CD5 and CD23 expression on B cells in peripheral blood and synovial fluid of rheumatoid arthritis patients: relationship with interleukin-4, soluble CD23 and tumour necrosis factor alpha levels

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Abstract

Methods. We have studied in peripheral blood (PB) and synovial fluid (SF) of 31 patients diagnosed with rheumatoid arthritis (RA), the expression of CD5 and CD23 antigens on B cells, and the levels of soluble CD23 (sCD23), interleukin-4 (IL-4) and tumour necrosis factor alpha (TNF-α). We have also correlated the results with the disease activity index.

Results. CD5+ B cells are expanded in SF and, moreover, show higher expression of CD23 than CD5− B cells. Twelve patients had detectable levels of IL-4 in plasma and 10 in SF (nine patients in both samples); the absence of IL-4 was related to a higher expression of CD23 on CD5+ B cells and with higher levels of sCD23. A negative correlation was found in SF between TNF-α and sCD23 levels.

Conclusion. There is no correlation between disease activity index and the different parameters studied (expression of CD5 and CD23 on B cells, sCD23, IL-4 and TNF-α levels) either in plasma/PB or in SF.

Key words: CD5+B cells, CD23, IL-4, TNF-α, Synovial fluid, Rheumatoid arthritis.

The origin and function of CD5+B cells are controversial [1, 2]. The description in rheumatoid arthritis (RA) patients of an expansion in peripheral blood (PB) of CD5+B cells [3] and the production by these cells of autoantibodies of rheumatoid factor type [4] had implicated this cellular population in the disease’s pathogenesis [5]. The studies carried out in PB of RA patients reveal contradictory results: some demonstrate expansion of the CD5+B-cell population compared to normal controls and correlation with disease activity [3, 4, 6–8], others do not find a correlation with disease activity but expansion in PB [9, 10] and, finally, other studies do not find differences with normal controls [11–14]. This discrepancy in the results could be explained by the idea that CD5+B cells are genetically regulated, so that some patients would have normal levels and others elevated levels of CD5+B cells [15].

CD23 antigen (the low-affinity receptor for IgE) is a membrane protein present in several cell types such as B lymphocytes, T cells, monocytes and eosinophils [16]. In B cells, the expression of CD23 in cellular membrane (mCD23) might regulate the main functions of B cells: antigen presentation to T cells and their differentiation to immunoglobulin-secreting cells [17]. The soluble form, sCD23, is a multifunctional cytokine that might participate in the inflammatory process and in the mechanisms of apoptosis [18, 19]. The expression and release of CD23 are upregulated by interleukin-4 (IL-4) [20, 21], and downregulated by interferon γ [22]. Several authors have described increased expression of mCD23 on B lymphocytes and increased levels of sCD23 in RA patients [23–26]. It has been suggested that the increased serum levels of sCD23 in RA patients are due to a high expression of mCD23 on CD5+B cells [27].

The majority of studies on the expression of CD5 and CD23 antigens on B cells of RA patients have been performed in PB; the studies in synovial fluid (SF) are scanty and have a low number of patients.

The aim of this work was to study, in PB and SF of RA patients, the CD5+B-cell population and the expression of mCD23 on CD5+ and CD5− B cells, quantify the levels of sCD23, IL-4 and pro-inflammatory cytokines such as tumour necrosis factor alpha.
(TNF-α), and correlate the results with the activity of the disease.

Patients and methods
Thirty-one patients diagnosed with RA according to the 1987 American College of Rheumatology (formerly American Rheumatism Association) revised criteria [28] were studied. Once informed consent was obtained, paired samples of PB and SF were obtained by venous puncture and by aseptic aspiration of the knee joint, respectively. The disease activity was measured using the modified Stoke index [29]. The disease index is an algorithm scoring inflammatory activity that includes clinical (proximal interphalangeal score, morning stiffness, Ritchie articular index) and biological data (erythrocyte sedimentation rate, C-reactive protein). The result is an index score ranging from 1 (minimal activity) to 17 (maximum activity). The modification introduced by us was to use the Thompson articular index [30] instead of the Ritchie articular index; the final score index was unchanged. The mean age (± s.e.m.) of the patients was 62.2 (± 2.2) yr, five patients were males and 26 females. Twenty-five patients had rheumatoid factor in their sera. The mean duration of the disease was 7.1 (± 1.5) yr, and the mean activity index 8.6 (± 0.7).

