Synovial membrane cellularity and vascularity

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Studies of the synovial membrane in joint inflammation, over many years, have proven of mixed benefit: while it is rarely of diagnostic value, clues to understanding the mechanisms involved both in the initiation and maintenance of the inflammatory response have been uncovered. While the path to understanding is fraught with difficulty, progress is slowly being made and, it is hoped, will lead in turn to novel and better treatment approaches. This review of synovial membrane cellularity and vascularity will describe our current understanding of the pathways to joint damage.

Normal cellularity and vascularity

Normal synovial tissue is characterised by a surface layer of cells, or intima, below which is a loose connective tissue which contains fibroblasts, macrophages, adipocytes, mast cells, nerve fibres, vascular endothelial cells, and occasional polymorphs and lymphocytes.¹ The intima contains two or possibly three cell types. Type A cells are of bone marrow origin and conform to the ultrastructural criteria for monocyte derived macrophages. They express macrophage markers including CD68 and CD14 and also stain with non-specific esterase.² Type B cells are fibroblastic in type and can be distinguished cytochemically and immunochemically from fibroblasts deeper in the tissues. Being involved in collagen synthesis, they express the β subunit of the enzyme prolyl hydroxylase.³ They are distinct in terms of their ability to express uridine diphosphoglucose dehydrogenase (UDPGD) which is an important enzyme involved in hyaluronan synthesis—a specialised synovial membrane function.³ The constitutive expression of vascular cell adhesion molecule (VCAM)-1 by type B synoviocytes is also an important distinguishing feature not shared by fibroblastic cells elsewhere in the synovial membrane.⁴ The exact function of this VCAM-1 expression remains a matter of speculation, but it suggests a role in trapping cells within the lining layer. The third type of intimal cell is dendritic in type, accounts for <1% of the total, is CD68 negative but expresses the class II related marker, RFD1.⁵

Studies on synovial vascularity date back to 1743, when Hunter described the plexus of vessels which supplied the synovial membrane and which he called the circulus articuli vasculosus. A more recent detailed study of normal synovial membrane has highlighted the high frequency of capillaries, with a peak density just below the lining layer between 6 and 11 μ m deep.⁶ Considerable variation in

vascularity is seen, depending on the type of membrane surface.

The cartilage-pannus junction, important in the development of joint erosion, has also been studied carefully in normal samples. Allard *et al* analysed the cellular composition both of the cells adjacent to bone and of the cells contained in the layer of tissue which extends over and merges with the cartilage, lining the joint cavity.⁷ Adjacent to bone, the cells stain predominantly with the monocyte/macrophage marker 63D3; in contrast, those cells lining the joint cavity express the monoclonal antibody 67, thought to be a marker for material surrounding type B synoviocytes.⁸

Synovial membrane in early rheumatoid arthritis

Most of the studies of rheumatoid arthritis (RA) synovial membrane have been performed on samples obtained from patients with long established disease, largely at arthroplasty. There have only been a few studies in early disease, when samples are more likely to reflect factors important in disease initiation rather than those contributing to chronicity. Schumacher and Kitridou described 24 patients with synovitis of recent onset, six of whom developed RA.⁹ They described variable (≤ 10 cells in depth) lining cell proliferation with largely a perivascular lymphocytic infiltration. There were prominent vascular changes, but no lymphoid follicles and only occasional polymorphs were observed. The intensity of the inflammation did not appear to correlate with the disease duration. Lindblad *et al* also studied arthroscopic biopsies from a small number of RA patients with disease duration between nine months and five years.¹⁰ Again, lining layer thickening was observed, with cells positive for both OKM1 and OKT9, suggestive of macrophage infiltration and acute proliferation. The sublining cells were largely of the helper T lymphocyte phenotype and focal aggregates of B cells were also seen.

More recent studies from our own group of 11 RA patients with a mean disease duration of 22 months have quantified the cellular infiltrate.¹¹ Again, variable lining layer thickening was seen (mean 4 cells in depth; range 2–32). In the sublining, 10 of the 11 patients had a diffuse mononuclear infiltrate comprising predominantly CD14 macrophages and CD5 T lymphocytes, with roughly equal numbers of CD4 and CD8 cells. Small numbers of B cells and plasma cells were also seen. Thus lining layer thickening, vascular proliferation and a diffuse sublining infiltrate made up of macrophages, T lymphocytes,

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more CD4 than CD8, and a small number of B cells, appear to be typical features of the involved synovial membrane in early RA.

The examination of synovial membrane obtained from clinically uninvolved joints is another approach to exploring changes early in the disease. The clinically uninvolved joint is histologically involved, though there is less lining layer hyperplasia and fewer mononuclear cells infiltrate the sublining compared with the involved joint.¹² In a further study, a vascular proliferation index and a high endothelial venule (HEV)-index were calculated both in the clinically involved and clinically uninvolved RA samples, in addition to postmortem controls.¹³ Of interest, vascular proliferation was not a feature of the uninvolved joint, and HEV-type vessels, readily seen in actively inflamed joints, were also not observed. This suggests that vascular changes, which may determine the rate of mononuclear trafficking, are important in the clinical expression of joint inflammation in early RA.

Adhesion molecule expression in RA

The receptor-ligand pairs important in the control of mononuclear trafficking into the synovial membrane have been the subject of a number of recent studies.¹⁴⁻¹⁶ The important adhesion molecules expressed on the endothelium include E-selectin, which plays a role in the initial margination and rolling of leucocytes, and both intracellular adhesion molecule-1 (ICAM-1) and VCAM-1, which are involved in leucocyte adhesion and penetration through the endothelial junctions. Studies of RA synovial membrane by our group and others have shown E-selectin expression to be vessel specific, the majority of vessels staining positive, particularly in the superficial synovial membrane.¹⁷ ICAM-1 expression is not vessel specific, cells in the lining and within the mononuclear infiltrates also staining positive. Perhaps surprisingly, vascular VCAM-1 expression is either minimal or absent, depending on the monoclonal antibody used. VCAM-1 is found in the lining, as it is in normal individuals, and occasional dendritic type cells within the infiltrates also stain positive.

In a recent study of biopsies taken from patients with actively inflamed joints (nine seropositive RA, three psoriatic arthritis, one ankylosing spondylitis), correlations were sought between the expression of adhesion molecules in the synovial membrane, measures of disease activity, and concentrations of soluble adhesion molecules in both sera and synovial fluid.^{18,19} A consistent positive correlation was identified between ICAM-1 concentrations in serum, synovial fluid, synovial membrane, and measures of disease activity (Ritchie articular index, erythrocyte sedimentation rate (ESR)), but this was not seen with VCAM-1 or E-selectin.¹⁸ However, a consistent negative relationship was identified between ICAM-1 and E-selectin expression in these patients.¹⁹ As serum levels of soluble E-selectin were greater in patients

with a shorter duration of disease, it may be that E-selectin expression is more upregulated in early disease, ICAM-1 then correlating with the intensity of the inflammatory response.

Synovial membrane in established RA

Most studies of RA synovial membrane have been performed on samples obtained at joint arthroplasty. While the immunohistological findings in established disease are not dissimilar from those seen earlier on, the mononuclear infiltrate is focal in about 70%, compared with about 10% of biopsy samples.²⁰ Duke *et al* described four different patterns of infiltrate: scattered/diffuse, perivascular, lymphoid follicles and germinal centres.²¹ Kurosaka and Ziff went on to study the infiltrates in more detail, and described lymphocyte rich areas where most cells stained positive for OKT4, and more peripheral transitional areas containing plasma cells, macrophages, and both OKT4 positive and OKT8 positive lymphocytes.²² More recently, Yanni *et al* analysed lymphoid infiltrates of different sizes and found that the composition of each aggregate depended on the total number of mononuclear cells it contained:²³ large aggregates contained a substantial number of B cells and cells bearing the CD45RA phenotype; in contrast, small aggregates contained few B cells and relatively larger numbers of T cells bearing CD8 and CD45RO.

Lining layer thickening is also a prominent feature of established RA. Considerable controversy exists as to whether this thickening is a result of macrophage recruitment or of synoviocyte proliferation. In favour of recruitment, mitotic cells are rarely seen, and staining for the proliferation marker Ki-67 is not observed in RA synovial membrane.²⁴ In addition, lining layer cells express a wide range of macrophage antigens, and after lethal irradiation and heterologous bone marrow transplantation, the synovial macrophages are replaced by cells of bone marrow donor strain.^{2,25} In favour of proliferation, some ³H-thymidine incorporation of synovial lining cells in RA is seen, and lining layer thickening without mononuclear infiltration occasionally observed.²⁶ In a recent study, Qu *et al* further addressed this question by examining synovial membrane samples for three markers of cell proliferation: proliferating cell nuclear antigen, C-myc proto-oncogene, and nucleolar organiser regions.²⁶ All three markers indicated that there was active proliferation in the synovial lining of RA patients. Double labelling experiments indicated that the proliferating cells were fibroblastic in type, as they were type I procollagen positive and CD68 negative. Thus lining layer thickening is likely to be the result of a combination of macrophage recruitment and fibroblast like synoviocyte proliferation.

