**LETTERS TO THE EDITOR**

**The HLA-B*2709 subtype in a patient with undifferentiated spondarthritides**

In 1998, in this journal, we reported the cases of two B27 positive patients with undifferentiated spondyloarthropathy (SpA) and showing dactylitis also affecting the synovial sheaths in the palm of the hand.1 Neither patient had axial disease but showed peripheral manifestations of spondyloarthropathy (SpA), such as peripheral arthritis, peripheral enthesitis, and dactylitis.

Recently, one of our two patients (No 2) was subtyped and found to be B*2709 positive. As far as we know this subtype has never been found in patients with SpA.

DNA typing of HLA class I alleles was performed using a DNA sample prepared from peripheral blood lymphocytes by the salting out procedure.2 The class 1 ABC SSP UNITRAY low resolution kit (Pel-Freez) was used. The primer sets amplify all alleles described by the International Nomenclature Committee of WHO in 19953 and in 1997.4 Polymerase chain reaction amplification with sequence-specific primers (PCR-SSP) was used. A control primer pair was present to verify the integrity of the PCR reaction. Molecular typing of B*2709 was carried out by a PCR-SSP technique with a DYNAL HLA-B27 kit (DYNAL AS, Oslo, Norway), which identifies all the phenotypically different HLA-B27 alleles, B*2701–11, recognised by the HLA Nomenclature Committee in 1973.5 The typing results for our patients were: HLA-A*0101-02, *3201-02; HLA-B*0801, *2709; HLA-C*0102-03, *0701-07.

To confirm these results HLA-B locus sequence based typing was performed. A unique DNA amplification, encompassing exon 1 to intron 3, and four fluorescent sequencing reactions, covering exon 2 and 3, were used.2 Two intronic amplification primers generated a 1 kb length product useful for direct sequencing. For complete subtyping of the alleles the sequence based typing was performed by PCR-SSP.

SpA has a strong association with the HLA-B27 molecule. Studies in humans and transgenic rodent models suggest a direct involvement of HLA-B27 in the pathogenesis of the disease. Thirteen subtypes of HLA-B27 (B*01–13) have been described, differing from each other by one or more amino acid changes, mainly in the peptide binding site. Of these B*2701, 02, 03, 04, 05, 07, 08, and 10 are associated with ankylosing spondylitis (AS). B*2711–13 are rare, which has precluded assessing their putative association with AS. B*2706 is not associated with AS in South East Asia. However some B*2706 positive patients with AS have been reported in China.6 It has been suggested that the B*2706 might protect against SpA. Recently, a study on families in which both B*2706 and B*2709 occurred has suggested that B*2706, although not associated with SpA, does not protect against SpA. B*2709 has been found in Sardinia and in continental Italy, where the frequency of HLA-B27 in the general population is around 2%. B*2709 accounts for 25% of HLA-B27 subtypes in Sardinia and 3% in continental Italy.7 D’Amato and coworkers have tested 35 Serardinian patients with AS and 40 Sardinian B27 positive healthy subjects by genomic typing.8 None of the patients with AS were found to be B*2709 positive, in contrast with 25% among the healthy controls. The authors suggested that B*2709 is not

**REFERENCES**


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associated with AS, B*2709 differs from B*2705 by a single substitution (His » Asp) at position 116, which is located in the F pocket of the peptide-binding site. In the opinion of D’Amato and his colleagues the substitution at position 116 might exclude the acceptance of arthrogenic peptide from the B*2709.

Our patient was born in the south of Italy, she is B27 positive, and has uSpA with an RA. Matters arising, Letters, Correction

Y chromosome microchimerism in rheumatic autoimmune disease

It is well known that some features of chronic graft-versus-host disease (GVHD) resemble those of other rheumatic autoimmune diseases, such as systemic sclerosis (SSc), Sjögren’s syndrome (SS), and primary biliary cirrhosis (PBC). Furthermore, the development of systemic lupus erythematosus (SLE)-like diseases has been seen in murine models of GVHD. The pathogenesis of rheumatic autoimmune diseases is still unknown. One possibility that has been suggested is that these diseases are associated with pregnancy because of their strong female predilection and, especially in SSc, a peak incidence after parturition. In 1996 Bianchi et al reported that fetal cells could survive in the maternal circulation for up to 27 years after parturition, a phenomenon termed fetal microchimerism. These observations led the hypothesis that persistent fetal cells in the maternal circulation could mediate a graft-versus-host reaction, resulting in autoimmune disease.

Nelson et al have previously carried out a quantitative assay for male DNA in women with SSc and normal women who had delivered at least one son. They indicated that the mean number of male cell DNA equivalents among controls was 0.38 cells/16 ml whole blood and 11.1 among patients with SSc. In addition, Artlett et al have shown Y-chromosome-specific sequences in the DNA extracted from peripheral blood in 32 of 69 women with SSc (46%) as compared with 1 of 25 normal women (4%). They also reported that those allo-cells were T lymphocytes and infiltrated lesional skin. These findings support the hypothesis that fetal microchimerism may contribute to the pathogenesis of SSc. However, this is still controversial because Murata et al have recently reported that there is no significant difference in the presence of fetal DNA in peripheral blood between Japanese patients with SSc and healthy women with non-quantitative assay. Here we report further studies of fetal microchimerism in SSc, SLE, and SS.

We assayed for a specific Y chromosome sequence in the DNA extracted from peripheral blood by a nested polymerase chain reaction (PCR) in 20 patients with SSc, 21 patients with SLE, 18 patients with SS, and 41 healthy volunteers. All patients and healthy volunteers were Asian-Japanese women who had delivered at least one son. The nested PCR was used with the primers Y1–1, Y1–2, Y1–3, and Y1–4, which are specific for a part of the Y chromosome sequence.

