Felix Bronner
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Bone and Osteoarthritis

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Preface

Osteoarthritis (OA) is a progressive and debilitating disease that affects some two thirds of people older than 65 years. Yet how the disease arises and what cellular and molecular changes the cartilage cells undergo in the course of the disease is not well understood. In recent years, bone and bone changes have been invoked as causing or contributing to cartilage destruction. Moreover, clinical care of osteoarthritic patients often falls on orthopaedic surgeons, as when an arthritic hip needs replacement. It therefore seemed logical for the series *Topics in Bone Biology* to devote a volume to this disease and its relationship to bone and bone metabolism, particularly because many matrix molecules and signaling pathways are common to both cartilage and bone.

In Chapter 1, Roach and Tilley discuss the pathobiology of OA, list the risk factors (aging, loading, genetics) and describe the clinical features (pain being the most prominent), the diagnostic procedures, and the changes in cartilage that occur during the disease process. They also refer to the role of subchondral bone, a topic more prominently discussed in Chapter 2. Roach and Tilley illustrate the microscopic changes that articular cartilage undergoes, including the Mankin scale for evaluating these changes, discuss apoptosis and necrosis of the cartilage cells, and then outline what can be done about the disease (risk factors, medication, surgical intervention). This chapter, like all the others, has an extensive list of references and full color illustrations.

In Chapter 2, Lajeunesse and Reboul discuss the role of bone in the development of OA. They point out that chondrocytes function under largely hypoxic conditions and that the response to severe hypoxia initiates angiogenesis, with the vessels that penetrate cartilage deriving from subchondral bone. Disease initiation and progression is characterized by dedifferentiation of the cartilage cells and changes in bone tissue that involve both osteoclasts and osteoblasts. Osseous outgrowths, termed osteophytes, occur at the margin of cartilage and bone, and may contribute to joint dysfunction and immobility. The authors hypothesize that some macrophages from the inflamed synovial membrane release growth factors that ultimately lead to osteophyte formation. Lajeunesse and Reboul then proceed to discuss risk factors, relating them to bone metabolism, review possible interaction and crosstalk between bone and cartilage, and conclude that "changes in subchondral bone and . . . [in] osteoblast metabolism are key in the initiation and progression of OA."

In Chapter 3, Goldring discusses the anabolic and catabolic roles of cytokines, growth factors, and bone-derived molecules that modulate cartilage cell behavior. When adult chondrocytes, which maintain cartilage structure and function, undergo phenotypic change to

become "abnormal," dysregulated signaling contributes to further disease progression. Topics discussed by Goldring include matrix destruction and synthesis in OA, mouse models of the disease, and detailed analyses of the role of such factors as the inflammatory cytokines, such as interleukins, and the chemokines. Growth and differentiation in OA are affected by the insulin-like growth factor, the transforming growth factors, the bone morphogenetic factors, and the fibroblast growth factors, which are discussed in detail, as are their interaction and influence on cartilage homeostasis and expression of matrix genes. The chapter concludes with a discussion of subchondral bone factors that may bring about abnormal chondrocyte responses.

In Chapter 4, Blom and van den Berg analyze the role of the synovium in OA, a tissue that has only recently been identified as having a role in the osteoarthritic process. Normally the synovium controls the environment within the joint by acting as gateway for substances that enter or leave the joint and by synthesizing hyaluronan and lubricin, two compounds that give the joint fluid its mechanical properties. Synovial macrophages, overexpressed in inflammation, induce osteophytes and thus contribute to joint dysfunction. Synovial macrophages also appear to induce cartilage changes. These changes are discussed in detail. Blom and van den Berg then proceed to analyze the catabolic and anabolic roles of cytokines in the osteoarthritic synovium and discuss possible gene therapy, inasmuch as OA is often local, expressed in only one or two sites in the body. Targeting and silencing the synovial macrophage may become one approach to treating some types of OA.

A characteristic aspect of osteoarthritis is the degradation of the normal cartilage resulting from a change in matrix composition involving collagen and other matrix molecules. As described in detail by Heinegård in Chapter 5, the matrix is indeed a network, with collagen molecules serving as cross-bridges, and when these are broken, different molecular domains are exposed, leading to different, often abnormal, binding, and providing sites that may now be accessible to degradative enzymes. To be sure, degradation is followed by increased synthesis of the degraded constituents, but this repair may be inadequate, leading to a decrease in joint strength and ultimately to the pathologic changes that characterize osteoarthritis. Heinegard discusses the major molecules involved, the various collagens, aggrecans, thrombospondins, biglycan, and decorin, and has included figures that indicate the spatial relationships and interactions of the various molecules.

