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CYTOKINES AND THEIR ROLE IN THE PATHOPHYSIOLOGY OF OSTEOARTHRITIS

Johanne Martel-Pelletier[1] , Nada Alaaeddine [2] and Jean-Pierre Pelletier [3]

[1] University of Montréal, Director - Osteoarthritis Research Unit, Centre hospitalier de l'Université de Montréal, Hôpital Notre-Dame, Montréal, Québec, Canada, [2] University of Montréal, Centre hospitalier de l'Université de Montréal, Hôpital Notre-Dame, Montréal, Québec, Canada, [3] University of Montréal, Head - Rheumatic Disease Unit, Director - Osteoarthritis Research Unit, Centre hospitalier de l'Université de Montréal, Hôpital Notre-Dame, Montréal, Québec, Canada

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1. ABSTRACT

The specific causative agent of the pathological process of osteoarthritis (OA) has not yet been identified, however, episodic inflammation at the clinical stage is now a well documented phenomenon and believed to be involved in the disease progression. Interleukin-1 beta (IL-1 beta) and tumor necrosis factor-alpha (TNF-alpha) are the predominant proinflammatory cytokines synthesized during the OA process. Other cytokines having proinflammatory properties or catabolic factors could also contribute to this pathological condition, and those having antiinflammatory properties may be able to counteract the negative effects of the former on the disease process. In this chapter, we will review cytokine interactions and their modulatory effects on joint articular tissue metabolism, including their stimulatory and/or inhibitory actions, and their potential relevance to OA. We will also briefly survey the major biological factors, in relation to cytokines, that look promising for future therapeutic approaches.

2. INTRODUCTION

Osteoarthritis (OA), the most prevalent disorder of the musculoskeletal system, is believed to be a consequence of mechanical and biological events that destabilize the normal coupling of degradation and synthesis within articular joint tissues. The disease process affects not only the articular cartilage, but also the entire joint structure including the subchondral bone, ligaments, capsule, synovial membrane and periarticular muscles. Typical radiological changes in OA include joint space narrowing, subchondral bone sclerosis, and cyst and osteophyte formation. Clinical features also include joint pain, tenderness, limitation of movement, crepitus, occasional effusion and variable degrees of inflammation.

OA is generally classified as idiopathic (primary) or secondary, according to the presence or absence of certain factors. Secondary OA frequently develops in joints with preexisting structural abnormalities or absence of etiological factors. In primary OA, no trauma or other predisposing factor is identified, and intrinsic alterations of the articular tissue, or response to normal cumulative stresses, are presumed responsible (1).

A series of biomedical and inflammatory phenomena and etiological agents describes the etiopathogenesis of OA. This disease process involves a disturbance in the normal balance of degradation and repair in articular cartilage, synovial membrane and subchondral bone. Once cartilage degradation has begun, the synovial membrane phagocytoses the breakdown products released into the synovial fluid. Consequently, the membrane becomes hypertrophic and hyperplasic. Several studies have reported inflammatory changes in the synovial membrane of patients with OA that, on occasion, were almost indistinguishable from those in patients with an inflammatory arthritis such as rheumatoid arthritis (RA) (2-5).

3. CYTOKINES AND OSTEOARTHRITIS

It is believed that cytokines and growth factors play an important role in the pathophysiology of OA. They are closely associated with functional alterations in synovium, cartilage and subchondral bone, and are produced both spontaneously and following stimulation by the joint tissue cells. Cytokines and growth factors appear to be first produced by the synovial membrane, and diffused into the cartilage through the synovial fluid. They activate the chondrocytes, which in turn could produce

catabolic factors such as proteases and proinflammatory cytokines. In OA synovial membrane, the synovial lining cells are key inflammatory effectors.

The major cytokines-(pro- and antiinflammatory) and antagonist - believed to be involved in OA pathophysiology include IL-1alpha, IL-1beta, IL-4, IL-6, IL-8, IL-10, IL-11, IL-13, IL-17, LIF, TNF-alpha as well as IL-1Ra. Several growth factors also appear to be involved in this disease, for example TGF-beta, FGF, PDGF and IGF. Some growth factors, such as TGF-beta, have a dual effect (synthetic or catabolic) that is dependent on the target cell, tissular location and concentration.

