number of months was the offset variable. We corrected for an overdispersed distribution by including a dispersion parameter based on the ratio of the deviance that corrects the standard errors and gives accurate P values as suggested by McCulagh and Nelder (17). The model was fit into the GENMOD procedure with the deviance over-dispersion correction in SAS. The parameters were exponentiated to express IDR. The General estimating equations (GEE) approach was used to account for the multiple correlated episodes in each child. For this model, we adjusted for age, gender, race, ethnicity, breastfeeding (BF), daycare (DC) attendance, cigarette smoke exposure (CSE), family history of OM, and number of pneumococcal conjugate vaccine (PCV7) doses. For the binary outcome of AOM (Yes vs. No) in Table 2. we fit a similar model using the GEE approach accept with a different distribution and link function (Binomial, logit link), and the exponentiated parameters were interpreted as odd ratios.

RESULTS

Included in this analysis were 242 children who were followed by the study group for 6–12 months or until they had tympanostomy tubes placed. Demographic and individual patient characteristics are shown in Table 1. DNA samples for the cytokine genotype analysis were available in 241 children for TNFα (in 1 case, genotype result was not conclusive) and 242 children for IL-6 genotypes. Sixty children (25%) were polymorphic for TNFα^{-308}, while 181 were normal for this allele. For IL-6^{-174} genotype, 73 (30%) children were polymorphic and 168 were normal. There were a total of 1235 URI episodes captured during the follow-up period of 2689 patient-months; 392 (32%) episodes of URI were complicated by AOM. There were 25 cases of AOM that were not associated with URI symptoms.

Polymorphisms vs URI Occurrences

We fit a general linear model using the GEE approach to model the number of URIs per child. The IDR for IL-6^{-174} polymorphism was 1.24 (95% CL: 1.0, 1.54) indicating that during the study follow-up period, polymorphic subjects were approximately 24% more likely to have URIs compared to normal subjects, after controlling for daycare status, age and breastfeeding duration. There was no association found for TNFα^{-308} polymorphism and URI occurrences (IDR=1.0, 95% CL: 0.81, 1.30).

Polymorphisms vs AOM Susceptibility

We applied the previous otitis susceptibility criteria (14) to the children in this cohort. Of 242 children, 213 (88%) met the criteria for either otitis susceptible or non-susceptible; of these, 68 children (32%) were classified as otitis susceptible and 145 (68%) were classified as non-susceptible. The remainder of the children did not fit into either category or completed the study before their 2nd birthday and therefore could not be considered for the non-susceptible designation.

Of the 60 children who were TNFα^{-308} polymorphic, 20 (33%) met the criteria for otitis susceptible, while of the 144 children with normal TNFα genotype, 36 (25%) were otitis susceptible (p=0.4). Of the 68 children with IL-6^{-174} polymorphism, 30 (44%) were otitis susceptible, compared to 37 (26%) of children with normal IL-6 genotype who were otitis susceptible (p<0.01). There was an equal number (eight) of otitis non-susceptible children and otitis susceptible children who had both TNFα^{-308} and IL-6^{-174} polymorphisms. Having either polymorphism increased the odds of being otitis susceptible: OR = 2.09 (1.1–3.8, P<0.02).

Of 168 children who were cared for at home, 44 (26%) were otitis susceptible compared to 33 (45%) of the 74 children who were in daycare ($p<0.001$).

Polymorphisms vs AOM Complicating URI

We fit a general linear model using the GEE approach to determine a child's odds of developing AOM after a URI (Table 2) and adjusted for age, gender, race, ethnicity, BF, DC attendance, CSE, family history of OM, and number of PCV7 doses. Children with TNFα^{-308} polymorphism had 42% greater odds of developing AOM after URI than children with normal genotype. IL-6^{-174} polymorphism alone did not increase the risk of AOM after URI. Combining TNFα^{-30} with IL-6^{-174} polymorphisms did not further increase the risk for AOM.

DISCUSSION

This was a prospective, longitudinal, cohort study designed to capture all symptomatic URI episodes during a one-year period in children at the peak age incidence of AOM to study the incidence and characteristics of AOM complicating URI. We show that children with IL-6^{-174} polymorphism have more frequent episodes of URI and recurrent AOM. Children with TNFα^{-308} polymorphism are at higher risk rate of developing AOM following a URI episode. We have previously reported an association between otitis susceptibility by history and TNFα^{-308} and IL-6^{-174} polymorphisms (14); however, that study was retrospective in nature relying principally on parental report of OM occurrences and chart review. In a recent retrospective study from the Netheralands, Emonts et al (18) found an association between frequent OM episodes with IL-6^{-174}. This study compared children with history of recurrent AOM to healthy controls using the Dutch adult blood donor pool. In our current report, we prospectively analyze the association of genetic polymorphisms with otitis susceptibility to URI and AOM. In addition, environmental and risk factor information was taken in real time at enrollment.

