Pathophysiology of Osteoarthritis

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Abstract: *Osteoarthritis (OA) in horses is a chronic, degenerative process. Affected horses typically have clinical evidence of synovitis, varying degrees of lameness, and progressive loss of joint function. The inciting cause of OA remains unclear; however, factors such as repeated episodes of trauma, joint instability, synovitis–capsulitis, hypoxia and neovascularization, genetic predisposition, and obesity have been related to its development. The biochemical mediators that are synthesized in affected joints are of an inflammatory nature and include catabolic cytokines and enzymes that degrade cartilage and subchondral bone matrix. Although horses with OA can be recognized clinically and treated symptomatically, it is also important for clinicians to understand the cellular and molecular mechanisms involved in the pathologic process. A thorough understanding of the pathophysiology of the disease can aid clinicians in managing osteoarthritic patients.*

At a Glance

Osteoarthritis (OA), also known as *degenerative joint disease*, is the most common joint disease that affects humans,[1] horses,[2,3] and dogs.[4] OA is a chronic, degenerative process characterized by progressive cartilage deterioration, subchondral bone remodeling, loss of joint space, marginal osteophytosis, and loss of joint function.[1–5] Although the etiology of OA may differ across species or among individuals within a species, some components of the pathophysiology of the disease are consistent.[1,3,4]

Several theories have been proposed to explain the origin of this disease. Independent of the initiating cause, however, the development of OA is consistently associated with a cascade of biochemical events mediated by cytokines, proteolytic enzymes, and other proinflammatory substances (e.g., prostaglandins, leukotrienes, nitric oxide). These mediators are responsible for the pathologic features of the disease, which include osteolysis, subchondral bone sclerosis, osteophytosis, articular cartilage erosion, and synovial membrane thickening.[5–7] Irrespective of the initiating cause or initial point of injury, eventually all components of the joint become involved in the process. This article summarizes the relevant molecular aspects of the etiopathogenesis of OA and potential therapeutic molecular targets for controlling this disease.

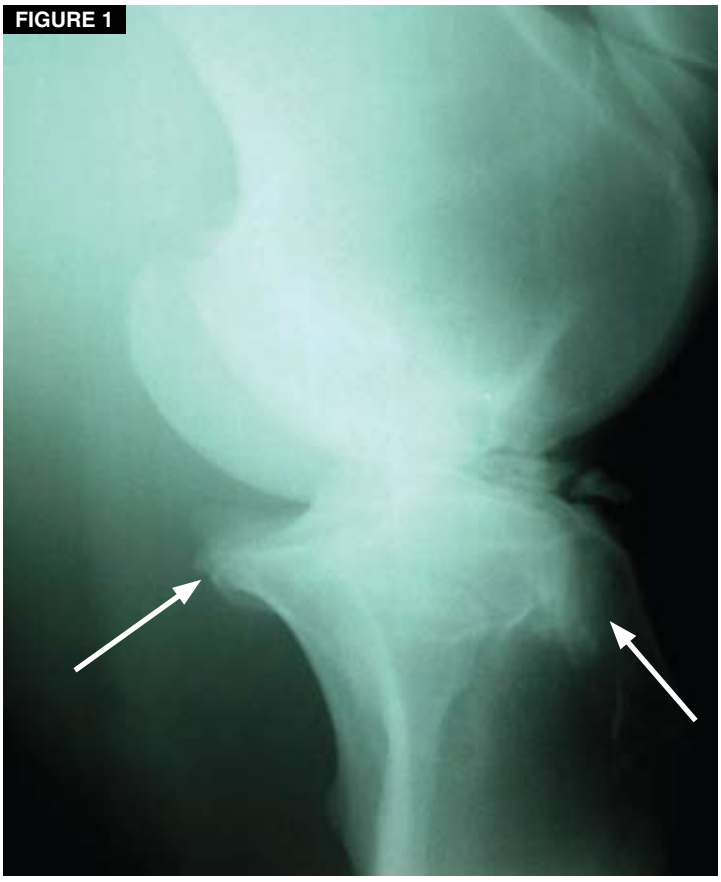
Pathogenic Mechanisms

Several pathogenic mechanisms have been proposed to be involved in the development of OA. They include subchondral bone overload, joint instability (loss of mechanical integrity), synovitis–capsulitis, hypoxia, body mass index as it relates to obesity, and heredity.

Subchondral Bone Overload (Mechanical Stress)

A typical finding in horses that exercise at speed is subchondral sclerosis of bone in joints subjected to high weight-bearing impact and shear forces (e.g., carpus and fetlock).[3,5] It has been postulated that articular overload, especially of subchondral bone, produces microtrauma, remodeling, hardening, and displacement of the osteochondral line.[5] These changes reduce the elasticity and energy-dissipation capacity of the articular cartilage during locomotion.[3] Furthermore, the injured tissue fails to heal because of the combined effects of high-impact exercise protocols, a lack of adequate warm-ups and post-exercise stretching, inadequate development of

FIGURE 1



Oblique 60˚, caudolateral–craniomedial radiographic projection of the left femorotibial joint of a 9-year-old intact Andalusian stallion with chronic joint instability due to ligamentous injury. Note the pronounced marginal osteophytosis on the proximal and caudal aspects (*arrows*) of the tibia.