3.1. Proinflammatory cytokines

Proinflammatory cytokines are believed to play a pivotal role in the initiation and development of this disease process, among which IL-1beta and TNF-alpha appear prominent. IL-1beta is extremely important to cartilage destruction, while TNF-alpha appears to drive the inflammatory process (6-8). IL-1 and TNF-alpha can induce joint articular cells, such as chondrocytes and synovial cells, to produce other cytokines such as IL-8, IL-6, LIF and their own production, as well as stimulate proteases and prostaglandin E_2 (PGE_2) production. IL-1beta and TNF-alpha have also been shown to increase osteoclastic bone resorption *in vitro* (9). On the one hand, it has been demonstrated using cultured synovial fibroblasts that blocking IL-1 activity with the IL-1 receptor antagonist (IL-1Ra) reduced IL-6 and IL-8 production, but not that of TNF-alpha (10). On the other hand, adding anti-TNF-alpha antibodies to synovial cells greatly reduced the production of other proinflammatory cytokines such as IL-1, GM-CSF, IL-6 (11).

IL-1beta is primarily synthesized as a 31 kilodalton (kD) precursor, pro-IL-1beta devoid of a conventional signal sequence, and released in the active form of 17.5 kD (12,13). In articular joint tissue, including synovial membrane, synovial fluid and cartilage, IL-1beta has been found in the active form, and *ex vivo* experiments have demonstrated the ability of the OA synovial membrane to secrete this cytokine (14). Several serine proteases can process the pro-IL-1beta to bioactive forms (15) but in mammals only one protease, belonging to the cysteine-dependent protease family and named IL-1beta converting enzyme (ICE or Caspase-1), can specifically generate the mature 17.5 kD cytokine (15,16). ICE is a pro-enzyme polypeptide of 45 kD [p45] (15,16) located in the plasma membrane, and belonging to the family of cysteine aspartate-specific proteases known as caspases. Active ICE is produced following proteolytic cleavage of the pro-enzyme p45, generating two subunits known as p10 and p20, both of which are essential for enzymatic activity (17).

The biological activation of cells by IL-1 is mediated through association with specific cell-surface receptors (IL-1R). Two have been identified and named type I and type II (18). In articular tissue cells, the type I receptor, which has a slightly higher affinity for IL-1beta than for IL-1alpha, appears responsible for signal transduction (19-21). Type II IL-1R has a greater affinity for IL-1alpha than IL-1beta. It is unclear whether the type II receptor can mediate IL-1 cell signaling or if it serves to competitively inhibit IL-1 binding to type I IL-1R. The number of type I IL-1R is significantly increased in OA chondrocytes and synovial fibroblasts (20.,21). This appears to be responsible for the higher sensitivity of these cells to stimulation by IL-1 (20), a process that increases proteolytic enzyme gene upregulation, which in turn enhances cartilage destruction.

Both types of IL-1R can also be shed from the cell surface, and exist extracellularly in truncated forms; they are named IL-1 soluble receptors (IL-1sR). The shed receptor may function as a receptor antagonist because the ligand binding region is preserved, thus enabling it to compete with the membrane-associated receptors of the target cells. Similarly, the shedding of surface receptors may decrease the responsiveness of target cells to the ligand. It is suggested that type II IL-1R serves as the main precursor for shed soluble receptors. The binding affinity of IL-1sR to both IL-1 isoforms and IL-1Ra differs. Type II IL-1sR binds IL-1beta more readily than IL-1Ra; in contrast, type I IL-1sR binds IL-1Ra with high affinity (19,22,23). Therefore, when IL-1Ra and type I IL-1sR are both present their individual inhibitory effects are abrogated; however, when type II IL-1sR and IL-1Ra are combined, the resulting effect appears extremely beneficial, showing an additive effect.

In OA, TNF-alpha also appears to be an important mediator of matrix degradation and a pivotal cytokine in synovial membrane inflammation, although this cytokine is detected in OA articular tissue at a low level. TNF-alpha is synthesized as a precursor protein with amino-terminal extensions that are cleaved from the mature sequence prior to secretion. The prosequence of human TNF-alpha comprises 76 amino acids, and proteolytic cleavage takes place at the cellular surface. This cleavage appears to occur via a TNF-alpha converting enzyme named TACE belonging to a subfamily of the adamalysin (24). This enzyme is also required for shedding the TNF receptors. An upregulation of TACE mRNA in human OA cartilage has recently been reported (25). Following cleavage, the 233-residue prohormone of human TNF-alpha is converted to the 157-residue (17 kD) secreted protein which oligomerizes to form trimers (26). The shape resembles a triangular cone with three subunits arranged edge to face.

