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Commentary
Current perspectives on synovitis

Paul P Tak and Ferdinand C Breedveld*

Academic Medical Center, Amsterdam and *Leiden University Medical Center, Leiden,
The Netherlands

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Introduction

The synovium lines the noncartilaginous surfaces of the diarthrodial joints, and synovial tissue is also found in tendon sheaths and bursae [1]. Several rheumatic diseases are characterized by synovial inflammation. In these conditions, descriptive studies of synovial biopsy specimens may contribute to an understanding of the events that take place *in vivo*, and they complement experimental animal studies as well as in-vitro studies. Examination of synovial tissue is generally more relevant than synovial fluid analysis, except, for example, the analysis of neutrophils and platelets, and studies of soluble mediators. Recently, there has been an enormous upsurge in investigations of the pathological changes in the synovium [2] because of the availability of new methods to obtain synovial biopsy samples [3,4] and because of the development of immunohistological methods, in-situ hybridization, and the polymerase chain reaction. Moreover, the complementary DNA microarray technology may hold great promise for synovial tissue analysis in the future [5].

## Methods to obtain and evaluate synovial biopsy samples

Synovial tissue may be obtained either at surgery, by blind needle biopsy, or at arthroscopy. It is likely that tissue obtained at joint replacement differs from that obtained by blind-needle biopsy or arthroscopy because of clear differences in patient selection. Obviously, surgery is inappropriate for studies on early rheumatoid arthritis (RA) or for serial investigations. The blind-needle biopsy technique is safe, well tolerated and is technically easy to perform. A limitation of this method is that its use in clinical practice is often restricted to the suprapatellar pouch of the knee joint. In addition, it is more difficult to obtain sufficient tissue from clinically uninvolved joints, for

example after successful treatment. Arthroscopic sampling of synovial tissue under direct vision is a similarly safe and well tolerated procedure, but is more complicated and expensive [3,6,7]. Most measures of inflammation in needle biopsies are similar to those selected at arthroscopy [4]. An advantage of arthroscopy is that it is always possible to obtain tissue in adequate amounts, even in clinically quiescent joints. Moreover, arthroscopy allows access to most joints and to most regions within the joint, including the pannus–cartilage junction.

There is large variability of synovial inflammation between individuals, different joints, and even within joints [2]. The degree of morphologic heterogeneity in synovial tissue samples obtained from a single joint could suggest that evaluation of synovial tissue is unreliable because of unavoidable sampling error. Several studies [8–10], however, have shown that, despite the degree of histologic variation, representative measures of several parameters of synovial inflammation may be obtained by examining a limited number of samples. For example, quantification of T-cell infiltration and activation in sections derived from at least six different biopsy specimens results in variance of less than 10% [10]. It is generally not necessary to know the macroscopic appearance of the rheumatoid synovium in order to obtain representative samples [4,8].

There are essentially three methods to quantify the features of synovial inflammation in biopsy samples: semi-quantitative analysis, quantitative analysis and computer-assisted analysis [11–13]. All three methods are reliable in experienced hands. It can be anticipated that digital image analysis will be increasingly important with the use of more advanced computer systems.

RA = rheumatoid arthritis; TNF = tumour necrosis factor.

Figure 1
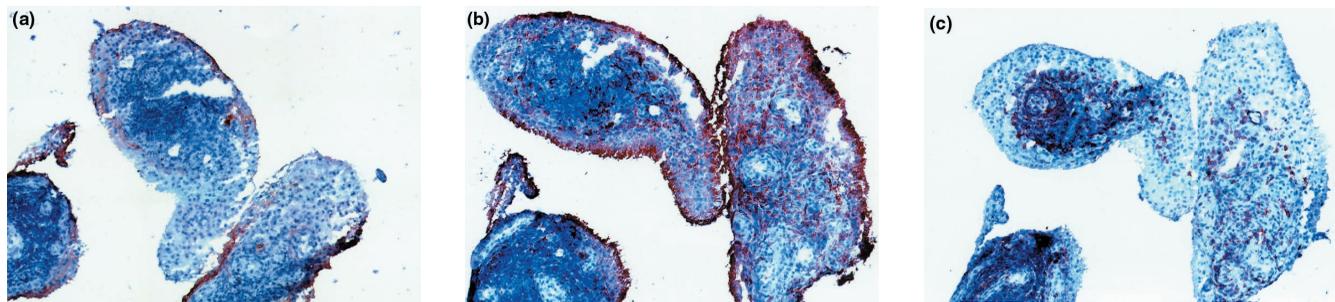
Rheumatoid synovial tissue showing **(a)** CD55+ fibroblast-like synoviocytes in the intimal lining layer; **(b)** CD68+ macrophages in the intimal lining layer and in the synovial sublining; and **(c)** CD3+ T cells in lymphocyte aggregates and in the diffuse leucocyte infiltrate in the synovial sublining. Original magnification ×100.