Owing to the impossibility of obtaining inflammatory SF from normal subjects, and because the aim of the study was to compare the results in PB and SF of RA patients, we have not included normal controls in this work.

Mononuclear cells were isolated either from venous blood or from SF samples by Ficoll-Hypaque density-gradient centrifugation. Plasma and cell-free SF obtained by centrifugation were kept at −80°C until studied.

A three-colour labelling procedure was performed to assess B-cell phenotype. Anti-CD20 Per-CP, anti-CD5 fluorescein isothiocyanate (FITC) and anti-CD23 phycoerythrin (PE) monoclonal antibodies from Becton Dickinson were used. After a standard labelling procedure, cells were analysed in a FacSort cytometer (Becton Dickinson).

Soluble CD23 was quantified in plasma and SF by a ‘sandwich’ enzyme immunoassay (The Binding Site, Birmingham, UK), the limit of detection was 0.7 ng/ml. Levels of IL-4 and TNF-α were measured in plasma and SF samples by ELISA (Endogen, Cambridge, MA, USA) following the manufacturer’s instructions. The limit of detection was 3 and 4 pg/ml, respectively.

Results are given as mean ± s.e.m. The two-tailed t-test, the non-parametric Mann–Whitney test and Pearson’s correlation coefficient were applied when appropriate. A P value of <0.05 was considered to indicate a significant difference between groups.

Results
The percentage of CD5+ B cells was significantly higher in SF (41.8 ± 3.8%) than in PB (25.7 ± 2.6%) (P < 0.01).

In total B cells, the expression of mCD23 was higher in PB than in SF (P < 0.001). On CD5+ and CD5− B cells, the expression of mCD23 was similar in PB; in both cases, mCD23 expression was higher in PB than in SF, but only with a significant difference on CD5− B cells (P < 0.001). CD5+ B cells expressed higher levels of mCD23 in SF than CD5− B cells (P < 0.05). Data are shown in Table 1.

The levels of IL-4 and sCD23 were determined in paired samples of plasma and SF of 28 patients, and the levels of TNF-α in 12. Twelve patients had detectable levels of IL-4 (>3 pg/ml) in plasma and 10 in SF (nine in both samples); there were no differences between plasma and SF levels (28.37 ± 12.56 vs 13.85 ± 4.57 pg/ml; P = 0.34). sCD23 was detected in all cases, the amount in plasma was slightly higher than that in SF (2.88 ± 0.41 vs 2.55 ± 0.29 μg/l; P = 0.21). The concentration of TNF-α was higher in SF than in plasma (2479 ± 574 vs 44.2 ± 9.2 pg/ml; P < 0.001).

We have found a significant negative correlation between TNF-α and mCD23 levels in SF (r = −0.555, P < 0.05); there was no correlation between plasma levels. IL-4 levels were not detectable either in plasma or in SF in the 12 cases in which TNF-α was determined.

Patients were divided into two groups: one with detectable levels of IL-4 both in plasma and SF, and the other with no detectable levels in either sample. There were no significant differences in the expression of CD5 on B cells between groups, either in PB (27.7 ± 4.3% vs 25.1 ± 3.9%; P = 0.65) or in SF (42.8 ± 6.2% vs 42 ± 5.6%; P = 0.93). On CD5+ B cells, mCD23 expression was lower in the group with detectable IL-4 levels, both in PB (12 ± 5.8% vs 31.3 ± 7.4%; P = 0.008) and in SF (8.1 ± 5.1% vs 17.2 ± 5.6%; P = 0.16) (Fig. 1). Conversely, on CD5− B cells, mCD23 expression was higher in the IL-4 detectable group, both in PB (25.8 ± 3.8% vs 17.8 ± 3.1%; P = 0.13) and in SF (9.1 ± 3.4% vs 2 ± 0.6%; P = 0.07). As occurred with mCD23 expression on CD5+ B cells, sCD23 levels were lower in the IL-4 detectable group, both in plasma (1.98 ± 0.13 vs 3.56 ± 0.68 μg/l; P < 0.01) and in SF (2.12 ± 0.67 vs 2.79 ± 0.27 μg/l; P < 0.05).

