Elevated levels of synovial fluid antibodies reactive with the small proteoglycans biglycan and decorin in patients with rheumatoid arthritis or other joint diseases

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Objectives. To determine whether patients with rheumatoid arthritis (RA) express humoral immunity to the small proteoglycans biglycan and decorin and to compare the response to that of patients suffering from other joint diseases. Methods. Serum and synovial fluid IgG and IgM antibody levels were determined by enzyme-linked immunosorbent assay. Antibodies to biglycan and decorin as well as to other known and extensively investigated cartilage matrix components such as type II collagen, aggrecan and fibronectin were investigated. Patients suffering from RA, osteoarthritis (OA), psoriatic arthritis and other seronegative spondylarthropathies were included in the study. Correlation between antibody levels and clinical/laboratory parameters was determined. Results. Patients with RA expressed an increased humoral immunity to biglycan, while patients with seronegative spondylarthropathies displayed elevated decorin-specific synovial antibody levels compared with OA patients. Conclusion. These results indicate a significantly higher immunity to small proteoglycans in RA and seronegative spondylarthropathies than in OA suggesting a possible involvement in the pathogenesis of inflammatory rheumatic diseases. Key words: Biglycan, Decorin, Aggrecan, Collagen, Fibronectin, Antibody, RA.

In the past few years key autoantigens for many autoimmune diseases have been successfully identified. However, in spite of extensive investigations, for rheumatoid arthritis (RA), one of the most frequent autoimmune diseases, the exact nature of the autoantigen is still debatable. Several microbial infections have been suggested over the years to play a role in the aetiopathogenesis of RA, including Epstein–Barr virus [1–3], cytomegalovirus [4] and some bacterial infections [5, 6]. Such infections are hypothesized to induce an initial immune response that may further become cross-reactive with self joint antigens.

Since hyaline cartilage is the primary target of autoimmune joint destruction, molecules of the articular cartilage are evident candidate autoantigens in RA. Their recognition by the immune system could maintain a chronic, progressive autoimmune inflammation. From the extracellular matrix of hyaline cartilage, recognition of collagen type II (CII) has been studied most extensively. Several works have shown the high prevalence of anti-CII antibodies in patients suffering from RA [7–9] and also the recognition of CII by T cells [10, 11]. However, the conclusion is that CII is recognized secondarily in RA. Data are also accumulating about the recognition of the cartilage large proteoglycan, aggrecan, by antibodies and T cells isolated from patients with various joint diseases [12]. Fibronectin is another, known component of the pericellular cartilage matrix as well as the free cartilaginous surface of RA patients that can be a target of immune recognition [13].

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Recently, research focused on HCgp39 as a candidate autoantigen in RA [14]. Hcgp39 was originally described as a major secreted glycoprotein of RA synovial cell cultures [15], later identified as a molecule produced also by chondrocytes, macrophages and neutrophil granulocytes [16]. However, recently even Hcgp39 has been excluded as the primary autoantigen since it has been shown that the major oligoclonal CD4 T cell expansions present in RA joints are neither specific for the dominant CII nor HCgp39 determinants [17]. So the question of the autoantigenic molecule in RA is still not answered convincingly.

Small proteoglycans of cartilage matrix, in spite of representing a small fraction of the total mass of proteoglycans, can be present in equivalent molar amount to the large proteoglycans because of their small molecular weight. They have homologous core proteins containing characteristic leucine-rich sequences. From this family of glycoproteins, decorin’s core protein is substituted with one chondroitin sulphate/dermatan sulphate chain while biglycan carries two [18, 19].

The lack of data on the immune response to small proteoglycans in rheumatic patients compelled us to investigate the humoral immune response to the small proteoglycans biglycan and decorin in RA patients and patients with other non-RA joint diseases.

**Materials and patients**

**Patients**

Sera and synovial fluid samples of 60 patients (15 males, 45 females) treated in the National Institute of Rheumatology and Physiotherapy (Budapest, Hungary) from January 1999 to December 2000 were investigated. Patients included in the study underwent an informed consent process and the study was approved by the ethical committee of the National Institute of Rheumatology and Physiotherapy (ORFI).

Both synovial fluid and serum samples were obtained from all patients included in the study as they all suffered from exudative synovitis. Thus the activity of the inflammatory processes was presumably higher in patients involved in the study than in those from whom synovial fluid could not be obtained (since no therapeutic aspiration was required). Patient selection involved no other bias.

