

## REPORT

## Are fibroblasts involved in joint destruction?

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Rheumatoid arthritis (RA) is a chronic systemic inflammatory disorder that primarily affects the joints and results in the progressive destruction of articular structures, particularly cartilage and bone. Notably, the joint destruction is a prominent feature of disease that not only distinguishes RA from other arthritides but also determines its outcome in the majority of affected individuals.

**SYNOVIAL FIBROBLASTS IN RA**

Synovial fibroblasts in the most superficial lining layer of the hyperplastic RA synovium have been assigned an important role in the pathogenesis of RA and associated with key processes leading to the degradation of extracellular matrix. It has been understood that these cells exhibit an altered morphology and are characterised by changes in their behaviour that show certain similarities to tumours. Therefore, the phenotype of these cells has been termed “transformed appearing”, “tumour-like”<sup>1</sup> or—reflecting the clear differences with malignancies—“activated”. As part of a cellular network, RA synovial fibroblasts are involved in the destruction of extracellular matrix both by direct mechanisms and through interaction with neighbouring cells such as macrophages. Thus, it has been demonstrated that RA synovial fibroblasts release large amounts of the ligand for the receptor activator of nuclear factor  $\beta$  (RANKL), which mediates the differentiation of bone resorbing osteoclasts from their macrophage precursors.<sup>2</sup> Direct degradation of extracellular matrix by RA synovial fibroblasts is mediated through the attachment to cartilage and subsequent release of matrix degrading enzymes, particularly matrix metalloproteinases (MMPs) and cathepsins.<sup>3</sup>

**ACTIVATION OF RA SYNOVIAL FIBROBLASTS**

Although inflammatory cytokines such as tumour necrosis factor  $\alpha$  (TNF $\alpha$ ) have been demonstrated to stimulate RA synovial fibroblasts to produce such matrix degrading enzymes, the activation of these RA synovial fibroblasts is maintained even in the absence of continuous stimulation by proinflammatory factors. This notion is derived from different in vitro studies as well as from data on the severe combined immunodeficient (SCID) mouse model of rheumatoid cartilage destruction.<sup>4</sup> Initially, this model was designed to investigate the fate of inflammatory cells, particularly lymphocytes in the inflamed RA synovium. However, by implanting rheumatoid synovial tissue into SCID mice, it was shown that lymphocyte infiltrates disappear with time, whereas lining layer synoviocytes survive.<sup>5,6</sup> Interestingly, these RA synovial fibroblasts not only survived in SCID mice recipients but also maintained their characteristic biological features. Based on these observations, the SCID mouse co-implantation model was developed as a new model for studying molecular mechanisms of rheumatoid joint destruction in vivo.<sup>7</sup> To imitate the situation in a rheumatoid joint, human RA synovium was implanted together with normal human cartilage under the renal capsule in SCID mice. It was shown that RA synovial tissue and normal human cartilage can be kept in SCID mice

for more than 300 days with the RA synovium maintaining its invasive growth and progressive cartilage destruction.<sup>7</sup> On histological examination, the vast majority of synovial cells found at sites of cartilage invasion resembled activated synovial fibroblasts.

To study specifically the molecular characteristics of these fibroblasts as well as their contribution to cartilage degradation, normal human cartilage was implanted together with isolated synovial fibroblasts from patients with RA in a next step. As SCID mice do not reject these implants, the SCID mouse model has helped to analyse the matrix degrading properties of RA synovial fibroblasts in the absence of both human lymphocytes and macrophages. In contrast to osteoarthritis or normal synovial fibroblasts, RA synovial fibroblasts maintained their aggressive phenotype, especially at sites of invasion and also maintained their ability to express vascular cell adhesion molecule (VCAM)-1 and other activation markers.<sup>8</sup> Using in situ hybridisation techniques to examine the presence of mRNA for matrix degrading enzymes a number of cartilage degrading proteases were demonstrated.<sup>8</sup> In contrast, much fewer or none of these matrix degrading enzymes could be found, when normal, osteoarthritis synovial fibroblasts, or dermal fibroblasts were examined. These results indicated an active role of synovial fibroblasts in the joint destruction of RA. Furthermore, they suggested that RA synovial fibroblasts were not just passively responding to stimuli from the inflammatory environment but also maintaining their activated and aggressive phenotype in the absence of continuous stimulation from human macrophage or lymphocyte derived cytokines.<sup>4</sup>