We have not found any significant correlation between the disease activity index and the different parameters studied (percentage of CD5+ B cells, mCD23 expression on CD5+ and CD5− B cells, and sCD23, IL-4 and TNF-α levels) either in plasma/PB or in SF (data not shown).

Table 1. Expression of mCD23 on B cells of PB and SF of RA patients

<table>
<thead>
<tr>
<th></th>
<th>PB (n = 31)</th>
<th>SF (n = 29)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total B cells</td>
<td>27.2 ± 2.4</td>
<td>10.1 ± 1.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD5+ B cells</td>
<td>23.4 ± 4.8</td>
<td>14.3 ± 3.7</td>
<td>0.14</td>
</tr>
<tr>
<td>CD5− B cells</td>
<td>22.1 ± 2.5</td>
<td>5.7 ± 1.6</td>
<td>&lt;0.001</td>
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</tbody>
</table>

Data express percentages (mean ± s.e.m.).
*CD5+ B cells express more mCD23 in SF than CD5− B cells (P < 0.05).
Discussion

In the present report, we describe an expansion of CD5+ B cells in SF of RA patients. This may be due to active traffic to SF of this cellular subtype or to an increase in local production of the CD5 molecule in this environment. There are few studies comparing the CD5+ B-cell population in PB and SF of RA patients. Sowden et al. [11] analysed in six RA patients the expression of CD5 on B cells of PB and SF and, as in our study, they found a higher proportion of CD5+ B cells in SF. Kipps [1], in his comprehensive review of the CD5+B cell, makes mention of an increase of CD5+ B cells in SF of RA patients without offering concise data.

Fernández-Gutiérrez et al. [25] have studied the expression of mCD23 on B cells of RA patients (24 cases in PB and nine cases in SF) and, as in our work, they found increased expression of mCD23 on PB B cells. Furthermore, we have analysed and compared the mCD23 expression on CD5+ and CD5− B cells. There were no differences in PB, yet in SF mCD23 expression was higher on CD5+ B cells; this could mean a higher activation state of this cellular population in SF.

Several authors have demonstrated increased serum levels of sCD23 in RA patients in comparison with normal controls; in general, this increase had no correlation with the disease activity [23, 24, 26, 27, 31], which is in agreement with our own results. Roussou et al. [32] have assessed the levels of sCD23 in serum and SF of 18 RA patients; as in our study, they found neither differences between serum and SF levels nor correlation with the disease activity.

In RA, IL-4 levels are considered to be low or absent [33], but not all studies show the same results [34–36]. We have detected the presence of IL-4 in plasma in 43% of our patients, and in SF in 36%. When comparing both groups (IL-4 detectable and not detectable), we have seen that mCD23 expression on CD5+B cells was higher in the absence of IL-4 (although only with a significant difference in PB); this finding might indicate a different functional behaviour of CD5+B cells with respect to IL-4. As occurred with mCD23 expression on CD5+B cells, sCD23 levels were higher in the absence of IL-4; this might indicate, as has been suggested by Ikizawa et al. [27], that CD5+B cells expressing mCD23 are the main source of sCD23 in RA patients. The lower expression of mCD23 on CD5+B cells and the lower amounts of sCD23 in the presence of IL-4 suggest that this is due to a decrease in production and not to an increased shedding of the molecule.

The capacity of human CD23 to activate monocytes/macrophages by binding to the β2integrins CD11b and CD11c, producing the release of pro-inflammatory mediators such as TNF-α, IL-1β and IL-6, has been described [37]. In a mouse model of collagen-induced arthritis, anti-CD23 antibody therapy has been shown to have a clear beneficial effect in inhibiting the clinical and histological progression of an established arthritis [38]. We have found a negative correlation in SF of RA patients between TNF-α and sCD23 levels; the meaning of this finding, if any, needs further investigation.

To the best of our knowledge, the study described here is the largest yet performed on SF of RA patients regarding the expression of CD5 and CD23 molecules on B cells. The CD5+B cells are increased in SF of RA patients and show a higher expression of mCD23 than CD5− B cells. The absence of IL-4 is related to a higher expression of mCD23 on CD5+B cells, and with higher levels of sCD23. There is a negative correlation in SF between TNF-α and sCD23 levels. There is no correlation between the disease activity index and the different parameters studied (CD5 and CD23 expression on B cells, and IL-4, sCD23 and TNF-α levels) either in plasma/PB or in SF.

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