Intercellular adhesion molecule 1 (ICAM-1) gene polymorphisms in Italian patients with rheumatoid arthritis

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Abstract

Aims
Rheumatoid arthritis (RA) has a wide range of clinical expressions which probably reflects different genetic backgrounds. Intercellular adhesion molecule-1 (ICAM-1) plays an important role in the inflammatory synovial activity in RA. The aim of this study was to examine the potential associations of ICAM-1 gene polymorphisms with RA and its severity.

Methods
Seventy-eight seropositive Italian RA patients with erosive disease entered the study. Radiographs of hands and feet 5 years after the diagnosis were available for 68 patients and were evaluated for the number of eroded joints. We obtained an erosive score for each patient by counting the number of joints with at least one erosion. Patients in the upper part of the distribution over the median were considered as fast eroders (FE) and the others as slow eroders (SE). Patients’ records were also evaluated for the presence of extra-articular features. 228 healthy subjects of the same ethnic origin were selected as a control group. All of the RA patients and controls were genotyped by polymerase chain reaction and allele-specific oligonucleotide techniques for ICAM-1 polymorphisms G/R at codon 241 (exon 4) and E/K at codon 469 (exon 6).

Results
The carriage rate of allele R241 was significantly higher in RA patients than in healthy controls (12.8% versus 5.7%, p = 0.039; odds ratio: 2.4 [95% CI 1.02 to 5.79]). The allele frequencies and carriage rate of the E 469 gene did not differ significantly between RA patients and the control group.

When we compared the control group with the patients with more or less severe disease (presence or absence of extra-articular features, SE and FE) we found that only the group of patients with the more favourable course maintained a significant difference in the carriage rate of R241 (16.7 vs 5.7%, p = 0.009 for patients without extra-articular features and 18.9 vs 5.7%, p = 0.004 for SE patients).

Conclusion
Our preliminary findings show that G/R 241 polymorphism of ICAM-1 is associated with RA, and that this confers a reduced risk of extra-articular manifestations and is associated with a slow rate of joint destruction.

Key words
Rheumatoid arthritis, ICAM-1 polymorphisms.
ICAM-1 gene polymorphisms in RA / P. Macchioni et al.

Introduction

Intercellular adhesion molecule-1 (ICAM-1) is a member of the immuno-globulin superfamily adhesion molecules which, with its ligands, integrins αM-β2 (MAC-1) and αL-β2 (LFA-1) (1), plays a key role in the recruitment and activation of leukocytes in the rheumatoid synovium (2). ICAM-1 is a heavily glycosylated single chain molecule with five tandem immunoglobulin-like domains. The first domain is the LFA-1 binding site while the third is the MAC-1 binding domain (1). High levels of this molecule have been described in inflammatory rheumatic disease in the activated endothelium, synovial lining synoviocytes, sublining macrophages and lymphocytes, and synovial fluid polymorphonuclear cells and lymphocytes (2).

The expression of ICAM-1 over the surface of chondrocytes induces the adhesion of T cells and eventually the death of the cells (2). Both the synovial and the serum soluble forms of the molecule are correlated to disease activity (Ritchie articular index and morning stiffness), erythrocyte sedimentation rate and C-reactive protein, but these data have not been confirmed by all authors (3-6).

Several drugs used in the treatment of rheumatoid arthritis (RA) induce both in vitro and in vivo the reduction of the expression of ICAM-1 on the cellular surface of inflammatory cells, which parallels the improvement of the indexes of clinical activity (7). It has been suggested in an open trial on RA patients that anti-ICAM-1 antibodies might have a beneficial effect on articular disease (8). Interestingly, during the treatment of RA patients with anti-TNF-α antibodies, a decreased concentration of ICAM-1 has been observed in the synovial tissue (9). The use of mice deficient in ICAM-1 showed a reduced susceptibility to collagen-induced arthritis both in heterozygous and homozygous deficient mice, suggesting that naturally occurring genetic variations in the expression of ICAM-1 might influence the susceptibility to RA in humans (10). Family studies have revealed that other genes outside the MHC region on chromosome 6 are implicated in the susceptibility to RA of humans (11).