proprioception, working musculoskeletal tissue while it is fatigued, poor neuromuscular training, and inadequate rest intervals.[8] The results of these forces are mechanical lesions that affect the joint tissue and its extracellular matrix (ECM),[3] which may account for the common finding of OA in fetlock joints of performance horses or in knee joints of human athletes.[5] However, OA also affects non–weight-bearing joints, such as those in the hands, spine, shoulders, and temporomandibular joints in humans and other mammals. Consequently, this theory does not completely explain the origin of these lesions, although either misalignment of articular surfaces or abnormalities of deep ligamentous components in the spinal and temporomandibular joints[9] may result in abnormal load distribution.

Joint Instability (Loss of Mechanical Integrity)

Joint instability can be due to increased ligament laxity, a tear or strain in a ligament (**FIGURE 1**), or poor conditioning of the muscles that affect the joints. An example of the latter is the increased incidence of OA in the knees of humans with poor development of their quadriceps muscles.[1] Training or racing can create episodes of increased joint laxity, especially when work is performed while an athlete is fatigued. It has been proposed that mechanoreceptors associated with joints lose their efficacy during fatigue, thereby impairing proprioceptive function and increasing the likelihood of injury.[10]

Joint instability can also occur as the result of intense synovitis that generates excessive amounts of synovial fluid.[1,3,5] It has been postulated that the increase in pressure within the joint may produce direct mechanical cartilage damage and anomalous overload forces of subchondral bone regions, thereby perpetuating synovitis.[3] In humans, subtle mechanical instability produced by partial traumatic transection of the cranial anterior ligament of the knee can produce OA changes 1 year after the traumatic event.[11] This may also occur in horses with posttraumatic arthritis with subtle mechanical impairment of soft tissue periarticular structures. Joint instability is an important cause of OA and should always be considered in affected patients, especially sport horses.

CriticalPoint

Osteoarthritis, also known as *degenerative joint disease*, is the most common joint disease that affects humans, horses, and dogs.

Osteoarthritis, a joint disease that affects a variety of species, is characterized by progressive loss of joint function, pain, cartilage destruction, subchondral bone remodeling, thickening of joint capsules, and increased synovial effusion.

Synovitis–Capsulitis

The synovitis (i.e., histologic evidence of synovial membrane inflammation) that occurs in horses with OA[2,3,7] can be a primary phenomenon, a consequence of joint trauma or articular overload, or an aftereffect of intraarticular drug injection or infection.[3,5,6] The cells that make up the synovial membrane are a rich source of several proinflammatory molecules that can incite and perpetuate articular deterioration if the underlying cause of the inflammation cannot be controlled.[1] Because the synovial membrane provides no mechanical protection to the joint, trauma or inflammation of adjacent structures (e.g., joint capsule, ligaments, muscles, tendons) could initiate synovitis and the subsequent development of OA.[3,5,6]

Hypoxia

A consistent finding during the development of OA is neovascularization, which initially involves the synovial membrane and subsequently the subchondral bone and cartilage.[6,7] Although this ingrowth of new vessels increases the delivery of nutrients to the stressed articular cartilage and subchondral bone,[1,6] it also contributes to the development of synovitis. In humans, hypoxia is a common component in the pathophysiology of OA and rheumatoid arthritis because the oxygen gradient across articular cartilage may be altered as a result of cartilage thinning and erosion, changes in ECM composition, and the development of cartilage fissures.[12]

In OA and rheumatoid arthritis, exaggerated expression and limited degradation of two nuclear hypoxia-inducible factors (α_1 and α_2) occur. The resulting increase in these factors promotes the expression of two angiogenic peptides called *vascular endothelial growth factor* and *platelet-derived cellular endothelial growth factor*. Both peptides increase neovascularization and promote vascular permeability in the joint tissue, resulting in edema, protein vascular leakage, inflammation, and cartilage damage.[6,12]

Body Mass Index/Leptin

Leptin, a cytokine produced by white adipocytes, regulates appetite, energy expenditure, and the activity of the physes and the metabolism of bone. Leptin also promotes cellular proliferation and increases the metabolic activity of chondrocytes.[9] It has recently been demonstrated that the plasma concentration of leptin is positively correlated with body mass index in humans with OA.[13] Furthermore, increased plasma concentration of leptin has been shown to correlate positively with the severity of articular damage in rats.[13] To date, there are no reported studies regarding the role of leptin in OA in horses. However, leptin is positively correlated with a high body mass index (obesity) in horses.[14]

Hereditary Osteoarthritis

In humans, OA occurs as a consequence of a genetic defect in collagen type-II (*Col-II*) assemblage, and there has been speculation regarding genetic mutations in other collagen-type codifying genes.[15] To date, there is no evidence of a genetic basis for the development of OA in horses.