Clinical studies

Histological features of the synovium have been documented in various clinical studies, describing associations with disease activity [14,15] and prognosis [16,17]. These studies underscore the important role of macrophages and macrophage-derived mediators of inflammation and destruction in RA. In addition, systematic comparison of synovial tissue from RA patients in different phases of the disease made it possible to define the cell infiltrate [14,15,18–20], as well as the expression of adhesion molecules [21], cytokines [14,22,23] and degrading enzymes [17,20,24] in early disease. A major conclusion from this work is that so-called early RA is already a chronic disease. This may explain the observation that a notable percentage of RA patients have signs of joint destruction at the time of initial diagnosis [25]. Preliminary work [26] has identified some immunohistological features that are characteristic for rheumatoid synovial tissue. More extensive future studies may provide helpful markers, which could be used for routine clinical practice.

Studies of synovial tissue may also play an important role in the development of rational therapies in which biotechnology products are used to influence defined pathogenetic mechanisms [27]. The design of optimal treatment regimens for interventions with agents such as monoclonal antibodies, soluble receptors, cytokines and peptides can be facilitated by information regarding the actual achievement of the biological effect at the site of inflammation. Such studies will also provide insight into the mode of action of such agents. Additionally, analysis of serial biopsy samples during treatment may provide useful alternative end points for both joint inflammation and joint destruction. This approach could lead to a rapid screening method that would require relatively low numbers of patients to predict the effects of novel antirheumatic strategies. Studies of the relation between a defined modification of inflammation and the clinical course could also produce information about the pathogenesis of rheumatic diseases.

Table 1

Inflammatory cells in synovial tissue from patients with rheumatoid arthritis

Macrophages

Fibroblast-like synoviocytes

T cells

Plasma cells

B cells

Interdigitating dendritic cells

Follicular dendritic cells

Natural killer cells

Mast cells

Neutrophils

Inflammatory cells in the synovium

The synovium comprises the intimal lining layer and the synovial sublining [1]. The intimal lining layer consists mainly of intimal macrophages and fibroblast-like synoviocytes. The synovium becomes hypertrophic and edematous in various arthritides. Angioneogenesis, recruitment of inflammatory cells under influence of chemokines, local retention and cell proliferation all contribute to the accumulation of cells in the inflamed synovium. The following discussion focuses on the major infiltrating cell populations.

Rheumatoid synovial tissue is characterized by marked intimal lining hyperplasia and by accumulation of T cells, plasma cells, macrophages, B cells, mast cells, natural killer cells and dendritic cells in the synovial sublining (Table 1, Fig. 1) [14,23,28–30]. Distinct patterns of lymphoid organization can occur in the synovium; diffuse, follicular and granulomatous variants have been distinguished [31]. In contrast to general belief, proliferation of synovial tissue is mainly due to changes in the synovial

sublining (Fig. 1). Important contributors are angioneogenesis, oedema, massive cell infiltration and fibrosis. The differences with other forms of arthritis are only gradual. There is, for example, on average stronger infiltration by macrophages, plasma cells and granzyme-positive cytotoxic cells in RA [23,26,30]. So-called pannocytes have been observed at the pannus–cartilage junction [32,33]. These cells exhibit phenotypic and functional features of both fibroblast-like synoviocytes and chondrocytes. Furthermore, cells with features of osteoclasts have been identified at the junction [34]; they are probably derived from the monocyte/macrophage lineage.