**ROLE OF SYNOVIAL FIBROBLASTS IN MATRIX DEGRADATION**

Interestingly some experiments in the SCID mouse model suggest that activated fibroblasts are capable of resorbing not only cartilage but also bone. This was initially shown for activated fibroblasts from the synovial-like interface membrane around loose joint prostheses, which show similarities to RA synovial fibroblasts.<sup>9</sup> Specifically, we demonstrated that fibroblasts developing at the interface of bone and prosthesis in aseptic loosening prosthesis are capable of superficial bone erosion both in vitro and in vivo. At co-implantation with femoral bone into SCID mice, such fibroblasts formed resorption lacunae and even reshaped the osseous matrix. The conclusion that osteoclasts are not required was derived from the experimental setting of the SCID mouse studies that excluded the presence of human macrophages and osteoclasts in the implants. In addition, immunohistological analysis using antibodies specific for human fibroblasts demonstrated the human nature of cells resorbing the co-implanted bone. These data were confirmed in vitro, where isolated fibroblasts produced resorption pits when cultured on dentine for four weeks. Compared with

**Abbreviations:** MMP, matrix metalloproteinase; RA, rheumatoid arthritis; SCID, severe combined immunodeficiency; TIMP, tissue inhibitor of metalloproteinase; TNF, tumour necrosis factor

osteoclasts, fibroblast generated resorption pits that were superficial and similar to early phases of osteoclastic bone resorption. A major question relating to the ability of fibroblasts to resorb bone was: How do these cells decalcify the osseous matrix prior to degradation? In this context, we have shown that upon stimulation, activated fibroblasts release acidic components that result in a significant drop of the pericellular pH.<sup>9</sup> To date, the mechanisms by which activated fibroblasts may produce acidic components have not been clarified, but the expression of a specific H<sup>+</sup> ATPase on their cell membrane may provide a potential explanation. This notion has been supported by the demonstration that specific inhibition of ATPases decreased the pericellular acidification.<sup>9</sup>

## PRODUCTION OF MMPs BY RA SYNOVIAL FIBROBLASTS

Although the ultimate cause for the stable activation of RA synovial fibroblasts remains to be clarified, several aspects of rheumatoid joint destruction have been elucidated over the past years. This is particularly true for mechanisms that are involved directly in the degradation of extracellular matrix. Thus, a number of reports have implicated MMPs in rheumatoid joint destruction (for a more comprehensive review on MMPs in RA see reference 10), and it has been shown that the expression of MMPs in RA synovial cells is regulated by several extracellular signals, including inflammatory cytokines, growth factors, and molecules of the extracellular matrix in the RA synovium. However, several lines of evidence also suggest that pathological expression of proto-oncogenes such as *ras*, *raf*, *sis*, *myb*, and *myc* in the RA synovium, and particularly in RA synovial fibroblasts, contributes to their aggressive potential.<sup>11–12</sup> Some of these proto-oncogenes appear to be involved directly in the regulation of MMPs, thus contributing directly to the increased expression of matrix degrading enzymes in RA and consecutive joint destruction. This notion has been supported by our group's most recent data demonstrating the relevance of Ras signalling in the regulation of MMP mediated cartilage destruction by RA synovial fibroblasts.<sup>13</sup> In this study, control fibroblasts most prominently invaded cartilage in the SCID mouse model, while RA synovial fibroblasts, which were retrovirally transduced with dominant negative (dn) mutants of *c-raf* or *c-myc* exhibited a marked reduction of invasion. This was accompanied by decreased expression of MMPs such as MMP 1 and MMP 13 in dn-*c-raf-1* transduced fibroblasts both in vitro and in vivo. No significant changes in apoptosis were seen in the dn-*c-raf-1* or dn-*c-myc* transduced cells. However, RA synovial fibroblasts that were transduced with both dn-*raf-1* and dn-*c-myc* rapidly underwent apoptosis. These results demonstrated that both *c-raf-1* and *c-myc* contribute to the activation of synovial fibroblasts in RA. The clear effect of dn-*c-raf-1* and dn-*c-myc* on the invasiveness of RA synovial fibroblasts in the SCID mouse model is also in line with the concept that the upregulation of relevant signalling pathways is maintained in RA synovial fibroblasts in the absence of human inflammatory cells.