Biochemical Events and Pathobiologic Consequences

At the molecular level, OA is the result of an imbalance between the peptides that promote the synthesis of components of the ECM of articular cartilage and those that induce remodeling of these components. It has been proposed that the overall health of a joint depends on adequate expression of several growth factors.[2,4,7] For example, transforming growth factor β (TGF-β)[16,17] and insulin-like growth factors (IGFs)[18–20] increase synthesis of the ECM. In contrast, cytokines such as interleukin-1 (IL-1) and tumor necrosis factor α (TNF-α) promote chemotaxis and degranulation of leukocytes and increased expression of additional proinflammatory mediators, including prostaglandin E_2 (PGE_2), leukotriene B_4 (LTB_4), bradykinin, and nitric oxide.[1–4]

CriticalPoint

At the molecular level, OA is the result of an imbalance between the peptides that promote the synthesis of components of the ECM of articular cartilage and those that induce remodeling of these components.

TABLE 1 Catabolic Cytokines Involved in Equine and Human Osteoarthritis

CYTOKINE	SPECIES	NATURAL SOURCE	STIMULUS	INHIBITION	MECHANISM OF ACTION
IL-1	Equine, human	Chondrocytes, synoviocytes, macrophages, lymphocytes, fibroblasts	Trauma, infection, TNF	IL-1ra, IL-4, IL-10, IL-11, IL-13, TGF-β, PGE_2	↑ MMPs, ↑ PGE_2, ↑ COX-2, ↑ NO, ↑ TNF, ↑ IL-6 and other catabolic cytokines, ↓ TIMPs, ↓ synthesis of ECM
IL-2	Human	Lymphocytes	IL-1, TNFs	IL-4, IL-10, IL-13, TGF-β, sIL-2R	↑ T and B lymphocyte proliferation, ↑ NK cellular activity, ↑ TNF, ↑ IFN-γ
IL-6	Equine, human	Chondrocytes, synoviocytes, macrophages, lymphocytes, fibroblasts	IL-1 (equine)	IL-4, IL-10, IL-13, sIL-6R	↑ Acute phase proteins, ↑ T and B lymphocyte and fibroblast proliferation, ↑ serine protease inhibitors
IL-8	Human	Macrophages, lymphocytes	IL-1	IL-4, IL-10, IL-13	↑ Neutrophil chemotaxis, ↑ neovascularization, ↑ free radicals
IL-12	Human	Macrophages	IL-1, TNFs	IL-10, IL-11	↑ IL-1, ↑ TNFs, ↑ IFN-γ, ↑ IL-18
IL-17	Human	T lymphocytes	IL-1, TNFs	—	↑ Osteoclast activity, ↑ PGE_2, ↑ NO, ↑ chemokines
IL-18	Human	Lymphocytes, synoviocytes	IL-1, TNFs	IL-10	↑ IL-1, ↑ TNF, ↑ IFN-γ, ↑ adhesion molecules
TNF-α	Equine, human	Chondrocytes, synoviocytes, macrophages, lymphocytes, fibroblasts	Trauma, infection, IL-1	IL-4, IL-10, IL-11, IL-13, TGF-β, sTNFRs	↑ PGE_2, ↑ COX-2, ↑ NO, ↑ IL-1 and other catabolic cytokines, ↓ TIMPs, ↓ synthesis of ECM

COX-2 = cyclooxygenase 2; *ECM* = extracellular matrix; *IFN-γ* = interferon γ; *IL* = interleukin; *IL-1ra* = IL-1 receptor antagonist; *MMPs* = matrix metalloproteinases; *NK* = natural killer; *NO* = nitric oxide; *PGE_2* = prostaglandin E_2; *sIL-2R* = IL-2 soluble receptor; *sTNFR* = TNF soluble receptor; *TGF-β* = transforming growth factor β; *TIMPs* = tissue inhibitors of metalloproteinases; *TNF* = tumor necrosis factor

IL-1 and TNF-α also increase the activity of several proteolytic enzymes that degrade articular cartilage, most notably the matrix metalloproteinases (MMPs).[21–23] Collectively, these substances perpetuate synovitis,[2,3,6] initiate articular cartilage damage,[1–5] and induce remodeling of subchondral bone.[2,8,9]

The pathogenesis of OA is orchestrated by a network of overlapping complex molecular mechanisms, resulting in damage to the tissue comprising the joint.[1–5] To facilitate the understanding of the most important molecules involved in these processes, we discuss them as either catabolic or anabolic molecules.

Catabolic Molecules

Degradation of articular cartilage in the osteoarthritic degenerative process is due to complex interactions and up-regulation of several catabolic molecules, as summarized in **TABLE 1**.[1–5] However, the exact mechanism that triggers the development of OA remains obscure.