Pathogenetic mechanisms in rheumatoid arthritis

Although the aetiology of RA remains elusive, immune-mediated mechanisms are probably of crucial importance. The evidence to support a role of CD4+ T cells in the immune response in RA patients [35,36] is substantial, but circumstantial. A subset of the CD4+ cells in the synovium shows phenotypic evidence of prior activation, but many of the T cells are small and few of them express activation molecules such as transferrin and the interleukin-2 receptor [22,37,38]. Of interest is that the percentage of interferon-γ producing T cells [22,39] and the detectable levels of T cell receptor-ζ protein [40,41] are significantly lower in RA synovium than in a chronic T-cell-mediated immunological reaction, such as tonsillitis or tuberculous pleuritis. These data indicate that T cells in RA synovium are in a peculiar activation state.

Lymphocyte aggregates (Fig. 1c) are observed in 50–60% of RA patients, and can be surrounded by coronas of plasma cells [14]. In these areas interdigitating dendritic cells are observed in proximity to CD4+ T-cells [42–45]. Human leucocyte antigen class II molecules and the costimulatory molecule CD86 (B7-2), which has an important role in antigen presentation, are expressed on these cells [46–48], suggesting that interdigitating dendritic cells could present antigen to CD4+ T cells. Whether this involves mainly endogenous autoantigens [49,50] or exogenous agents, such as bacteria [51] and viruses [52], remains to be elucidated. Recent studies [51,53,54] have shown that there is a much higher load of bacterial DNA and peptidoglycans in the synovium than previously expected. Conceivably, the T-cell response is directed at an array of different antigens, which might well be a secondary phenomenon.

When the perivascular lymphocyte aggregates are large, substantial numbers of B cells can be found in close association with CD4+ cells and follicular dendritic cells [14,28]. Of importance is that fibroblast-like synoviocytes also have intrinsic properties of follicular dendritic cells [55]. The aggregates that consist mainly of CD4+ T cells and B cells resemble germinal centers, although they are morphologically not identical to the germinal centers in lymphatic organs [45,56]. The microenvironment suggests a close functional relationship between follicular dendritic cells and B cells in RA synovium, allowing activation and maturation of the humoral immune response.

It has become clear that cells other than lymphocytes, in particular activated macrophages (Fig. 1b) and fibroblast-like synoviocytes (Fig. 1a), play a critical role as effector cells in chronic disease [14,37,57,58]. Both cell types are highly activated and secrete a variety of cytokines [59,60], as well as matrix metalloproteinases [61,62]. Fibroblast-like synoviocytes can also produce other factors, such as proteoglycans and arachidonic acid metabolites [58,63]. The increase in the numbers of fibroblast-like synoviocytes can be explained in part by proliferation and by impaired apoptosis. Although proliferation probably contributes to some extent [64,65], inhibition of apoptosis in particular provides an important explanation for the increased cellularity [66]. Very few apoptotic cells are found in the synovium of RA patients [67,68], despite the presence of fragmented DNA in the intimal lining layer [67,69]. Various mechanisms may be involved in causing inadequate apoptosis: the development of mutations of the p53 suppressor gene [70–72]; deficient functional Fas ligand expression [73], overexpression of antiapoptotic molecules, such as sentrin [74]; and activation of nuclear factor-κB [75–77]. The marked increase in the expression of granzymes A and B in RA patients [30,78,79] could be a reactive attempt to induce apoptosis in synovial cells. Interestingly, fibroblast-like synoviocytes from RA patients exhibit many features of transformed cells [58,72]. The presence of these 'transformed' cells in the synovium may contribute to the autonomous progression of pannus and joint destruction in a subset of RA patients.

