Immunohistochemical demonstration of CD23 expression on lymphocytes in rheumatoid synovitis

E A Hellen, D C Rowlands, T T Hansel, G D Kitas, J Crocker

Abstract
The leucocyte antigen CD23 is expressed by B lymphocytes following activation by a number of stimuli and functions as an IgE receptor, and in its soluble form, as a putative B cell growth factor. The expression of CD23 on the surface of lymphocytes in paraffin wax sections of synovial biopsy specimens was studied using a novel mouse monoclonal antibody, BU38. Specimens were investigated from nine cases of rheumatoid arthritis, six cases of osteoarthritis, and eight cases of chronic inflammation in articular and non-articular tissues. CD23 was expressed on a high proportion of lymphocytes in all forms of chronic inflammation and was not specific for rheumatoid arthritis. It may be a characteristic feature of any chronic inflammatory response. As CD23 was found on the surface of lymphocytes in many cases of these arthritides, sCD23 in serum or synovial fluid may yet prove a useful marker for the severity of the inflammatory infiltrate.

Rheumatoid arthritis is a chronic inflammatory disease which predominantly affects synovial tissues, resulting in irreversible articular damage and loss of function. Most patients with rheumatoid arthritis have serum rheumatoid factors consisting of immunoglobulins with specificity for the Fc fragment of IgG. Synovial lymphocytes have the capacity to produce rheumatoid factors, and the rheumatoid synovium is infiltrated with activated B and T cells.1 2 A lymphocytic infiltrate is also seen, however, in other chronic synovitides.3 4 Although the aetiology of rheumatoid arthritis is unknown, there is an association with HLA DR4,5 and a defect in cellular immunity with abnormal production of and response to cytokines has been postulated.7 8 Certain infectious agents including Epstein Barr virus (EBV) have also been implicated in the pathogenesis of the disease.9 11

The low affinity IgE receptor (CD23) is a 45 kilodalton molecule expressed on IgM/IgD bearing B lymphocytes following activation,12 13 as well as on a number of other cell types including follicular dendritic cells and Langerhans' cells.12 14-16 CD23 expression on B lymphocytes is particularly increased following infection by EBV,17 but also occurs after activation by a number of other stimuli such as interleukin 4 and so is not specific to EBV infection.12 CD23 also exists in a 25–30 kilodalton soluble form (sCD23) that is secreted or can be formed by cleavage of surface CD23.18 This sCD23 has been suggested to have autocrine B lymphocyte growth factor activity,19 20 although gene cloned sCD23 does not have this property.21

In this study we investigated the expression of CD23 by lymphocytes in paraffin wax embedded synovial biopsy specimens from patients with rheumatoid arthritis and osteoarthritis. Several specimens of other tissues showing chronic inflammation were also studied. A novel mouse monoclonal antibody, BU38, with the particular advantage of being able to visualise CD23 in paraffin wax sections22 was used.

Methods
Archival paraffin wax embedded synovial biopsy specimens were obtained from the Department of Histopathology, East Birmingham Hospital. All sections of each specimen were reviewed, and those showing changes of chronic inflammation with a prominent chronic inflammatory cell infiltrate were chosen for further study. These included nine cases of rheumatoid arthritis, six of osteoarthritis, and three cases of non-specific synovial inflammation. The clinical case notes of all these patients were reviewed to confirm these diagnoses. Particular note was made of the rheumatoid factor concentration, radiological changes, the presence of morning stiffness, haemoglobin concentration and erythrocyte sedimentation rate (ESR) as indices of disease activity at the time of the biopsy.

Five biopsy specimens showing chronic inflammation in other tissues were also chosen for study. These consisted of non-specific dermatitis, chronic cholecystitis, branchial cyst, Crohn's disease of the ileum and chronic gastritis.