Interestingly, chondrocytes that reside in diseased cartilage are metabolically hyperactive. In discussing anabolic mediators that may play a role in raising cartilage metabolism, Fukui and Sandell, in Chapter 6, point out that increased anabolism of cartilage cells may prevent disease progress, but, by inducing "abnormal" proteins, may also accelerate the progress of the disease. The authors describe and discuss the various anabolic molecules, for example, aggrecan and collagens II, IX, and XI. They then analyze the significance of hyperanabolism in terms of possible degradative and repair mechanisms, calling attention to the fact that enhanced metabolism simply may constitute a reparative response to matrix damage. There follows a discussion of various factors that may be involved in the hyperanabolic response, calling attention to, among others, leptin, a hormone that only recently has been identified as also involved in bone metabolism. The authors conclude by raising the question, crucial to an understanding of the disease, at what time point the normal repair

process involving removal of damaged tissue and its replacement changes to the hyperanabolic state, that is, overproduction of proteins such as aggrecan or the matrix metalloproteinases.

In Chapter 7, Shapiro, Adams, Srinivas, and Freeman discuss the hypertrophy of the cartilage cell—a feature characteristic of OA—and its ultimate death. They point out that cell volume increases in response to changes in the extracellular matrix and that changes in cell volume lead to changes in the osmotic pressure of the cartilage territorial matrix fluid. In turn, the membrane of the cartilage cell changes in response sensitivity, and the cell hypertrophies and ultimately dies. After a detailed description of the events involved in cell hypertrophy and death, the authors advance two hypotheses, one of which relates to hypertrophy, stating that the late-stage cells are maintained in a catabolic state they term *autophagy,* with cell death following. The number of cells is thus reduced. Autophagy and related processes are thought to be regulated by a signaling molecule, mTOR. The second hypothesis is that initiation of the phenotypic changes of the cartilage cell that lead to the disease is the result of a variant of ischemia reperfusion, that is, a pathologic increase in joint vascularity.

All diseases seem to have a genetic basis, sometimes the result of a single mutation, more often involving several genes, with even that gene combination accounting for only a portion of disease susceptibility and incidence. In the case of OA, Chapman and Roach, in Chapter 8, first discuss the predisposing genes, reviewing family and twin studies. They then proceed to analyze the various candidate genes, for bone density and mass and for effects on the extracellular matrix, and describe the results of linkage scans, focused in particular on chromosome 2. The second portion of the chapter deals with epigenetic changes, that is, heritable changes that do not alter the DNA sequence. These arise when genes, not normally expressed by a given cell, are "unsilenced," as by demethylation, leading to the expression of "abnormal" proteins, for example, aggrecan or the matrix metalloproteins. Epigenetic changes are increasingly recognized as of importance for development and as involved in many chronic diseases, and their role in OA may also have significance for therapy.

Animal models are important for the study of human disease, with OA no exception. Whether the disease occurs in an animal species spontaneously or is induced, its progress, because it is so much faster than in the longer living human, can be studied more effectively and more readily subjected to therapeutic trials. Bendele, in Chapter 9, discusses the various animal models that have been utilized to study OA, the use of partial meniscectomy and transection of the anterior crucial ligament in dogs, unilateral medial meniscal tear in older rats, intraarticular injection of iodoacetate in rats, as well as spontaneous and induced arthritis in mice. Throughout, the text emphasizes the need to match expectation of what may be achieved with the model or lesion that has been selected before testing pharmacologic agents. Guinea pig and rabbit models of OA are discussed in detail. The chapter closes with a discussion on the differences between animal models and the human disease and, as also stressed throughout the chapter, by emphasizing both the limitations and advantages of animal models in the search for therapeutic approaches.

How the mechanical aspects of joint function are modulated by molecular signals emitted by the articular cartilage and subchondral bone, and how joint injury and OA affect these mechanical functions is discussed by Chai, Stevens, and Grodzinsky in Chapter 10. After briefly discussing how the structure of the extracellular matrix of cartilage is designed to

withstand dynamic forces, the authors describe the clinical features of the disease and then proceed to elaborate on the various types of loads applied to the joint and how these can be tested in vitro. They discuss confined and unconfined loading, and point out the need for clearly defining the rate of loading, the peak stress, and final strain, as these define the threshold of tissue damage. The chapter then discusses the effect of mechanical loading and injury on chondrocyte apoptosis and necrosis, on gene expression, and how mechanoresponsiveness is compromised by mechanical injury. The authors conclude by pointing to new research and therapeutic directions that may emerge from proteomic analysis of tissue inflammation and matrix degradation.