The gene of ICAM-1 is localized at chromosome 19 and two single base polymorphisms of the gene have been described at positions 241 (GGG or AGG) and 469 (AAG or GAG) which lead to an aminoacid change to the ICAM-1 protein sequence (Lys or Glu in position 241 and Gly or Arg in position 469). The polymorphism at codon 241 is located in exon 4 which codes for the Ig-like domain 3, binding site of the MAC-1 integrin, and the polymorphism at codon 469 is located in exon 6 which codes for Ig-like domain 5 whose binding activity is not yet known (12).

We have evaluated the frequency of ICAM-1 polymorphism at codons 241 and 469 in a consecutive series of 78 RA patients of Italian origin who tested positive for rheumatoid factor on at least two occasions and who presented an articular erosive disease. They were compared with a group of 228 healthy people from the same geographic area. Subsequently, we divided the patients into subgroups with more or less severe disease (presence of extra-articular manifestations, and faster articular erosive disease) and compared them with the control population for the same gene polymorphism.

Patients and methods

Study population

Seventy-eight consecutive seropositive RA patients fulfilling the 1997 ARA criteria (13) with erosive disease, seen during a 2-month period in our rheumatology department (a secondary care center), were studied. All of these patients were of Italian origin. The medical records of the patients were reviewed for the presence of rheumatoid factor (patients were considered seropositive if the Waaler-Rose test had a titre > 1:64 or the nephelometric RA determination was > 40 IU/ml on two or more occasions), the presence of antinuclear antibody, age at disease onset, RA duration and presence of extra-articular features (EAFs).

As EAF we considered the following: subcutaneous nodules, pulmonary involvement (fibrosing alveolitis and pleuritis), cardiac involvement (pericarditis), cutaneous vasculitis, Sjögren’s syndrome, Felty’s syndrome, neuropathy,
and amyloidosis. The diagnostic definition of each EAF is reported in a previous study (14).

Radiographs of the hands and feet taken after 5 years from the appearance of symptoms of RA were available for 68 patients and were read separately by two authors (PM, CS) who did not have any knowledge of the identity of the patients. A blind consensus result was given when necessary. We evaluated the presence of erosions in metacarlo-phalangeal joints, proximal inter-phalangeal, wrists, metatarso-phalangeal joints and the inter-phalangeal of the big toes. We obtained an erosive score for each patient by counting the number of joints with at least one erosion. Patients in the upper part of the distribution over the median were considered as fast eroders (FE) and the others as slow eroders (SE).

**Molecular analysis of ICAM 1 polymorphism**

Genomic DNA was isolated from 500 µl whole blood collected in edetic acid. To detect the substitution (arginine for glutamic acid (K/E 469), was detected as described elsewhere (12). The amplified fragment was digested with 5 U Bst Ul, which cut product from the E469 allele but not from the K469 allele, and was then subjected to a 8% polyacrylamide gel electrophoresis gel. The control group consisted of 228 healthy subjects who were unrelated volunteer blood donors.

**Statistical analysis**

Statistical analysis was done using the SPSS statistical package (SPSS Inc., Chicago, Illinois). The frequencies of the alleles and genotypes among the patient and control groups were determined and were then compared by the χ² test. Odds ratio were calculated, together with their 95% confidence intervals.

**Results**

Table I shows the clinical and demographic characteristics of 78 RA patients at the time of the study.

<table>
<thead>
<tr>
<th></th>
<th>Fast eroders (n = 31)</th>
<th>Slow eroders (n = 37)</th>
<th>All (n = 78)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>61.6 ± 11.4</td>
<td>62.3 ± 11.8</td>
<td>61.7 ± 11.2</td>
</tr>
<tr>
<td>Female sex</td>
<td>61%</td>
<td>78%</td>
<td>68%</td>
</tr>
<tr>
<td>Age at disease onset (yrs.)</td>
<td>54.0 ± 11.1</td>
<td>53.5 ± 12.2</td>
<td>53.4 ± 12.0</td>
</tr>
<tr>
<td>Duration of follow-up (months)</td>
<td>61 ± 2</td>
<td>62 ± 2</td>
<td>117.2 ± 89.3</td>
</tr>
<tr>
<td>ANA positivity</td>
<td>18%</td>
<td>32%</td>
<td>28%</td>
</tr>
<tr>
<td>Patients with extra-articular features</td>
<td>38%</td>
<td>35%</td>
<td>36%</td>
</tr>
<tr>
<td>Mean erosive score</td>
<td>11.6 ± 5.5</td>
<td>1.4 ± 1.4</td>
<td>9.3 ± 9.2</td>
</tr>
<tr>
<td>Elderly onset rheumatoid arthritis</td>
<td>30%</td>
<td>35%</td>
<td>32%</td>
</tr>
</tbody>
</table>

