Cytokine Gene Polymorphism in Recurrent Acute Otitis Media

Veli-Pekka Joki-Erkkilä, MD; Heikki Puhakka, MD; Mikko Hurme, MD

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 studied the polymorphisms of tumor necrosis factor (TNF), interleukin (IL) 1α, IL-1β, 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-1α-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-1α gene polymorphism may be associated with recurrent middle ear infections in a subgroup of patients without allergic disorders.

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 unsel ected 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'-AGGCAAATGTTTTGAGGCCAT-3' and 5'-TCCCTCCTGGTCCTCAGTTCG-3'. The amplified product was digested with NcoI and analyzed using electrophoresis on 9% polyacrylamide gel.

Interleukin 1α-889

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

Interleukin 1β-3953

The region that contains the Aval polymorphic site at position -3953 within exon 5 of the IL-1β gene was amplified using PCR. The oligonucleotides 5'-CGGAGCAACACTCTTAT-3' and 5'-CCCTGGTCTGCAGGTAA-3' were used as primers in PCR. The final PCR product was analyzed using electrophoresis on 9% polyacrylamide gel stained with ethidium bromide.

Interleukin 1Ra

The IL-1Ra exon 2 polymorphism was analyzed as described previously. Oligonucleotides 5'-CTCAGCACACTCTCAT-3' and 5'-TTCTGGTCTGCGATT-3' were used as primers in PCR. The final PCR product was analyzed using electrophoresis on 9% polyacrylamide gel stained with ethidium bromide.

STATISTICAL ANALYSIS

The distribution of proinflammatory cytokine alleles (TNF-α-308, IL-1α-889, IL-1β-511, and IL-1β+3953) and anti-inflammatory IL-1Ra alleles (IL-1α, IL-1β, and IL-1 receptor antagonist (IL-1Ra)) genes in patients with rAOM.

Table 1. Characteristics of 20 Families With rAOM

<table>
<thead>
<tr>
<th>Family members, No.</th>
<th>Adults</th>
<th>Children</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (range), y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children per family, mean (range), No.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenoidectomy/tonsillectomy. total No. (range)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>History of mastoiditis, No.</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Secretary otitis media, No.</td>
<td>4</td>
<td>16</td>
<td>20</td>
</tr>
<tr>
<td>Allergic disorder, No.</td>
<td>4</td>
<td>13</td>
<td>17</td>
</tr>
</tbody>
</table>

*42 adults and 3 children did not undergo surgery.
†In every patient, the primary condition was 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 mastoideectomy 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-1α-889, IL-1β-511, and IL-1β+3953) (Table 2) and anti-inflammatory IL-1Ra alleles (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
IL-1α-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-1α-889 differed significantly from those in the control group (allele 1: 0.78 vs 0.67; \(P = .03\)).

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

<table>
<thead>
<tr>
<th>Cytokine and Group</th>
<th>Genotype</th>
<th>Allele Frequency</th>
<th>(P) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.1</td>
<td>1.2</td>
<td>2.2</td>
</tr>
<tr>
<td>TNF-α-308</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rAOM</td>
<td>38</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>Controls</td>
<td>275</td>
<td>120</td>
<td>5</td>
</tr>
<tr>
<td>IL-1α-889</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rAOM</td>
<td>32</td>
<td>29</td>
<td>2</td>
</tr>
<tr>
<td>Controls</td>
<td>167</td>
<td>201</td>
<td>32</td>
</tr>
<tr>
<td>IL-1β-511</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rAOM</td>
<td>27</td>
<td>31</td>
<td>5</td>
</tr>
<tr>
<td>Controls</td>
<td>146</td>
<td>182</td>
<td>72</td>
</tr>
<tr>
<td>IL-1β+3953</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rAOM</td>
<td>36</td>
<td>25</td>
<td>2</td>
</tr>
<tr>
<td>Controls</td>
<td>206</td>
<td>164</td>
<td>30</td>
</tr>
</tbody>
</table>

*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*

<table>
<thead>
<tr>
<th>Group</th>
<th>Genotype</th>
<th>Allele Frequency</th>
<th>(P) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.1</td>
<td>1.2</td>
<td>1.3</td>
</tr>
<tr>
<td>rAOM</td>
<td>38</td>
<td>18</td>
<td>7</td>
</tr>
<tr>
<td>Controls</td>
<td>199</td>
<td>156</td>
<td>37</td>
</tr>
</tbody>
</table>

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

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-1β, IL-2, TNF-α, interferon γ,\(^{10}\) IL-6,\(^{15}\) and IL-8\(^{26}\)) and from nasopharyngeal secretions (IL-1β, IL-6, and TNF-α),\(^{16}\) 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-1β, 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 cells;\(^{29}\) 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. Pelling 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 al\(^{9}\) 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 co-inherited 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-1α, IL-1β, 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-1β-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-1α-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 reported 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, bacterial-viral interactions, and individual immunologic reactions. Reanimation is involved in middle ear infections, but the genetic foundation of susceptibility to rAOM is unclear. In this finding should be considered cautiously. On 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.

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REFERENCES