In Chapter 11, Bingham discusses medications used to treat the signs of symptoms of OA, with emphasis on the diminution of pain. Because the efficacy of analgesics is limited and arthroplasty has high cost and not insignificant mortality and morbidity, it is important to have and develop drugs that slow disease progression. Bisphosphonates are one class of drugs that are coming into use with OA, largely because they have proved effective in osteoporosis and in Paget's disease and because these compounds act on subchondral bone. Bingham then discusses other inhibitors of osteoclasts such as interleukin-1, diacerein, as well as inhibitors of interleukin-1. The chapter concludes with an analysis of the role matrix metalloproteinase and cathepsin inhibitors can play in treatment of the disease.

Developing this book entailed bringing together bone and rheumatology specialists and encouraging a common language. We thank the authors for making this book possible and for their informative and timely contributions. Special thanks go to Dr. H.I. Roach, not only for making available information on the most current research on OA and thus guiding the editors, but also for her willingness to coauthor two chapters. Springer-UK, the publishers of this series, have, as in the past, produced a handsome book, and, by making it possible to publish color illustrations, have markedly enhanced the usefulness of this volume.

M.C.F.-C. wishes to dedicate this volume to her friend and childhood mentor, Mrs. Kathryn W. Moore, who passed away in 2005 after decades of enduring the constant pain of osteoarthritis with her characteristic cheer and dignity.

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1.

The Pathogenesis of Osteoarthritis

Helmtrud I. Roach and Simon Tilley

1.1 Introduction: What is Osteoarthritis?

Osteoarthritis (OA) is defined by the American College of Rheumatology as a "heterogeneous group of conditions that lead to joint symptoms and signs which are associated with defective integrity of articular cartilage, in addition to related changes in the underlying bone at the joint margins." Osteoarthritis is usually classified as *primary* or *idiopathic* when there is no obvious predisposing cause, and *secondary* when there is some clearly defined predisposing pathology [6]. Idiopathic OA is the most common form of arthritis and is a debilitating progressive disease that affects 60% of men and 70% of women over the age of 65 [79] with enormous socioeconomic costs, rivaling those of ischemic heart disease. As the Baby Boomers reach middle age and obesity is on the increase in the general population, OA will have an even greater impact on society in the future. Primary OA is a frustrating disease for both patient and clinician, because neither cause nor cure is known and, once started, the disease cannot be halted, even though individual rates of joint degradation can vary considerably [13]. Understanding of the various aspects of the disease has advanced sufficiently, however, to give rise to cautious optimism that other treatments beside joint replacement may become available. This chapter provides an introduction to the pathogenesis of OA, concentrating on primary or idiopathic OA.

1.2 Risk Factors for Osteoarthritis

1.2.1 Aging

Osteoarthritis used to be considered an inevitable consequence of old age due to "wear and tear." Indeed, aging is a major risk factor for both OA and osteoporosis; however, neither disease is a necessary outcome of aging. Daily use actually preserves rather than "wears out" articular cartilage and inadequate use is the commonest cause of cartilage degeneration, as noted by Harrison et al [37] more than 50 years ago. It is of particular interest that OA and osteoporosis are almost mutually exclusive: people who develop the former do not generally suffer from osteoporosis and vice versa [21]. Part of the reason may be anthropometric differences: OA patients generally have a stronger body build with wider geometrical bone measurements, increased bone mineral density (BMD) and a higher peak bone mass. Their greater bone mass may explain why OA patients have no or a very low incidence of osteoporotic fragility fractures. On the other hand, it is not clear why osteoporotic patients maintain full-thickness, fully functional articular cartilage into their eighties or nineties. It is possible that OA patients, as compared to patients with osteoporosis, have a higher rate of cellular senescence; that is, their cartilage cells do not replicate, repair, or maintain their matrix as well [54,100].