Data are expressed as percentages or means (± SD)
Association between the R241 allele and RA severity

After the division of the RA group according to the presence or absence of features of disease severity (presence of EAFs, presence of faster erosive disease) we compared the genotype frequencies of these RA subgroups with healthy controls. We found a significant difference in the frequencies of the carriage rate of R241 only in the RA subgroups with less severe disease (Table III). Only 2 of the 10 RA patients carrying the R241 variant had EAFs during their disease course (subcutaneous nodules) and none presented severe EAFs (vasculitis, polyneuropathy, pulmonary or cardiac involvement). The comparison between the two RA subgroups (with or without EAFs) did not reach statistical significance. The same increased frequency of the carriage rate of R241 was present only in the subgroup of SE patients (the group of RA patients in the lower half of the distribution of erosive activity) compared with normal controls. The carriage rate of R241 was not significantly different between the SE and FE patient subgroups (SE 18.9% vs FE 7.7%, p = ns).

When we compared patients with disease onset before or after the age of 60 years and each subgroup with healthy controls we did not find any significant differences (data not shown).

Discussion

RA is a relatively heterogeneous disease with probably different genetic and environmental bases. The group of patients with erosive articular disease and positive rheumatoid factor may represent a relatively homogeneous subset. In this study we decided to evaluate only patients with these characteristics and with a sufficient follow-up to confirm the diagnosis. Several genes either of the MHC or non-MHC regions have been found to be associated with the occurrence of RA in various populations (18). The genes of the MHC region include DR4, DR1, and DR10 (18) and the non-MHC genes linked to the occurrence of RA are the TCR (19), immunoglobulin VH (20), NRA MPI (21), prolactin (22) and corticotropin release hormone genes (23). In our RA population we have identified a new non-MHC gene polymorphism (R/G ICAM-1 polymorphism) which seems to be associated with the occurrence of the disease.

A significantly different frequency of the ICAM-1 gene variants has been described in three other autoimmune disease, i.e. Crohn’s disease (CD), ulcerative colitis (UC)(24) and multiple sclerosis (MS) (25). In particular, CD patients with the R241 variant have an increased prevalence of a positive ANCA test while in UC the same R241 variant is associated with ANCA negativity. In MS an increased prevalence of the other ICAM-1 gene variant, K469 homozygosity, has been reported, while E469 homozygosity was significantly reduced. In a recent study we found that in patients suffering from polymyalgia rheumatica (PMR) the same allelic variant had a significantly higher prevalence than in a control population (26).

ICAM-1 is a molecule involved in several steps of the recruitment and activation of leucocytes at the site of inflammation (1, 2). In RA the activation and recruitment of synoviocytes is partially dependent on the interaction of ICAM-1 with its counter-receptor integrins LFA-1 and MAC-1 (2). The 241-Lys/Arg substitution is located at the third domain of the ICAM-1 molecule, which is the counterpart of MAC-1. This substitution can modify the functional activity of the ICAM-1 molecule leading to a different recruitment and activation of the inflammatory cells (12). However, evidence that this substitution can modify molecular activity is still lacking.