Proinflammatory Cytokines

Interleukin 1

IL-1 is actually a family of three cytokines composed of two agonist peptides, IL-1α and IL-1β, and the IL-1 receptor antagonist protein (IL-1ra). The biologic effects of the two IL-1 agonist peptides are initiated by binding to a specific receptor.[24] Although this same receptor can bind IL-1ra with a similar affinity, it does so without initiating a biologic effect.[25] It has been proposed that IL-1β is one of the most important catabolic cytokines involved in OA.[1,2,6] The proform of IL-1β is converted inside the cell by IL-1–converting enzyme (also called *caspase 1)* to produce the active form of

IL-1β.[1] The active form of IL-1β then promotes expression of an important transcription factor called *nuclear factor* κ-β.[4] This factor moves into the nucleus, where it interacts with the promoter regions of several genes and participates in up-regulation of genes, including those that produce secondary proinflammatory peptides (e.g., IL-6, IL-8, IL-12), chemokines, LTB_4, PGE_2, MMPs, and nitric oxide.[1,4,21] IL-1β also inhibits the metabolic pathways in chondrocytes that are used to repair damaged ECM_2; releases proteoglycans from ECM into the synovial fluid[3]; inhibits collagen type II (*Col-II*), IX (*Col-IX*), and XI (*Col-XI*) synthesis; stimulates production of abnormal proteoglycan molecules; and down-regulates expression of the natural inhibitors of MMPs, called *tissue inhibitors of metalloproteinases* (TIMPs).[4,21,26–28]

While IL-1 has been considered the main stimulator of the degenerative joint disease process, recent information may refute this hypothesis. In an experimental model of OA that used knockout mice that lacked the IL-1 gene, lesions and evidence of accelerated cartilage catabolism developed in the lateral tibial plateau of stifle joints that had not undergone surgery.[29] Furthermore, synthesis of MMP-3 initially increased when human chondrocytes were cultured with alginate beads and IL-1, and then synthesis decreased.[30] In addition, recent evidence from studies using an in vitro model of equine cartilage degeneration[31] shows that catabolic cytokines (i.e., IL-1 and TNF-α) are solely responsible for initiation of focal cartilage degeneration in OA.[31] These findings suggest that IL-1 plays an important regulatory role in maintaining normal homeostasis in cartilage turnover but that its role in OA initiation and progression is perhaps not as critical as previously thought.

An important part of the research on OA has been IL-1β blockade. The use of gene therapy with the IL-1ra encoding gene has been evaluated in experimentally induced OA in horses. Although the results of this research were encouraging, the effects were transient (28 days), and synovial inflammation occurred as a secondary complication.[32] In a different approach, promising results have been obtained using an IL-1–converting enzyme inhibitor (pralnacasan) in an experimental model of OA in rats.[1]

Osteoarthritis is a multifactorial disease influenced by (1) mechanical overload during high-impact physical activity, joint instability, and repeated trauma and (2) metabolic processes associated with aging, body mass index related to obesity, and hereditary factors.

Tumor Necrosis Factor α

TNF-α is secreted by macrophages, chondrocytes, synoviocytes, and osteoclasts as a membrane-bound precursor (latent form) that is activated by a specific TNF-α–converting enzyme.[26–28] There is evidence that this enzyme is present in an increased concentration in humans with OA[27]; the same may be true in horses. TNF-α induces its biologic effects by interacting with two families of membrane receptors—TNF receptor types 1 and 2. There is convincing immunologic evidence for the presence of type 1 TNF receptors in the ECM of cartilage in osteoarthritic humans[27] and in synovial membrane and noncalcified cartilage in an endotoxin-induced model of synovitis/arthritis in horses.[28]

Although TNF-α seems to have catabolic effects similar to those of IL-1 on articular cartilage,[1–3,26] it appears that TNF-α plays a more important role in the pathophysiology of rheumatoid arthritis.[1,6,26] In fact, an important way of reducing the effects of TNF-α activity on articular cartilage in humans with rheumatoid arthritis has been administration of specific anti–TNF-α antibodies.[27] These antibodies have not been used in treating OA.

Extracellular Matrix–Degrading Enzymes

Several enzymes that degrade ECM are up-regulated by IL-1β and TNF-α. The principal

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TABLE 2 Enzymes Associated with Extracellular Matrix Degradation in Osteoarthritis

ENZYMATIC GROUP	ENZYME	INHIBITORS	CARTILAGE SUBSTRATES SUSCEPTIBLE TO DEGRADATION
Matrix metalloproteinases	**Collagenases** MMP-1, MMP-8, MMP-13	TIMPs, α_2-Mg	*Col-II* and *X*, aggrecan, link protein, denaturalized *Col-II* *Col-II*, aggrecan, link protein *Col-II, V, IX,* and *X*, aggrecan, fibronectin
	Gelatinases MMP-2 MMP-9	TIMPs, α_2-Mg	*Col-X* and *XI*, elastin, denaturalized *Col-II* *Col-X* and *XI*, aggrecan, decorin, elastin, procollagens, link protein
	Stromelysins MMP-3 MMP-10 MMP-11	TIMPs, α_2-Mg	*Col-IV, IX, X,* and *XI*, aggrecan, decorin, elastin, laminin, denaturalized *Col-II* procollagens, link protein (MMP-3 and 10) Proteoglycans, fibronectin, laminin, denaturalized *Col-II*
Serine proteases	Cathepsin G	α_1-PI, α_2-Mg	*Col-II*, aggrecan, elastin, denaturalized TIMPs
	Plasmin	α_2-Mg, α_2-AP	Active pro-MMPs
Aspartic proteases	Cathepsin D	α_2-Mg	Aggrecan, denaturalized *Col-II*
Cysteine proteases	Cathepsin B	Cystatins, α_2-Mg	Aggrecan, *Col-II*, procollagenase activity
	Cathepsin K	Cystatins, α_2-Mg	*Col-II*

