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I-24 THE ROLE OF ACTIVATED SYNOVIAL MACROPHAGES IN OSTEOARTHRITIS

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Purpose: To investigate the role of synovial macrophages in driving inflammatory and degradative responses in osteoarthritis.

Methods: We have used a model of cultures of synovial cells from digested OA (or RA) synovium. These cells spontaneously produce both pro- and anti-inflammatory cytokines, including TNFα, Il-1 and Il-10, as well as MMPs. If adenoviral gene transfer is used in this model, all cell types are effectively infected, including the synovial macrophages. Specific depletion of synovial macrophages from these cultures of OA synovial cells could be achieved using anti-CD14-conjugated magnetic beads. In other experiments, specific neutralisation of the endogenous production of TNFα and/or Il-1β was done using incubation with the p75 TNF soluble receptor Ig fusion protein etanercept (Enbrel), a neutralizing anti-Ill-1β antibody, or a combination of Enbrel and anti-Ill-1β.

Results: Using an adenovirus transferring the inhibitory subunit 1xIkB, it was possible to selectively inhibit NFκB in synovial cell cocultures. While macrophage-produced TNFα and Il-1β was strongly NFκB dependent in the culture of Enbrel and adenoviral transfer of 1xIkB did not affect Il-1β production and had only a partial effect on TNFα. Effects on other cytokines were similar in RA and OA synovium, with Il-6 and Il-8 both being NFκB dependent. In addition, MMP-1, 3, and 13 were strongly NFκB dependent in both RA and OA, but TIMP-1 was not.

CD14-depleted cultures of OA synovial cells no longer produced significant amounts of macrophage-derived cytokines like TNFα and Il-1β.

There was also significant inhibition of several cytokines produced mainly by synovial fibroblasts, like Il-6 and Il-8, and significant downregulation of MMP-1 and MMP-3. To investigate the mechanisms involved in this macrophage driven stimulation of inflammatory and degradative pathways in the OA synovium, we went on to use specific neutralisation of TNFα and/and/or Il-1β in the cultures of OA synovial cell. As could be expected, TNFα production was effectively neutralised by Enbrel treatment, and Il-1β by treatment with the anti-Ill-1β antibody. There was no effect of Enbrel on Il-1β production, nor did the anti-Ill-1β antibody affect the production of TNFα. Both Enbrel and the anti-Ill-1β antibody inhibited Il-6 and Il-8, with 60% inhibition achieved when both Il-1β and TNFα were neutralized. Neither Enbrel nor the anti-Ill-1β antibody had an impressive effect on MMP-1 and MMP-3, but combination of the two led to significant inhibition both on the mRNA and protein levels. ADAMTS4 mRNA was significantly inhibited by Enbrel, and more potently inhibited by a combination of Enbrel and the anti-Ill-1β antibody, but there was no effect on ADAMTS5 mRNA.

Conclusions: The differential effect of NFκB downregulation on the spontaneous production of TNFα and Il-1β in RA and OA would indicate that the regulation of at least one key intracellular pathway differs fundamentally between these diseases. Both TNFα and Il-1β have functional NFκB elements on their promoters and in various macrophage models, there are both NFκB dependent and NFκB independent ways of inducing TNFα and Il-1β.

Depletion of OA synovial macrophages leads to potent downregulation of several fibroblast-produced cytokines and MMPs, as well as ADAMTS4. This effect is mediated via a combination of Il-1β and TNFα. This finding, along with recent results indicating a key role for macrophages in murine models of OA, would point out synovial macrophages and their cytokines as potential therapeutic targets in human OA.

In contrast to the situation in RA, Il-1β in not TNFα driven in the OA synovium. This finding indicates yet another difference in macrophage cytokine biology between RA and OA: whereas TNFα is the ‘boss cytokine’ in the RA synovium, regulating the production of Il-1β, there is a redundancy between these two cytokines in the OA synovium.

I-25 DIFFERENTIATION OF SYNOVIAL FLUID MACROPHAGES INTO OSTEOCLASTS


Purpose: Synovial Fluid (SF) is dialysate of plasma, which, in uninflamed joints, contains relatively few inflammatory cells. In joint conditions such as rheumatoid arthritis (RA), pyrophosphate arthropathy (PPA), and some cases of inflammatory osteoarthritides (OA), the SF contains numerous inflammatory cells including macrophages, neutrophil polymorphs and lymphocytes.

Methods: Osteoclasts can be formed from monocytes and macrophages when these cells are cultured in the presence of macrophage-colony stimulating factor (M-CSF) and soluble receptor activator for nuclear factor-κB ligand (RANKL). Osteoclasts exhibit a number of specific phenotypic characteristics, being CD14+, vitronectin receptor (VNR) +, tartrate-resistant acid phosphatase (TRAP) + and are uniquely capable of lacunar bone resorption.