Others genes seem to be associated with the expression and severity of the dis-

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**Table II.** Allele and genotype frequencies of G/R 241 and E/K469 ICAM-1 polymorphisms in case patients and controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>RA patients (n = 78)</th>
<th>Healthy controls (n = 228)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alleles</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>10/156 (0.064)</td>
<td>14/456 (0.031)</td>
<td>0.05</td>
</tr>
<tr>
<td>G</td>
<td>146/156 (0.936)</td>
<td>442/456 (0.969)</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>66/154 (0.429)</td>
<td>200/456 (0.439)</td>
<td>NS</td>
</tr>
<tr>
<td>K</td>
<td>88/154 (0.571)</td>
<td>256/456 (0.561)</td>
<td></td>
</tr>
<tr>
<td><strong>Genotypes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RR</td>
<td>0/78 (0.0%)</td>
<td>1/228 (0.4%)</td>
<td>0.039</td>
</tr>
<tr>
<td>GR</td>
<td>10/78 (12.8%)</td>
<td>12/228 (5.3%)</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>68/78 (87.2%)</td>
<td>215/228 (94.3%)</td>
<td></td>
</tr>
<tr>
<td>EE</td>
<td>11/77 (14.3%)</td>
<td>34/228 (14.9%)</td>
<td></td>
</tr>
<tr>
<td>EK</td>
<td>44/77 (57.1%)</td>
<td>132/228 (57.9%)</td>
<td>NS</td>
</tr>
<tr>
<td>KK</td>
<td>22/77 (28.6%)</td>
<td>62/228 (27.2%)</td>
<td></td>
</tr>
</tbody>
</table>

*Data on K/E 469 were missing for one case with RA.*

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**Table III.** ICAM-1 G/R genotype: Comparisons between RA patient subgroups and controls.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>RA patients without extra-articular features (n = 48 pts)</th>
<th>RA patients with slow radiological erosive progression (n = 37 pts)</th>
<th>Healthy controls (n = 228)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A (16.7%)</td>
<td>B (18.9%)</td>
<td>C (5.7%)</td>
</tr>
<tr>
<td>RR+GR</td>
<td>8</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>GG</td>
<td>40 (83.3%)</td>
<td>30 (81.9%)</td>
<td>215 (94.3%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>P value</th>
<th>Relative risk (Conf. interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.009</td>
<td>3.3 (1.3 - 8.5)</td>
</tr>
<tr>
<td>0.004</td>
<td>3.8 (1.4 - 10.4)</td>
</tr>
</tbody>
</table>
ease. In particular the presence of DR4, DR1 or DR10 or the shared epitope of the third region of hypervariability of the α chain of the DRB1 gene have been found to be linked to the development of more severe articular and extra-articular manifestations (27-34). In a recent study on a population of RA patients from northern Italy, we observed that the presence of the rheumatoid epitope is associated with more severe radiological disease (29).

Two genes located in the MHC region were found to have a protective effect on the radiological and EA manifestations of RA. The first is the HLA-DR2 gene, which was associated in one study with less severe joint damage and a lower prevalence of EAFs (35). The second is the TNF-α promoter gene, of which at least 4 allelic variants have been disclosed at positions -376, -308, -244, and -238. The -238A polymorphism was associated with less severe joint damage in a series of RA patients followed during the first 3 years of disease (36). The MHC region is located at chromosome 6p21 and other non-MHC genes related to the development or clinical expression of RA (namely the TCR (19), immunoglobulin VH (20), NRAMP1 (21), proactin (22) and corticotropin release hormone genes (23) are located at other chromosomes and a linkage with the ICAM-1 gene variants is improbable.

Our study shows that the presence of an arginine residue at position 241 has a favourable effect on RA disease expression, in terms of both the presence of EAFs and the aggressivity of erosive joint damage.

PMR is a clinical syndrome of elderly patients characterised by proximal symptoms of synovitis in both the articular and extra-articular membranes. Synovitis is less severe in PMR than in RA, principally because of the absence of erosive joint damage. Recently articular synovitis and articular bone erosions have been considered as two different pathological process which are not closely connected (37).

Our findings support the possibility that the presence of this allelic variant may represent both a predisposing factor for the development of synovitis and at the same time may drive the articular inflammatory toward a less aggressive form. Other genes located in the same area of chromosome 19, in close proximity to the ICAM-1 gene may influence the inflammatory response, are the genes of ICAM-3 (38), MadCAM-1 (39), IL-11 (40) and human heat shock protein 40 (41). We cannot exclude the possibility that our findings may be related to some form of linkage with some of these genes.

In conclusion, this preliminary report shows that G/R ICAM-1 gene polymorphism may contribute to susceptibility to RA in the Italian population of seropositive patients and could drive the synovitis toward a less aggressive form. Further evaluation of RA seronegative and/or non-erosive patients may better define this linkage in a wider disease expression group. This analysis could confirm the protective role of ICAM-1 gene variants on the clinical severity of RA.

References