TNF-alpha acts by binding to two specific receptors on the cell membrane. TNF-R are expressed on most tissues and in most cell lines with the exception of red blood cells, resting lymphocytes and a number of transformed B cells (27-29). These two TNF-R have apparent molecular masses of 55 to 60 kD and 75 to 80 kD (30.,31), and are named according to their molecular weight; TNF-R55 and TNF-R75. Their extracellular domains share 28% identity. This homology is also shared with other cell surface proteins, including the nerve growth factor receptor, Fas antigen, Bp 50, Ox 40 and CD27 (32,33). Interestingly, there is a complete absence of

homology between the intracellular domains of the two TNF-R and any other known protein receptor (30,31,34,35). In articular tissue cells, TNF-R55 seems to be the dominant receptor responsible for mediating TNF-alpha activity. In OA chondrocytes and synovial fibroblasts, enhanced expression of TNF-R55 has been reported (36,37).

TNF-alpha induces multiple biological activities, and several distinct mechanisms of signal transduction may explain this diversity of action. First, although it is accepted that TNF-R55 is biologically relevant for several cell types, both receptor types appear to be actively involved in signal transduction (36,38-41). Indeed, in a given cell such as lymphocytes, each receptor type has been shown to induce a specific subset of TNF-alpha activities (42,43). It is suggested that the membrane receptors TNF-R55 and TNF-R75 are linked to distinct intracellular second-messengers. Second, it has been reported that TNF-R75 may regulate the rate of TNF-alpha association to TNF-R55 (44). TNF-R75/TNF-alpha complex may exhibit enhanced and/or specific intracellular function. Third, heterogeneity in the TNF-alpha response may also be caused by different postreceptor signal transduction pathways (45). Finally, TNF-alpha is a nondisulfide-bonded trimer that induces receptor trimerization by binding to its receptors. Each subunit makes contact with two adjacent receptor molecules, thus stabilizing the receptor trimer. It is not clear, however, if 1) receptor trimerization is necessary for activation, or 2) whether receptor dimerization is sufficient, or 3) if receptor trimerization triggers other and/or additional intracellular pathways.

Adding to the complexity of this cytokine, proteolytic cleavage of the extracellular domain of each TNF-R produces TNF-alpha soluble receptors (TNF-sR). The two soluble receptors, TNF-sR55 and TNF-sR75, are produced spontaneously by OA synovial fibroblasts and chondrocytes (36,46). These pathological cells have been found to release a significantly elevated level of the TNF-sR75 (36,46). TNF-sR are also found in the fluid of patients with different forms of arthritis, in quantities that vary according to disease status, with a higher ratio of TNF-sR75/TNF-sR55 noted in the more severe cases (47-49). It has been suggested, although not yet proven, that the biological role of the TNF-sR depends on its concentration in the joints. Thus, at low concentrations, TNF-sR stabilize the trimeric structure of TNF-alpha, thereby increasing the half-life of bioactive TNF-alpha (50), while at high concentrations, TNF-sR reduce the bioactivity of TNF-alpha by competing for TNF binding with cell-associated receptors (51). However, as the affinity of both TNF-sR is similar to that of the plasma membrane receptor, large amounts of these inhibitors are required to decrease TNF-alpha activity. To address this issue *in vivo*, chimeric proteins were generated between TNF-sR and IγG domains (52). These fusion proteins were used in animal models and have been shown to display a high affinity. They have also been proven useful in the experimental therapy of septic shock, listeriosis and collagen arthritis (53-56).

The balance between cytokine-driven anabolic and catabolic processes determines the integrity of articular joint tissue. As previously mentioned, not all negative catabolic activity in OA articular tissue can be attributed to IL-1beta and TNF-alpha; other cytokines may also be involved. A shift in the balance between pro- and antiinflammatory cytokines is believed to contribute to the destructive processes in OA. Other proinflammatory cytokines including IL-8, LIF, IL-11, IL-6 and IL-17 have been shown to be expressed in OA tissue, and have therefore been considered potential contributing factors in the pathogenesis of this disease.

