Cellular immunohistopathology of acute, subacute, and chronic synovitis in rheumatoid arthritis

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SUMMARY Cellular inflammation in rheumatoid arthritis (RA) synovial membrane was studied in biopsy specimens taken at different stages of synovitis and disease. Patients were classified into three subgroups: acute RA, subacute RA, and chronic RA. Inflammatory cells were characterised by a histochemical esterase method and immunohistochemical peroxidase–antiperoxidase (PAP) and avidin-biotin-peroxidase (ABC) staining.

The amounts and distribution of inflammatory cells were different in various stages of the synovitis. In acute onset RA monocytes and granulocytes predominated, suggesting that the beginning of rheumatoid inflammation is similar to inflammatory reaction in general. The presence of T cells and also of plasma cells in subacute RA suggests underlying subclinical changes also in apparently healthy joints in RA. The most typical feature of prolonged synovitis in chronic RA was its intensity, characterised by the presence of large T cell and plasma cell infiltrates. Our findings suggest that the immunological mechanisms are secondary to the tissue damage caused by the initial inflammatory events of unknown cause. However, the immunological mechanisms may still play a central role in the aetiopathogenesis, because findings in chronic RA suggest a defective down-regulation of the immune response.

Inflammation in rheumatoid synovial tissue in chronic RA has been exhaustively characterised by different histological, histochemical, immunological, and immunohistochemical methods.1-8 However, the histopathological pattern observed in chronic RA may present an end stage in a prolonged disease. This is supported by two earlier publications on synovitis of recent onset, where histopathological findings quite different from those observed in chronic synovitis in RA were reported.9 10 These studies by Kulka et al.9 and Schumacher and Kitridoul10 dealt with the morphological findings at light microscopic and electron microscopic level. We therefore decided to extend their work to cellular immunohistopathology of synovitis of recent onset. This is the final report of a collaborative study started at the end of 1980 between Helsinki University Central Hospital and the Finnish Rheumatism Foundation Hospital for evaluation of cellular inflammation in acute, subacute, and chronic synovitis in RA by modern (immuno)histochemical methods.

Materials and methods

PATIENTS AND BIOPSIES RA was divided into three different entities on the basis of the clinical evaluation of the duration of the disease and synovitis at the time of histopathological analysis (Table 1). We separated synovitis of recent onset into two subgroups based on the duration of the underlying RA. In cases with acute RA the duration of both the synovitis and disease was clinically less than three months. Patients with subacute RA had a well established chronic RA but no clinical symptoms or signs in the knee joint.
examined; symptoms occurred less than three months before biopsy was performed for analysis.

In each patient rheumatoid synovial tissue was taken from the knee joint. In acute and subacute RA at least three biopsy specimens were studied. Clinical data of the patients studied are given in Table 2.

A follow up of all patients continued for at least three years after biopsy. Patients classified as having acute or subacute RA developed a classic sero-positive, erosive RA during the follow-up period.

HISTOCHEMICAL &-NAPHTHYL ACETATE ESTERASE (ANAE) STAINING

The synovial biopsy specimens were fixed in Baker's formalin calcium for 20 h, washed in Holt's solution for 4-6 h, dehydrated in acetone for 2-4 h, and cleared in xylene for 4 h (all steps at 4°C) before paraffin embedding for 1-2 h at 55°C. Deparaffinized sections were incubated in a medium consisting of 35-6 ml of 0.067 M phosphate-buffered saline (PBS, pH 7.6), 2.4 ml of hexaazotised p-nrosaniline, and 16 mg of &-naphthyl acetate (Sigma Chemical Co., St Louis, MO) in 2 ml of ethylene glycol monomethyl ether acetate. The pH of the mixture was adjusted to 6.1. The incubation time was 2 h and counterstaining was performed in a 1% aqueous solution of toluidine blue for 5 min before dehydration, clearing, and mounting. Histochecmical staining of ANAE disclosed two different staining patterns: one or more cytoplasmic dots (T pattern) and diffuse cytoplasmic activity (M pattern).

IMMUNOHISTOCHEMICAL PAP STAINING

For the immunoperoxidase demonstration of intracellular immunoglobulins (plasma cells) the synovial specimens were fixed in Bouin's fluid for 4-8 h. The tissues were then dehydrated in absolute ethanol, cleared in xylene, and embedded in paraffin according to a standard histological technique.5 Paraffin blocks were cut into 6 µm thick sections. After deparaffinisation the peroxidase-antiperoxidase (PAP) method was applied. Briefly, the endogenous peroxidase activity was destroyed by pretreating the sections in methanol-H2O2 and then treating them sequentially with: (a) normal swine serum; (b) rabbit antihuman IgG, antihuman κ or antihuman λ light chains; (c) swine antirabbit IgG; and (d) rabbit antihorseradish peroxidase–horseradish peroxidase complexes (PAP). All these antisera were purchased from Dakopatts, Copenhagen, Denmark. Finally, the sections were stained according to the method of Graham and Karnovsky13 and counterstained with haematoxylin.

