

Cytokine Gene Polymorphism in Recurrent Acute Otitis Media

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Background: There is increasing evidence that a strong genetic component is involved in the predisposition to recurrent acute otitis media (rAOM). Cytokines play a key role in the pathogenesis of otitis media. Constitutional polymorphisms in cytokine genes may lead to individual variations in cytokine secretion.

Objective: To elucidate the role of cytokine gene polymorphisms in rAOM.

Setting: University hospital.

Participants and Methods: Blood samples for genetic analysis were obtained from 63 individuals with rAOM from 20 different families and from 400 healthy blood donors. The medical history of the rAOM group was based on medical records and interview data. We stud-

ied the polymorphisms of tumor necrosis factor α , interleukin (IL) 1 α , IL-1 β , and IL-1 receptor antagonist genes.

Results: The distribution of cytokine alleles in the rAOM group did not differ significantly from that of the control group. However, in patients with rAOM without a history of allergic disorders, allele frequencies of IL-1 α -889 differed significantly from those of controls ($P=.03$).

Conclusions: There is no clear association between the polymorphism of studied cytokine genes and rAOM. However, the IL-1 α gene polymorphism may be associated with recurrent middle ear infections in a subgroup of patients without allergic disorders.

Arch Otolaryngol Head Neck Surg. 2002;128:17-20

ACUTE OTITIS media (AOM) is one of the most common childhood illnesses. Although nearly all children experience at least one otitis episode, only about 15% of children experience recurrent attacks.¹ Recurrent AOM (rAOM) is a challenging clinical problem consuming many medical resources. Moreover, frequent otitis episodes are also associated with a child's developmental delay.²

Recurrent AOM has a multifactorial background that is still only partly understood. Epidemiologic data have revealed several external factors predisposing to otitis media, including day care outside the home, use of a pacifier, and parental smoking.³ However, recent twin and triplet studies^{4,5} have suggested that there is a strong genetic component involved in middle ear infections. This is supported by earlier findings⁶ of incidence differences of AOM in different ethnic groups and epidemiologic analyses³ that have shown that a positive family history of AOM is the most im-

portant risk factor for AOM. Genetically determined immunoglobulin markers have also been studied in children with rAOM, and allotype G2m(23) has been linked with otitis proneness.^{7,8} Moreover, an association between the HLA-A2 antigen and rAOM has been reported but not between HLA-A2 and secretory otitis media.^{9,10}

Cytokines are bioactive proteins that widely mediate host responses to inflammatory stimuli. In the pathogenesis of an inflammation in the respiratory tract, they regulate proliferation, chemotaxis, and the activation of inflammatory cells.¹¹ Constitutional polymorphisms in cytokine genes may lead to individual variations in cytokine secretion.^{12,13} The outcomes of many infectious diseases, such as malaria,¹⁴ meningococcal meningitis,¹⁵ and Epstein-Barr virus,¹⁶ have been associated with these polymorphisms. Cytokines also play a key role in the pathogenesis of otitis media.¹⁷⁻¹⁹ The aim of the present work was to study the polymorphisms of tumor necrosis factor (TNF) α ,

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PARTICIPANTS AND METHODS

This study was conducted at Tampere University Hospital, Tampere, Finland, and included 20 different families with a high occurrence of AOM. In each family, at least 1 parent and all the children had a history of rAOM. The criterion for rAOM was at least 6 AOM episodes within 12 months or at least 10 AOM episodes during a lifetime. The medical history was based on medical records and interview data. A blood sample for genetic analysis was obtained from 63 patients with rAOM.

Control blood samples were obtained from 400 unselected healthy blood donors (The Finnish Red Cross Blood Transfusion Centre, Tampere) living in the same area as the study group members. Donors were aged 18 to 60 years. The history of AOM in the control population was not known.

The study was approved by the ethical committee of Tampere University Hospital, and written informed consent was received from the parents in each family with rAOM.

ANALYSIS OF CYTOKINE GENE POLYMORPHISMS

DNA specimens from citrated whole blood samples were prepared using standard methods.

Tumor Necrosis Factor α -308

A 107-base pair fragment of the TNF- α gene promoter region containing the G→A substitution was amplified using polymerase chain reaction (PCR) primers 5'-AGGCAATAGGTTTGTAGGGCCAT-3' and 5'-TCCTCCCTGCTCCGATTCCG-3'. The amplified product was digested with *NcoI* and analyzed using electrophoresis on 9% polyacrylamide gel.²⁰

Interleukin 1 α -889

The base exchange at position -889 of the IL-1 α gene was analyzed as previously described.²¹ Oligonucleotides

5'-AAGCTTGTT CTACCACCTGAAGTAGGC-3' and 5'-TTACATATGAGC CTTCCATG-3' flanking the polymorphic site were used as primers in PCR. The products were digested with *NcoI*, and the resultant products were analyzed using 9% polyacrylamide gel electrophoresis.

