The Cytokine Network in Osteoarthritis

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Osteoarthritis (OA) could be defined as a complex or interactive degradative and repair process in cartilage and subchondral bone with secondary components of synovial membrane inflammation. The etiopathologic processes involve various factors including mechanical, biochemical, and genetic. In the course of time, the chondrocytes react to these injuries by elaborating degradative enzymes and developing inappropriate repair responses. Much recent research implicates the enzymatic breakdown of the cartilage as a key feature of the disease progression.

The main developments over the last few decades have been the change in the basic concepts concerning the pathophysiology of the disease, which have moved from a fairly mechanical hypothesis of wear and tear to include a number of interactive pathways explaining the structural changes. While the disease process is a fairly complicated one, involving three important tissues (synovial membrane, cartilage, and subchondral bone), there are now a number of pathways identified as being responsible for the different structural changes seen during the evolution of the disease process. There is now evidence of global cross-talking among these tissues, further complicating the whole process more than may have been thought.

The extracellular matrix structure plays an integral role in the function of cartilage. In this tissue, matrix homeostasis is controlled by the chondrocytes through a balanced regulation of synthesis and degradative events, with the rate of new matrix synthesis being equal to the rate of matrix degradation. Both processes are controlled by a variety of extracellular messenger proteins, termed growth factors and/or cytokines. Disturbance or alteration in the net effects of multiple growth factors and cytokines may compromise the macromolecule synthesis and degradation pattern, therefore being responsible for the development of pathological conditions such as OA.

Even if articular tissue destruction characterizes the OA condition, synovial inflammation is of fundamental importance in the progression of cartilage lesions in this disease (1). At the clinical stage of OA, there are changes in the synovial membrane and an inflammatory reaction is often seen. A hypothesis explaining the pathological development of OA may be summarized as follows: the cartilage matrix breakdown produced by proteolytic enzymes releases increased amounts of matrix fragments into synovial fluid. Synovial cells ingest the cartilage breakdown products through phagocytosis, inducing an inflammatory process that leads to the production of proteases and soluble inflammatory mediators. These factors, through diffusion into the cartilage, increase the catabolic process and create a vicious cycle with more cartilage being degraded and subsequently provoking more inflammation.

Several soluble mediators have been identified in articular tissues from OA disease. Of the proinflammatory cytokines, IL-1β (interleukin-1beta) and TNF-α (tumour necrosis factor-alpha) appear to be the principal mediators of joint destruction (2). Yet it is claimed, and substantiated by studies on animal models (3-5), that IL-1β is of pivotal importance in cartilage destruction and considered to be the principal mover of the enzyme system. It has also been claimed that TNF-α drives the inflammatory process, making these two cytokines prime targets for therapeutic approaches.

These proinflammatory cytokines are able to increase other inflammatory factors such as some prostanoids, nitric oxide (NO), and the synthesis of enzymes, to inhibit the synthesis of the major physiological inhibitors of these enzymes, and also, to inhibit the synthesis of matrix constituents such as collagen and proteoglycans. Thus, the actions of IL-1β and TNF-α on other inflammatory mediators and on the enzyme process, combined with the suppression of matrix synthesis, result in the severe degradation of cartilage and the appearance of conditions that we know to be characteristic of OA.

IL-1β and TNF-α are synthesized as an inactive precursor, and have to be activated by an enzyme to be released extracellularly in their active forms. For IL-1β, only one protease belonging to the cysteine-dependent

protease family and named IL-1β converting enzyme (ICE or caspase-1) can specifically generate the mature IL-1β. Our laboratory showed that ICE is produced by both human synovial membrane and cartilage, with a marked and significant increase in expression and synthesis in OA tissues (6). An immunohistochemical study also revealed that in human articular cartilage this enzyme is preferentially located at the superficial zone of this tissue. The proteolytic cleavage of membrane-bound pro-TNF-α appears to occur, at least in part, via TNF-α converting enzyme (TACE), an enzyme belonging to a subfamily of adamalysin (7). An up-regulation of TACE expression has also been shown in OA human articular cartilage (8, 9).