Results: It has been shown that SF macrophages (CD14+) isolated from the knee joint SF of patients with OA, RA and PPA, when cultured with M-CSF and RANKL, can differentiate into multinucleated cells which express the cytochemical and functional features of osteoclasts. Under these conditions more resorption is seen in RA than OA SF macrophage cultures. Macrophage-osteoclast differentiation can also occur by a RANKL-independent mechanism in which other TNF superfamily members, such as tumour necrosis factor-α (TNFα) and LIGHT, substitute for RANKL. LIGHT (but not TNFα) is present in high concentration in inflammatory (compared to non-inflammatory) OA synovial fluid, and LIGHT-treated OA SF macrophage cultures induces osteoclast formation and resorption equal to that seen in RA SF macrophage cultures.

Conclusions: SF macrophages are thus capable of differentiating into mature osteoclasts capable of lacunar resorption, and significantly more osteoclast formation occurs in SF containing increased numbers of inflammatory cells. The increase in macrophage number and the amount of LIGHT and other osteoclastogenic factors in inflammatory SF, may play a role in joint destruction in OA and other arthritic conditions by promoting SF macrophage-osteoclast differentiation.

I-26 ACTIVATION OF INFLAMMATORY SIGNALLING IN CARTILAGE BY SOLUBLE AND PHYSICAL STIMULI

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Purpose: The ability of soluble mediators such as proinflammatory cytokines (Il-1, TNF) or bacterial lipopolysaccharide (LPS) to activate inflammatory signalling pathways in cartilage is well known. Typically such stimuli activate NFκB and the MAP kinase pathways to cause expression of inflammatory response genes and the molecular mechanisms coupling the cell surface receptors for the soluble stimuli to the downstream pathways are known in some detail. One important result is that the chondrocytes are activated to degrade their extracellular matrix. There is good evidence that such proteolytic degradation of cartilage matrix is an important early feature of osteoarthritis but to what extent the degenerative process is driven by known soluble inflammatory stimuli is unclear. It is less widely appreciated that inflammatory signalling pathways are also rapidly activated by simple physical injury to tissues; such a process is potentially relevant to osteoarthritis, where tissue injury is a well-known predisposing factor.

Simple wounding of porcine articular cartilage with a scalpel, either by scoring its surface, or dissecting tissue explants, rapidly activates proteolytic enzymes such as matrix metalloproteinase-1 and -3. To investigate the mechanisms involved in this macrophage-driven activation of inflammatory signalling pathways in cartilage is well known. Typically such stimuli activate NFκB and the MAP kinase pathways to cause expression of inflammatory response genes and the molecular mechanisms coupling the cell surface receptors for the soluble stimuli to the downstream pathways are known in some detail. One important result is that the chondrocytes are activated to degrade their extracellular matrix. There is good evidence that such proteolytic degradation of cartilage matrix is an important early feature of osteoarthritis but to what extent the degenerative process is driven by known soluble inflammatory stimuli is unclear. It is less widely appreciated that inflammatory signalling pathways are also rapidly activated by simple physical injury to tissues; such a process is potentially relevant to osteoarthritis, where tissue injury is a well-known predisposing factor.

Simple wounding of porcine articular cartilage with a scalpel, either by scoring its surface, or dissecting tissue explants, rapidly activates the MAP kinase and NFκB pathways and also induces inflammatory response genes. In part, this cell activation is due to release of basic fibroblast growth factor (FGF-2) which is stored in a pericellular pool bound to perlecan. However FGF-2, while augmenting the response, does not itself activate NFκB or the stress kinase c-jun N-terminal kinase (JNK). The activation of JNK, for example, occurs within seconds of injury. It has been proposed that damaged cells release contents that are pro-inflammatory (e.g., heat shock proteins); however, we have been unable to find evidence of any soluble factor released from wounded cartilage that causes inflammatory signalling as indicated by JNK activity. Rather, chondrocytes seem to directly ‘sense’ matrix injury. We have explored the possibility that such sensing could be integrin mediated. Although activation and phosphorylation of focal adhesion kinase (FAK) occurs upon sharp injury to the tissue, there is also additional major tyrosine phosphorylation of cellular proteins, some of it consistent with major activation of src family kinase(s). Experiments with inhibitors of protein kinases suggest that the FAK/src activation is not upstream of inflammatory signalling.

Our current model is that disruption of the extracellular matrix (and probably collagen fibres in particular) directly activates multiple parallel cell signalling pathways including src and the MAP kinases. Our lack of understanding of how tissue damage activates inflammatory signalling is a major gap in our knowledge of the inflammatory response.