The central role of the T cell in the initiation and maintenance of the inflammatory response in RA has often been stressed.²⁷ It is of interest, though, that T cell products such as interferon gamma and interleukin-2 (IL-2) are difficult to

find in RA synovial fluid and membrane, and that gene expression for these lymphokines is also sparse. In contrast, monocyte/macrophage derived products are plentiful in RA synovial membrane with cells, mostly of macrophage origin, staining positive with antibodies against IL-1 α , tumour necrosis factor (TNF) α , TNF β , and IL-6, in addition to both the type 1 IL-1 receptor (IL-1R1) and the TNF receptors (p55TNF-R and p75TNF-R).²⁸⁻³² While IL-1 receptor antagonist (IL-1ra) expression is also seen, it has been suggested that insufficient amounts are produced by RA synovial membrane to neutralise IL-1 activity.³¹ Finally, in vitro production of cytokines IL-1 β and IL-6 by RA synovial membrane explants was compared in two groups of arthroplasty samples, those with focal and those with diffuse infiltrates.²⁰ Focal infiltrates occur more frequently in arthroplasty samples, and both IL-1 β and IL-6 production was significantly enhanced in those with focal disease. This suggests that lymphoid follicle formation is associated with enhanced IL-1 β and IL-6 production, which in turn leads to greater synthesis of the proteases involved in joint destruction.

Cartilage-pannus junction in established RA

Two main histological patterns at the cartilage-pannus junction have been recognised in established RA.³³ These patterns are based on the presence or absence of a transitional fibroblastic zone overlying the cartilage and separating the cartilage from pannus which contains macrophages, fibroblasts, and endothelial cells. This transitional fibroblastic zone stains positively for keratin sulphate and type II collagen, suggesting that it is of chondrocytic origin. In a further study, Allard *et al* examined the association between cartilage-pannus junction pattern, joint involved, cartilage degradation (proteoglycan depletion and chondrocyte necrosis), and radiological appearance.³⁴ The transitional zone occurred more commonly in the hip and knee compared with the metatarsophalangeal joint. When the zone was absent and a distinct, well defined cartilage-pannus junction was present, cartilage degradation appeared to be enhanced and joint erosion more common. Thus it was suggested that these different cartilage-pannus junction patterns may explain the predominance of erosive change in small joints, compared with joint space narrowing found in large joints in RA.

The cytokine profile of the cartilage-pannus junction has also been studied in these two histological patterns.³⁵ In those samples in which a distinct junction was seen, there was plentiful IL-1 α , IL-1 β , TNF α , and IL-6, in addition to both IL-1R1, p55TNF-R and p75TNF-R.^{31 32 35} In contrast, these cytokines and receptors were not seen in samples which contained the transitional fibroblastic zone. Transforming growth factor β , which is thought to inhibit the immune response and to promote tissue repair, is also found at the

cartilage-pannus junction and elsewhere in the synovial membrane, and it appears to be the dominant cytokine in samples containing a transitional fibroblastic zone. Finally, IL-1ra, seen in 35% of lining cells in RA, is seen in <10% of cells at the cartilage-pannus junction.³¹ Particularly in samples of invasive cartilage-pannus junction, there appears to be a predominance of proinflammatory cytokines capable of promoting release of factors involved in joint destruction.

Follow up studies of RA synovial membrane and effects of treatment

It has long been recognised that certain joints may deteriorate radiologically even in the absence of clinical inflammation. Follow up studies in RA have confirmed that radiological deterioration occurs despite significant improvement in parameters of disease activity.³⁶ In a study of patients for 40 months after total lymphoid irradiation, a significant and sustained decrease in peripheral blood T cell numbers was observed, accompanied by a continued, gradual radiological deterioration.³⁷ Rooney *et al* compared synovial membrane immunohistological features with the clinical outcome of disease over a one year period.³⁸ In those patients in whom there was a measurable clinical improvement, there was a reduction in spontaneous in vitro IgM rheumatoid factor production, a decrease in the intensity of the T cell infiltrate, and reductions in the T helper cell to T suppressor cell ratio and the number of samples that contained B cells. These observations were consistent with the suggestion that some of the immunopathogenic events in RA may be arrested or reversed by treatment.

A more recent study has sought correlations between clinical and radiological outcome over one year and synovial membrane features obtained before treatment in RA patients.¹² While a significant correlation was observed between the number of macrophages and the degree of joint erosion, there was none with infiltrating T or B lymphocyte populations. In addition, the number of synovial tissue macrophages correlated with lining layer cellularity, which in turn correlated with synovial fluid concentrations of IL-6. These findings suggest that synovial tissue macrophages are critical in the pathogenesis of joint erosion, which is perhaps mediated through release of cytokines such as IL-6.

