Interleukin-6, soluble interleukin-2 receptor and soluble interleukin-6 receptor in the sera of patients with different histological patterns of rheumatoid synovitis

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Abstract

Objective
The present study was conducted to investigate whether the serum levels of interleukin 6 (IL-6), soluble IL-2 receptor (sIL-2R) and sIL-6R are associated with the morphological appearance of rheumatoid arthritis (RA).

Methods
Using the ELISA technique we measured the IL-6, sIL-2R and sIL-6R concentrations in the serum of 34 patients with RA and 28 patients with osteoarthritis (OA). Histological analysis of synovial samples distinguished 2 types of rheumatoid synovitis. Twenty-one RA specimens presented diffuse infiltrates of mononuclear cells without any specific microanatomical organization. In remaining 13 samples the formation of lymphocytic follicles with germinal center-like structures was found.

Results
Serum levels of IL-6, sIL-2R and sIL-6R were elevated in patients with RA compared to the OA control group (p < 0.001, p < 0.001 and p < 0.05 respectively). Concentrations of IL-6 and sIL-2R were highest in the serum of RA patients with follicular synovitis in comparison to patients with diffuse synovitis (p < 0.001 and p < 0.01 respectively) and could distinguish RA patients with these two histological variants of the disease. Serum levels of IL-6 and sIL-2R correlated with markers of disease activity such as ESR and CRP levels. In addition, the clinical data suggest a more severe disease among RA patients with follicular synovitis.

Conclusion
Distinct histological types of rheumatoid synovitis associated with unique serum concentrations of IL-6 and sIL-2R reflect levels of disease activity and confirm the concept of RA heterogeneity.

Key words
IL-6, sIL-2R, sIL-6R, rheumatoid arthritis, histology, heterogeneity.
Introduction

Several studies indicate the genetic, biological and clinical heterogeneity of rheumatoid arthritis (RA) (1-3); in addition, the histological diversity of RA has been suggested (4-8). Rheumatoid synovium is infiltrated by lymphocytes, macrophages and synoviocytes; these cells are thought to contribute to synovial tissue destruction processes via several mechanisms (9-12). The majority of rheumatoid synovia display only diffuse infiltrates of mononuclear cells, without any further microanatomical organization, and may be classified as diffuse synovitis (8). Increased angiogenesis and proliferation of the synovium-lining layer may be also seen. In about one-third of RA synovia the formation of lymphoid follicles has been reported (5,7,8). Such T-B cell conglomerates, which sometimes form germinal-like centres, seem to play an important role in the pathogenesis of RA (13-15). These RA synovia may be categorized as follicular synovitis (8). Only individual RA synovia have revealed necrotic granulomas with a fibrinoid necrotic centre lined by a collar of histocytes. The coexistence of lymphoid follicles and granulomatous necrobiosis in a single patient has not been detected (8).

Patients exhibiting the presence of follicular structures seem to have a greater degree of immunological activation, and greater potential for joint tissue destruction (7, 8, 16, 17). Furthermore, different histological types of rheumatoid synovitis were found to be associated with the unique pattern of cytokine production in the synovium (7, 8) and serum cytokine (16) or matrix metalloproteinase (MMP) (17) levels. Therefore, in addition to genetic, biological and clinical heterogeneity, a histological heterogeneity of RA has been postulated (4-8, 16, 17). Determination of the RA variants might be important for the further exploration of new and more selective therapeutic methods. The aim of this study was to investigate whether the serum concentrations of interleukin 6 (IL-6), soluble IL-2 receptor (sIL-2R) and sIL-6R reflect differences in the histological appearance of the RA synovitis.

Materials and methods

Patients and controls

We studied 34 patients who fulfilled the American College of Rheumatology 1987 revised criteria for RA (18) and 28 patients with osteoarthritis (OA) constituting the control group. Synovial samples were obtained during hip or knee joint orthopaedic surgery from all RA and OA patients entered into the study. Study procedures were approved by ethical committee.

Clinical and laboratory data

The analysis included the number of tender joints (Ritchie’s index) (19), the number of swollen joints, the erythrocyte sedimentation rate (ESR), the C-reactive protein (CRP) concentration measured by a radial immunodiffusion kit (Nanorid, The Binding Site Ltd., Birmingham, UK) and rheumatoid factor (RF) levels. Steinbrocker’s criteria were used for the radiological assessment of joint destruction (20).

