Immunocytology of synovial fluid cells may be of diagnostic and prognostic value in arthritis

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SUMMARY Cells of the synovial fluid (SF) have been examined by immunocytocological methods. Samples were aspirated from four groups of patients with knee effusions: (a) seropositive inflammatory arthritis (n=9); (b) seronegative inflammatory arthritis (n=9); (c) osteoarthritic patients (n=5); and (d) patients with traumatised knees (n=4). The proportions of lymphocyte and macrophage subsets within the SF were determined with a panel of monoclonal antibodies. Patients with inflammatory arthritis had significantly larger proportions of activated T cells (RFT2+) and macrophages with the phenotype of interdigitating cells (RFD1+). No significant difference between groups could be found on differential count or when T4+/T8+ subset ratios were calculated. No significant difference in proportions of lymphocyte or macrophage subsets was found between the groups with seropositive and seronegative inflammatory arthritis. In two of three patients, where immunocytocchemical analysis was performed before and after intra-articular steroids, reductions in the proportions of RFT2+ T cells and RFD1+ macrophage like cells were seen. It is suggested that such analysis may be of diagnostic or prognostic value.

Key words: immunology, rheumatoid arthritis, monoclonal antibodies.

A growing number of reports have focused attention on the immunopathology of the synovial membrane in arthritic patients.1-3 Further studies have shown that signs of local cell mediated immunological reactivity can be identified in patients with rheumatoid and psoriatic arthritis,4 5 and in patients with ankylosing spondylitis.6 A quite distinct immunopathology was observed in the synovia of patients with degenerative arthropathies.7 Staining of tissue sections with monoclonal antibodies (McAbs), with immunohistological techniques, has shown that T and B lymphocytes, plasma cells, and various subpopulations of macrophage like cells are all present in these tissues.8 More specifically, it has been shown in the synovial membrane of patients with rheumatoid arthritis (RA) that subpopulations of T cells (T4+ and T8+) both occur, but in a ratio one to the other that is abnormal, with an excess of T4+ cells.6 It was further shown that within these T4+ lymphocyte clusters non-lymphoid dendritic cells occurred in abnormally large numbers.7 These dendritic cells were shown to have the phenotype of interdigitating cells, a cell type normally residing in the T cell areas of lymph nodes and thought to be responsible for antigen presentation and the induction of immune responses.4 Such observations add weight to the hypothesis that the basis of the chronicity of RA associated inflammation is the result of a local breakdown of lymphocyte/macrophage immunoregulation.8 Further evidence in support of the suggestion that this is a local phenomenon comes from the examination of synovial tissue from osteoarthritic patients and rheumatoid nodules taken from RA patients. In osteoarthritis (OA) the immunohistological analysis found only few T cells, with T4+/T8+ ratios normal and a marked absence of interdigitating cells.4 5 Again within the rheumatoid nodules the immunopathological picture did not reflect that seen in the...
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lymphocytes with rheumatoid arthritis expressed signs of evidence of local immunohistopathological grounds from degenerative arthropathies where no arthritis and those relating to the interdigitating with patients activated these tissue were inflamed or arthropathies.5

With monoclonal antibodies directed respectively against cells synovium.4 9 More recently, similar immunohistological studies showed that a significant proportion of T lymphocytes within the synovial stroma of patients with rheumatoid arthritis expressed signs of activation.5 With monoclonal antibodies directed against class II major histocompatibility complex (MHC) antigens and a P40 antigen preferentially expressed on T blasts (McAbs RFDR2 and RFT2 respectively) it was found that 25% of T cells in this inflamed tissue were RFT2 positive, and all of these RFT2 cells were RFDR2+. Furthermore, all of these activated cells were present in the synovia of patients with RA, anklosing spondylitis (AS), and psoriatic arthritis but not present on degenerative arthropathies or in rheumatoid nodules.5 These observations and those relating to the interdigitating cells referred to earlier offer cogent evidence in support of the hypothesis that inflammatory arthritis can be clearly distinguished on immunohistological grounds from degenerative arthropathies where no immunohistopathological evidence of local immunoregulatory dysfunction is present. Such studies, however, demand the use of arthroscopic biopsy to obtain tissue for analysis. As this procedure is not always available and indeed may result in a degree of morbidity the present study concentrated on an analysis of cells within synovial fluid, which may be obtained repeatedly by aspiration. Our intention was to determine whether similar cellular changes indicative of the ongoing disease process could be identified within the synovial fluids obtained from patients presenting with clinically defined arthropathies.

