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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 joints of patients with rheumatoid arthritis (RA), either autonomously or in concert with T cells and macrophages. RA synovial fibroblasts are a major component of the invasive pannus, a vascular and fibrous granulation tissue arising from the joint recessus and extending onto the surface of the cartilage. A number of studies indicate that these fibroblasts are morphologically altered and, in addition, highly activated — phenotypic features that have been interpreted either as signs of irreversible cellular transformation or as the result of strong, but reversible stimulation originating from the surrounding inflammatory microenvironment.

Activation of fibroblasts in vitro is known to generate several functional responses, such as production of matrix components or soluble mediators, or enzymes that could contribute significantly to joint pathology in chronic RA in vivo. Activation of 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.

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. These different "immediate-early response" genes are rapidly but transiently induced by a variety of activating stimuli and can be regarded as markers of cell activation, regardless of whether the final outcome is proliferation, transformation, or secretion of cellular products.

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, closely matching the distribution of macrophages in these regions. 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.

The nature of the cells positive for the proto-oncogenes c-fos, jun-B, and c-myc has
been identified both by exclusion, as they
do not express markers for macrophages
(CD14, CD68) or for T cells (CD3),6,8
and by positive characterisation through
parallel, massive expression of mRNA for α5(I)
and α1(III) collagens,46 or procollagen I
proteins.47

These findings indicate that fibroblasts are
virtually the only cell type within the RA
synovial membrane to undergo massive acti-
vation, 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,46
conceivably reflecting baseline stimulation
linked to normal functioning of the joint.
In addition, proto-oncogenes are found in synovial
fibroblasts of the osteoarthritis synovial mem-
brane; as in RA, they are mostly expressed in
areas with inflammatory infiltration, although
the absolute number of positive cells is
considerably smaller.46

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.8,20-23 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 fibro-
blasts during the course of chronic RA. A study
on the expression of the proto-oncogene
p53 in the proliferating cell nuclear antigen,
and the nuclear larolar region,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 pre-
viously 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,24 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 layer4,8,12-15 28-31 or directly at the
cartilage-pannus junction.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 fibro-
blasts positive for proto-oncogenes1,4,8,10,12-15,28-31
—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—I,4,8,10,12-15,28-31
after exposure to various activating stimuli
such as interleukin-1 (IL-1),3,4 tumour
necrosis factor α (TNFα),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,46 55-56 and to that of matrix
degrading enzymes,4,8,10,12-15,28-31

In parallel with Jun-B and c-Fos, RA fibro-
blasts like cells express massive amounts of
mRNA for α5(I) and α1(III) collagens.46 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
Activation of synovial fibroblasts in rheumatoid arthritis

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,47 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, irreversible 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 mediators48 and after transfection with the c-fos proto-oncogene.49 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,46 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,67 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.48 This observation supports the hypothesis that the activation of fibroblasts may result from reversible but sustained paracrine stimulation originating from the neighbouring inflammatory milieu.48,49,50 Potential mediators for such 'cross talk', such as IL-1, TNFa, and PDGF, are in turn abundantly and most easily available in the lining layer, or in the synovial fluid of RA joints.48

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.49,50

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' 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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References


Fibroblasts from synovial tissue of patients with rheumatoid arthritis (RA) exhibited increased expression of c-fos and c-myc genes compared to normal fibroblasts. This upregulation was associated with the pathology of RA, including cartilage destruction and synovial hyperplasia. The authors suggest that these immediate-early genes play a crucial role in the pathogenesis of RA.

The study highlights the potential of these genes as therapeutic targets for RA, as their inhibition could lead to the regression of pathological changes in synovial tissue. Further research is needed to explore the mechanism of action of these genes and to develop targeted therapies for RA.

References: