REPORT

Toll-like receptors in rheumatoid arthritis joint destruction mediated by two distinct pathways

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Novel therapies, in particular biological agents, have resulted in major breakthroughs in the treatment of rheumatoid arthritis (RA). However, none of the new and promising biologicals has resulted in an American College of Rheumatology (ACR) 70 above 50%, and none of them has shown sustained benefit after termination of therapy. These results are based on the fact that all these agents target the inflammatory cells in the joint, including macrophages, T and B lymphocytes, or vascular endothelium but not the activated synovial fibroblasts. RA synovial fibroblasts (RA-SFs) have to be considered key cells in joint destruction since they differ in their morphology and their biological behaviour from normal synoviocytes and, most importantly, show invasive growth into adjacent tissue.

We have shown that RA-SFs maintain their activated phenotype independently of inflammatory cells and cytokines, considering that they invade human cartilage in the severe combined immunodeficiency (SCID) mouse model even in the absence of cytokine producing macrophages and T and B cells.[1] By analysing cytokines, matrix degrading enzymes, and signalling molecules in the SCID mouse model, we explored the factors leading to synovial activation.

Characterisation of RA-SFs in detail has shown that cellular activation is driven and maintained by proinflammatory cytokines as well as by cytokine independent pathways of activation such as endogenous retroviral elements. In these studies, it was shown for the first time that a specific isoform of the p38 family, namely p38δ is induced through the expression of L1 retrotransposable elements in RA-SFs in a cytokine independent pathway[2 3] (fig 1). In RA synovial tissue, p38δ is predominantly expressed at sites of invasion and bone destruction, whereas other members of the p38 family, such as p38α, are found in the non-attached lining and sublining layers.

These data and the results from the SCID mouse model clearly indicate that at least two pathways of joint destruction are operational in RA.[4] The cytokine dependent pathway is mainly driven by inflammatory cells and targeted by the biologicals, whereas RA-SFs play a key role in the cytokine independent pathway.

Most recently, our work has focused on the search for additional stimulating factors in the activation of RA-SFs in RA. On the basis of the fact that bacterial DNA containing CpG motifs and bacterial cell wall fragments (peptidoglycans) have been detected in the synovial fluid of patients with RA, we have been searching for the presence of the germ line encoded pattern recognition receptors, called toll-like receptors (TLRs), to initiate activating signals in these cells.[5 6]

The TLR family comprises at least 11 members, however, TLR-11 was identified only very recently.[7] Even though the diverse ligands and signalling pathways of most TLRs are well characterised, it is still not clear how and where exactly they interact with their ligands and whether they possibly contribute to the development of autoimmune diseases.

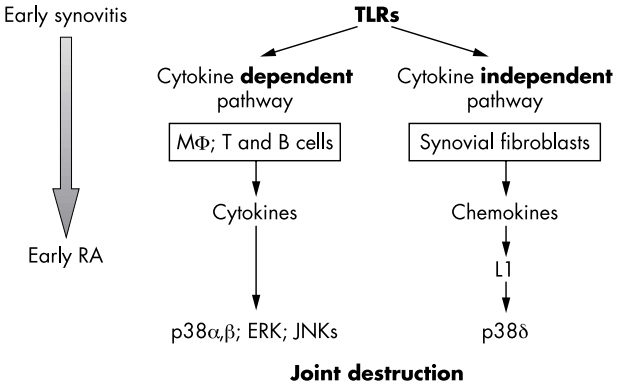

Figure 1 Cellular activation pathways in rheumatoid arthritis joint destruction. EKR, extracellular regulating kinase; JNK, c-Jun-N-terminal kinase; TLR, toll-like receptor.

We have shown that RA-SFs express TLR2 in culture and in the lining layer of synovial tissue, especially at sites of invasion into cartilage and bone.[8] TLR2 recognises a variety of microbial components, but it is critical for the recognition of peptidoglycans. In agreement with these data, cultured RA-SFs have been stimulated with peptidoglycans, but not with CpG DNA, which is a ligand for TLR9.[9] TLR2 expression in RA-SFs was upregulated by stimulation with proinflammatory cytokines and after exposure with synthetic lipopeptides and lipopolysaccharides.

More recently, we used microarray techniques to characterise genes that were induced in RA-SFs after stimulation with peptidoglycans. We demonstrated that TLR2 signalling in these cells results not only in the upregulation of nuclear factor (NF)-κB, certain matrix metalloproteinases, and cyclooxygenase-2, but also, most impressively, in the massive upregulation of several potent chemokines.[10] These chemokines were subsequently detected in synovial tissue as well as in synovial fluid of patients with RA. Most striking is the fact that monocyte chemoattractant protein (MCP)-2 and granulocyte chemotactic protein (GCP)-2, which were among the most strongly induced genes, have never been investigated in arthritis before. Since both are found in significantly higher levels in the synovial fluid of patients with RA compared with patients with osteoarthritis, it can be presumed that these chemokines have an important role in the pathogenesis of RA.

A novel TLR2 fusion protein has been designed to block TLR2 mediated signalling and thus inhibit the activation of RA-SFs. Furthermore, new potential binding ligands can be identified by these means.[6]

Abbreviations: RA, rheumatoid arthritis; RA-SF, RA synovial fibroblast; SCID, severe combined immunodeficiency, TLR, toll-like receptor

Altogether, these data point to a cytokine independent pathway of RA-SF activation by TLR ligands that probably leads to chemoattraction of inflammatory cells at an early stage of disease. Through the expression of various TLRs, RA-SFs act as part of the innate immune system. At an early stage of the disease, they recognise microbial structures or yet unknown endogenous ligands and initiate an inflammatory response.

Of special interest is the observation that the expression of TLR2 and the downstream signalling molecules mediating joint destruction are mainly found at sites of synovial invasion into cartilage and bone, where oxygen supply is low. By examining the hypoxia induced cellular pathways, Kurowska and Distler demonstrated that hypoxia can be detected at sites of destruction in joints in RA.[11] Furthermore, they showed that hypoxia induces a number of interesting molecules, including Id-2, a negative regulator of the basic-Helix-Loop-Helix (bHLH) transcription factor. Id-2 is induced in RA-SFs by hypoxia and proinflammatory cytokines in a hypoxia inducible factor (HIF-1) independent way and mediates the upregulation of the receptor activator of NF-κB ligand (RANKL). Since RANKL promotes the differentiation of osteoclasts, this pathway potentially facilitates osteoclast mediated bone destruction in RA.[12]

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