α_2-AP = α_2 antiplasmin; *α_2-Mg* = α_2 macroglobulin; *α_1-PI* = α_1-plasminogen inhibitor; *Col-n* = collagen type n; *MMPs* = matrix metalloproteinases; *TIMPs* = tissue inhibitors of metalloproteinases

enzymes associated with degradation of ECM in cartilage are the MMPs, aggrecanases, serine proteases, aspartic proteases, and cysteine proteases. Whereas MMPs and serine proteases act at a neutral pH, aspartic proteases and cysteine proteases have greater activity at an acid pH.[1,2,6] Relevant details about these enzymes and their capability to degrade ECM substrates in cartilage are summarized in **TABLE 2**. An imbalance in the production of these enzymes, namely excessive and uncontrolled production, results in irreversible damage to joint tissues and self-perpetuation of the vicious cycle.

Metalloproteinases

MMPs belong to a zinc-dependent group of endopeptidases and can be secreted by synoviocytes, chondrocytes, macrophages, and neutrophils. Several members of this family, including collagenases (MMP-1, MMP-8, MMP-13), gelatinases (MMP-2, MMP-9), and stromelysins (MMP-3, MMP-10, MMP-11), are involved in the pathophysiology of OA.[21–23,33] These enzymes are secreted as inactive zymogens that are activated by enzymatic cleavage of their catalytic region, which contains the active zinc-binding site. The effects of the specific MMP depend on the activity levels of the enzymes and the presence of inhibitors such as TIMPs and α_2-macroglobulin.[2,3]

MMP-1 and MMP-13 play prominent roles in the development of OA.[7,21,33] MMP-1 is produced primarily by synovial cells that line the joints, and MMP-13 is a product of chondrocytes that reside in the cartilage. MMP-13 degrades the proteoglycan molecule aggrecan, giving it a dual role in matrix destruction.[22] Up-regulation of other MMPs, such as MMP-2, MMP-3, and MMP-9, is also increased in OA, and these enzymes degrade noncollagen matrix components in joints. Although there has been considerable effort to design compounds that effectively inhibit either the synthesis or activity of MMPs and thereby minimize connective tissue destruction within joints, these efforts have not been successful.[22]

Aggrecanases

Aggrecanases, which are also called *a disintegrin and metalloproteinase with thrombospondin motifs* (ADAMTS), include 19 members that are numbered ADAMTS 1 through 20; there is no ADAMTS-11 because early reports

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of it were later found to describe ADAMTS-5. Aggrecanase-1 (ADAMTS-4) and aggrecanase-2 (ADAMTS-5) are the proteolytic enzymes that appear to degrade aggrecans in cartilage in OA.[34] Aggrecanases cleave the aggrecan core protein and thus play an important role in the pathophysiology of OA. Synthesis of ADAMTS-4 and ADAMTS-5 in chondrocytes appears to be regulated by IL-1β, and there is convincing evidence that IL-1β down-regulates the synthesis of aggrecanase-1 under certain conditions.[1,4] In an experimental model of canine OA, it was observed that a dual inhibitor (licofelone) of the lipoxygenase and cyclooxygenase pathways also inhibited the synthesis of aggrecanases.[35] To our knowledge, this inhibitor has not been used in horses.

Serine Proteases

Plasminogen activator, bradykinin, plasmin, trypsin, cathepsin G, and elastase are important members of the serine protease family,[3] as they can directly cleave ECM molecules. However, the principal catabolic effect of these enzymes is to activate latent proteases, such as the pro-MMPs.[2] There is evidence that IL-1β may promote the action of these enzymes through PGE_2-mediated up-regulation of plasminogen activator.[3] Bradykinin is an important mediator of synovitis, and a specific bradykinin B_2-receptor antagonist has recently yielded encouraging results in treating OA in human knees.[1]

Eicosanoids

Eicosanoids, such as prostaglandins, thromboxanes, and leukotrienes, are metabolites of arachidonic acid that are produced by inflammatory cells, chondrocytes, and synoviocytes. These substances are present in inflamed joints, primarily as the possible result of up-regulation of cyclooxygenase-2 (COX-2) by catabolic cytokines.[1–4] PGE_2 has important effects in the inflammatory process because it promotes vascular dilation, reduces the threshold for painful stimuli, facilitates up-regulation of plasminogen activator, and promotes degradation of proteoglycans.[3,21] However, PGE_2 also has anti-inflammatory effects by up-regulating expression of antiinflammatory cytokines (**TABLE 3**) and down-regulating expression of catabolic cytokines and MMPs.[3] Therefore, it has been postulated that PGE_2 is a necessary component in controlling the inflammatory process.[36]