Two-thirds or more of the cells in the hyperplastic intimal lining layer in RA are macrophages, where they are particularly observed in the more superficial parts [80]. It is generally believed that they originate from bone marrow-derived monocytes that have migrated in response to chemotactic factors [81]. Relatively little is known about the factors that influence the specific retention of macrophages in the intimal lining layer. It has recently been suggested that the ligand pair CD55–CD97 could be involved [82]. Fibroblast-like synoviocytes can be distinguished from other fibroblasts by the marked expression of CD55 or complement decay accelerating factor. CD55 can act as a cellular ligand for the sevenspan-transmembrane molecule CD97, which is expressed by nearly all intimal macrophages [82]. The microarchitecture of the intimal lining layer strongly suggests that intimal macrophages and fibroblast-like synoviocytes may specifically interact via this ligand pair. Of note is that intimal macrophages exhibit stronger expression of CD97 than macrophages in the synovial sublining, illustrating the highly activated phenotype of the intimal macrophages in

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rheumatoid synovial tissue. The exact role of the CD55–CD97 interaction in the pathogenesis of RA remains to be elucidated.

The macrophages often also constitute the majority of the inflammatory cells in the synovial sublining [14]. Macrophage infiltration occurs preferentially in areas adjacent to the articular cartilage [4]. Of interest is that most cells in areas where synovial cells display tumour-like morphology are macrophages [83]. The preferential accumulation of macrophages at the pannus–cartilage junction is probably related to the expression of a range of adhesion molecules by macrophages [84–86] and to the effects of selective chemotactic factors [87–89].

The importance of these cells and their soluble mediators is supported by clinical observations. Local disease activity is particularly associated with the number of macrophages and the expression of cytokines, such as tumour necrosis factor (TNF)-α and interleukin-6, in synovial tissue [14,15]. There is also a significant positive correlation between intimal lining layer depth and cell counts for macrophages in the synovial sublining on the one hand, and radiographic signs of joint destruction after follow up on the other [16]. The pivotal role of TNF-α, at least in the majority of RA patients, has been confirmed by the impressive effects of specific therapeutic strategies targeting the TNF-α molecule [90–92]. The importance of cytokines, which are mainly derived from macrophages, is also illustrated by the effects of treatment aimed at blocking the effects of interleukin-1 [93,94] and interleukin-6 [95].

These observations have stimulated studies of the factors that drive the production of proinflammatory cytokines, such as TNF-α. It has been suggested [96] that cytokine-stimulated T cells may contribute to the excessive production of TNF-α in synovial tissue. Among the cytokines that can promote a Th1-like proinflammatory response in the synovium are interleukin-12 [97,98] and probably also IL-18 [99]. Interleukin-15 is another cytokine that has drawn a lot of attention as a potential factor implicated in the interaction between T cells and TNF-α-producing macrophages [100–102]. In addition to cytokines, cell-surface molecules may also play a role in driving the production of proinflammatory cytokines and matrix metalloproteinases by macrophages and fibroblast-like synoviocytes [103,104].

Conclusion

There has been increased interest in the pathological changes at the site of inflammation in patients with various forms of arthritis. Several studies have focused on methodological matters concerning synovial biopsy procedures, sampling error and the methods used to quantify synovial inflammation. This has led to the first steps in the development of quality control systems and the standardization of methodology.

Preliminary studies have identified features of synovial tissue that are associated with specific arthritides. More extensive studies could yield important information for differential diagnosis and estimating prognosis. Moreover, studies on serial biopsy samples after experimental therapy may help to understand the mechanism of action of specific interventions. Such studies may also provide insight into the role of specific cells and molecules in the pathogenesis. Based on these and other investigations, macrophages and fibroblast-like synoviocytes have been recognized as key players in the effector phase of rheumatoid arthritis.

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Author addresses: Paul P Tak (Division of Clinical Immunology and Rheumatology, Academic Medical Center, Amsterdam, The Netherlands) and Ferdinand C Breedveld (Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands)

Correspondence: Professor Paul P Tak, Division of Clinical Immunology and Rheumatology F4, Department of Medicine, Academic Medical Center, PO Box 22700, 1100 DE Amsterdam, The Netherlands. Tel: +31 20 566 2171; fax: +31 20 691 4904; e-mail: P.P.Tak@amc.uva.nl