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SECTION 2: PATHOLOGY AND CLINICAL ASPECTS Guest editor: J C W Edwards

Activation of synovial fibroblasts in rheumatoid arthritis

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Several reports have proposed the hypothesis that synovial fibroblasts play a major role in the crippling destruction of cartilage and bone in the inflamed joints of patients with rheumatoid arthritis (RA),^{1-4a} either autonomously¹⁻⁴ or in concert with T cells and macrophages.^{1-3 4a 5} RA synovial fibroblasts are a major component of the invasive pannus,³ a vascular and fibrous granulation tissue arising from the joint recessus and extending onto the surface of the cartilage.⁶ A number of studies indicate that these fibroblasts are morphologically altered^{1-4 7} and, in addition, highly activated^{1 2 4a 8 9}—phenotypic features that have been interpreted either as signs of irreversible cellular transformation^{1-4 7} or as the result of strong, but reversible stimulation originating from the surrounding inflammatory microenvironment.^{2 4a 5}

Activation of fibroblasts *in vitro* is known to generate several functional responses, such as production of matrix components,¹⁰ soluble mediators,¹¹ or enzymes^{1 2 4 8 11} that could contribute significantly to joint pathology in chronic RA *in vivo*.^{1-4 8 11-15}

The present paper discusses the *in situ* characteristics and functional properties of activated RA synovial fibroblasts in relationship to the possible mechanisms of their alteration, and to their potential role(s) in the pathogenesis of the disease.

The *in situ* phenotype of RA synovial fibroblasts

Morphological analysis of the RA synovium has demonstrated that fibroblasts in the lining layer have abundant cytoplasm and a large, pale nucleus with multiple prominent nucleoli, reminiscent of some tumours.⁷

Several studies have indicated that RA synovial fibroblasts express proto-oncogenes and other transcription factors, such as *c-fos*, *jun-B*, *myc/c-myc*, *myb*, *ras*, and the zinc factor *Z-225/egr-1*.^{1 2 4 4a 8 9} These different 'immediate-early response' genes are rapidly but transiently induced by a variety of activating stimuli,^{16 17} and can be regarded as markers of cell activation, regardless of whether the final outcome is proliferation, transformation, or secretion of cellular products.¹⁷

Protein, mRNA, or both, of the proto-oncogenes *c-fos*, *jun-B*, and *c-myc* are massively expressed in both lining layer and diffuse infiltrates of the RA synovial membrane,¹⁸ closely matching the distribution of macrophages in these regions;¹⁹ interestingly, there is a clear gradient of expression from the surface to the stroma of the synovial membrane, with the latter showing sparse and weak staining.^{4a}

The nature of the cells positive for the proto-oncogenes *c-fos*, *jun-B*, and *c-myc* has

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been identified both by exclusion, as they do not express markers for macrophages (CD14, CD68) or for T cells (CD3),^{4a, 18} and by positive characterisation through parallel, massive expression of mRNA for α_2 (I) and α_1 (III) collagens,^{4a} or procollagen I protein.¹⁸

These findings indicate that fibroblasts are virtually the only cell type within the RA synovial membrane to undergo massive activation, although a lesser degree of activation of T cells and macrophages cannot be excluded because of limitations in the sensitivity of the technique used. More importantly, the high expression of proto-oncogenes in RA synovial fibroblasts supports the hypothesis that these cells may not be in their normal, baseline functional status, but undergo pathological stimulation, either paracrine or autocrine.

We found that the proto-oncogenes *c-fos* and *jun-B* are also expressed in a small number of fibroblasts in normal synovial membrane, randomly distributed within the synovium,^{4a} conceivably reflecting baseline stimulation linked to normal functioning of the joint. In addition, proto-oncogenes are found in synovial fibroblasts of the osteoarthritis synovial membrane; as in RA, they are mostly expressed in areas with inflammatory infiltration, although the absolute number of positive cells is considerably smaller.^{4a}

