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Analysis of synovial biopsy samples: opportunities and challenges

It has become clear in recent years that the synovium is the primary site of inflammation and a major effector organ in a variety of joint diseases, including rheumatoid arthritis (RA). As a result, there has been increased interest in studies of the pathological changes of the synovium.¹ There are, however, several caveats that need to be recognised. Many of the older studies have examined synovial tissue obtained at surgery. In these patients inflammation is not necessarily a prominent feature. Moreover, patients requiring joint surgery obviously represent a highly selective group, in whom specific pathogenetic mechanisms may be operative that are associated with the process of destruction. For instance, mutations in the tumour suppressor gene p53 were demonstrated in fibroblast-like synoviocytes from synovium of patients with RA with longstanding, destructive disease.² It has been suggested that the resulting loss of p53 function may contribute to the autonomous progression of pannus and joint destruction. Conceivably, p53 mutations are not present in earlier, less destructive phases of the disease,^{3,4} though this remains to be determined. In line with this hypothesis, intimal lining layer hyperplasia and p53 expression by fibroblast-like synoviocytes also seem to be more pronounced in tissue obtained at surgery than in arthroscopic samples.^{5,6} In addition, synovial tissue from patients with end stage, destructive RA may be distinct as a result of other mechanisms. For instance, the release of fragments secondary to the degradation of bone and articular cartilage may theoretically influence the synovial infiltrate. The advent of blind needle biopsy techniques and needle arthroscopy has created the opportunity to obtain synovial tissue samples of patients with active synovial inflammation in earlier stages of the disease in a safe and well tolerated way.⁷⁻⁹

In addition to the stage of the disease, the use of drugs provides another possible source of bias in studies of synovial tissue. It has been shown in many studies that it is possible to influence the features of synovial inflammation by antirheumatic treatment.¹⁰ Consequently, the analysis of serial biopsy samples has been used as a screening method to test new treatments. It has been suggested that the changes in serial synovial biopsies are more sensitive to change than for example the ACR 20% criteria for clinical improvement.^{11,12} Therefore, a control group of patients, matched for drug treatment, is ideally included when different patient groups are being compared.

In this issue of the *Annals of the Rheumatic Diseases* Baeten *et al* present interesting data on the features of arthroscopic biopsy samples of patients with RA compared with disease controls.¹³ The authors should be complimented for this laborious and important work. They con-

firmed a previously suggested relation between synovial inflammation and local disease activity.^{14,15} Subsequently, they attempted to minimise confounding by stratification for disease activity. Using this approach, they demonstrated increased lymphocyte infiltration in RA synovium compared with the synovial tissue of patients with ankylosing spondylitis, psoriatic arthritis, and undifferentiated spondyloarthropathies. These observations are in line with a previous report showing a specific and significant increase in the mean scores for lymphocytes in rheumatoid synovial tissue compared with synovium from patients with reactive arthritis.¹⁶

Descriptive studies of rheumatoid synovium may contribute to an understanding of the events that take place *in vivo*. Thus the study by Baeten *et al* raises the question of which lessons can be learnt about the pathogenesis of RA based on their study. The increased infiltration by CD4+ T cells and CD20+ B cells in rheumatoid synovium is compatible with the hypothesis that specific immune recognition takes place in the joints of patients with RA. This is among the strongest arguments for a pathogenetic role of CD4+ T cells in the pathogenesis of RA, together with the association of disease susceptibility and outcome with the presence of the "shared epitope". Clearly, however, additional data from experimental studies are required to prove the role of T cells and B cells in various stages of the disease.

A second question that arises is whether synovial tissue analysis might have any value in differential diagnosis. Baeten *et al* have shown that it is possible to assess differences in the synovial cell infiltrate when synovium from patients with RA is compared with that from patients with spondyloarthropathy. The interpretation of the features of synovial inflammation for diagnostic purposes has been complicated, however, by the great variability between individual patients.^{14,17} In addition, many of the pathological changes in rheumatoid synovium, such as vascular congestion, intimal lining layer hyperplasia, mononuclear cell infiltration, and fibrin depositions, can be seen in disorders other than RA.¹ A recent study suggested that examination of synovial biopsy samples has diagnostic potential in distinguishing early RA from other forms of early arthritis.¹⁸ Multivariate models could predict a diagnosis of RA solely on the basis of synovial tissue examination with an accuracy of 85% when massive infiltration by plasma cells and macrophages in the synovial sublining was present, and a diagnosis other than RA in even 96% of the cases when minimal infiltration by these cells was found. A limitation of this study was, however, the small number of patients with psoriatic arthritis and ankylosing spondylitis

included. Especially in these conditions, increased infiltration by both plasma cells and macrophages has been reported.^{19,20} Therefore, examination of synovial biopsy specimens may be of limited value in distinguishing RA from spondyloarthritis. Future studies in consecutive patients with clinical features which do not allow an immediate confident diagnosis are warranted to clarify further the role of synovial biopsy in differential diagnosis.

Baeten *et al* also examined the effect of disease duration on the features of the synovium in patients with active synovial inflammation who did not require joint surgery. Investigating the changes in the synovium may provide insight into the mechanisms that are operative in the early phases of RA. Furthermore, information about synovial inflammation in various stages of the disease could be important for the development of antirheumatic treatments. A systematic comparison of synovium from 16 patients with RA of less than one year's duration with that from 14 patients with RA of more than one year's duration showed no major macroscopic or microscopic differences.¹³ This is remarkable, as these patient groups could not be completely matched for drug treatment. Six of the patients with late RA used disease modifying antirheumatic drugs (DMARDs), whereas only one patient with early RA used a DMARD. Presumably, the effects of treatment were minimised by matching for disease activity. The results confirm a previous study comparing synovial tissue samples from patients with RA of less than one year's duration with those from patients with RA of more than five years' duration.¹⁴ Similar results were obtained when only patients with a disease duration of less than three months were included. These studies show that so-called early RA represents already a chronic phase of the disease. This view is supported by the observation that signs of articular damage can be found early in the course of RA.²¹ Of interest, synovial inflammation has also been described in clinically quiescent joints from patients with RA.¹⁵ This may represent an earlier stage of the disease in some patients. The chronicity of synovial inflammation at initial presentation of the patient with RA is not unexpected in light of the data obtained in animal models of arthritis. Activation of the transcription factor nuclear factor- κ B, increased cell infiltration, and p53 overexpression in response to DNA damage in the inflammatory environment have all been described in the synovium in the preclinical phase.²² These data support the concept that asymptomatic synovitis precedes clinically manifest arthritis. Therefore, it might be difficult to draw any firm conclusions about the initiating pathogenetic events on the basis of synovial tissue analysis in so-called early RA until we have the methods to identify patients who will get RA.

As shown in this issue of the *Annals*, descriptive studies of rheumatoid synovium may contribute to an understanding of the events that take place *in vivo* and complement experimental animal studies and *in vitro* studies. The availability of new methods to obtain synovial biopsy specimens and the development of immunohistological methods, *in situ* hybridisation, the polymerase chain reac-

tion, and cDNA microarray technology have created new opportunities and challenges for studies of the site of inflammation in patients with RA.

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