Histological analysis. Synovial specimens were subjected to routine staining with hematoxylin and eosin. Morphological evaluations, which included assessment of the mononuclear cell infiltrate density and their microanatomical organization, were conducted as previously described (8, 16).

Serum specimens. Blood samples were clotted for 30 minutes and then centrifuged for 10 minutes at 1000 x g. Serum aliquots were frozen at -80°C immediately after collection.

IL-6, sIL-2R and sIL-6R assays. Serum interleukin 6 (IL-6), soluble IL-2 receptor (sIL-2R) and sIL-6R concentrations were tested by commercial ELISA kits from Bender MedSystems (Vienna, Austria). Measurements were carried out according to the manufacturer’s instructions. The sensitivity of the assays was 1.4 pg/ml (IL-6), 36 pg/ml (sIL-2R) and 20 pg/ml (sIL-6R).

Statistical analysis

The normally distributed data were analysed by the unpaired Student’s t-test. The Mann-Whitney U test was used to evaluate the differences between non-normally distributed data of ESR,
CRP, IL-6 and sIL-2R values. The probability of differences in frequency distributions was determined by Fisher's exact test. Correlations between studied parameters were defined using Spearman's rank order test. P values less than 0.05 were considered statistically significant.

**Results**

**Histological findings**

In RA synovia samples mononuclear cell infiltrates consisting predominantly of lymphocyte- and macrophage-like cells were found. Twenty-one samples revealed diffuse lymphocyte infiltration without any additional microanatomical organization, and were classified as diffuse rheumatoid synovitis. The formation of lymphocyte follicular aggregates, sometimes with germinal centre-like structures, was demonstrated in 13 specimens. Such RA synovia were categorized as follicular synovitis. Other histological finding in RA samples included synovial lining layer proliferation, rare giant-like cells and new vessel formation. The presence of necrobiotic granulomas was not found. OA synovial specimens demonstrated only mild mononuclear cell infiltrates. Representative examples of OA and two various histological forms of rheumatoid synovitis are presented in Figure 1.

**Demographic and clinical results**

Differences in sex, age or disease duration between patients with either histological form of RA or with OA were not found. The ESR and the CRP concentrations were higher in the RA group than in the OA group (in all cases p < 0.001), especially in patients with follicular rheumatoid synovitis (Table I). RA patients with the follicular histological type of disease were also characterized by a higher number of swollen joints than those with diffuse synovitis (in both cases p < 0.01). Approximately 67% and 77% of the RA patients, respectively, with the diffuse and follicular morphological forms of the disease were seropositive (Table I). All patients had been taking nonsteroidal anti-inflammatory drugs (NSAIDs). Disease modifying anti-

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**Fig. 1.** Histological findings in RA and OA synovia. Representative specimens are presented for the analysed groups of patients. (A) RA synovium sample displaying diffuse lymphocyte infiltrates without additional specific microanatomical organization. (B) RA specimen with the presence of lymphocytic follicular conglomerates. (C) OA synovium with mild mononuclear cell infiltration. Original magnification x 200.
rheumatic drugs (DMARDs) were more often used by patients with follicular synovitis, but only in the case of methotrexate was the difference significant (p < 0.05) (Table I). More advanced joint destruction (stage III or IV according to Steinbrocker’s radiological classification) was more often demonstrated in patients with lymphocytic follicular conglomerates compared to those without (p < 0.05) (Table I).

Serum levels of interleukin 6 (IL-6), soluble IL-2 receptor (sIL-2R) and sIL-6R

Our main goal was to evaluate whether serum concentrations of interleukin 6 (IL-6), soluble IL-2 receptor (sIL-2R) and sIL-6R are associated with various morphological forms of rheumatoid synovitis. IL-6 was found in higher levels in the serum of all RA patients (mean ± SD, 43.3 ± 22.9 pg/ml) and of those with diffuse or with follicular synovitis than in OA serum (5.9 ± 5.1 pg/ml) (p < 0.001 for all comparisons) (Fig. 2). IL-6 dominated in sera of patients with follicular type of synovitis (60.2 ± 18.9 pg/ml), clearly distinguishing them from those with diffuse synovitis (32.8 ± 18.6 pg/ml) (p < 0.001). As shown in Figure 3, also the concentrations of sIL-2R were increased in all RA patients (mean ± SD, 4431 ± 1099 pg/ml) and with both histological types of synovitis relative to OA patients (1953 ± 764 pg/ml) (p < 0.001 for all comparisons). sIL-2R dominated in RA group with follicular synovitis (5215 ± 968 pg/ml) and could differentiate them from RA patients with diffuse synovitis (3946 ± 886 pg/ml) (p < 0.01).