Interleukin-8 is a potent chemotactic cytokine for polymorphonuclear neutrophils (PMN), stimulating their chemotaxis and generating reactive oxygen metabolites (57). This chemokine is synthesized by a variety of cells including monocytes/macrophages, chondrocytes and fibroblasts (58-61). TNF-alpha can stimulate the release of IL-8 by these cells (61), and it is possible that IL-8 plays an important role in the acute inflammatory reaction. In synovial culture, TNF-alpha has been shown to stimulate the production of IL-8 in a time- and dose-dependent manner (61). In OA patients, IL-beta, IL-6, TNF-alpha and IL-8 coexist in the synovial fluid. IL-8 can enhance the release of inflammatory cytokines in human mononuclear cells, including that of IL-1beta, IL-6 and TNF-α, which may further modulate the inflammatory reaction (57). Deleuran et al (62) reported that the strongest expression of IL-8, in both OA and RA patients, was detected in the blood vessels and lining cell layers of the resected synovial membrane. The presence of IL-8 in the lining cell layers may well result in delivery to the synovial fluid, and could explain the high amount of IL-8 in this location. IL-8 is also present in the chondrocytes, and has been shown to enhance the production of oxidative and 5-lipoxygenase products (63). Lotz et al (64) have shown that human articular chondrocytes, stimulated by certain agents generated during response to cartilage injury (i.e. TNF-alpha), express the IL-8 gene and secrete bioactive IL-8.

Leukemia inhibitory factor (LIF) is a single-chain glycoprotein that has diverse effects, including induction of acute-phase protein synthesis and the inhibition of lipoprotein lipase activity. The LIF receptor is comprised of a combination of at least two chains: gp 130 (common to the IL-6 and IL-11 receptors) and gp 190 (65). A higher LIF level has been detected in synovial fluid of OA patients (66). LIF has been shown to enhance IL-1beta and IL-8 expression in chondrocytes, and IL-1beta and TNF-alpha in synovial fibroblasts (67). On various cell types, including those from articular joint tissue, IL-1beta and TNF-alpha upregulate LIF production (68-72). LIF regulates the metabolism of connective tissue such as cartilage and bone, and induces both the resorption and the formation of bone (73,74). LIF can induce expression of collagenase and stromelysin by human articular chondrocytes without affecting the production of the specific tissue inhibitor of metalloproteases, TIMP (69). This cytokine stimulates cartilage proteoglycan resorption (75) as well as nitric oxide (NO) production. These observations support the

hypothesis that LIF may be directly and/or indirectly involved in the development of cartilage destruction and joint inflammation.

The IL-11 receptor shares the gp 130 domain with the LIF and IL-6 receptors, suggesting that they may have similar actions. This cytokine was originally identified as a stromal cell-derived lymphoietic and hematopoietic factor, but can also be induced in articular chondrocyte and synovial fibroblast cultures (76,77). IL-11 can be regulated on the transcriptional as well as the translational levels by various cytokines and growth factors (77). In addition, in articular chondrocytes and synovial fibroblasts, IL-11 does not increase the production of stromelysin, but is capable of inducing *de novo* synthesis of TIMP (77). Moreover, IL-11 has been found to decrease the release of PGE_2 from OA synovial fibroblasts (78), suggesting that, contrary to the action of LIF, IL-11 can prevent the excessive extracellular matrix degeneration induced by synovial inflammation.

IL-6 has also been proposed as a contributor to the OA pathological process by: 1) increasing the number of inflammatory cells in synovial tissue (79); 2) stimulating the proliferation of chondrocytes; and 3) inducing an amplification of the IL-1 effects on the increased synthesis of metalloproteases (MMP) and inhibiting proteoglycan production (80). However, as IL-6 can induce the production of TIMP (81), and not MMP, it is believed that this cytokine is involved in the feedback mechanism that limits proteolytic damage.

IL-17 is a newly discovered cytokine of 20-30 kD present as a homodimer with variable glycosylated polypeptides (82). The tissue distribution of IL-17R appears ubiquitous, and it is not yet known whether all cells expressing IL-17R respond to its ligand. IL-17 upregulates a number of gene products involved in cell activation, including the proinflammatory cytokines IL-1beta, TNF-alpha and IL-6, as well as MMP in target cells such as human macrophages (83). IL-17 also increases the production of NO in chondrocyte cultures (84,85).

