Soluble HLA class I antigens in serum and synovial fluid from patients with rheumatoid arthritis and other arthropathies


Rheumatology and Immunology Units, Hospital Universitario La Paz, Universidad Autónoma de Madrid, Madrid, Spain

Abstract

Objectives. To investigate the presence of soluble HLA class I (s-HLA) antigens in serum and synovial fluid (SF) from a large cohort of rheumatic patients.

Methods. We studied clinical and analytical data and serum samples from 300 patients [122 patients with rheumatoid arthritis (RA), 38 with osteoarthritis or osteoporosis, 29 with seronegative spondyloarthropathies, 45 patients with other rheumatic diseases and 66 healthy controls. In addition, we studied 25 paired samples of serum and SF from these groups of subjects. In RA patients, we examined whether the levels of s-HLA in serum and SF were related to the activity of the disease.

Results. The mean concentrations of s-HLA molecules in serum were slightly higher in RA patients (1.2 µg/ml) than in the other four groups (1.08, 1.01, 1.09 and 0.94 µg/ml respectively). We found no correlation between serum s-HLA levels and any variable of inflammatory disease activity in RA patients. s-HLA molecules were found in SF and at levels that correlated with those found in serum (P = 0.04; r = 0.4). Furthermore, s-HLA levels were higher in SF from patients with RA (1.3 µg/ml) or crystal-induced arthritis (0.98 µg/ml) than in SF from those with osteoarthritis (0.38 µg/ml) (P < 0.05 and P < 0.005 respectively), and these levels were correlated inversely and significantly with the score on the visual analogue scale of pain (P = 0.02), the number of painful joints (P = 0.05) and the level of C-reactive protein (P = 0.03) in RA patients.

Conclusions. This is the first report to demonstrate the presence of s-HLA molecules in SF at levels that correlate with serum levels. The mean levels of s-HLA molecules were significantly higher in SF from patients with RA and crystal-induced arthritis than in SF from cases of osteoarthritis, and correlated inversely with certain variables of disease activity in RA patients.

KEY WORDS: s-HLA, Arthritis, Rheumatoid arthritis.
Levels of s-HLA molecules also rise during episodes of graft-versus-host disease in recipients of some types of transplants. As the increase seems to precede the clinical evidence of graft rejection in these cases, the measurement of s-HLA antigens may be a non-invasive method of diagnosing it [16–18].

Increased levels of s-HLA have also been reported in other pathological conditions, such as active tuberculosis [19], haemorrhagic fever with renal syndrome [20] and childhood atopic dermatitis [21]. In other situations, the levels vary during the course of the disease, as occurs in gastric cancer [22], malignant liver disease [23], infection with *Trichuris trichiura* [24] and other diseases [16, 18, 25, 26].

The major mechanisms that have been suggested to explain the immunomodulatory effects of s-HLA class I molecules are the inhibition of cytotoxic T cells [27] and of natural killer cells [28].

Concentrations of s-HLA have been studied in autoimmune diseases, such as rheumatoid arthritis (RA) [29–31], systemic lupus erythematosus (SLE) [31–33], multiple sclerosis [34, 35] and Graves disease [36]. However, s-HLA antigens have never been measured in a large series of rheumatic patients and data concerning their presence in synovial fluid (SF) are scarce. The purposes of this study were to determine the levels of s-HLA in a large cohort of rheumatic patients, mainly presenting with RA, and to investigate whether these molecules could be found in soluble form in SF.

**Patients and methods**

**Patients**

The study population consisted of 300 subjects distributed in five groups: 122 RA patients [83.6% women, 64.8% positive for rheumatoid factor (RF), mean age 55 yr, range 22–79 yr] who fulfilled the 1987 American College of Rheumatology criteria for the disease [37], 38 patients with non-inflammatory disorders (25 with osteoarthritis, 12 with osteoporosis, one with chondromalacia patellae, 79% women, mean age 61 yr, range 42–74 yr), 29 with seronegative spondyloarthropathies (60.7% men, mean age 40 yr, range 24–71 yr), 45 patients with other arthropathies (five cases of gout, 14 of SLE, two of mixed connective tissue disease, one of CREST syndrome (calcinosis, Raynaud’s phenomenon, oesophageal dysmotility, sclerodactyly, telangectasias), four of scleroderma, 11 of Paget’s disease, three of calcium pyrophosphate deposition disease, three of vasculitis, one of Behçet’s disease and one of primary Sjögren’s syndrome; this group consisted of 32 women and 13 men with a mean age of 60 yr, range 32–85 yr), and 66 age-matched healthy blood donors as controls.

We collected data from these subjects at the time of blood sampling, using a clinical protocol. Informed consent was obtained from patients and controls. In RA patients, the protocol included the following parameters of clinical disease activity: morning stiffness, a 10-cm visual analogue scale of pain, grip strength of both hands, number of painful and swollen joints, Ritchie’s index, erythrocyte sedimentation rate and serum levels of C-reactive protein, haemoglobin and RF.