Interleukin 1 β -511

The region that contains the *AvaI* polymorphic site at position -511 of the IL-1 β gene was amplified using PCR.²² The oligonucleotides 5'-TGGCATTGATCTGGTTCATC-3' and 5'-GTTTAGGAATCTTCCCACTT-3' were used as primers. Fragments were analyzed using electrophoresis on 9% polyacrylamide gel stained with ethidium bromide.

Interleukin 1 β +3953

Position +3953 within exon 5 of the IL-1 β gene has a single base pair polymorphism.²³ The polymorphic region containing the *TaqI* restriction site was amplified using the following primers: 5'-GTTGTCATCAGACTTTGACC-3' and 5'-TTCAGTTCATATGGACCAGA-3'. Fragments were analyzed using electrophoresis on 9% polyacrylamide gel stained with ethidium bromide.

Interleukin 1Ra

The IL-1Ra exon 2 polymorphism was analyzed as described previously.²⁴ Oligonucleotides 5'-CTCAGCAACACTCCTAT-3' and 5'-TCCTGGTCTGCAGGTAA-3' were used as primers in PCR. The final PCR product was analyzed using electrophoresis on a 2% agarose gel stained with ethidium bromide.

STATISTICAL ANALYSIS

The distribution of the TNF- α -308, IL-1 α -889, IL-1 β -511, IL-1 β +3953, and IL-1Ra allele frequencies in the rAOM families was compared with that of healthy blood donors. Statistical analysis was performed using the χ^2 test.

interleukin (IL) 1 α , IL-1 β , and IL-1 receptor antagonist (IL-1Ra) genes in patients with rAOM.

RESULTS

The families included in the study were extremely prone to otitis (**Table 1**), but none of the patients had been known to have an immunologic deficiency. In every patient, the primary condition was rAOM, but secretory otitis media later developed in 20 (32%). Three patients also experienced subacute or chronic mastoiditis, and mastoidectomy was performed in 2 patients. In the study group, 18 patients were adults and 45 were children; 57% were male (n=36) and 43% were female (n=27). Seventeen patients from 7 different families had a history of allergic disorders (atopic dermatitis, asthma, or allergic rhinitis): 10 in 2 families and 7 in 5 families.

The distribution of proinflammatory cytokine alleles (TNF- α -308, IL-1 α -889, IL-1 β -511, and IL-1 β +3953) (**Table 2**) and anti-inflammatory IL-1Ra al-

Table 1. Characteristics of 20 Families With rAOM*

	Adults	Children	Total
Family members, No.	18	45	63
Age, mean (range), y	35.5 (28-42)	6.2 (1-16)	15.0
Children per family, mean (range), No.	NA	NA	2.5 (2-5)
Adenoidectomy/tympanostomy, total No. (range)	18 (0-3)	78 (0-11)	96 (0-11)†
History of mastoiditis, No.	2	1	3
Secretory otitis media, No.	4	16	20‡
Allergic disorder, No.	4	13	17

*rAOM indicates recurrent acute otitis media; NA, not applicable.

†Four adults and 3 children did not undergo surgery.

‡In every patient, the primary condition was rAOM.

les (**Table 3**) did not differ significantly in the rAOM group vs the control group. When calculating the children's data separately, the result remained basically the same. The greatest difference was in the distribution of

Table 2. Polymorphism of TNF- α -308, IL-1 α -889, IL-1 β -511, and IL-1 β +3953 in 63 Patients With rAOM vs 400 Controls*

Cytokine and Group	Genotype			Allele Frequency		P Value
	1.1	1.2	2.2	Allele 1	Allele 2	
TNF- α -308						
rAOM	38	25	0	0.80	0.20	.23
Controls	275	120	5	0.84	0.16	
IL-1 α -889						
rAOM	32	29	2	0.74	0.26	.12
Controls	167	201	32	0.67	0.33	
IL-1 β -511						
rAOM	27	31	5	0.67	0.33	.08
Controls	146	182	72	0.59	0.41	
IL-1 β +3953						
rAOM	36	25	2	0.77	0.23	.24
Controls	206	164	30	0.72	0.28	

*TNF- α indicates tumor necrosis factor α ; IL, interleukin; and rAOM, recurrent acute otitis media.

Table 3. Polymorphism of IL-1Ra in 63 Patients With rAOM vs 400 Controls*

Group	Genotype					Allele Frequency			P Value
	1.1	1.2	2.2	1.3	2.3	Allele 1	Allele 2	Allele 3	
rAOM	38	18	7	0	0	0.75	0.25	0	.56
Controls	199	156	37	7	1	0.70	0.29	0.01	

*IL-1Ra indicates interleukin 1 receptor antagonist; rAOM, recurrent acute otitis media.