3.2. Nitric oxide (NO): a catabolic factor

It has also been proposed that the inorganic free radical NO is a potential factor in the promotion of cartilage catabolism in OA. Compared with the normal state, OA cartilage produces a large amount of NO, both under spontaneous and proinflammatory cytokine-stimulated conditions (86). A high level of nitrite/nitrate has been found in the synovial fluid and serum of arthritis patients (87,88). This is caused by an increased level of the inducible form of NO synthase (iNOS), the enzyme responsible for NO production (88,89). A chondrocyte-specific iNOS cDNA has been cloned and sequenced, and encodes a protein of 131 kD (90). The deduced amino acid sequence exhibits about 50% identity and 70% similarity to endothelial and neuronal forms of iNOS.

NO inhibits the synthesis of cartilage matrix macromolecules and enhances MMP activity (91,92). Moreover, elevated NO reduces the synthesis of IL-1Ra by chondrocytes (86). As such, an increased level of IL-1, in conjunction with a decreased IL-1Ra-level, may cause an over-stimulation of OA chondrocytes by this factor, leading to enhanced cartilage matrix degradation. Interestingly, a selective inhibitor of iNOS administered *in vivo* proved to exert positive therapeutic effects on the progression of lesions in an experimental canine OA model (93).

3.3. Antiinflammatory cytokines and cytokine antagonist

OA is characterized by progressive cartilage degradation, in which matrix integrity is no longer maintained and the homeostasis of catabolic cytokines (i.e. IL-1beta, TNF-alpha), anabolic cytokines (i.e. IGF, TGF-beta) and antiinflammatory cytokines or antagonist (i.e. IL-4, IL-10, IL-13 and IL-1Ra) is disturbed. Three antiinflammatory cytokines (IL-4, IL-10, IL-13) have been shown to be spontaneously elaborated by synovial membrane and cartilage, and are found in increased levels in the synovial fluid of OA patients. The antiinflammatory properties of these cytokines include decreased production of IL-1beta, TNF-alpha and MMP, upregulation of IL-1Ra and TIMP-1, and inhibition of PGE_2 release (94-101). Although these cytokines share biological activities, their effects depend on the target cell of interest. For example, it was found that IL-10 modulated TNF-alpha production by increasing the release of the TNF-sR from monocytes in culture, while downregulating the receptor surface expression (99). In human OA synovial fibroblasts, IL-10 also downregulated the TNF-R density, while increasing the release of TNF-alpha-induced TNF-Rs75. In these cells, however, IL-4 upregulated TNF-R, and enhanced TNF-alpha-induced TNF-sR75 (101). This concurs with data from mononuclear cells from RA synovial fluid, in that both TNF-R55 and TNF-R75 were upregulated by IL-4 (102), and contrasts with findings from monocytes where this antiinflammatory cytokine downregulated both the membrane and soluble TNF-R (103). IL-13 inhibits lipopolysaccharide (LPS)-induced TNF-alpha production by mononuclear cells from peripheral blood and OA synovial fibroblasts, but not in cells recovered from the synovial fluid of OA and RA patients (104). In addition, the TNF receptor system does not appear to be a target for IL-13 in OA synovial fibroblasts (101).

Although only recently discovered, IL-13 has been shown to have important biological activities such as inhibiting the production of a wide range of proinflammatory cytokines in monocytes/macrophages, B cells, natural killer cells and endothelial cells, while increasing IL-1Ra production (104,105). In human synovial membrane specimens from OA patients treated with LPS, *in vitro* IL-13 inhibited the synthesis of IL-1beta, TNF-alpha and stromelysin, and increased production of IL-1Ra (100).

IL-1Ra is a competitive inhibitor of IL-1R. This molecule does not bind to IL-1, is not a binding protein, nor does it stimulate target cells. IL-1Ra can block many of the effects observed during the pathological process of OA, including PGE_2 synthesis in synovial cells, collagenase production by chondrocytes, and cartilage matrix degradation. Three forms of IL-1Ra were found, one

extracellular and termed soluble IL-1Ra (IL-1sRa), and two intracellular, icIL-1RaI and icIL-1RaII (19). Both the soluble and icIL-1Ra can bind to IL-1R, but with about 5-fold less affinity for the latter. Although intensive research is underway, the biological actions of icIL-1Ra remains elusive. *In vitro* experiments have revealed that an excess of 10-100 times the amount of IL-1Ra is necessary to inhibit IL-1beta activity whereas, *in vivo*, 100-2000 times more IL-1Ra is needed (14,19). This may likely explain why, even though a higher level of IL-1Ra is found in OA articular tissue, there is a relative deficit of IL-1Ra to IL-1beta in this tissue. This in turn may cause the increased level of IL-1beta activity.