Cell signaling by pro-inflammatory cytokines such as IL-1β and TNF-α occurs through binding to specific membrane receptors. For IL-1β and TNF-α two types of receptors have been identified (10, 11). For IL-1, the receptors are named type I and type II. Data from our laboratory showed that for synovial fibroblasts and chondrocytes, it is type I which is responsible for mediating the signal, and that this receptor type level is significantly increased in OA tissues (12, 13). Two receptors also exist for TNF-α, and are named TNF-receptor 55 and 75 (TNF-R55, TNF-R75) according to their molecular weight. In addition it was demonstrated that TNF-R55 is responsible for signal transduction in both chondrocytes and synovial fibroblasts, and its level is significantly increased in these OA cells (14, 15).

Natural or physiological inhibitors capable of directly counteracting the binding of cytokines to cells or reducing the proinflammatory level have been identified. In these tissues, these inhibitors could be divided into three categories based on their mode of action. The first category is a receptor antagonist that interferes with the binding of the ligand to its receptor by competing for the same binding site. To date, such an inhibitor has been found only for the IL-1 system, and named IL-1 receptor antagonist (IL-1Ra). The second category includes the soluble forms of the proinflammatory cytokine receptors that bind to free cytokines. These are truncated forms of the receptor and are named soluble receptors (sR). Both IL-1 and TNF-α are named according to the classification of their receptor. Thus, for IL-1, they are named types I and II IL-1sR; and for TNF-α, TNF-sR55 and TNF-sR75. The third category is molecules able to reduce proinflammatory cytokine production and/or activity: these molecules are named antiinflammatory cytokines. Three such cytokines, namely IL-4, IL-10 and IL-13, have been identified.

IL-1Ra was found to be present in both synovial membrane and articular cartilage. Laboratory data suggests that a relative deficit of IL-1Ra in relation to IL-1β exists in these arthritic joint tissues. By *ex vivo* experimentation in human OA synovial membrane, it has been shown that IL-1Ra induces a dose-dependent decrease in MMP (metalloprotease) production in this tissue (16). In addition, by using two experimental OA models, it was demonstrated that increasing the *in vivo* level of IL-1Ra in the diseased joints reduced the tissue progression of lesions (3, 17, 18).

The second category of physiological inhibitors of the proinflammatory cytokines included the soluble receptors. Indeed, both receptor type forms of IL-1β and TNF-α can be shed from the cellular membrane and released extracellularly as soluble receptors (19, 20). These soluble receptors bind to the free cytokines, thus functioning as receptor antagonists, and being capable of competing with the membrane-associated receptors for the cytokines. The shedding of surface receptors may decrease the responsiveness of target cells to the ligand. For the IL-1 system, it is suggested that type II IL-1sR serves as the main precursor for shed soluble receptors. Interestingly, the binding affinity of the type I and type II IL-1sR differs between IL-1 and IL-1Ra and is of crucial importance if it is to be used *in vivo* (21, 22). Indeed, type II IL-1sR binds more readily to IL-1β than IL-1Ra. Conversely, the type I IL-1sR binds IL-1Ra with the same affinity as IL-1β. *In vitro* experiments have revealed that the simultaneous addition of both IL-1Ra and soluble type II IL-1sR appears to be extremely beneficial. On the other hand, the individual inhibitory effects of both soluble type I IL-1sR and IL-1Ra are abrogated when present concurrently. For TNF-α, both membrane receptors may also be shed from chondrocytes and synovial fibroblasts and by binding to the free TNF-α, diminish the availability of this cytokine for their specific cellular receptors. Contrasting with the IL-1 system, both TNF-sR, appear to have similar affinity. Although these TNF-sR are released from the cellular membrane in arthritis, there is a relative imbalance between their level and that of the free TNF-α (14, 23). In OA synovial fibroblasts, although both TNF-sR are increased extracellularly, a statistically significant up-regulation in the release has been found only for the TNF-sR75. It is suggested that TNF-sR function as inhibitors of cytokine activity by rendering the cells less sensitive to the activity of the ligands or by scavenging ligands not bound to cell surface receptors.