Serum sIL-6R levels were also higher in all RA patients (117.4 ± 33.7 ng/ml) as compared to OA patients (96.8 ± 36.5 ng/ml) (p < 0.05) (Fig. 4). However, sIL-6R concentrations did not differ significantly between patients with the diffuse (114.3 ± 38.2 ng/ml) and follicular (122.5 ± 25.4 ng/ml) morphological patterns of rheumatoid synovitis.

### Table I. Patient characteristics. Data presented as means ± SD unless otherwise stated.

<table>
<thead>
<tr>
<th></th>
<th>OA</th>
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<th>Follicular rheumatoid synovitis</th>
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<td>Gender (F/M)</td>
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<td>17/4</td>
<td>10/3</td>
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<td>Age (years)</td>
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<td>53.1 ± 11.8</td>
<td>58.0 ± 15.4</td>
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<td>Disease duration (years)</td>
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<td>18.4 ± 7.6</td>
<td>14.8 ± 5.5</td>
<td>NS</td>
</tr>
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<td>ESR (mm/h)</td>
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<td>48.8 ± 11.2</td>
<td>63.9 ± 21.1</td>
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<td>CRP (mg/l)</td>
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<td>33.0 ± 11.7</td>
<td>44.6 ± 13.3</td>
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<td>Swollen joints</td>
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<td>16.9 ± 3.5</td>
<td>0.01</td>
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<tr>
<td>Ritchie’s index</td>
<td>-</td>
<td>13.3 ± 3.0</td>
<td>15.2 ± 2.7</td>
<td>NS</td>
</tr>
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<td>RF-positive patients (%)</td>
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<td>76.9</td>
<td>NS</td>
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<td>DMARDs* (%)</td>
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<td>84.6</td>
<td>NS</td>
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<td>Oral steroids* (%)</td>
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<td>Radiological stage III or IV* (%)</td>
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<td>84.6</td>
<td>0.05</td>
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SD: standard deviation; OA: osteoarthritis; NS: not significant; ESR: erythrocyte sedimentation rate; CRP: C reactive protein; RF: rheumatoid factor; DMARDs: disease modifying antirheumatic drugs.

*Treatment in the last 3 months prior to the surgery. #Radiological stage of rheumatoid arthritis according to Steinbrocker.

**Fig. 2.** Serum concentrations of interleukin 6 (IL-6) in patient groups. Measurement was based on the ELISA technique. Box plots represent the median (line), and 25th and 75th percentiles (box), and whiskers indicate the 10th and 90th percentiles.
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**Fig. 3.** Serum concentrations of soluble interleukin 2 receptor (sIL-2R) in RA and OA patients. The assessment of sIL-2R was carried out and is presented as described in the legend to figure 2.

**Fig. 4.** Serum concentrations of soluble interleukin 6 receptor (sIL-6R) were determined and shown as described in the legend to figure 2.

**Relationship between serum levels of interleukin-6 (IL-6), soluble IL-2 receptor (sIL-2R) and/or sIL-6R and clinical findings**

Correlations between clinical parameters of disease activity and serum IL-6, sIL-2R or sIL-6R levels in all RA patients were presented in Table II. No associations between patient age, disease duration or rheumatoid factor and serum IL-6, sIL-2R or sIL-6R concentrations were noticed (data not shown).

**Discussion**

Rheumatoid arthritis (RA) is a chronic inflammatory disease involving the synovium and periarticular tissues with several systemic manifestations. It is a multi-gene disorder with genetic polymorphisms that influence a wide spectrum of its clinical presentations. Disease progression, the pattern of joint involvement and extra-articular manifestation are highly variable (1-3). Several studies suggest also the histological heterogeneity of RA (4-8). Most rheumatoid synovia are characterized by diffuse infiltrates of mononuclear cells of varying density, without any further specific microanatomical organization. Such synovia may be classified as diffuse synovitis (8). In about one-third of RA synovia, categorized as follicular synovitis (8), the formation of lymphocytic follicles was found (5, 7, 8). Such T-B cell structures, which sometimes form germinal-like centres, seem to be involved in the pathogenesis of RA (13-15).