4. FUTURE THERAPEUTIC APPROACHES

A novel and interesting approach to controlling proinflammatory cytokine production and/or activity is the use of biological molecules possessing antiinflammatory properties. As such, recombinant human IL-4 (rhIL-4) has been tested *in vitro* on OA synovial tissue, and has been shown to suppress the synthesis of both IL-1beta and TNF-alpha in the same manner as low-dose dexamethasone (106). To date, of the antiinflammatory cytokines, only IL-10 is employed in clinical trials for the treatment of RA in humans. Results from IL-13 experimentation on human synovial membrane from OA patients (100) indicate it is potentially useful in the treatment of this disease. The capacity of IL-1Ra to reduce *in vitro* and *in vivo* cartilage degradation, MMP production and the progression of OA lesions (6,107) has elicited much attention concerning the use of this molecule in OA therapy, and more particularly in regard to gene therapy. Using the MFG retrovirus, the IL-1Ra gene has been successfully transferred into animal and human synovial cells using an *ex vivo* technique (108,109). One such study using the experimental dog model of OA showed *in vivo* that the progression of structural changes of OA was significantly reduced (107). It has also been demonstrated *in vitro* that the human IL-1Ra gene can be successfully transferred into chondrocytes using the Ad.RSV adenovirus, and that the resulting increase in production of IL-1Ra can protect the OA cartilage explants from degradation induced by IL-1 (109).

Although it is reasonable to focus therapeutic research on events responsible for initiating the catabolism cascade, benefits may also be realized by interrupting the general inflammatory process. One example of a potentially beneficial intervention would be to inhibit the activity of NO. Indeed, and as mentioned previously, findings regarding the action of NO on the biological functions of joint tissue suggest that controlling the production of this factor would have a potentially therapeutic value in OA. A recent study examining the *in vivo* effect of a selective inhibitor of iNOS on the progression of experimental OA showed that under prophylactic conditions, such an agent could reduce the progression of early lesions. In addition, the inhibition of NO production, correlated with a reduction of MMP activity in cartilage (93), was also associated with a reduced level of proinflammatory mediators (i.e. IL-1beta, PGE_2 and NO) in the synovial fluid, and a marked reduction in the volume of joint effusion (93).

5. PERSPECTIVE

Although the primary etiology of OA remains undetermined, it is now believed that cartilage integrity is maintained by a balance obtained from cytokine-driven anabolic and catabolic processes. An excess of proinflammatory cytokines is thought to be responsible for many clinical manifestations of OA. Studies examining the contribution of cytokines to the pathogenesis of arthritic disease, including OA, have focused mainly on two proinflammatory cytokines, IL-1beta and TNF-alpha. In OA, the specific causative for the pathological process has not been identified, but synovial inflammation at the clinical stage is now a well-documented phenomenon. Other cytokines having inflammatory properties could contribute to this pathological process; therefore, these cytokines may represent a therapeutic way to prevent the consequences of inflammation in OA.

The therapeutic agents currently available to treat OA do not appear to block the major catabolic pathways; consequently, different cytokine-related therapies are being considered. Elucidating the mechanisms of action/interaction of ambient cytokines (both pro- and antiinflammatory) in OA articular joint tissue cells may provide the future foundation for targeting critical pathways through which drugs will be able to effectively block the proinflammatory action. This control may directly and/or indirectly decrease the release of degradative factors and inhibit the sequelae of the pathological process. Trends indicate a move toward the employment of antiinflammatory molecules. This change in philosophy is largely a result of the increasing interaction between basic and clinical research, which has been instrumental in specifically identifying the catabolic pathways, as well as providing opportunities to define new therapeutic approaches.

Despite the explosive growth of knowledge, our understanding as to how the individual proinflammatory cytokine players are regulated and orchestrated remains incomplete. Determining the regulation of proinflammatory cytokine activity is therefore critical to understanding articular joint degeneration. Progress in identifying the diverse pathogenic factors will eventually lead to more selective targets, superseding the current treatments for OA.

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Send all correspondence to: Johanne Martel-Pelletier, Ph.D., Osteoarthritis Research Unit, CHUM Hôpital Notre-Dame, 1560 rue Sherbrooke est, Montréal, Québec, Canada H2L 4M1. Tel.:514-281-6000 ext. 6658, Fax: 514-896-4680, E-mail: jmartelpelletier@arthrolab.qc.ca

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