Background & objectives: Interleukin-1 receptor antagonist (IL-1Ra) is a naturally occurring anti-inflammatory molecule that blocks action of IL-1. Polymorphism in IL-1Ra gene intron 2 results in differences in production of IL-1Ra. These polymorphisms are reportedly associated with autoimmune disease susceptibility in different studies. However, such data are lacking from India. We undertook this study to examine the IL-1Ra polymorphism as a susceptibility marker in patients with rheumatoid arthritis (RA).

Methods: DNA samples from 107 patients with RA and 111 healthy controls were used to study genotypes of the IL-1RA gene by PCR. Allelic frequencies and carriage rates were calculated and compared in both the groups.

Results: Among the 107 patients with RA, 93 were females and 75 per cent were seropositive for rheumatoid factor. The frequencies of IL-1RA alleles in controls were as follows: Allele 1 (IL-1RN*1) was 83.33 per cent, IL-1RA allele 2 (IL-1RN*2) was 16.21 per cent and allele 3 (0.46%). In RA patients the allele frequencies were 84.11 per cent for IL-1RN*1, 14.95 per cent for IL-1RN*2, 0.47 per cent each for IL-1RN*3 and IL-1RN*4. There was no difference in frequency of different alleles between the two groups. However, homozygosity for allele 2 was more frequent in controls (9.91%) as compared to patients (4.67%).

Interpretation & conclusion: Our findings indicated that IL-1RA polymorphism was not a susceptibility marker in RA nor did it show any association with seropositivity, Sjögren’s syndrome or subcutaneous nodules. Further studies with large sample need to be done to confirm these findings.

Key words Cytokine antagonist - interleukin-1 receptor - rheumatoid arthritis - Sjögren’s syndrome

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease characterized by synovial inflammation, bone and cartilage destruction with a variety of extra-articular features. It affects approximately 1 per cent of world population including India. RA is a complex multi-factorial disease in which genetic susceptibility affects disease occurrence and severity. HLA class II DR4 alleles are known to be associated with more severe disease, however, HLA complex represents only
30-50 per cent of the total genetic susceptibility. Thus, non-HLA genes including cytokine gene polymorphism may be important in RA.

Interleukin-1 (IL-1) plays an important role in mediating joint inflammation and destruction in RA. The IL-1 gene family contains three related genes, IL-1A, IL-1B and IL-1RN, which encode the pro-inflammatory cytokines IL-1α, IL-1β and their naturally occurring antagonist, interleukin-1 receptor antagonist (IL-1Ra), respectively. IL-1Ra acts by competitive blockade of IL-1 receptor without affecting downstream signaling. IL-1Ra knockout mice spontaneously develop inflammatory arthritis resembling RA, and exogenous administration of IL-1Ra prevents bone damage in animal models and joint erosion in patients of rheumatoid arthritis. Recombinant IL-1Ra (Anakinra) is now an established therapeutic agent in the management of RA. The gene for IL-1Ra (IL-1RN) exists in 5 allelic variants corresponding to 2, 3, 4, 5 and 6 copies of 86-base pair sequence repeat located in intron 2. Since three potential protein binding sites are located in this 86-bp sequence, the number of repeats may influence gene transcription and protein production. Moreover, one allele IL-1RN*2 has been associated as a risk and/or severity factor with various autoimmune diseases like systemic lupus erythematosus, Sjögren’s syndrome, vasculitis and interstitial disease were collected from hospital records. Patients having RF seropositivity and/or extra-articular features were classified as having severe disease.

PCR for IL-1RN VNTR polymorphism: Genomic DNA (1 µg) was amplified in 20 ml reaction volume using Taq polymerase (0.5 units, Invitrogen, USA), dNTP (200 µM, Invitrogen, USA), oligonucleotide primers (1 µM each of the two primers, 5’-CTCAGCAACACTCTATGGA-3’ and 5’-GTGTCGCTGCAGTAA-3’ Genosys, India) and PCR buffer 10X (100 mM Tris HCl, 500 mM KCl and 15 mM magnesium chloride). Forty cycles with each cycle having denaturation at 95°C for 30 sec, annealing at 62°C for 30 sec and extension at 72°C for 30 sec were run. Ethidium bromide-stained 2 per cent agarose gels were used to visualize the PCR products and the product size was calculated relative to 100-bp DNA ladder. The product size of different alleles was as follows: I IL1RN*1 410 bp; II L1RN*2 240 bp; III IL1RN*3 500 bp; IV IL1RN*4 325 bp; and V IL1RN*5 595 bp.

Statistical analysis: Allelic frequencies (number of copies of a specific allele divided by the total
number of alleles in the group) and carriage rates (number of individuals with at least one copy of a specific allele divided by the total number of individuals within the group) were calculated. The inter-group comparison of frequencies was done using chi square and Fisher’s exact test. Odds ratio (OR) were calculated for disease susceptibility or severity in carriers of specific alleles. The 95 per cent confidence intervals (95% CI) for the OR were also determined.

Results

Among 107 patients with RA there were 93 females. The mean age was 45 ± (23-71) yr and mean age of onset of disease was 36.74 (17-58) yr. Rheumatoid factor was present in 80 patients (75%), 39 patients (36.44%) had extra-articular features with 24 having subcutaneous nodules, 21 secondary Sjögren’s syndrome, 9 interstitial lung disease and 2 had vasculitis.

The frequencies of different alleles were similar in controls and patients. Genotype frequencies showed a trend towards lesser frequency of allele II homozygosity in patients. No difference was seen in the carriage rate of different alleles (Table I).