Our data confirm that male DNA is found more commonly in women with SSc than in normal women. Interestingly, DYZ1 was not detected in patients with SLE and there was no significant difference between patients with SSc and healthy volunteers. These data suggest that fetal microchimerism may be a phenomenon which is strongly associated with the pathogenicity of SSc and not with the related autoimmune diseases, SLE and SS.

Table 1 Patients’ characteristics

<table>
<thead>
<tr>
<th>SS</th>
<th>SLE</th>
<th>SSx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years, mean (range))</td>
<td>56.1 (44–74)</td>
<td>50.2 (34–82)</td>
</tr>
<tr>
<td>Duration of illness (years, mean (range))</td>
<td>10.2 (1–26)</td>
<td>11.9 (1–24)</td>
</tr>
</tbody>
</table>

DYZ1 positive (%) | 10% (50) | 0% (0) | 6% (33) |

*p=0.017, systemic sclerosis (SSc) vs healthy volunteers.

Table 2 Comparison of clinical findings of DYZ1 positive and negative systemic sclerosis groups

<table>
<thead>
<tr>
<th>DYZ1</th>
<th>Positive (n=10)</th>
<th>Negative Total (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barnett’s type, I</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>II</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>III</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Autoantibodies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antinuclear factor</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Tissue transglutaminase</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Centromere (PBC*)</td>
<td>3 (3)</td>
<td>8 (0)</td>
</tr>
<tr>
<td>RNP</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>SSA (S)</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>SSA (E)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>RA</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>SS-A (E)</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Smooth muscle</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

*p=0.028, healthy volunteers and systemic lupus erythematosus (SLE). 

SSx = Sjögren’s syndrome.

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 Marker of erosive progression in RA

Urokinase plasminogen activator (uPA) catalyses the activation of the proteolytic enzyme plasmin, which plays a part in tissue degradation and remodelling, and seems to have an important role in the erosive growth of pannus in rheumatoid arthritis (RA). The action of uPA is localised and intensified by a cell bound receptor (uPAR), expressed by some malignant cells and some inflammatory cell types, including activated synoviocytes in the marginal zone between pannus and cartilage in RA.

The uPAR may become cleaved at the cell surface bound anchor, forming a free soluble receptor (suPAR) which is detectable in accessible plasma marker of erosive progression in RA. In a pilot study we followed up outpatients with RA to evaluate the relation between suPAR and disease activity. Plasma suPAR was measured and other clinical and paraclinical variables of disease activity determined in these patients on two or more occasions during a 12 month period. The present study included all patients (n=16) for whom comparable radiographs of the wrists and hands were obtainable, and also, when relevant, other symptomatic joints, taken before and after the period of suPAR measurements. The x ray films of participating patients were read independently by a radiologist unaware of the patient's clinical status and suPAR values. An enzyme linked immunosorbent assay (ELISA) was used to measure suPAR in plasma, as previously described.

The study group comprised 11 women and five men with a median age of 53.5 years (range 25–80) and a median disease duration of 57 months (range 5–360). Fifteen patients were rheumatoid factor positive and 10 had bony erosions on pre-study radiographs. Antirheumatic treatment included methotrexate (11 patients), hydroxychloroquine (two), sulfasalazine (one), and low dose steroids (eight). Clinical evaluation and measurement of suPAR, erythrocyte sedimentation rate (ESR), and C reactive protein (CRP) were done a median number of three times, and the time interval between radiographs was a median of 22 months.

Table 1 shows the results of the study. We found significantly higher suPAR concentrations (p<0.05) in plasma from those patients with RA whose x ray findings showed disease progression than in the patients who had no radiographic signs of progression, but the differences in ESR, CRP, and clinical variables were not significantly different.

This study was a pilot study in a clinical setting and conclusions must be drawn cautiously. The main problems, apart from the small number of patients, are, firstly, that in some of the patients pre-study radiographs were one to two years old. However, this would tend to diminish the differences found between the erosive progressive and non-erosive progressive groups as patients in remission, or with low activity in the study period, could be classified as progressive due to previous activity. Secondly, another possible bias, tending to increase the difference in suPAR between the two groups in this study, is that patients with high clinical activity would probably have had more extensive x ray examinations, increasing the chance of finding new erosions. We did not, however, find a difference in the number of radiographically investigated joints between our two groups of patients.

In conclusion, we find that this study indicates that plasma suPAR may be an easily accessible plasma marker of erosive progression in RA, and further studies on the subject are warranted.

<table>
<thead>
<tr>
<th>suPAR (ng/l)</th>
<th>Erosive progression (n=5)</th>
<th>No erosive progression (n=11)</th>
</tr>
</thead>
</table>
| <i>p<0.05</i>, non-parametric Mann-Whitney test. suPAR = soluble urokinase plasminogen activator in plasma; CRP = C reactive protein; ESR = erythrocyte sedimentation rate.

Table 1 - Period average values of corresponding paraclinical and clinical variables of 16 patients with rheumatoid arthritis followed up prospectively divided subsequently into two groups with or without progressive erosive changes on radiographs. Values are medians with range

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**CORRECTION**


The Editor of the *Annals* regrets that we inadvertently published a reply to Dr Barnsley from Drs Ferrari and Russell that contained some misinformation, and offers his apologies to Dr Barnsley.

Possibly, Drs Ferrari and Russell were confusing Dr Barnsley with someone else. Firstly, Dr Barnsley is a man and not a woman, as they stated. Secondly, Dr Barnsley did not attend the World Whiplash Congress in Vancouver and has not read the transcripts of it and thus could not be, as Drs Ferrari and Russell commented, “well aware of an impressive study presented there”.

(Note: Corrections appear in the journal, but do not appear on the *Annals* web page (www.annrheumdis.com) and are linked to the original publication.)