The distribution of patients by diagnosis was as follows: 28 patients with RA (3 males, 25 females), 10 with psoriatic arthritis (PsA) (5 males, 5 females), 10 with other seronegative spondylarthritides (SNSA, 5 males, 5 females) and 12 with OA (2 males, 10 females). All RA patients met the 1987 American Rheumatism Association criteria [20] and PsA patients the Moll–Wright criteria [21]. All SNSA patients had sacroiliitis, 1 woman had ulcerated colitis-associated SNSA, 4 patients (3 males, 1 female) were suspected of suffering from ankylosing spondylitis (AS), but did not meet the AS New York criteria [22]. OA patients did not show any sign of inflammation, their erythrocyte sedimentation rates were under 30 mm/h.

The mean (S.D.) ages of RA, PsA, SNSA and OA patients were 52.1 (13.2), 47.5 (11.7), 45.2 (15.9) and 62.6 (11.6) yr, respectively. The average disease durations were 102.0 (138.6), 59.9 (60.8), 81.6 (135.1) and 88.5 (118.7) months, respectively.

**Serum and synovial fluid samples**

Native blood and synovial fluid samples were collected under sterile conditions, pelleted at 2000 rpm for 20 min and aliquots were stored at −20°C until use.

**Enzyme-linked immunosorbent assays (ELISAs)**

Nunc-Immunoplates (Maxisorp, Nunc Intermed Ltd, Copenhagen, Denmark) were coated with antigens (0.2 mg protein/well). Antigens included aggrecan from bovine articular cartilage (Sigma-Aldrich Kft, Hungary), biglycan from bovine articular cartilage (Sigma), collagen type II from chicken sternal cartilage (Sigma), decorin from bovine articular cartilage (Sigma) and fibronectin from bovine plasma (Sigma).

Free binding capacity of the polystyrene surface was blocked with 200 ml of 1% bovine serum albumin (Sigma-Aldrich). Based on preliminary experiments, serum and synovial fluid samples were tested at a 1:100 dilution. This was followed by incubation with affinity isolated, horseradish peroxidase-conjugated immunoglobulins to human IgG and IgM immunoglobulins (Sigma-Aldrich) in 1:30 000 and 1:50 000 dilution, respectively. Colour reaction was developed as described previously [23].

The unavailability of synovial fluid samples from completely normal individuals made it impossible to define the upper limit of ‘normal’ for antibodies in synovial fluid. Therefore we defined prevalence as frequency (%) of samples with a value above the mean + 2 s.d. value of OA samples (a degenerative disease to serve as a control for inflammatory diseases).

**Statistics**

The experimental design including the approximate patient group size was based on earlier studies testing serum and/or synovial antibody levels reactive to cartilage matrix components or other antigens in rheumatology patients leading to statistically significant intergroup differences [24, 25].

Statistical analysis of the data was performed using SPSS. Intergroup differences were investigated using ANOVA. Post hoc comparisons were performed by Tukey test. To indicate significance, *(P < 0.05), **(P < 0.01) and ***(P < 0.001) labels were used.

As a measure of association between clinical/laboratory findings and antibody levels as well as between antibody levels specific to various cartilage matrix components, Spearman’s rank order correlation coefficients were calculated. In order to avoid false-positive findings potentially arising from multiple testing, stringency was increased and correlation was considered significant at *P < 0.01 (labelled as *) and P < 0.001 (labelled as **).

**Results**

**Comparison of IgG and IgM levels in sera and synovial fluid samples of different groups of patients**

In order to obtain information about the disease specificity of elevated antibody levels we compared patients suffering from RA, OA, PsA and other SNSAs.

In serum we found no significant intergroup differences of cartilage antigen-specific antibodies (data not shown). On the contrary, synovial fluid antibody levels clearly distinguished certain groups of patients (Table 1).
Recognition of biglycan by synovial fluid antibodies (both IgG and IgM) was common and highly variable in different joint diseases. However, RA patients expressed a significantly higher synovial fluid IgM reactivity than patients with OA ($P < 0.001$).

The group of other SNSAs was distinguished by a significantly higher synovial fluid anti-decorin IgG level compared with that of OA patients ($P < 0.05$).

Among the other classic cartilage matrix antigens included in the test, recognition of native collagen type II was the most prominent. In synovial fluid both IgG and IgM levels were significantly higher in the RA group than among patients suffering from OA ($P < 0.001$ and $P < 0.01$, respectively) as well as patients suffering from PsA ($P < 0.05$).

The fibronectin-specific IgG level in synovial fluid was also elevated in RA compared with OA ($P < 0.05$), while no significant intergroup differences were detected in the levels of aggrecan-specific synovial antibodies.