IMMUNOHISTOCHEMICAL ABC STAINING

The avidin–biotin–peroxidase (ABC) staining method of Hsu et al.14 was used to identify the cell subsets in the biopsy specimens. For ABC staining

Table 1 Origin of the RA synovial tissue obtained for analysis

<table>
<thead>
<tr>
<th>RA</th>
<th>Duration of the disease</th>
<th>Number of patients studied</th>
<th>Method by which samples were obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute</td>
<td>&lt;3 months</td>
<td>&lt;3 months</td>
<td>4</td>
</tr>
<tr>
<td>Subacute</td>
<td>&gt;3 years</td>
<td>&lt;3 months</td>
<td>3</td>
</tr>
<tr>
<td>Chronic</td>
<td>&gt;3 years</td>
<td>&gt;3 years</td>
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Table 2 Clinical data of the patients studied

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<th>ARA* criteria</th>
<th>RF</th>
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<th>Synovial fluid</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Leucocytes</td>
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<td>55</td>
<td>Female</td>
<td>6</td>
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<tr>
<td>Subacute</td>
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<td>5</td>
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*ARA = American Rheumatism Association; MNC = mononuclear cells; PMN = polymorphonuclear leucocytes.

† No erosions in the joint from which the biopsy was obtained.
the tissue specimens were embedded in OCT compound (Lab-Tek Products, Division Miles Laboratory, Elkhart, IN) and immediately frozen in isopentane at −50°C. Cryostat sections were fixed in cold acetone for 5 min. Cell surface markers were demonstrated by applying: (a) monoclonal antiserum (OKT3, OKT4, OKT8, OKIa; Ortho Pharmaceuticals Co., Raritan, NJ); (b) biotinylated horse antimouse IgG (Vector Laboratories, Burlingame, CA); and (c) ABC complexes (Vector Lab.). All antiserum incubations were performed in a humidified chamber and room temperature for 30 min. The peroxidase binding sites were shown with a fresh solution of 3,3′-diaminobenzidine tetrahydrochloride (0.033%) and H2O2 (0.003%) in PBS for 5 min. Tissue sections were counterstained in haematoxylin, dehydrated, cleared, and mounted.

Results

Patients with clinically different forms of RA were also segregated histopathologically into three distinct patterns (Table 3). However, because of interindividual and intrasample variations, grading of the intensity of cellular inflammation (instead of numerical evaluation as percentages of all cells) was the most rational way of expressing the findings in these three different subgroups of RA.

**Acute RA**

In acute RA the most prominent feature was the abundant occurrence of mononuclear phagocytes, especially in the sublining layer (Fig. 1). Granulocytes were also often present in patchy infiltrates and entrapped in the fibrin layer covering synovial villi (Fig. 2). Only a sparse T cell infiltrate was observed in the subsynovial layer.

**Subacute RA**

Subacute RA was histopathologically quite distinct from the acute RA. In subacute RA lymphocytes were always observed in nodular perivascular infiltrates in the subsynovium (Fig. 3). Mononuclear phagocytes were also abundant in subacute RA, especially below the lining layer as shown in Fig. 3. In the perivascular lymphocyte rich infiltrates the ANAE+, T3+ T lymphocyte was the predominant cell. There was only a slight augmentation of T4+ cells over T8+ cells (Fig. 4) as compared with the normal 2:1 ratio in the peripheral blood. Many of the local lymphocytes were Ia+ (Fig. 5). In contrast to acute synovitis plasma cells also were observed in situ in subacute RA, but they were not as numerous as in chronic RA.

**Chronic RA**

An abundance of plasma cells was the most characteristic feature of chronic RA as opposed to acute and subacute synovitis, and they often formed dense infiltrates composed almost exclusively of plasma cells (Fig. 6). Mononuclear phagocytes were not as frequent as in the more acute forms of synovitis. T cell was the predominant inflammatory cell in situ in chronic RA.

**Histopathological features of patients**

To give some idea of variations in histology between members of the same group of patients a short description of the histopathological features in each individual patient is given below:

![Fig. 1 Mononuclear phagocytes in acute synovitis of rheumatoid arthritis. (Esterase staining, × 250).](image-url)
Kontinen, Bergroth, Nordström, et al.

Fig. 2 A granulocyte-rich infiltrate in acute synovitis of rheumatoid arthritis. (Esterase staining, × 1000).

Fig. 3 Mononuclear phagocytes and a slight lymphocyte infiltrate in subacute synovitis of rheumatoid arthritis. (Esterase staining, × 250).

Acute RA
Patient No 1. Moderate hyperplasia and hypertrophy of synoviocytes, fresh fibrin on synovial surface, oedema, proliferating capillaries, diffuse infiltrates of monocytes, some small focal aggregates of lymphocytes.

Patient No 2. Slight hyperplasia of synoviocytes, proliferating fibroblasts, diffuse infiltrates of monocytes, diffuse infiltrates of granulocytes in sub-synoviocyte tissue.