Patients and methods

Patients

A total of 27 patients in four groups was studied. One group contained nine patients with classic seropositive rheumatoid arthritis.10 Group 2 comprised nine patients with seronegative inflammatory arthropathies, of which five were clinically defined as RA and two as psoriatic arthritis. One case of Reiter’s syndrome was included and one mono-arthropathy presented in a patient with leukaemia. All patients fulfilled recognised clinical criteria for the respective disease.

Group 3 contained five patients with clinically defined degenerative osteoarthritis (OA), and group 4 was composed of four patients with knee effusions resulting from trauma. None of these group 4 patients had any history of arthritis. In three cases (see ‘Results’) a repeat aspiration of SF was obtained two weeks after the intra-articular injection of Depo-Medrone 80 mg into the joint space.

Aspiration

Aspiration of SF was carried out with an 18 gauge needle. A medial approach was used after lateral displacement of the patella to increase the patello-femoral gap. The volume of fluid removed ranged from 10 to 100 ml. No samples containing blood or aspirates of less than 10 ml were used in this study. Total cell counts ranged from $6.2\times10^8$ cells/ml ($6.2\times10^9$) to $4.8\times10^8$ cells/ml ($4.8\times10^9$).

Preparation of cytospins

The aspirated SF cells were washed twice with phosphate buffered saline (PBS) and the cells suspended in PBS at a final concentration of $5.8\times10^8$ nucleated cell/ml ($5.8\times10^9$). Viability as determined by trypan blue exclusion was consistently > 85%. A differential cell count was performed on each sample.

Cytospin preparations of these suspensions were made with a Shandon Cytospin II cytocentrifuge. Prepared slides were air dried, fixed for 10 minutes in chloroform/acetone (1:1), wrapped in cling film, and stored at $-20^\circ$C until used.

Immunocytochemistry

Discrete subpopulations of cells were identified with McAbs by indirect immunoperoxidase methods.11 The McAbs used and the cell types identified are listed in Table 1. In some studies the cytochemical reaction to identify lysosomal acid phosphatase activity was used.12

Quantification

For each immunocytochemical and histochemical reaction random high power fields from duplicate cytospins of each reaction were examined with an eyepiece grid, and the proportions of positive cells were enumerated. Not less than 200 cells were counted in each case. If no positive cells were detected this was recorded as < 1%. The mean values of the proportions of cells in the SFs of each group of patients were calculated, and Student’s $t$ test for non-paired data was used to determine significance where relevant.

Results

Differential counts were performed on all SF cell populations. The proportion of polymorphonuclear cells (PMNs) was very variable in cases of seropositive RA, ranging from 3% to 87% (Fig. 1). Although less variable, the numbers of PMNs in seronegative RA and OA both ranged from 2% to 37%. In the four traumatised cases high numbers of PMNs were recorded. The proportions of macrophage like cells (MLCs) also varied considerably from patient to
Table 1  Tests used for analysis of SF cells

<table>
<thead>
<tr>
<th>Reagent used</th>
<th>Source</th>
<th>Method</th>
<th>Cells identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>RFT mix (cocktail of RFT 1, 8, 11)</td>
<td>RFHSM</td>
<td>Indirect immunoperoxidase</td>
<td>All T cells</td>
</tr>
<tr>
<td>RFT2</td>
<td>RFHSM</td>
<td>Indirect immunoperoxidase</td>
<td>Activated T cells</td>
</tr>
<tr>
<td>RFT8</td>
<td>RFHSM</td>
<td>Indirect immunoperoxidase</td>
<td>Suppressor/cytotoxic type T lymphocytes</td>
</tr>
<tr>
<td>OKT4</td>
<td>Orthopharmaceuticals</td>
<td>Indirect immunoperoxidase</td>
<td>Helper/inducer type T lymphocytes</td>
</tr>
<tr>
<td>RFD7</td>
<td>RFHSM</td>
<td>Indirect immunoperoxidase</td>
<td>Mature macrophages</td>
</tr>
<tr>
<td>RFD1</td>
<td>RFHSM</td>
<td>Indirect immunoperoxidase</td>
<td>Dendritic cells</td>
</tr>
<tr>
<td>Acid phosphatase activity (ACP)</td>
<td>—</td>
<td>Histochemistry</td>
<td>Monocytes/macrophages</td>
</tr>
</tbody>
</table>

A consistently high number of MLcs was found in cases of OA, while the SFs from traumatic cases contained significantly fewer MLcs. Greater proportions of T cells were found in both seropositive and seronegative RA than in OA or traumatic arthropathies (Fig. 1), though the difference between RA and OA cases did not reach statistical significance (p>0.05).

**T4/T8 Ratios**

When the proportions of $T_4^+$ (helper type) T cells and $T_8^+$ (suppressor/cytotoxic type) T cells were calculated no significant differences in their ratio could be detected between any of the groups studied (Fig. 2), and wide variations were seen.