Relative frequency of elevated antibodies recognizing different cartilage matrix antigens

To gain an insight into the relative frequency of patients with elevated antibodies reactive to various cartilage matrix components, we set a threshold as a mean + 2 S.D. detected in the OA group (serving as a control for the inflammatory arthritides) (Fig. 1).

The highest (79.16%) prevalence was found in the case of native type II collagen-specific IgG antibodies in the synovial fluid of patients suffering from RA. This value is much higher than what has been found either in PsA (40%) or other SNSAs (33.33%) for collagen-specific antibodies.

Interestingly, in both RA and SNSA patients, elevated synovial IgM levels specific for decorin proved to be the most prevalent. Furthermore, in the group of SNSA patients, decorin-specific IgG antibody in synovial fluid also represented the leading specificity.

On the other hand, in patients suffering from PsA, an overall lower prevalence of cartilage matrix-specific antibodies was seen. This may be partially explained by the fact that the primary lesion in PsA is enthesitis, whereas synovitis is a secondary process [26].

Correlation between antibody levels and clinical/laboratory parameters in patients with RA

We failed to identify any correlation between serum antibody levels and clinical/laboratory parameters of patients within the RA group (data not shown).

Table 1. ELISA results of synovial fluid IgG and IgM antibodies reactive to a selected group of cartilage matrix molecules in different groups of patients

<table>
<thead>
<tr>
<th></th>
<th>RA $n=28$</th>
<th>AP $n=10$</th>
<th>SNSA $n=10$</th>
<th>OA $n=12$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>S.D.</td>
<td>mean</td>
<td>S.D.</td>
</tr>
<tr>
<td>Biglycan</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td>0.331</td>
<td>0.221</td>
<td>0.403</td>
<td>0.268</td>
</tr>
<tr>
<td>IgM</td>
<td>0.215</td>
<td>0.055</td>
<td>0.141</td>
<td>0.047 ***</td>
</tr>
<tr>
<td>Aggrecan</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td>0.280</td>
<td>0.119</td>
<td>0.351</td>
<td>0.225</td>
</tr>
<tr>
<td>IgM</td>
<td>0.206</td>
<td>0.069</td>
<td>0.162</td>
<td>0.067</td>
</tr>
<tr>
<td>Collagen II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td>0.389</td>
<td>0.117</td>
<td>0.279</td>
<td>0.118 ***</td>
</tr>
<tr>
<td>IgM</td>
<td>0.437</td>
<td>0.140</td>
<td>0.332</td>
<td>0.081</td>
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<tr>
<td>Fibronectin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td>0.286</td>
<td>0.106</td>
<td>0.257</td>
<td>0.125</td>
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<tr>
<td>IgM</td>
<td>0.258</td>
<td>0.077</td>
<td>0.207</td>
<td>0.087</td>
</tr>
<tr>
<td>Decorin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td>0.178</td>
<td>0.064</td>
<td>0.150</td>
<td>0.033</td>
</tr>
<tr>
<td>IgM</td>
<td>0.197</td>
<td>0.088</td>
<td>0.173</td>
<td>0.080</td>
</tr>
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</table>

Mean OD 492 nm and S.D. values are presented.

* $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$. 

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On the contrary, type II collagen-reactive IgG level in synovial fluid was correlated with serum rheumatoid factor content ($P < 0.01$).

Our attempt failed to find a significant correlation between synovial fluid antibody levels and the age of the patient, the duration of the disease, the number of swollen or tender joints, erythrocyte sedimentation rate, white blood cell and platelet counts and the serum C-reactive protein levels (data not shown).

Correlation between antibody levels specific to various cartilage matrix components

We also compared antibody levels to different antigens to test for correlation. The correlation matrix for patients suffering from RA is shown in Table 2.

Unexpectedly, we found a strong correlation between the type II collagen-specific and fibronectin-specific antibody levels. A clear correlation is also observed between aggrecan- and biglycan-specific antibodies. The same correlations also proved to be significant when we tested data of all patients included in this study (data not shown).

Discussion

The present study focused on the question of whether small proteoglycans (biglycan and decorin) receive special attention by the immune system of patients suffering from RA.

In RA the inflamed synovial membrane is characterized by the presence and abundance of lymphatic follicles with germinal centres. This strongly suggests local B-cell activation and differentiation to plasma cells secreting antibodies with presumably relevant specificity [8].

Since in most cases T-B cell co-operation is essential for antibody production, synovial fluid antibody composition could also indirectly reflect the antigen-specificity pattern of locally activated T-helper cells.

Furthermore, data from experimental models of arthritis strongly suggest an important role for both antibodies and T cells in the pathomechanism of joint inflammation. Thus antibody specificity could provide information about the hierarchy of target molecule recognition in hyaline cartilage matrix with possible functional implications.