The study has also pointed to a prominent role of individual members of the MMP family for joint destruction in RA. One example is MMP 1 (collagenase I) that is found in the synovial membranes of all RA patients but only in about 55–80% of synovial samples from trauma patients.<sup>14</sup> Synovial lining cells produce most of the MMP 1 in the diseased synovium, and MMP 1 is released from these cells immediately after production (for review see reference 15). As a result, expression of MMP 1 in the synovial fluid correlates with the degree of synovial inflammation.<sup>16</sup> Recently, a close correlation between the expression of MMP 1 and the

invasive growth of RA synovial fibroblasts has been found,<sup>17</sup> but the specific contribution of individual MMPs to matrix degradation is only partly understood. Using gene transfer of ribozymes to MMP 1 we demonstrated recently that the specific inhibition of MMP 1 significantly reduces the production of these enzymes in RA synovial fibroblasts and inhibits the invasiveness of the cells in the SCID mouse model.<sup>18</sup> In this study, transduction of RA synovial fibroblasts with ribozymes to MMP 1 decreased significantly the production of MMP 1 without affecting other MMPs such as MMP 9 and MMP 13. MMP 1 specific ribozymes reduced not only the spontaneous production of MMP 1 but also prevented the lipopolysaccharide (LPS) and TNF $\alpha$  induced increase in MMP 1 production. Inhibition of MMP 1 was maintained for at least two months and accompanied by a reduction of the invasiveness of RA-SF in the SCID-mouse model by about 50%.

Normally, MMP activity is balanced by the naturally occurring tissue inhibitors of metalloproteinase (TIMPs) that interact irreversibly with MMPs. TIMPs are synthesised and secreted by chondrocytes, synovial fibroblasts, and endothelial cells.<sup>19–21</sup> In situ hybridisation studies demonstrated striking amounts of TIMP 1 mRNA in the synovial lining of patients with RA,<sup>19</sup> but it is widely accepted that the molar ratio of MMPs to TIMPs rather than the absolute levels of TIMPs are crucial for joint destruction. In RA, the amount of MMPs outweighs that of TIMPs, allowing destruction to take place. In line with this concept, we have shown that the overexpression of TIMP 1 and TIMP 3 by gene transfer may result in a marked reduction of the invasiveness of RA synovial fibroblasts.<sup>22</sup> Interestingly, TIMP 3 not only inhibits the degradation of extracellular matrix but shows a number of features that are distinct from other TIMPs. Thus, TIMP 3 can prevent the shedding of cell membrane proteins such as the TNF receptor,<sup>23</sup> the interleukin (IL)-6 receptor,<sup>24</sup> and of TNF $\alpha$ -converting enzyme (TACE).<sup>25</sup> Another interesting feature of TIMP 3 is its ability to induce apoptosis in different cell types.<sup>26–29</sup> In this context, we have shown most recently that in addition to its general pro-apoptotic function, TIMP 3 can sensitise RA synovial fibroblasts to apoptosis induced by Fas ligand when expressed through adenoviral gene transfer.<sup>30</sup> Moreover, adenoviral delivery of TIMP 3 reversed completely the inhibiting effects of TNF $\alpha$  on apoptosis in RA synovial fibroblasts. These findings indicate that overexpression of TIMP 3 in RA synovial fibroblasts may have beneficial effects both by inhibiting matrix degradation and by facilitating cell death.

## CONCLUSION

Collectively, these data suggest that RA synovial fibroblasts contribute significantly to the joint destruction in RA and exhibit features of stable cellular activation of which the increased production of MMPs is a prominent one. Inhibition of MMPs, therefore, is an promising strategy to interfere with the destructive potential of RA synovial fibroblasts. Strategies to achieve this goal include the inhibition of signalling molecules that regulate the expression of MMPs as well as the delivery of molecules that inhibit MMPs directly—either by interfering with its mRNA or by acting as antagonists.

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