Proliferation and programmed cell death of synovial fibroblasts

There have been several attempts to assess the proliferation rate of RA synovial cells, in particular fibroblasts, using ³H-thymidine incorporation or immunohistochemical detection of proliferation-specific markers.^{18, 20-23} Most of the studies agree that the proliferation rate in RA synovial membrane is less than 5%²⁰⁻²³—a modest figure that could nevertheless be compatible with gradual expansion of fibroblasts during the course of chronic RA. A study on the expression of the proto-oncogene *c-myc*, the proliferating cell nuclear antigen, and the nucleolar organiser regions,¹⁸ has recently suggested that proliferation rates for synovial fibroblasts exceed 17%. However, a direct comparison of the expression of the latter markers with that of the Ki-67 antigen,²³ a marker that appears more specific for proliferating cells,²⁴ has confirmed the previously known figures (<5%);²⁰⁻²² these are also more compatible with the paucity of mitoses in the tissue.²⁰ More importantly, only 1% of the fibroblast like cells positive for proto-oncogenes are in parallel positive for the Ki-67 antigen,^{4a} indicating that, in situ, most of the activated RA synovial fibroblasts are not proliferating.

Another factor that may influence the degree of expansion of synovial fibroblasts in RA is their rate of programmed cell death; this may be in fact relatively low, as only lining cells in the RA synovial membrane, but not in the osteoarthritis synovial membrane, express the *bcl-2* gene, capable of suppressing programmed cell death.²⁵ This mechanism,

however, appears to be counteracted by over-expression on RA synovial fluid cells of the Apo-1/Fas antigen, which can promote programmed cell death.²⁶

On the basis of the above findings it is possible that disruption of the delicate balance between cell proliferation, however low, and cell death contributes to synovial hyperplasia.

Contribution of synovial fibroblasts to joint pathology

HUMAN RA

Several proteolytic enzymes of the metallo-, cysteine, and serine protease classes have been implicated in the destruction of cartilage in the RA inflamed joint,²⁷ and identified in situ in the lining layer^{1, 4, 12-15, 28-31} or directly at the cartilage-pannus junction.^{1, 28-31} The presence of uncommon fragments of the large aggregating cartilage protein (aggrecan) in the RA synovial fluid suggests that yet other, so far unknown proteases, may also participate in joint destruction in RA.³²

Most of the enzymes found in fibroblasts are distributed in the RA synovial membrane in a pattern that grossly coincides with that of fibroblasts positive for proto-oncogenes^{1, 4, 4a, 12-15, 28-31}—that is, strong expression in the lining layer and much weaker signal in the underlying stroma. This coincidence appears compatible with the hypothesis that activated fibroblasts produce large quantities of the above enzymes, thereby promoting tissue degradation.

In vitro, RA synovial fibroblasts produce several proteolytic enzymes^{1, 2, 4, 8, 11, 27, 31, 33-35} after exposure to various activating stimuli^{2, 33-35} such as interleukin-1 (IL-1),^{2, 34} tumour necrosis factor α (TNF α)³⁴ and platelet derived growth factor (PDGF).³⁵ Interestingly, these cytokines are distributed within the RA synovium in a pattern similar to that of Jun-B and *c-Fos* protein,^{4a, 35-38} and to that of matrix degrading enzymes.^{1, 4, 4a, 12-15, 28-31}

In parallel with Jun-B and *c-Fos*, RA fibroblast like cells express massive amounts of mRNA for α_2 (I) and α_1 (III) collagens.^{4a} As the Jun/Fos heterodimer AP-1 can induce transcription of the genes for these types of collagens,³⁹ activated RA synovial fibroblasts may produce excessive amounts of collagens, thereby contributing to interstitial fibrosis, another pathological feature of longstanding RA.¹⁹

ANIMAL MODELS

Direct evidence for involvement of synovial fibroblasts in joint destruction derives from several animal models.