Patient No. 3. Slight hyperplasia of synoviocytes, oedema, villi, proliferating capillaries, diffuse infiltrates of monocytes with some lymphocytes and granulocytes.

Patient No 4. Marked hyperplasia of synoviocytes, oedema, proliferating fibroblasts, diffuse infiltrates of monocytes.

Subacute RA
Patient No 5. Moderate hyperplasia and hypertrophy of synoviocytes, oedema, proliferating capillaries, proliferating fibroblasts, focal aggregates of lymphocytes and monocytes with occasional plasma cells.

Patient No. 6. Slight hyperplasia of synoviocytes, villi, proliferating fibroblasts, focal aggregates of lymphocytes, occasional monocytes and plasma cells.

Patient No 7. Moderate hyperplasia of synoviocytes, fresh fibrin on synovial surface, proliferating fibroblasts, large infiltrates of lymphocytes, diffuse infiltrates of monocytes.

Chronic RA
Patient No 8. Marked hyperplasia and hypertrophy of synoviocytes, villi, moderate proliferation of fibroblasts, diffuse infiltrates of lymphocytes,
Cellular immunohistopathology of synovitis in rheumatoid arthritis

Numerous plasma cells, proliferating capillaries, occasional monocytes and granulocytes.

**Patient No 9.** Small deposits of older fibrin scattered on synovial surface, marked hyperplasia of synoviocytes, villi, fibrosis, focal aggregates of lymphocytes and plasma cells, occasional monocytes.

**Patient No 10.** Marked hyperplasia and hypertrophy of synoviocytes, villi, proliferating fibroblasts, fibrosis, large infiltrates of lymphocytes and plasma cells.

**Patient No 11.** Slight hyperplasia of synoviocytes, fibrosis, thick walled blood vessels, diffuse infiltrates of plasma cells and lymphocytes, occasional granulocytes.

**Patient No 12.** Fibrin on synovial surface, marked hyperplasia of synoviocytes, villi, oedema, pro-
The host inflammatory response applies to the cellular immunohistopathology of rheumatoid arthritis.

Our findings indicate that rheumatoid arthritis develops distinct stages depending on both the duration of the disease and the duration of the synovitis in the respective joint. More specifically, in RA arthritis and synovitis of recent onset phagocytic cells are predominant. The situation is different in synovitis of recent onset in chronic RA (called subacute RA in the present study), where mononuclear phagocytes and granulocytes are not as abundant, but T lymphocytes and also some plasma cells are present in situ. This suggests that in cases of prolonged RA the reasons for the marked differences observed in the histopathological patterns of these two entities are subclinical synovial changes. The inflammation in prolonged synovitis in chronic RA is compatible with an active T cell dependent, B cell mediated immunoglobulin synthesis with an abundance of plasma cells as described earlier.

Immunological mechanisms are usually believed to have a central role in the aetiopathogenesis of RA. Our findings on the cellular immunohistopathology of RA synovium indicate that the target organ in RA undergoes sequential changes during the course of the disease. Definite proof of these changes would require repeated biopsy specimens from the same patients and the same joint during different stages of the disease and synovitis, but for ethical and practical reasons this would be difficult to perform. In addition, the possibility of sampling error when using needle biopsy must be considered, but because the findings in different forms of RA were clearly segregated into distinct subgroups, we concluded that they present different phases of the disease and synovitis. This finding and earlier observations on synovitis of recent onset suggest that immunological mechanisms are not the initial unknown cause of RA. Our findings suggest rather that cellular immune mechanisms become involved as a reaction to the tissue damage caused by the initial inflammatory attack. This applies in some other diseases with known exogenous pathogenic agents which also activate the immunological response of the host against autologous but damaged tissue.

The close contact between HLA-D/DR* inter-digitating cells and T4* helper cells so typical of RA has also been observed in non-specific synovitis, such as traumatic and crystal arthritis. This suggests that immunological mechanisms are not necessarily the initial unknown cause of RA, but they may still have a central role in the aetiopathogenesis of RA. Therefore, the crucial question ‘How does RA start?’ should perhaps be replaced by ‘Why doesn’t it stop?’. Certainly the most characteristic feature of RA synovitis is the intensity and chronicity of the inflammation. This might be due to defective down-regulation of the immune response in RA. Actually, experiments in vitro have shown that the
production of both suppressor T cells\textsuperscript{22, 23} and also cytotoxic T cells\textsuperscript{24, 25} is deficient in RA. Furthermore, we have recently observed that the activation of T8\textsuperscript{+} suppressor/cytotoxic cells is defective in chronic RA in vivo.\textsuperscript{26} This is not the case in reactive arthritis (manuscript in preparation). In addition, the cellular immunohistopathology of RA at different developmental phases does not show any disease-specific changes. These observations indicate further that deficient production of terminal effector cells may be the cause of (the chronicity of) RA.

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