In 25 of the 300 subjects (seven with RA, 11 with osteoarthritis, three with acute gout, two with calcium pyrophosphate deposition disease arthritis, one with Behçet’s syndrome and one with SLE), we collected SF simultaneously with blood sampling at the time of the clinical evaluation. SF samples from patients with osteoarthritis were used as non-inflammatory controls with respect to SF samples from patients with inflammatory arthritis.

All sera and SF samples were stored at −80°C until analysis.

**s-HLA assay**

The assay was a modified version of that previously described by us [38], which was validated in the First International Workshop on Soluble HLA Antigens [39]. In this method, s-HLA molecules were measured by sandwich ELISA (enzyme-linked immunosorbent assay) involving the recognition of the heavy chain by the monoclonal antibody W6/32 bound to the microtitre well, followed by the recognition of β2-microglobulin by an anti β2-microglobulin monoclonal antibody labelled with peroxidase. After the addition of substrate (1,2 phenylenediamine), the amount of s-HLA was proportional to the intensity of colour developed (492 nm). Standardization of the method has been reported elsewhere [38, 40]. Papain-solubilized HLA, purified as described earlier [40], was used as standard.

**Statistical analysis**

We compared the differences in s-HLA levels in the five groups by analysis of variance. To determine the relationship between each parameter of disease activity of RA and the serum s-HLA level, we used simple regression analysis. The same method was used to compare the SF and serum levels of these antigens. In order to assess differences in s-HLA concentrations found in the 25 SF samples from patients with different diseases, we used the Kruskal–Wallis test with *post hoc* pairwise comparisons when appropriate. s-HLA levels in SF were compared with the clinical parameters of RA activity by simple regression.

**Results**

The results of s-HLA quantification in sera from all groups are summarized in Table 1. The mean serum concentration of s-HLA was slightly higher in RA patients than in the other four groups, but these differences did not reach statistical significance.

We found no correlation between serum s-HLA concentration and any of the clinical and analytical parameters of disease activity recorded in RA patients. s-HLA levels did not correlate with the presence of RF and did not differ between RA patients with and without extra-articular features of the disease.
Soluble HLA class I antigen in arthropathies

Table 1. Concentrations of soluble HLA class I antigens (μg/ml) in serum

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>Range</th>
<th>S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA (n = 122)</td>
<td>1.209</td>
<td>0.15–5.6</td>
<td>0.92</td>
</tr>
<tr>
<td>Non-inflammatory arthropathies (OA, OP) (n = 38)</td>
<td>1.089</td>
<td>0.15–5</td>
<td>0.93</td>
</tr>
<tr>
<td>Seronegative spondyloarthropathies (n = 29)</td>
<td>1.01</td>
<td>0.25–6</td>
<td>1.1</td>
</tr>
<tr>
<td>Other diseases (n = 45)</td>
<td>1.096</td>
<td>0.1–4.4</td>
<td>0.95</td>
</tr>
<tr>
<td>Healthy controls (n = 66)</td>
<td>1.06</td>
<td>0.2–4</td>
<td>0.6</td>
</tr>
</tbody>
</table>

OA, osteoarthritis; OP, osteoporosis.

Table 2. Concentrations of soluble HLA class I antigens (mean and range, μg/ml) in paired samples of serum and synovial fluid

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum</th>
<th>Synovial fluid</th>
<th>S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA (n = 7)</td>
<td>1.2 (0.61–2)</td>
<td>1.3 (0.5–2)</td>
<td>0.54</td>
</tr>
<tr>
<td>OA (n = 11)</td>
<td>0.8 (0.2–3)</td>
<td>0.38 (0.15–0.75)</td>
<td>0.18</td>
</tr>
<tr>
<td>Crystal arthritis (n = 5)</td>
<td>1.72 (0.87–3.8)</td>
<td>0.98 (0.61–1.55)</td>
<td>0.41</td>
</tr>
<tr>
<td>Others (n = 2)</td>
<td>0.8 (0.75–0.85)</td>
<td>0.5</td>
<td>0</td>
</tr>
</tbody>
</table>

The overall differences in the mean concentration in SF between different groups was significant (P = 0.0007). When compared differences between groups the significance between RA vs OA (P < 0.05) and crystal arthritis vs OA (P < 0.005) remained. There was no significant difference between RA and crystal arthritis patients.

This group included three patients with acute gout and two with acute arthritis, diagnosed on the basis of the presence of calcium pyrophosphate crystals.

This group included one patient with SLE and one with Behcet’s disease.

OA, osteoarthritis.