IL-1 α -889 alleles, but this did not reach statistical significance ($P=.07$). However, in a subgroup of patients with rAOM without a history of allergic disorders (atopy, asthma, or allergic rhinitis), allele frequencies of IL-1 α -889 differed significantly from those in the control group (allele 1: 0.78 vs 0.67; $P=.03$).

COMMENT

Cytokines are of central importance in the pathogenesis of inflammation. Three main proinflammatory cytokines—TNF- α , IL-1, and IL-6—and anti-inflammatory cytokines such as IL-1Ra and IL-10 down-regulate the inflammatory process.^{12,13} The production of cytokines is regulated by genetic elements. Polymorphisms associated with these genes are thought to lead to individual variations in cytokine secretions and are inherited in mendelian fashion.^{12,13} Cytokine gene polymorphism is associated with the outcomes of various infectious diseases.¹⁴⁻¹⁶

In the past few years, several studies have been published regarding cytokines and otitis media, and it has been possible to determine various types of cytokines from middle ear effusions (IL-1 β , IL-2, TNF- α , interferon γ ,¹⁹ IL-6,²⁵ and IL-8²⁶) and from nasopharyngeal secretions (IL-1 β , IL-6, and TNF- α),¹⁸ indicating that cytokines play a key role in the pathogenesis of AOM. High concentrations of TNF- α in middle ear effusions correlate with the persistence of secretory otitis media.²⁷ Furthermore, IL-1 β , IL-6, and TNF- α concentrations measured from the nasopharyngeal secretions of children with rAOM are lower compared with healthy children.¹⁸ It has also been

postulated that children with rAOM may have low IL-2 production, leading to lower serum IgA levels in mucosal secretions.²⁸ On the other hand, inflammatory cytokines can also increase bacterial adherence to and invasion of cells²⁹; hence, if a respiratory tract virus infection, which usually precedes an AOM attack, induces too high or prolonged cytokine production, this might theoretically increase the risk of developing AOM.

According to results of twin and triplet studies,^{4,5} the weight of genetic factors in middle ear infections is 0.45 to 0.64 among males and 0.74 to 0.79 among females. The genetic foundation of susceptibility to rAOM is unclear. Prellner et al found that the genetically determined immunoglobulin marker G2m(23) was present in 95% of children with rAOM but in only 64% of children with no history of AOM ($P=.01$).^{7,8} Kalm et al⁹ revealed that the HLA-A2 antigen occurred significantly more often in the rAOM group than in controls (80% vs 56%; $P<.01$) and that the frequency of the HLA-A3 antigen was lower. The TNF- α gene is located on the short arm of chromosome 6 in the major histocompatibility complex between HLA class I and III loci and can be coinherited with certain HLA antigens.³⁰ However, in the present study, we could not find differences in allele frequencies of TNF- α -308 between the study groups.

The IL-1 gene family includes IL-1 α , IL-1 β , and IL-1Ra, which are situated on the short arm of chromosome 2.³¹ In the present study, the allele frequencies of the IL-1 gene did not differ significantly in the rAOM group vs controls. The greatest difference was in the allelic distribution of the IL-1 β -511 gene, but it did not

reach statistical significance ($P=.08$). However, in a subgroup of patients with rAOM who did not have any allergic disorders, there were significant differences in the IL-1 α -889 allele frequencies. Because these differences were not so great and because the number of patients in the subgroup analysis was small, the value of this finding should be considered cautiously. On the other hand, it is possible that the pathogenesis of middle ear inflammation in allergic patients differs from that in nonallergic patients.

In the present study we paid extra attention to selecting families who were extremely prone to otitis; however, the control population was not ideal because the occurrence of AOM in this population was not known. We have no reason to assume that our control population would have had an unusually high occurrence of AOM. Also, the frequency of TNF alleles reported²⁰ in other populations did not differ from that in our control population.

Otitis media has a multifactorial background. The pathogenesis of AOM is regulated by many external and internal factors, including normal flora,³² bacterial-viral interactions,³³ and individual immunologic reactions. Recent studies have shown that a strong genetic component is involved in middle ear infections,^{4,5} but the genetic foundation of susceptibility to rAOM is unclear. In the present work, we studied the polymorphisms of TNF- α -308, IL-1 α -889, IL-1 β -511, IL-1 β +3953, and IL-1Ra genes in patients with rAOM, but we did not find any clear association between the studied cytokine genes and susceptibility to recurrent middle ear infections, except in a subgroup of patients without any allergic disorders. More data are needed to assess the value of this finding.

Accepted for publication August 16, 2001.

This study was supported by a grant from The Medical Research Fund of Tampere University Hospital.

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