In the present study we showed that circulating IL-6 dominate in RA patients with follicular synovitis and may distinguish them from those with diffuse synovitis. Serum IL-6 level correlated with the ESR, CRP levels, the number of swollen joints and the Ritchie’s index. We also found associations between serum concentrations of IL-6 and sIL-2R and a weak association with sIL-6R. Several studies revealed higher IL-6 levels in the serum of patients with RA than in those with OA or in healthy controls (21-25). Some investigators also observed a positive correlation of serum IL-6 with the number of swollen joints (26), Ritchie’s index (24), ESR (21, 26-28), CRP (21, 26-29), sIL-2R (30) and sIL-6R (24). However, in other reports circulating IL-6 in RA patients was not significantly higher than in controls (31). Furthermore, others found no association between the serum IL-6 concentration and clinical markers of disease activity such as the ESR (29, 30) or CRP (30).

Increased sIL-2R levels in RA serum (21,22,31-34) compared to OA patients and healthy individuals have been already demonstrated. Serum sIL-2R was also reported to correlate with number of swollen joints (21), ESR (27, 33-35), CRP (21, 27, 35), circulat-
ing IL-6 (30) and negatively with haemoglobin concentration (21, 30). However, others failed to correlate sIL-2R with markers of disease activity such as ESR and CRP (29, 32). Our study showed the serum sIL-2R levels to be higher in all RA patients in comparisons with OA group. sIL-2R dominated in patients with follicular synovitis and could differentiate them from those with diffuse synovitis. Furthermore, we observed the correlation of serum sIL-2R with IL-6 and with such markers of disease activity like ESR, CRP, the number of swollen joints and Ritchie’s index. Taken together all these findings suggest that RA is more severe in patients with follicular synovitis. Here we also report weakly elevated serum sIL-6R levels in all patients with RA and with follicular synovitis in comparison with OA patients. However, the sIL-6R concentration did not differ significantly between the two morphological forms of RA. Some investigators found increased sIL-6R levels in RA serum compared to controls (24, 36). However, in other reports circulating sIL-6R in RA patients was not significantly higher than in OA patients or in healthy individuals (23). In our study we found a weak association of serum levels of sIL-6R with ESR and circulating IL-6. We did not find sIL-6R to correlate with other markers of disease activity. Some studies have also found a correlation of sIL-6R with IL-6 in RA patients (24). However, others failed to correlate sIL-6R with markers of disease activity such as ESR or CRP (26, 27).

Disease-modifying antirheumatic drugs (DMARDs), especially methotrexate therapy, may downregulate the production of IL-6 and sIL-2R (21, 22). Although our patients with follicular synovitis used DMARDs more frequently, the difference was significant only in the case of methotrexate. Moreover, serum levels of IL-6 and sIL-2R were especially increased in patients with the follicular histological type of RA. Therefore, more aggressive therapy in those patients simply seems to reflect a greater severity of the disease. Furthermore, advanced articular destruction (stage III or IV according to Steinbrocker’s radiological criteria) was also more frequently observed among patients with follicular synovitis. We found no correlations between the sex, patient age or disease duration and serum levels of IL-6, sIL-2R or sIL-6R (data not shown).

In our study we showed significantly elevated levels of IL-6, sIL-2R and sIL-6R in RA serum as compared to OA patients. These molecules, with the exception of sIL-6R, dominated in patients with the follicular type of rheumatoid synovitis, clearly distinguishing them from patients with the diffuse histological form of RA. Moreover, circulating IL-6 and sIL-2R concentrations correlated with laboratory and clinical markers of disease activity. Our report confirms the greater disease activity in RA patients with follicular synovitis than in those with diffuse synovitis. Therefore, serum levels of these molecules might be used to determine the morphological patterns of rheumatoid synovitis. These findings support the theory of RA heterogeneity and suggest the possibility of various responses to disease treatment. Therefore, RA heterogeneity should be considered in the design of the therapy.

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**References**


11. KLIMIUK PA, YANG H, GORONZY JJ, WEY-