The definition of an otitis-prone (susceptible) child has been debated for the past several decades. Early otitis investigators (19) observed that children who had at least two or more episodes of AOM during the first year of life had at least twice the total number of episodes than children who had one or no episode during the first year of life. In later studies, age at the first AOM episode in at risk children was reduced to 6 months (20). Children with a family history of recurrent otitis (21) were especially at risk. More recently, several authors have, by convention, expanded the criteria to: 4 AOM episodes in one year, 3 in six months or, the first AOM episode prior to age 6 months to define the otitis susceptible child (20,22). These definitions are limited because it does not allow us to predict early in life which child is at risk to be otitis susceptible. Furthermore, AOM occurs most commonly as a complication of URI; these otitis-prone definitions do not take into account the number of URI episodes experienced by the child or the proportion of AOM complicating URI. For example, a child in daycare who has 10 colds in a year but only 3 cold episodes develop into AOM may be less otitis-prone than a child who only has 3 colds per year but each one develops into AOM.

Interestingly, IL-6^{-174} polymorphism alone is not associated with increased risk of AOM following and episode of URI. However, children with IL-6^{-174} polymorphism were more likely to have frequent episodes of URI and AOM. The study was not designed as a viral surveillance study, therefore, we can not comment on whether viral attack rate in polymorphic children was more or less than in non-polymorphic children. Considering that previous literature has shown an association between IL-6 concentrations in nasal secretions and enhanced respiratory symptoms (4), one possible mechanism by which children with IL-6^{-174} polymorphism have higher rate of symptomatic URI could be because of higher

production of IL-6 in these polymorphic individuals. Children who are high acute-phase cytokine producers from various cytokine polymorphisms may be susceptible to recurrent AOM by different mechanisms.

In this study we did not find an additive effect of having both TNFα^{-308} and IL-6^{-174} polymorphisms. This is likely due to having only 16 children with both polymorphisms. We did find a significant increase in OM susceptibility and tympanostomy tube placement in a previous study (14) with a cohort of 500 children, 35 of who had both polymorphisms.

It is well known that children in daycare have more URI episodes per year than children who do not attend daycare (23,24); we also showed the same finding in this present report. In addition, we showed that children in daycare were more likely to be AOM susceptible than children who are not in daycare regardless of polymorphic cytokine genotypes. This finding supports the data that daycare attendance by itself is an independent risk factor for AOM susceptibility which is likely due to the increased rates of virus exposure and nasopharyngeal bacterial colonization in these children.

Further studies are needed to evaluate the interaction of the acute-phase cytokine gene polymorphisms with modifiable environmental risk factors such as day care attendance and breastfeeding. In addition, further studies are required to determine the mechanisms by which cytokine polymorphisms predispose children to URI and AOM. The understanding of these mechanisms, in turn, will allow interventions to prevent AOM in these high risk children.

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Table 1

Demographic and Individual Characteristics of 242 Children

	All Cases N= 242 (% of total)	TNF^{-308} N=241 (%)		$IL\text{-}6^{-174}$ N=242 (%)	
		Normal N=181	Pol* N=60	Normal N=184	Pol* N=58
Males	123 (51%)	93 (51%)	30 (50%)	93 (51%)	30 (52%)
Females	119 (49%)	88 (49%)	30 (50%)	91 (49%)	28 (48%)
Race					
Asian	6 (2%)	3 (2%)	3 (5%)	5 (3%)	1 (2%)
African American	73 (30%)	51 (28%)	22 (37%)	55 (30%)	18 (31%)
Caucasian	143 (59%)	111 (61%)	31 (52%)	109 (59%)	34 (58%)
Other (mixed race)	20 (8%)	16 (9%)	4 (7%)	15 (8%)	5 (9%)
Hispanic/Latino Ethnicity	111 (46%)	91 (50%)	20 (33%)	94 (51%)	17 (30%)
Breast-fed 8 weeks or longer	83 (34%)	64 (35%)	19 (32%)	61 (33%)	22 (38%)
Daycare attendance	74 (31%)	55 (30%)	18 (30%)	56 (30%)	18 (31%)
Exposure to cigarette smoke	79 (33%)	63 (35%)	16 (27%)	59 (32%)	20 (34%)
Mean (Median) age at enrollment	13.7 (12)				

*POL = polymorphism. Includes both heterozygous and homozygous polymorphism.

Table 2

Adjusted Odds of AOM Complicating URI in Polymorphic Children Compared to Non-Polymorphic Children

Comparison (n=number of subjects)	Adjusted OR (CI)
TNFα^{-308} POL vs NL (n=60 vs 181)	1.42 (1.00–2.00)
IL-6^{-174} POL vs NL (n=73 vs 169)	1.19 (0.83–1.72)
TNFα^{-308} and IL-6-^{174} POL vs NL (n=16 vs 121)	1.40 (0.83–2.49)
TNFα^{-308} or IL-6-^{174} POL vs NL (n=92 vs 150)	1.43 (1.01–2.02)
TNFα^{-308} and/or IL-6-^{174} POL vs NL (n=121 vs 120)	1.43 (1.02–2.00)

POL = polymorphic children (heterozygous and homozygous polymorphism); NL = non-polymorphic (normal) children. Among the adjusting factors (age, gender, race, ethnicity, BF, DC attendance, CSE, family history of OM, and number of PCV7 doses), AOM was only significantly associated with young age (P<0.003); no other factors showed significant association with AOM.