Leukotrienes, which are produced via the lipoxygenase pathway, cause vasodilation and chemotaxis. There is convincing evidence for the involvement of leukotrienes, specifically LTB_4, in the pathogenesis of arthritis. For example, the density of LTB_4 type II receptors is increased in synovial membranes of humans with rheumatoid arthritis,[37] suggesting that leukotrienes have an important role in the development of synovitis. Furthermore, a positive correlation has been identified between the number of leukocytes and the concentration of LTB_4 in synovial fluid in horses with joint disease.[38] Collectively, these findings would lend credence to studies comparing LTB_4 receptor density in synovial membranes in normal horses and in horses with OA as well as to studies designed to test the efficacy of specific lipoxygenase inhibitors or LTB_4 receptor antagonists in horses with OA.[4]

Inhibition of eicosanoid synthesis is a cornerstone in treating OA in humans and animals. NSAIDs and corticosteroids have been used for this purpose and produce symptomatic relief of pain and synovial effusion. However, neither form of treatment alters progression of the disease. It is well recognized that corticosteroids are potent antiinflammatories, but they also have effects on not only joints but also metabolism and immunologic responses at a systemic level. Furthermore, corticosteroids produce catabolism of articular cartilage, especially when they are administered repeatedly.

Nitric Oxide

Nitric oxide, an inflammatory mediator synthesized by several cell types in joints, diminishes the deposition of sulfate into glycosaminoglycan chains, reduces collagen synthesis, interferes with up-regulation of the IL-1 receptor antagonist,[7,39] decreases the activity of growth factors such as TGF-β and IGF-I, and has been postulated to be associated with aberrant apoptosis of chondrocytes in the pathogenesis of OA.[40,41] Stimulation of chondrocytes by either endotoxin or IL-1β activates the inducible form of nitric oxide synthase (iNOS) and its associated enzymes.[1] Although it does not appear that local iNOS expression plays a key role in the development of synovitis in horses,[39] positive correlations (that were not apparent in normal horses) have been demonstrated among artic-

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TABLE 3 Growth Factors and Antiinflammatory Cytokines Associated with Equine and Human Osteoarthritis

CYTOKINE	SPECIES	SOURCE IN OA	STIMULUS	MECHANISM OF ACTION
IGFs	Equine, human	Articular cartilage	ECM PG depletion, leptin	↑ ECM synthesis, ↑ catabolic cytokines, ↓ PG degradation
IL-1ra	Equine, human	Monocytes, synoviocytes	IL-4, IL-10, IL-13, TGF-β	Antagonize IL-1 catabolic effects
IL-4	Human	T lymphocytes	PGE_2	↓ IL-1, ↓ TNF, ↓ IL-8, ↓ IFN-γ
IL-10	Human	T lymphocytes	PGE_2	↑ Lymphocyte proliferation, ↑ immunoglobulin synthesis, ↑ IL-1ra, ↑ serine protease inhibitors, ↓ IL-1, ↓ TNF, ↓ IL-8, ↓ IFN-γ, ↓ PGE_2, ↓ NO, ↓ MMPs, ↓ PLA_2
IL-11	Human	—	PGE_2	↑ Acute phase proteins, ↓ IL-1, ↓ TNF, ↓ IL-12, ↓ IFN-γ
IL-13	Human	T lymphocytes	PGE_2	↑ B lymphocyte proliferation, ↑ IL-1ra, ↓ IL-1, ↓ TNF, ↓ IL-8, ↓ IFN-γ, ↓ PGE_2
IFN-γ	Human	T lymphocytes	IL-2, IL-12, IL-18	Immunoregulatory cytokine
Leptin	Human	Adipocytes	Obesity, PG depletion?	↑ TGF-β, ↑ IGFs
TGF-β	Equine, human	Articular cartilage	Leptin	↑ ECM synthesis, ↑ catabolic cytokines, ↓ PG degradation, ↑ imbalance in synovial membrane PG synthesis, ↑ osteophytes

ECM = extracellular matrix; *IFN-γ* = interferon γ; *IGF* = insulin-like growth factor; *IL* = interleukin; *MMPs* = matrix metalloproteinases; *NO* = nitric oxide; *PG* = proteoglycan; *PGE_2* = prostaglandin E_2; *PLA_2* = phospholipase A_2; *TGF-β* = transforming growth factor β; *TNF* = tumor necrosis factor

ular cartilage damage, chondrocyte apoptosis, and high immunoreactivity to nitrotyrosine (a protein that is closely associated with cellular production of nitric oxide) in osteoarthritic horses.[40] In addition to nitric oxide, other free radicals, including superoxide, peroxide, and hydroxyl, are produced as part of the inflammatory response within joints. In turn, these mediators can act on chondrocytes and synovial fibroblasts, modifying their biosynthesis of proteoglycans, collagen, and hyaluronan as well as promoting release of catabolic mediators.[3,6] The administration of nitric oxide synthase inhibitors in experimentally induced OA has resulted in reduction of synovial inflammation and destruction of cartilage and bone.[1]