All the specimens had been fixed for at least 18 hours in 10% formalin-saline and then processed as for routine histological examination. Sections were cut at 3 μm thickness, attached to glass slides, and incubated at 40°C for 18 hours and then dewaxed and
rehydrated. After the addition of trypsin, and
and blocking non-specific antibody binding with
normal swine serum at a dilution of 1 in 20,
the BU38 monoclonal antibody (The Binding
Site Ltd, Birmingham) was applied to the
sections at a dilution of 1 in 100. Control
sections of the same specimens were stained
with another ascitic fluid antibody, BU31,
which recognises an antigen associated with
nuclear envelopes. A standard alkaline
phosphatase anti-alkaline phosphatase (APAAP)
detection system was used. The bridging
antibody used was a rabbit anti-mouse
immunoglobulin reagent (Dakopatts; No
Z259) at a dilution of 1 in 50. The APAAP
complex (Dakopatts; No D651) was used at a
dilution of 1 in 100. A second cycle of bridg-
ing antibody and APAAP complex was per-
formed. All antibody incubations were per-
formed for 30 minutes. The chromogen was
prepared immediately before use by dissolving
25 mg naphthol AS-BI phosphate (Sigma; No
N2250) in 1 ml dimethylformamide, adding
this to 50 mg levamisole (Sigma; No L9756)
in 50 ml 0·05 M TRIS buffer (pH 8·7), and
then adding 50 mg Fast Red TR salt (Sigma;
No F1500). The sections were incubated in
this solution for 20 minutes at room temper-
ature, washed, counterstained in Mayer’s
ahematoxylin and then mounted in an
aqueous medium.

All the sections were examined by two
pathologists (EH and DCR) who assessed the
degree of positivity (negative, weak, moderate
or strong) and the percentage of lymphoid
cells stained (none, less than 50%, more than
50% but less than 90% and more than 90%).

Table 1 gives a summary of the clinical data
corning all the patients with joint disease
included in this study. All of the patients who
had been classified as having rheumatoid arth-
ritis on clinical grounds were positive for
rheumatoid factor, and all but one had
radiological changes consistent with
rheumatoid arthritis.

The results of the immunohistochemical
staining on all of the specimens investigated is
summarised in table 2. CD23 expression
was moderately or strongly positive in 50% of
more lymphocytes in six of the nine cases of
rheumatoid arthritis. Two of the cases of
rheumatoid arthritis, however, were negative
for CD23, and in the remaining case only a
small proportion of the lymphocytes in the
inflammatory infiltrate were positive (figure).

There was no apparent relation between CD23
positivity and age, sex, or disease activity, as
measured by haemoglobin concentration and
ESR at the time of the biopsy.

In the group of six biopsy specimens from
patients with osteoarthritis one showed no
CD23 staining, one had weak staining, two
showed moderate staining and two showed
strong staining in more than 50% of the
lymphocytes. There was also a spectrum of
weak to strong CD23 positivity in the three
synovial biopsy specimens considered to show
non-specific inflammation. There was moderate
or strong CD23 positivity of more than 50% in
all of the specimens showing chronic inflamma-
tion in non-articular tissues. None of these
patients had any evidence of joint disease.

In both synovial and non-articular tissues
that had pronounced CD23 positivity in
lymphocytes, there was CD23 positivity in
endothelial cells of small vessels within the
region of the inflammation. Vessels away from
areas of lymphoid infiltrate and in biopsy
specimens with no or only weak CD23
positivity did not show this CD23 staining.

Table 1  Summary of clinical data and investigations performed in conjunction with synovial biopsy

<table>
<thead>
<tr>
<th>Case No</th>
<th>Age (y)</th>
<th>Sex (M/F)</th>
<th>Morning stiffness</th>
<th>RF concentration</th>
<th>ESR (mm/h)</th>
<th>Biopsy site</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>61</td>
<td>F</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Lx</td>
</tr>
<tr>
<td>2</td>
<td>73</td>
<td>F</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>38</td>
<td>F</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>57</td>
<td>M</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>77</td>
<td>M</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>46</td>
<td>F</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>7</td>
<td>35</td>
<td>M</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>8</td>
<td>56</td>
<td>F</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>28</td>
</tr>
<tr>
<td>9</td>
<td>34</td>
<td>F</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>13·1</td>
</tr>
</tbody>
</table>

Osteoarthritis:

<table>
<thead>
<tr>
<th>Case No</th>
<th>Age (y)</th>
<th>Sex (M/F)</th>
<th>Morning stiffness</th>
<th>RF concentration</th>
<th>ESR (mm/h)</th>
<th>Biopsy site</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>43</td>
<td>F</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>R Hip</td>
</tr>
<tr>
<td>11</td>
<td>77</td>
<td>F</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>R Hip</td>
</tr>
<tr>
<td>12</td>
<td>87</td>
<td>F</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>R Hip</td>
</tr>
<tr>
<td>13</td>
<td>90</td>
<td>F</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>R Hip</td>
</tr>
<tr>
<td>14</td>
<td>62</td>
<td>F</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>R Hip</td>
</tr>
<tr>
<td>15</td>
<td>68</td>
<td>F</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>R Hip</td>
</tr>
</tbody>
</table>

Non-specific joint inflammation:

<table>
<thead>
<tr>
<th>Case No</th>
<th>Age (y)</th>
<th>Sex (M/F)</th>
<th>Morning stiffness</th>
<th>RF concentration</th>
<th>ESR (mm/h)</th>
<th>Biopsy site</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>12</td>
<td>F</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>R Ankle</td>
</tr>
<tr>
<td>17</td>
<td>18</td>
<td>F</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>R Ankle</td>
</tr>
<tr>
<td>18</td>
<td>28</td>
<td>M</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>R Ankle</td>
</tr>
</tbody>
</table>

RF = rheumatoid factor; Lx = Latex agglutination test; R-W = Rose-Waller test; Hb = haemoglobin concentration in grams per decilite; ESR = erythrocyte sedimentation rate in millimetres per hour; ND = not done; RA = diagnostic or consistent with rheumatoid arthritis, OA = diagnostic or consistent with osteoarthritis; NAD = nothing abnormal discovered.

Results

Table 1 gives a summary of the clinical data
corning all the patients with joint disease
included in this study. All of the patients who
had been classified as having rheumatoid arth-
ritis on clinical grounds were positive for
Table 2  Assessment of CD23 positivity in lymphocytes from each of the biopsy specimens studied

<table>
<thead>
<tr>
<th>Case No</th>
<th>Nature of biopsy</th>
<th>Site of biopsy</th>
<th>Percentage of lymphocytes positive</th>
<th>Degree of positivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rheumatoid arthritis:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Synovium</td>
<td>R Knee</td>
<td>50%-90%</td>
<td>+++</td>
</tr>
<tr>
<td>2</td>
<td>Synovium</td>
<td>L Knee</td>
<td>50%-90%</td>
<td>+++</td>
</tr>
<tr>
<td>3</td>
<td>Synovium</td>
<td>R Wrist</td>
<td>50%-90%</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Synovium</td>
<td>L Wrist</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Synovium</td>
<td>R Elbow</td>
<td>90%</td>
<td>+++</td>
</tr>
<tr>
<td>6</td>
<td>Synovium</td>
<td>R Wrist</td>
<td>&lt;50%</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Synovium</td>
<td>R Knee</td>
<td>50%-90%</td>
<td>+++</td>
</tr>
<tr>
<td>8</td>
<td>Synovium</td>
<td>R Hand</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Synovium</td>
<td>L Knee</td>
<td>50%-90%</td>
<td>+++</td>
</tr>
<tr>
<td>Osteoarthritis:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Synovium</td>
<td>R Hip</td>
<td>&gt;90%</td>
<td>+++</td>
</tr>
<tr>
<td>11</td>
<td>Synovium</td>
<td>R Hip</td>
<td>&gt;90%</td>
<td>+++</td>
</tr>
<tr>
<td>12</td>
<td>Synovium</td>
<td>L Hip</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Synovium</td>
<td>L Hip</td>
<td>50%-90%</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td>Synovium</td>
<td>R Knee</td>
<td>50%-90%</td>
<td>+</td>
</tr>
<tr>
<td>15</td>
<td>Synovium</td>
<td>R Hip</td>
<td>&lt;50%</td>
<td>+</td>
</tr>
<tr>
<td>Non-specific joint inflammation:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Synovium</td>
<td>R Ankle</td>
<td>&lt;50%</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Synovium</td>
<td>R Knee</td>
<td>&lt;50%</td>
<td>+++</td>
</tr>
<tr>
<td>18</td>
<td>Synovium</td>
<td>R Ankle</td>
<td>&gt;90%</td>
<td>+++</td>
</tr>
<tr>
<td>Non-articular lesions:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Skin</td>
<td></td>
<td>50%-90%</td>
<td>+++</td>
</tr>
<tr>
<td>20</td>
<td>Gallbladder</td>
<td></td>
<td>50%-90%</td>
<td>+++</td>
</tr>
<tr>
<td>21</td>
<td>Branchial Cyst</td>
<td></td>
<td>&gt;90%</td>
<td>+++</td>
</tr>
<tr>
<td>22</td>
<td>Stomach (Gastritis)</td>
<td></td>
<td>&gt;90%</td>
<td>+++</td>
</tr>
<tr>
<td>23</td>
<td>Ileum (Crohn's)</td>
<td></td>
<td>50%-90%</td>
<td>+</td>
</tr>
</tbody>
</table>