Involvement of a cartilaginous key antigen in RA is strongly suggested by the observation that synovectomy results in a transient remission only, whereas arthroplasty (removal of articular cartilage) leads to a complete remission [27].

Any cartilage matrix molecule can be theoretically implicated as either a primary target antigen or as one recognized by the immune system of arthritic patients secondarily, as a result of intermolecular determinant spreading. In an effort to determine the position of an antibody response specific to small proteoglycans in cartilage matrix B-cell epitope hierarchy in RA and other rheumatic diseases we set out to determine antibody levels reactive to an array of cartilage matrix molecules.

All samples tested in this study were collected from patients with high disease activity (as suggested by their exudative synovitis) and, thus, epitope specificity of synovial fluid antibodies was expected to be relevant to the disease.

This comparative study showed predominant recognition of type II collagen in patients with RA (highest prevalence of antibodies in synovial fluid, significantly different synovial fluid IgG and IgM levels compared with those in OA patients, strongest correlation with clinical and laboratory parameters).

The present study found that the anti-biglycan IgM antibody levels in synovial fluid were significantly higher in RA than in OA. Also, in RA the relative frequency of elevated decorin-specific IgM antibodies in synovial fluid was the highest among elevated IgM antibodies reactive with various cartilage molecules. Since IgM antibodies are excellent activators of the complement system, the contribution of biglycan- and decorin-specific synovial fluid antibodies may be significant in the pathomechanism of joint inflammation.

Another exciting aspect of the question is related to published immunomodulatory functions of decorin. Such functions include binding C1q and inhibiting the activity of the C1 complex [28], as well as inhibiting the effect of transforming growth factor-$\beta$ [29]. Binding of cartilage decorin by antibodies in patients might interfere with the above functions of the molecule and thus modulate inflammatory processes.

As an unexpected finding of this study, we showed a striking correlation between type II collagen- and
**Table 2. Spearman’s correlation of antibody levels specific to various cartilage matrix components in synovial fluid of patients suffering from RA**

<table>
<thead>
<tr>
<th></th>
<th>Biglycan</th>
<th>Decorin</th>
<th>Aggrecan</th>
<th>Collagen II</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>IgG</td>
<td>IgM</td>
<td>IgG</td>
<td>IgM</td>
</tr>
<tr>
<td></td>
<td>Se</td>
<td>Sy</td>
<td>Se</td>
<td>Sy</td>
</tr>
<tr>
<td>Decorin</td>
<td>0.264</td>
<td>0.24</td>
<td>0.504</td>
<td>0.580</td>
</tr>
<tr>
<td></td>
<td>0.325</td>
<td>0.301</td>
<td>0.386</td>
<td>0.131</td>
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<td></td>
<td>0.341</td>
<td>0.078</td>
<td>0.148</td>
<td>-0.206</td>
</tr>
<tr>
<td>Aggrecan</td>
<td>0.413</td>
<td>0.477</td>
<td>-0.002</td>
<td>-0.264</td>
</tr>
<tr>
<td></td>
<td>0.325</td>
<td>0.301</td>
<td>0.148</td>
<td>-0.206</td>
</tr>
<tr>
<td></td>
<td>0.473</td>
<td>0.441</td>
<td>0.728</td>
<td>0.368</td>
</tr>
<tr>
<td>Collagen II</td>
<td>0.503</td>
<td>0.521</td>
<td>0.533</td>
<td>0.880**</td>
</tr>
<tr>
<td></td>
<td>0.559</td>
<td>0.056</td>
<td>0.181</td>
<td>0.341</td>
</tr>
<tr>
<td></td>
<td>0.470</td>
<td>0.447</td>
<td>0.541</td>
<td>0.513*</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>0.042</td>
<td>0.181</td>
<td>0.363</td>
<td>0.334</td>
</tr>
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<td></td>
<td>0.004</td>
<td>0.027</td>
<td>0.774</td>
<td>0.297</td>
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<td>0.486</td>
<td>0.262</td>
<td>0.072</td>
<td>0.15</td>
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<td>0.457</td>
<td>0.447</td>
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<td></td>
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<td>0.389</td>
<td>0.696</td>
<td>0.669**</td>
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<td>0.669**</td>
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<td></td>
<td>0.346</td>
<td>0.125</td>
<td>0.125</td>
<td>0.125</td>
</tr>
</tbody>
</table>

Se, serum; Sy, synovial fluid. Significant correlations are indicated by bold type. Correlations were considered significant at *P < 0.01 and **P < 0.001.

**Acknowledgment**

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Small proteoglycan-specific antibodies