We found a significant correlation between the SF and serum levels of s-HLA (P = 0.04, r = 0.4) (Fig. 1). The mean concentrations of s-HLA in SF and serum are shown in Table 2. When we compared these data using the Kruskal–Wallis test, we found significant differences between the groups (P = 0.0007). The post hoc pairwise comparisons showed that the concentration of s-HLA molecules was significantly higher in SF from RA patients and crystal-induced arthritis (acute gout and pseudogout) than in samples from osteoarthritis patients (P < 0.05 and P < 0.005 respectively).

We then studied the possible relationship between s-HLA molecules in SF from patients with RA and each of the parameters of disease activity recorded. In spite of the small number of cases in which this study was carried out, we found a negative and significant correlation between the levels of s-HLA antigens in SF and the score on the visual analogue scale of pain (P = 0.02, r = 0.925), the number of painful joints (P = 0.05, r = 0.878) and the serum level of C-reactive protein (P = 0.03, r = 0.905). Nearly significant relationships with morning stiffness (P = 0.07, r = 0.838) and erythrocyte sedimentation rate (P = 0.1, r = 0.796) were observed.

Discussion

The levels of s-HLA have not been examined in large cohorts of rheumatic patients. For this reason, our purpose was to investigate s-HLA levels in several rheumatic diseases, and their relationship with inflammatory activity in RA patients. In addition, we attempted to determine whether these soluble antigens could be found in SF. We failed to demonstrate a significant increase in s-HLA levels in sera of any group of patients when compared with sera from healthy controls; however, RA patients had the highest mean concentration. We found no correlation between levels of s-HLA molecules in sera and the parameters of inflammatory activity of RA. In our review of the literature, we found only five articles in which s-HLA antigens had been investigated in rheumatic patients [29–33]. Adamashvili et al. [30] studied s-HLA in serum of 25 black patients with RA and the first-degree relatives of some of these patients. They found significantly higher amounts in RA sera than in normal sera of black individuals (P < 0.05), but when they compared RA patients with the healthy general population, they observed no significant differences (P < 0.065). This finding agrees with our results. In the same study, the authors described higher levels in RA patients with active multisystem involvement than in those with moderately active disease (P < 0.015), a difference that we could not confirm. Tsuchiya et al. [31] found significantly increased levels of s-HLA in RA patients than in controls in a white Japanese population.
but their series included only women and their results could show wide dispersion. In addition, 68% of the Japanese population are positive for HLA-A24, an allotype that is associated with high concentrations of s-HLA in this population [41]. In Spain, as in other countries, no relationship has been found between RA allotypes and class I antigens. Moreover, it is known that certain class I specificities could influence levels of s-HLA, as is observed with HLA-A2 (suppressing) or HLA-A9, -A23, -A24 and -A29 (increasing) [42]. Although we have not carried out typing, it is known that some 47% of the Spanish population are positive for HLA-A2, and this could explain the differences between studies. Incidences as high as 5% for HLA-A23, 13% for HLA-A24 and 17% for HLA-A29 have been found in the same population, and therefore our results would not be modulated by allotyping in serum [43].

s-HLA levels in SF have never been measured. Stevenson et al. [29] are the only authors to have reported, in some individuals, the presence of HLA class I molecules complexed to class II molecules but no free class I molecules. We found s-HLA in SF from all groups of patients studied and we quantified them. When we compared SF s-HLA levels with those detected in serum, we found a linear correlation. Furthermore, when we compared SF from patients with osteoarthritis with SF samples from patients with inflammatory arthritis (RA, crystal-induced arthritis, SLE and Behçet’s disease), we found significantly increased s-HLA levels in the latter patients, suggesting local production of s-HLA antigens in the synovial space in patients with inflammatory arthritis. This is not surprising, given that the chronic inflammatory arthropathies are associated with the local activation of proinflammatory molecules [44]. We found that the levels of these molecules in SF correlated inversely with some parameters of disease activity of RA, in spite of the small number of cases in our study. This finding could be explained by the facts that s-HLA molecules have an inhibitory effect on the T-lymphocyte response [27] and that T cells are important for disease activation in RA. Thus, an increase in s-HLA could be related to the suppression of T-lymphocytes.

In conclusion, to our knowledge this is the first report of the measurement of s-HLA antigens in serum samples from patients with a wide range of rheumatic diseases and in paired serum and SF samples. We found increased levels of these molecules in SF from patients with inflammatory arthropathies, and possible inverse correlations with parameters of RA disease activity. The significance of these findings and the role of s-HLA antigens in the mechanism of inflammation remain to be clarified in further studies.

References


20. Park CW, Yun SN, Yang CW et al. Serum and urine soluble HLA class I antigen concentrations are increased
42. Adamashvili IM, Fraser PM, McDonald JC. Association of serum concentrations of soluble class I HLA with HLA allotypes. Transplantation 1996;61:984–7.