- = negative; + = weakly positive; ++ = moderately positive; +++ = strongly positive.

The synovial fluid from patients with rheumatoid arthritis has been shown to contain interleukin-1,23-25 granulocyte-macrophage colony stimulating factor,26 macrophage colony stimulating factor27 and a protein with similar properties to murine B cell growth factor 2.28 In contrast, γ interferon29 and interleukin-27β are found only in small quantities in the synovial fluid from these patients. Similar changes in the concentrations of these cytokines, however, are found in other chronic arthritides such as psoriatic and juvenile arthritis.26 Thus changes in the concentrations of these cytokines are not specific to rheumatoid arthritis.

CD23 is a B lymphocyte activation marker and its soluble derivative has been shown to have effects on B cell growth and differentiation.12 B cell activation has been suggested as an important pathogenic factor in rheumatoid arthritis, so CD23 is a candidate for involvement in the inflammatory cell infiltrate in the disease.

Many monoclonal antibodies have been raised against CD23, but are limited in their use as reagents for immunohistochemistry because they do not work on sections of paraffin wax embedded tissues. The antibody used in this study is unique in that it shows CD23 antigen in this type of specimen. Two distinct forms of the CD23 molecule have been shown, but these differ only in their intracytoplasmic regions.29 BU38 recognises an epitope on the extracellular IgE binding part of the molecule, and so would be expected to bind to both forms of the membrane bound protein and to soluble CD23.

This study found CD23 positivity on lymphocytes in synovial biopsy specimens from most but not all patients with rheumatoid arthritis. There was no apparent correlation between the degree of CD23 staining and the activity of the disease as assessed by the ESR and the haemoglobin concentration. There was also no difference in CD23 staining between rheumatoid arthritis and other conditions showing chronic inflammation, both articular and non-articular. These findings suggest that CD23 expression is not of any specific pathogenetic importance in rheumatoid arthritis, but may be a characteristic feature of any chronic inflammatory response.

The finding of cytoplasmic endothelial staining in regions of CD23 positive cellular infiltrate is similar to that found in a previous study.23 It is believed that this phenomenon is due to uptake of soluble CD23 by endothelial cells.

The results of this study suggest that CD23 is found in all forms of chronic inflammation.
and is not likely to be specific for any particular form of chronic inflammatory disease. As CD23 was found to be on the surface of lymphocytes in many cases of these arthritides, however, sCD23 in serum or synovial fluid may still be a useful marker for the severity of the inflammatory infiltrate.

This study was supported by a grant from the West Midlands Regional Health Authority.


29 Firestein GS, Zvaifler NJ. Peripheral blood and synovial fluid monokine activation in inflammatory arthritis. II. Low levels of synovial fluid and synovial tissue interferon suggest that gamma interferon is not the primary macrophage activating factor. *Arthritis Rheum* 1987;30:364–71.

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