There was no difference in allele or genotype frequency in patients with rheumatoid factor and those without it, patients with and without subcutaneous nodules or Sjögren’s syndrome (Table II) and those with early (<40 yr; n=61) or late age of onset (n=46). Patients with Sjögren’s syndrome had a lower frequency (4.76%) of allele 2 as compared to those who were negative for it (17.44%) but this difference was not statistically significant.

<table>
<thead>
<tr>
<th>IL-1RN allele</th>
<th>RA (n =107) no. (% )</th>
<th>Controls (n =111) no. (% )</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>180 (84.11)</td>
<td>185 (83.33)</td>
<td>1.06 (0.62-1.82)</td>
</tr>
<tr>
<td>II</td>
<td>32 (14.95)</td>
<td>36 (16.21)</td>
<td>0.91 (0.52-1.57)</td>
</tr>
<tr>
<td>III</td>
<td>1 (0.47)</td>
<td>1 (0.46)</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>1 (0.47)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

**Genotype:**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>RA (n =107) no. (% )</th>
<th>Controls (n =111) no. (% )</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I / I</td>
<td>78 (72.90)</td>
<td>85 (76.58)</td>
<td>0.82 (0.43-1.59)</td>
</tr>
<tr>
<td>I / II</td>
<td>22 (20.57)</td>
<td>14 (12.61)</td>
<td>0.79 (0.82-3.97)</td>
</tr>
<tr>
<td>I / III</td>
<td>1 (0.93)</td>
<td>1 (0.90)</td>
<td>1.034 (0 –38.13)</td>
</tr>
<tr>
<td>I / IV</td>
<td>1 (0.93)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>I / V</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>II / II</td>
<td>5 (4.67)</td>
<td>11 (9.91)</td>
<td>0.45 (0.13-1.45)</td>
</tr>
</tbody>
</table>

**Carriage rate:**

<table>
<thead>
<tr>
<th>Carriage rate</th>
<th>RA (n =107) no. (% )</th>
<th>Controls (n =111) no. (% )</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>102 (95.32)</td>
<td>100 (90.09)</td>
<td>1.87 (0.61-5.94)</td>
</tr>
<tr>
<td>II</td>
<td>27 (25.23)</td>
<td>25 (22.52)</td>
<td>1.16 (0.59-2.27)</td>
</tr>
<tr>
<td>III</td>
<td>1 (0.93)</td>
<td>1 (0.90)</td>
<td>1.034 (0 –38.13)</td>
</tr>
<tr>
<td>IV</td>
<td>1 (0.93)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>0</td>
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</tbody>
</table>
**Discussion**

Cytokines are key players in pathogenesis of synovitis and subsequent joint damage in RA. Difference in levels of various cytokines among different individuals can be a possible explanation for differences in disease susceptibility and severity. These variations are mostly due to polymorphism in the genes encoding for cytokines. IL-1, a cytokine produced by monocytes mediates cartilage and bone destruction in RA. IL-1α, IL-1β and their naturally occurring antagonist, IL-1Ra are coded by genes located within a 430KB region. IL-1Ra counteracts the action of IL-1 by binding to IL-1 receptor without activating it.

No difference was found in allele frequency, genotype frequency and carriage rate for IL-1Ra between patients with RA and healthy controls. There was a trend towards less frequency of IL1RN*2 homozygosity in patients with RA.

Among our population, the 4 repeat (IL1RN*1, allele 1) and 2 repeat (IL1RN*2, allele 2) were found in majority of patients and controls. The frequency of IL1RN*1 allele was more common in Indians than in French, British and Turkish population but was comparable to other south-eastern population like Koreans and Chinese (Taiwan). Alleles IL1RN*3 (allele 3), IL1RN*4 (allele 4) were seen only in a few patients and this frequency was similar to that reported in Korean and Taiwanese.

Absence of any difference in allelic frequency between patients with RA and controls has been previously reported in studies from Europe and Taiwan. We found a trend towards a lower
frequency of homozygosity of allele 2 in patients with RA as compared to controls even though there was no difference in allele frequency of allele 2. Allele 2 is associated with increased production of IL-1Ra and homozygosity may further increase the amount of IL-1Ra produced. High IL-1Ra downmodulates the inflammatory signals and may thus reduce susceptibility to RA. In a study from Korea a lower frequency of allele 2 was found in patients (2.5%) as compared to controls (7.1%). This is in contrast to other autoimmune diseases like SLE, Sjögren’s syndrome and juvenile idiopathic arthritis where increased frequency of allele 2 is reported and it may be related to linkage with promoters of IL-1 gene complex.

No significant difference was found in allele frequencies, carriage rates or genotype distribution between patients with or without extra-articular features, seropositive or seronegative groups, early versus delayed age of onset (< 40 yr) in our study. Our results corroborate with an earlier study by Cantagrel et al who also did not find any association of IL-1RA VNTR polymorphism with disease severity. Even IL-1RN+2017T to C polymorphism did not show any association with disease severity. It is possible that once the clinical disease sets in, the level of pro-inflammatory cytokines is persistently higher than the anti-inflammatory cytokines, therefore, these polymorphisms do not make much difference.

Huang et al found that two individuals with allele 4 had low inflammatory activity of RA. We had only a single patient with this allele and thus could not study this association.

In conclusion, the IL-1RA polymorphism does not contribute to susceptibility or presence of extra-articular features of RA in Indian patients. In this study lower prevalence of allele 2 was found in patients with RA and secondary Sjögren’s syndrome and overall decreased homozygosity for allele 2 in patients with RA which needs to be confirmed in a study with a larger sample size.

References


23. Hurme M, Santila S. IL-1 receptor antagonist (IL-1Ra) plasma levels are co-ordinately regulated by both IL-1Ra and IL-1beta genes. *Eur J Immunol* 1998; 28: 2598–602.


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