Clinical Signs Associated with Osteoarthritis Catabolic Molecules

It has been postulated that horses with OA have different degrees of pain, synovial effusion, and functional impairment,[3] reflecting the effects of the aforementioned catabolic molecules and tissues that comprise joints.[3,6] Pain is generally manifested as lameness, which is the result of joint inflammation, exposure of subchondral bone, neovascularization and neoreinnervation, and increased osseous intramedullary pressure.[6] There is no correlation between the apparent degree of pain and the severity of articular lesions.[5] The primary network of nociceptors in joints (polymodal mechanoreceptors) is localized in the joint capsule, with receptor types I, II, and III predominating. In contrast, subchondral bone and synovial membrane have a more discrete distribution of type IV (unmyelinated endings) nociceptors, which play an important role in the perception of pain in patients with OA.[6,42]

Type IV nociceptors are stimulated by lactate, potassium ions, quinines, serotonin, PGE_2, and histamine.[6,43] These stimuli result in the production of several tachykinins[41] (e.g., substance P,[43] neurokinin A, neuropeptide Y), calcitonin gene-related peptide, vasoactive intestinal peptide, and other substances.[42,44,45] These substances stimulate the release of inflammatory mediators that perpetuate the inflammatory response, which is called *neurogenic inflammation*.[1,6,42–44] Substance P is the main neuropeptide related

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to inflammation of articular cartilage.[1,6,40] Substance P and other neuropeptides have been immunolocalized in the synovial subintimae of healthy and arthropathic horses.[43] Substance P intensifies articular catabolism and synovial inflammation because it causes up-regulation of IL-1, MMPs, and PGE_2.[42–44] Substance P also produces synovial hyperplasia, local vasodilation, and extravasation of leukocytes and protein in innervated areas.[43] The results of a study involving a canine model of OA suggest that antagonism of substance P with an analogue of the anticonvulsant gabapentin reduced synthesis of MMPs 1, 3, and 13 and iNOS without causing adverse systemic effects.[45] These findings suggest that substance P may be a viable therapeutic target in treating OA.

Joint effusion occurs as a consequence of synovitis; as blood flow increases, plasma proteins leak into the interstitium, and synovial fluid production increases.[6] Although moderate synovitis may have a positive effect on the nutrition of articular cartilage, severe synovial effusion adversely affects joint function.[5,6] Moreover, this degree of effusion can lead to fibrosis of the joint capsule, which, in turn, impairs joint function, thereby causing mechanical lameness.[3,5]

The end result of osteoarthritis is loss of joint homeostasis as the body attempts to repair damaged tissue. The reparative process becomes ineffective, and a self-perpetuating cycle results in structural modifications of joint tissue.

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Anabolic Molecules

During the development of OA, an array of growth factors and cytokines are produced to counteract the catabolic effects exerted primarily by IL-1β and MMPs.[1] Unfortunately, the effects of the catabolic molecules predominate, resulting in the development of severe OA. The principal peptide growth factors that have anabolic and antiinflammatory effects in joints are summarized in **TABLE 3**.

Growth Factors

Growth factors are multifunctional peptides that have anabolic and proliferative effects on chondrocytes and their surrounding ECM. Of the many growth factors identified in the pathophysiology of OA process, the most important appear to be the families of IGF and TGF-β.[6–20] It has been postulated that lesser known anabolic growth factors, such as platelet-derived growth factor and fibroblastic growth factor, could also be important in the disease.[2]

Insulin-like Growth Factors

IGFs are two molecules (IGF-I and IGF-II) that belong to the insulin family and are produced primarily by the liver; these factors are also synthesized by other cell types, including those in cartilage.[18–20] The primary reserve of IGFs is plasma. IGF-I is transported by six binding proteins that modulate its biologic action.[46] Although IGF-I is expressed in abundance in foal cartilage, its level of expression is diminished in older horses.[19] IGF-I promotes differentiation of fetal chondrocytes and maintenance of ECM synthesis. In adult cartilage, IGF-I antagonizes IL-1β and reduces catabolism of ECM.[2,19,20] With aging, the concentration of IGF-I required to maintain adequate synthesis of ECM increases dramatically.[47] Although the results of in vitro studies indicate that the supraphysiologic concentration of IGF-I does not affect either chondrocyte survival or the quality of the ECM, intraarticular injection of IGF-I promotes tissue repair.[2] This approach was tested in horses with experimentally induced cartilage defects, and the results indicated that intraarticular injection of IGF-I produced better evidence of cartilage repair than was seen in joints that were not injected with IGF-I.[48] Anabolic and mitogenic effects of IGF-II have been demonstrated in IL-1–conditioned equine cartilage, suggesting that this peptide may have positive effects in horses.[20] To date, however, no in vivo studies using IGF-II have been conducted in horses.

It is important to note that although the IGF-I concentration is increased in horses with naturally occurring OA,[49] expression of a specific IGF-binding protein that reduces

the activity of IGF-I is also increased.[50] It is possible that antagonism of this binding protein may provide an alternative therapeutic approach to the use of IGF-I alone.