Subcutaneous injection into nude Balb/c mice of a single cell suspension of RA synovial cells, whether or not deprived of non-adherent lymphoid cells in culture, leads to the formation of a persisting pannus like tissue in vivo; cells enzymatically released from this tissue are capable of producing collagenase and prostaglandin E₂—properties analogous to those of fibroblast like cells from the RA synovial membrane.⁴⁰

In mice transgenic for the human proto-oncogene *c-fos*, induction of antigen induced arthritis is accompanied by a severe synovitis, in which infiltration of fibroblast like cells predominates, while lymphocytes appear to be absent.⁴¹

Grafting of synovial tissue explants from RA patients into the knee joint of severe combined immunodeficiency disease (SCID).bg mice, which lack functional T, B, and natural killer cells, leads to formation of a mixed mouse/human pannus and to erosion of cartilage and bone;⁴² this is not observed after implantation of control tissues, such as normal human synovial membrane or thymus. Interestingly, intra-articular injections of RA peripheral blood mononuclear cells, synovial mononuclear cells, or even T cell lines reactive to mouse or rat type II collagen, fail to induce pannus tissue in the mouse knee joint.⁴²

In a similar model, human RA synovial tissue and normal human cartilage have been coimplanted under the renal capsule of SCID mice.⁴³ Only the implantation of RA synovial tissue, not that of normal synovial membrane, induces erosion of the adjacent cartilage; also in this case, fibroblast like cells appear to be predominant at the cartilage-pannus junction.⁴³ The use of virtually pure RA synovial fibroblasts embedded in gel foam, instead of whole synovial tissue, also results in erosion of the cartilage within 48 days; control implantation with human skin fibroblasts, in contrast, does not induce any erosion.⁴⁴ Interestingly also in this model, there does not seem to occur any infiltration of murine T and B cells, or even macrophages.⁴⁴

All these observations combine to indicate that RA synovial fibroblasts are theoretically capable of inducing destruction of cartilage and bone without the influence of inflammatory cells. However, definite conclusions should be drawn with caution, as host B cells⁴⁵ and potentially functional macrophages⁴⁶ may potentiate the aggressive character of the implanted RA synovial fibroblasts by producing immunoglobulins,⁴⁵ or releasing species cross reactive cytokines.

Conclusions

Altered cellular morphology and massive expression of proto-oncogenes by fibroblast like cells in the hyperplastic RA synovial membrane have been regarded as an indication for a tumour like transformation of these cells.^{1-4 7-9} However, reversible morphological changes of fibroblasts, which may represent the counterpart of those observed in situ in the RA synovial membrane, have also been observed in vitro, both upon activation with inflammatory mediators⁴⁷ and after transfection with the *c-fos* proto-oncogene.⁴⁸ Moreover, proto-oncogenes do not unequivocally characterise tumoural cells, as they are non-specific signalling molecules common to a variety of (patho)physiological processes in the cell,¹⁷ as demonstrated in this particular case by their expression in normal synovial membrane and in osteoarthritis,^{4a} an unquestionably non-

tumoural joint alteration. More importantly, the in situ expression of proto-oncogenes in RA synovial fibroblasts does not appear to reflect cell proliferation,^{4a} thereby rendering unlikely the hypothesis of a tumour like transformation, at least in the sense of a highly proliferative malignant neoplasm.

Activated fibroblasts in the RA synovial membrane have a rather close spatial relationship with surrounding inflammatory cells and their mediators, especially macrophages, as they are distributed in a gradient fashion from the lining layer to the stroma.^{4a} This observation supports the hypothesis that the activation of fibroblasts may result from reversible but sustained paracrine stimulation originating from the neighbouring inflammatory milieu.^{34 49 50} Potential mediators for such 'cross talk', such as IL-1, TNF α , and PDGF, are in turn abundantly and most closely available in the lining layer, or in the synovial fluid of RA joints.³⁵⁻³⁸

The induction of cartilage erosion by grafting of RA synovial tissue or fibroblasts in SCID mice⁴²⁻⁴⁴ indicates that these cells may play a primary, potentially exclusive role in the development of arthritis. Therefore, it cannot be excluded that limited molecular transformation of the fibroblasts, reflected by massive expression of proto-oncogenes, is transferred to the host and consecutively maintained by reciprocal cooperation with host inflammatory cells.^{45 46}

Future studies should clarify whether the activation of RA synovial fibroblasts is an irreversible or reversible feature, addressing the question whether these cells are 'initiators' or 'perpetuators'³ of the RA disorder. As most investigations are carried out on samples derived from longstanding RA, complementary analysis of samples from early RA⁵¹⁻⁵⁴ will perhaps give clearer indications as to the genuine role of this cell type in the pathogenesis of human RA.

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