**Proportion of Activated T Cells**

The McAb RFT2 identifies an antigen (mol. wt 40 000) which has been shown to be preferentially expressed on activated T cells.
expressed on activated T cells. Numbers of RFT2+ cells were counted and expressed as a percentage of total T cells determined by applying a ‘pan T’ reagent (T mix) to the cytospins. The results (Fig. 3) showed that significantly higher proportions of T cells were activated in the SF of rheumatoid patients (mean 25%, seropositive; 18%, seronegative) than were seen in OA SFs (2.5%) and in traumatic knees (mean <1%) (p<0.01 in both cases).

**Proportions of Subsets of Macrophage Like Cells**

Cytospins of SF cells from all groups were subsequently analysed with McAbs, RDF7 and RDF1, and the proportions of positive cells were recorded. RDF7 identifies mature ‘classical’ macrophages, while RDF1 identifies interdigitating cells (ID cells, see Table 1). A lysosomal acid phosphatase (ACP) reaction was also performed on some cytospins. This histochemical reaction is positive on all monocytes/macrophages but negative on ID cells. The results are presented in Fig. 4. SFs of patients with OA contained larger proportions of ACP+ cells than all other groups (p<0.01). SF from traumatic knees showed smaller proportions of ACP+ cells (p<0.05), while no difference in the proportions of ACP+ populations was recorded in the seropositive and seronegative rheumatoid arthropathies. In contrast, however, the SFs of both these groups contained significantly larger proportions of RDF1+ ID cells than SFs from either OA or traumatised patients (p<0.01 in each case).

**Effect of Treatment**

In three cases the above analysis was performed before and two weeks after the intra-articular injection of 80 mg Depo-Medrone. One male patient (No 1) presented with pain and swelling of both knees and with morning stiffness lasting up to two hours. He had a two year history of intermittent arthropathy that had deteriorated over the six weeks before admission. Past history included episodes of urethritis and a penile rash but no other mucocutaneous lesions or eye manifestations. He was taking piroxicam 30 mg in the morning and Indocid suppositories 100 mg at night. On 5 June 1984 his erythrocyte sedimentation rate (ESR) was 84 mm/1st h.

On 13 June 1984 both knees were swollen, and the effusions were aspirated. SF cells were collected, and the first immunocytochemical analysis was performed. Each knee was injected with 80 mg Depo-Medrone. On 27 June 1984 a recurrence of the effusions occurred, which were aspirated again, and a second immunocytological analysis was performed. Depo-Medrone was again injected into each knee. After one more occurrence of swelling on 19 July 1984 (again treated) a subsequent progressive improvement took place with no more effusions, and on 7 August 1984 his ESR was 40 mm/1st h and no morning stiffness was being experienced. He was discharged on 9 August 1984 and has been followed up in outpatient clinic, without recurrence of symptoms.

The results of the immunocytochemical analysis showed that before treatment >40% of T cells expressed signs of activation (RFT2+) and 25% of the SF cells were RDF1+ dendritic cells (Table 2). Two weeks later the proportion of RFT2+ cells was halved despite no change in total T cells and <1% of cells were RDF1+. Repeat SF immunocytochemistry was performed on two further cases (Nos 2 and 3, both with seropositive RA). The results in case No 2 showed no change in the proportion of RFT2+ T cells or RDF1+ cells after treatment with intra-articular steroids. In case No 3 a reduction in the proportion of T cells expressing RFT2+ was re-

**Fig. 3** The proportion of total T cells that were activated (RFT2+) in each patient studied. Each point represents the number of RFT2+ cells expressed as a percentage of total T cells. The mean figure in each case is identified with a horizontal line. Groups as for Fig. 1.
The percentage of total cells that were ACP⁺ (○), identifying macrophages and monocytes; RFD7⁺ (▼), identifying mature macrophages only; and RFD1⁺ (■), identifying dendritic interdigitating cells. The bars represent the mean percentage of cells positive for each reaction on each group of patients. Groups as for Fig. 1.