Transforming Growth Factor β

Members of the TGF-β superfamily include TGF-β_1, TGF-β_2, TGF-β_3, and a variety of bone morphogenetic proteins.[2] It has been postulated that TGF-β has anabolic and proliferative effects on articular cartilage and antagonizes the catabolic effects of IL-1β; however, TGF-β is less potent than IGF-I or IGF-II in this regard.[18–20] There is conflicting information about the anabolic effects of TGF-β,[4,6] primarily because this peptide has been associated with disorders in ECM synthesis (e.g., imbalances in proteoglycan assemblage) and promotes the synthesis of fibromodulin over that of biglycan and decorin.[51] In addition, TGF-β promotes osteophyte formation in joints.[6] Although osteophytes can perpetuate the local inflammatory response, it has been postulated that they promote stability of joints.[6] Thus, TGF-β may have two roles in the pathophysiology of OA.[4,6]

Antiinflammatory Cytokines

Several antiinflammatory cytokines are produced as part of the inflammatory response and modulate the effects of catabolic cytokines and other inflammatory metabolites. The most important of these include IL-1ra, IL-4, IL-10, and IL-13[2,26,27] (**TABLE 3**). IL-1ra blocks IL-1 catabolic effects by coupling its membrane receptor. IL-4, IL-10, and IL-13 up-regulate IL-1ra expression. The use of an equine autologous conditioned serum rich in IL-1ra was evaluated in an equine OA model by Frisbie et al.[52] This treatment significantly improved clinical lameness and the histologic appearance of the synovial membrane of the treated horses compared with those of the placebo group.

Therapeutic Use of Growth Factors

Anabolic growth factors, specifically IGF-I and TGF-β_1, and certain antiinflammatory cytokines, most notably IL-1ra have been evaluated in several animal models of OA for their ability to induce regenerative changes in joints. These growth factors have been tested either by direct intraarticular injection of recombinant peptides or by gene therapy.[32] Although the results obtained with purified growth factors and gene therapy are promising, significant economic restrictions are likely to reduce their use in equine practice. Additional research is needed to determine optimal doses of growth factors, and gene therapy has been used only experimentally.

Platelet concentrates have been used as an autologous source of growth factors, primarily TGF-β_1 and IGF-I, in humans undergoing maxillofacial or orthopedic surgery.[53,54] This approach has been used in horses, with intraarticular injection of platelet concentrates being used to treat horses with severe joint disease.[55] The results of this study were encouraging, with the treated horses having evidence of reduced lameness and joint effusion for 8 months without additional therapies. As with other forms of treatment, larger clinical trials that include appropriate control treatments will need to be conducted to determine whether this treatment will be effective in horses with OA.

Conclusion

Appreciation of the molecular mechanisms involved in the pathophysiology of OA makes it easier to understand why many symptomatic approaches to the treatment of this disease have failed. It is important to note, however, that many of the relevant studies to date have been conducted using either in vitro systems or laboratory animal models. Because OA is common in horses, future equine studies may provide the best means of evaluating new treatments[7] that could be used in humans. There is considerably more to learn regarding the pathophysiology and treatment of OA. Biologic manipulation of cells, peptides, and genes directly related to this disease is providing exciting new ways to arrest the progression of OA and restore joint function.

CriticalPoint

Several antiinflammatory cytokines are produced as part of the inflammatory response and modulate the effects of catabolic cytokines and other inflammatory metabolites. The most important of these include IL-1ra, IL-4, IL-10, and IL-13.

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1. Which molecule is one of the most important catabolic cytokines implicated in the pathophysiology of OA?
a. TGF-β
b. IL-6
c. IL-1
d. IL-4

2. Which molecules stimulate production of MMPs in equine joint disease?
a. PGE_2 and nitric oxide
b. LTB_4 and cathepsin K
c. IL-6 and TGF-β
d. IL-1 and TNF-α

3. Which metabolite is associated with chondrocyte apoptosis?
a. nitric oxide
b. PGE_2
c. IL-6
d. cathepsin K

CE TEST CONTINUED ON PAGE 40.

CE TEST CONTINUED FROM PAGE 39.

4. Which peptide is associated with the development of osteophytes in OA?
- **a.** TNF-α
- **b.** IL-4
- **c.** lymphotoxin
- **d.** TGF-β

5. Which MMPs have collagenase activity?
- **a.** MMPs 1, 8, and 13
- **b.** MMPs 2 and 9
- **c.** MMPs 3, 10, and 11
- **d.** MMPs 2 and 13

6. Which of the following describes the main effect of serine proteases in OA?
- **a.** ECM degradation
- **b.** up-regulation of IL-1 expression
- **c.** increasing the effects of PGE_2
- **d.** pro-MMP activation

7. Which metabolite can down-regulate IL-1 and MMP expression?
- **a.** nitric oxide
- **b.** LTB_4
- **c.** PGE_2
- **d.** TNF-α

8. Which organ/tissue is the main producer of IGFs?
- **a.** the liver
- **b.** the kidneys
- **c.** the gut
- **d.** bone

9. Which growth factor could be associated with imbalanced proteoglycan synthesis in OA?
- **a.** IGF-I
- **b.** IGF-II
- **c.** TGF-β
- **d.** vascular endothelial growth factor

10. Which of the following describes the effects of leptin in OA?
- **a.** up-regulation of IL-1 expression
- **b.** diminishment of proteoglycan synthesis
- **c.** promotion of synovial neovascularization
- **d.** up-regulation of IGF-I and TGF-β expression