Short Communication

CCR2 and CCR5 gene polymorphisms in children with recurrent respiratory infections

A. IANNI, S. MAJORE, D. ARZANI, I. CARBONI, G. M. CORBO AND V. ROMANO-SPICA

Institute of Hygiene and Department of Respiratory Physiology, Catholic University Medical School, Rome, Italy

Introduction

Recurrent lower respiratory infections in infancy represent a major health problem because of their high incidence and possible consequences on lung function (1, 2). On the basis of data acquired from a recent epidemiological study carried out in Italy on 18737 children aged 6–7 years, lifetime pneumonia in 1999 showed a 7.9% frequency (SIDRIA project, unpublished data).

Several environmental risk factors have been associated with lower respiratory infections, but knowledge about genetic predisposition is very limited (3). Analysis of polymorphic genes involved in regulation of the immune system is disclosing promising perspectives. In particular, chemokines and their receptors are involved in host inflammatory response against invading pathogens (4).

Chemokines are a family of pro-inflammatory cytokines that attract and activate specific types of leukocytes (5). Their actions are mediated by subfamilies of G-protein-coupled receptors, indicated as CCR or CXCR (6). A common 32-bp deletion in the CCR5 gene (CCR5D32) and a G-to-A nucleotide substitution in the CCR2 gene at position 190 (CCR264I) have been described (7, 8). These variant alleles have been first associated with resistance to HIV-1 infection and delay in the progression to AIDS (9–12). Recently, their involvement has been investigated in asthma, allergic diseases and insulin-dependent diabetes mellitus (13–15).

Since CCR2 and CCR5 allelic forms have an important role in regulating the immune response during the infectious process we evaluated the distribution of these genetic polymorphisms in children with recurrent respiratory tract infections.

Methods

Case selection was made by means of strict clinical criteria regarding lower respiratory infections: we enrolled Caucasian children less than 7 years old (mean age: 4–6 years) showing more than three episodes of low tract respiratory infection per year, for at least 2 years. A total of 180 genomic DNA samples were analysed, including 50 children (60% males, 40% females) that met the selection criteria and 130 controls selected from the general population after excluding the presence of asthma or recurrent respiratory infections. It was excluded the presence of cystic fibrosis patients or mutation carriers by clinical evaluation or reverse hybridization analysis. After obtaining informed written consent, a mouthwash sample was taken for DNA extraction, and CCR2 and CCR5 genotypes were determined on amplification products, as previously described (16). Fisher’s exact test and χ² test were employed in order to evaluate differences in allelic counts between cases and controls and verify the Hardy–Weinberg equilibrium in both groups.

Results and discussion

We observed a different distribution of CCR264I mutation between cases and controls. The allelic frequency of CCR264I mutant allele was 0.2 for cases and 0.07 for controls, respectively, showing a statistically significant difference among the two groups (P = 0.001).

CCR2 and CCR5 genotypes and allelic frequencies in the whole study population are summarized in Table 1. We found that 20 (40%) out of 50 cases were classified as heterozygous CCR2/CCR264I, whereas the remaining 30 cases (60%) were homozygous for the wild-type allele. Among controls, 17 (13-1%) of 130 subjects were heterozygous CCR2/CCR264I, 112 (86-1%) were homozygous CCR2/CCR2 and 1 (0.8%) of 130 controls was found to be homozygous CCR264I/CCR264I.

The following CCR5 genotype distribution was observed among cases: 46 (92%) of 50 cases were homozygous for CCR5 wild-type allele and four cases (8%) were heterozygous CCR5/CCR5D32, with a 0.04 frequency of...
CCR5Δ32 allele. Among controls, 120 (92.3%) of 130 subjects were homozygous CCR5/CCR5, nine (6.9%) were heterozygous CCR5/CCR5 Δ32, and one (0.8%) was found to be homozygous CCR5Δ32/CCR5Δ32, with a 0.04 frequency of CCR5 mutant allele. CCR5 allelic counts did not show statistically significant difference between cases and controls (\( P = 0.8 \)). CCR5 Δ32 allelic frequency in the general population was in accordance with previous reports (16, 17). Both CCR2 and CCR5 genotype distributions were in equilibrium according to the Hardy–Weinberg equation.

CCR-family genes play a critical role in modulating the immune response and acting as viral co-receptors (18). On the basis of our preliminary results, a significantly higher frequency of CCR2Δ4I allele has been observed in children with recurrent respiratory tract infections, suggesting a possible role in determining individual susceptibility. Larger studies are in progress and further investigation will contribute to clarify biological mechanisms and verify the predictive value of CCR alleles.

### References


### Table 1. CCR2 and CCR5 allelic frequencies

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCR2</td>
<td>CCR2/CCR2</td>
<td>30 (60%)</td>
</tr>
<tr>
<td>Genotypes</td>
<td>CCR2/CCR2Δ4I</td>
<td>20 (40%)</td>
</tr>
<tr>
<td></td>
<td>CCR2Δ4I/CCR2Δ4I</td>
<td>0</td>
</tr>
<tr>
<td>CCR2 alleles</td>
<td>CCR2</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>CCR2Δ4I</td>
<td>0.2</td>
</tr>
<tr>
<td>CCR5</td>
<td>CCR5/CCR5</td>
<td>46 (92%)</td>
</tr>
<tr>
<td>genotypes</td>
<td>CCR5/CCR5Δ32</td>
<td>4 (8%)</td>
</tr>
<tr>
<td></td>
<td>CCR5Δ32/CCR5Δ32</td>
<td>0</td>
</tr>
<tr>
<td>CCR5 alleles</td>
<td>CCRD</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>CCR5Δ32</td>
<td>0.04</td>
</tr>
</tbody>
</table>