Table 2 Proportions of RFT2⁺ T cells and RFD1⁺ dendritic cells before and after intra-articular steroid treatment

<table>
<thead>
<tr>
<th>Patient No</th>
<th>Diagnosis</th>
<th>Systemic treatment</th>
<th>Pre- and Poststeroid (80 mg Depo-Medrone)</th>
<th>Follow up (months)</th>
<th>Current clinical status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total T cells*</td>
<td>RFT2⁺ cells†</td>
<td>RFD1⁺ cells*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pre  Post  Pre  Post  Pre  Post</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Reactive arthritis, Reiter's</td>
<td>Piroxicam 30 mg/day,</td>
<td>55  53   41  22   25  &lt;1   15</td>
<td>Improved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>syndrome</td>
<td>Indocid supp. 100 mg at night</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Seropositive RA</td>
<td>Pencillamine 375 mg/day</td>
<td>50  53   11  13   10  6   12</td>
<td>Deteriorated</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Seropositive RA</td>
<td>Prednisolone 5 mg/day</td>
<td>16  23   62 &lt;4   13  9   1</td>
<td>No change</td>
<td></td>
</tr>
</tbody>
</table>

*As a percentage of total cells.  †As a percentage of T cells.

corded after steroid treatment, but no effect was seen on the proportion of RFD1⁺ cells. The follow up in this last case is as yet too short for any firm conclusions to be drawn. Indeed these results are presented as preliminary observations.

Discussion

The data presented in this paper show the presence of activated T cells and dendritic cells with the phenotype of antigen presenting interdigitating cells within the SF of patients with RA, and in other inflammatory arthropathies. These populations were not identified in any significant numbers in patients with degenerative OA or in effusions taken from traumatised knees. On the other hand, differential cell counts or the quantification of T4/T8 ratios failed to discriminate between these patient groups.

There is now increasing evidence that the pathogenic tissue changes associated with inflammatory arthritis stem from dysfunction of macrophage/lymphocyte immunoregulation.⁸ This suggestion has been strengthened by the observation that the T cell infiltrates of the synovial tissue contain a disproportionately large number of the T4⁺ subset,⁹ and that large dendritic cells expressing high concentrations of class II MHC antigens are present in close association with these cells.⁷ More recently it has been observed that many T cells within the inflamed synovia are activated.⁵ As it has also been reported that interleukin-2 (IL-2) production of...
these cells may be reduced,\textsuperscript{13} the possibility emerges that stimulation of T4\textsuperscript{+} helper cells in the absence of concomitant IL-2 induced stimulation of T8\textsuperscript{+} suppressor cells results in progressive inflammation and the uncontrolled activation of local B cells.

The immunohistological studies that showed these abnormal cellular associations in inflammatory arthritic synovium also confirmed that no similar changes occur in the synovium of patients with degenerative arthropathies.\textsuperscript{5} Furthermore, similar studies of rheumatoid nodules from RA patients showed that the abnormalities seen within the synovial membranes were not reflected in these nodules.\textsuperscript{9} Such observations show that the cellular changes of increased numbers of activated T4\textsuperscript{+} cells and the high concentrations of dendritic cells are specifically localised in the synovial stroma, and that such changes may be characteristic only of patients with inflammatory arthritis. It follows, therefore, that the identification of these cell types may have diagnostic significance and may be of potential value in determining the level of immunological reactivity occurring locally.

The aspiration of SF is an atraumatic procedure that may be repeated at intervals for clinical reasons and offers a convenient source of cells intimately involved in the joint inflammation. The SF of patients with RA has already been shown to contain T cells that express class II MHC antigens\textsuperscript{14,15} and dendritic cells,\textsuperscript{16} and IL-2 production from SF cells has been reported.\textsuperscript{17} These data suggest that the cells of SF may reflect the situation seen in the synovial stroma. The results in this paper go further in demonstrating that the RFT2\textsuperscript{+} activated lymphocytes and RFD1\textsuperscript{+} dendritic cells only appear as significant proportions of T cells and macrophage like cells respectively in the SF of patients with inflammatory arthritis but not in the SF of patients with OA and traumatised joints. It is accepted that total numbers of these cells may vary due to variation in the overall number of cells within the SF. Previous studies have shown, however, that it is the difference in proportion of one cell subset to another, rather than total numbers, that is related to pathogenesis.\textsuperscript{5,6}

In the cases where immunocytocchemical analysis was repeated after treatment with intra-articular steroids the numbers of both activated T cells and dendritic cells were both found to be reduced in one case, unchanged in another, and with a reduction seen only in RFT2\textsuperscript{+} cells in a third. All these observations were made at times when no clinical improvement was manifest. Although the one case where reductions in both immunocompetent cell types were seen has subsequently improved, it is far too early to draw any conclusions. We feel, however, that these preliminary results on repeat aspirations warrant further investigation.

With regard to the direct immunocytocological investigation of SF samples, however, we conclude that this analysis may offer some diagnostic or prognostic indication of the immunological basis of the ongoing arthropathy. Furthermore, the similar results obtained with seropositive and seronegative patients suggest that this serological distinction may have no direct bearing on the inflammatory process in the joint.

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References


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