For the third category, the properties of the anti-inflammatory cytokines IL-4, IL-10 and IL-13 include a decreased production of proinflammatory cytokines such as IL-1β and TNF-α, PGE_2 (prostaglandin E_2) and an up-regulation of IL-1Ra. IL-10 also up-regulated the specific inhibitor of MMP - TIMP.

The balance between cytokine-driven anabolic and

catabolic processes determines the integrity of articular joint tissue. However, not all catabolic activity in OA articular tissue can be attributed to IL-1β and TNF-α, as other cytokines may also be involved. Other proinflammatory cytokines include IL-6, LIF (leukemia inhibitory factor), IL-17, IL-18 and IL-8. All of these cytokines have been shown to be expressed in OA tissue, and have, therefore, been considered potential contributing factors in the pathogenesis of this disease. However, their exact role of in this disease process is not yet clearly established. Moreover, it has also not yet been determined if they act independently or in concert, and whether a functional hierarchy exists between them. An example could be that in the TNF-α-induced PGE_2 production, IL-8 synergizes and LIF potentiates TNF-α-induced PGE_2 (24). Another example is that the IL-17-induced NO production in human OA chondrocytes was independent or complementary to LIF or TNF-α production, but common to that of the IL-1β production (25). Indeed, treatment of these cells with both IL-1β and IL-17 induced no additional effect than IL-1β alone, but the addition of IL-17 with TNF-α or LIF synergized or produced an additional response of NO production, respectively.

Osteoarthritis also involves changes in the surrounding bone, and subchondral bone sclerosis is a well-recognized manifestation in human OA. Recent data underlines the concept that abnormal subchondral bone cell functions may contribute to the onset/progression of OA (26, 27). Recent work also suggests that very early in the OA process, biological and morphological disturbances occur at the subchondral bone and may have a role in the modulation of articular cartilage metabolism. Indeed, evidence indicates that the altered subchondral bone metabolism in OA is possibly caused by abnormal osteoblast behavior. This includes altered expression of biomarkers such as alkaline phosphatase activity and osteocalcin production and abnormal response to parathyroid hormone (PTH) challenge due to down-regulation of PTH receptors (28). Furthermore, cytokines and inflammatory mediators including IL-6 and PGE_2 that regulate subchondral bone remodeling/resorption are also elevated in OA subchondral osteoblasts (29).

In summary, the morphological changes observed in OA, at the clinical stage of the disease, include alteration in the cartilage, synovial inflammation and subchondral bone. The changes in the cartilage are believed to be related to a complex network of biochemical factors, which lead to the breakdown of the cartilage macromolecules. Proinflammatory cytokines such as IL-1β, locally produced, also contribute to these changes. It is now clearer that the progression of OA is associated to the influence of tissue cross-talking. It appears that there is a movement of factors and cytokines from the different joint tissues to the cartilage. In this regard, the cytokines appear to be an interesting link in OA as they are responsible for important structural changes in joint articular tissues. Our current understanding of the factors involved in OA demonstrated that a disturbance in the synthesis of proinflammatory cytokines might accelerate the progression of OA. A relative deficit in the production of IL-1Ra coupled with an up regulation of the receptor level, as well as imbalances in the soluble IL-1R or TNF-R and/or anti-inflammatory cytokines and proinflammatory cytokines are additional factors that favor the enhancement catabolic effects of OA. Other proinflammatory cytokines although not necessarily playing a central role in OA could greatly contribute to the production of catabolic factors responsible for articular joint tissue destruction.

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