Mulherin *et al* have examined the same question in a prospective study of 58 RA patients followed over a mean of 6.1 years.^{36 39 40} In that study, all clinical measures of disease activity improved, while the mean Larsen score deteriorated significantly. Synovial membrane was obtained at review from 28 patients and analysis showed that sublining layer CD14 counts and lining layer thickness correlated with both the percentage change and the final Larsen scores. Additional correlations were seen between lining layer CD68 count and final Larsen score, sublining CD14 count and both lining layer thickness and lining layer CD14 counts, and between

lining CD68 count and both sublining CD68 count and lining layer CD14 count. While no correlation was found between T cells or T cell subsets and radiological outcome, these consistent positive correlations underscore the important role of the macrophage in articular damage.

Current treatment strategies in RA involve the use of a number of drugs which are considered to modify the course of the disease. These drugs do appear to modify indices of disease activity, but support for their ability to alter the radiological outcome has not been convincing. In addition, only a few studies have examined the effects of these agents on the synovial membrane. Corkill *et al* examined synovial membrane biopsy samples after two and 12 weeks of treatment with intramuscular gold.⁴¹ Interestingly, the number of vessels expressing E-selectin was significantly reduced after two and 12 weeks. As IL-1 β expression was also shown to decrease, the reduction in E-selectin expression could have been due to this decrease in IL-1 β .⁴²

The mechanism of action of methotrexate was also examined in a recent serial synovial membrane biopsy study of eight patients who were beginning oral therapy.⁴³ All patients noted a clinical improvement, with a reduction in ESR and a variable though slight reduction in synovial membrane inflammation. Given the importance of macrophages and metalloproteinases in joint destruction, collagenase gene expression was significantly decreased after methotrexate therapy. Tissue inhibitor of metalloproteinase-1 and stromelysin gene expression were not changed. As methotrexate did not alter the in vitro collagenase gene expression by IL-1 β stimulated fibroblast like synoviocytes, it was suggested that its effects in vivo are probably the result of an alteration in synovial membrane cytokine profiles.

Synovial membrane in psoriatic arthritis

Certain clinical features in patients with psoriatic arthritis (PsA) are helpful in diagnosis: the presence of psoriasis, an asymmetric joint distribution, nail dystrophy, distal interphalangeal joint involvement and dactylitis. Radiological features are also different and help distinguish the disease from RA; in particular the presence of bone proliferation is common in PsA.⁴⁴ The immunohistological features of PsA synovial membrane have recently been described and quantified.¹⁷ Compared with RA controls, there was significantly less lining layer hyperplasia, fewer macrophages and a greater number of blood vessels in PsA synovial membrane. In addition, E-selectin expression was less intense in PsA synovial membrane, while there was no difference in expression of ICAM-1 and VCAM-1. Numbers of T cells, T cell subsets and B cells were similar in both groups. With these findings, it is tempting to suggest that with the reduction in E-selectin, fewer macrophages traffic into the synovial membrane and out to the lining layer. It may also be suggested that the reduction in macrophage numbers and

lining layer thickness in PsA explains the different radiographic features observed.

Summary

Both inflammation and destruction of the joint are the hallmarks of RA. While the conventional model of RA suggests that synovial inflammation is T cell mediated and that this in turn stimulates lining layer thickening and pannus formation leading to joint damage, Zvaifler and Firestein have recently postulated an alternative model of joint destruction.⁴⁵ In this model they suggest that synoviocyte proliferation, pannus formation and inflammation comprise an autonomous process independent of T cell derived factors. In support of their suggestion, they point out that the RA synoviocyte expresses features of a transformed phenotype: evidence of active proliferation, ability to invade cartilage, the expression of oncogenes, and the secretion of metalloproteinases. In addition, T cell products and lymphokine gene expression are not a prominent feature in RA, animal models of arthritis have shown that joint damage can occur in the absence of lymphocytic infiltration, and T cell targeted therapies have largely been disappointing.

Studies reported in this review lend support to the suggestion that mechanisms of joint inflammation and destruction operate at least in a semi-autonomous fashion. With the treatments currently available, clinical features of joint inflammation in RA improve and yet joint damage worsens. A consistent relationship is seen between radiological deterioration and synovial membrane macrophage infiltration and lining layer thickening. In PsA, a disease with different radiological features compared with RA, there is a reduction in macrophage numbers and in lining layer thickening. The macrophage, lining layer cells and their products clearly play a role in joint destruction, and current treatment strategies do not appear to affect this process. The treatments do modify inflammation which is mediated both by cells in the lining and by the sublining infiltrate, but new approaches are needed which are aimed at altering the more destructive potential of the disease.

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