1.2.2 Abnormal Loading

The normal joint is well adapted to withstand physiologic loads, but abnormal loading can increase the risk of OA. For example, trauma, heavy manual labor, and obesity all carry an increased risk of OA. Workers in certain occupations, such as coal miners, dockyard workers, and farmers have an increased risk of hip and knee OA [61]. Similarly, in obese men and women, with a body mass index of 30 to 35, the risk of developing arthritic knees increases approximately fourfold [61]. Physiologically normal loads applied to a pathologically impaired joint will also lead to OA, as will subluxation, malalignment of the joint, and crystal deposition diseases. Former soccer or football players have a high prevalence of knee OA; this has been attributed to the high incidence of meniscectomy and cruciate ligament injuries [61]. Such injuries can lead to instability or sublaxation of the joint, along with abnormal impact and load distribution. Sublaxation also results if the collagen fibrils in tendons or ligaments are structurally altered, as is the case when there is a lack of molecules for fibril assembly. This is the case in biglycan/fibromodulin deficient mice, in whom absence of these small proteoglycans hinders collagen assembly. As a result, tendon stiffness is reduced, leading to subluxation and decreased joint flexibility. The mice develop gait impairment, as well as severe early OA [7]. It is not known, however, whether or to what extent abnormalities in these small proteoglycans contribute to human OA.

1.2.3 Genetic Factors

It has been known for some time that osteoarthritis runs in families, but to what extent this is due to shared genetic factors or to family environment is still uncertain. In recent years, there has been a lot of interest in searching for the genetic basis of OA. The disease is clearly multifactorial and polygenetic; that is, it results from the interaction of a number of genes. This fact and the late onset of the disease make linkage studies and identification of susceptibility genes very difficult. Genes coding for the extracellular matrix proteins of cartilage have been obvious candidates. A defect in type II collagen has been demonstrated in a Finnish family with a very early onset of OA [96], but this is not a general defect in OA [10,50]. Moreover, the disease in the Finnish family was probably secondary to mild skeletal dysplasia. Indeed, OA forms part of several inherited chondrodysplasias, conditions in which mutations in collagen genes have been identified (reviewed in [71]). In recent years, the search for genetic susceptibility genes has turned to study genes whose expression is involved in cartilage development and homeostasis. An example is the gene for secreted frizzled-related protein 3, involved in the *wnt* signaling pathway. Other examples are the genes for asporin or calmodulin 1, molecules involved in maintaining cartilage matrix. For further discussion, see Chapter 8.

1.3 Clinical Features of Osteoarthritis

Pain is the most common presentation of an osteoarthritic joint. The nature of the pain is often described as dull and ill defined; this is especially true for hip disease. Pain is exacerbated by joint use and relieved by rest. In advanced cases, however, pain also persists at rest and at night, because the protective muscle splinting mechanism around the joint has been lost. Joint pain is typically accompanied by morning stiffness and generally lasts less than an hour. The phenomena of "start-up" pain are commonly described by patients in the early stages of the disease, as is the transient stiffness due to "articular gelling." The latter usually only lasts for a few flexion-extension cycles and is especially prevalent in lower limb disease in the elderly.

The origin of pain is poorly understood. Hyaline cartilage lacks nociceptors, but neighboring structures do possess them. Pain from articular cartilage lesions results from mechanical irritation of loose flaps of cartilage, from synovial and capsular inflammation, and from subchondral bone sclerosis that acts on the periarticular nerve endings. The stimuli causing pain are related to, but fundamentally different from, those producing cartilage loss [29].

As the disease progresses, the patient notices a decreased range of motion due to joint space incongruity, muscle spasm and contracture,

capsular shrinkage, and mechanical block that results from osteophytes or loose bodies [79]. Certain signs that point to the involvement of specific joints are not pathognomonic, but help to confirm the diagnosis. These include varus or bow-legged knees due to medial compartment collapse. The presence of bone spurs that form on the dorsal aspect of the distal interphalangeal joints of the fingers, known as Heberden's nodes, are also commonly seen in patients with established OA. On examination, patients with classic disease often demonstrate localized tenderness along the joint line most severely affected by the degeneration. This is especially true of the medial joint line in knee OA. There is often no demonstrable effusion, or increased local temperature. Osteophytes may be palpable around the affected hand, knee, foot, and ankle joints.

1.3.1 Macroscopic Changes in the Joints

The loss of cartilage from the weight bearing or previously traumatized area is usually clearly seen in osteoarthritic joints (Fig. 1.1). This loss is often associated with collapse of neighboring subchondral bone, resulting in a deformed irregular articular surface. Cystic areas due to synovial intrusion into bone are a common finding, especially in the subchondral region of the acetabulum. These areas need to be enucleated prior to cementing the acetabular component. Similarly, the dense subchondral sclerotic areas in the acetabular and tibial pleateau regions need to be aggressively reamed, cut, and drilled to provide a good grip for prosthesis cementation. The removal of the largely avascular sclerotic areas is even more important when uncemented, hydroxyapatite-coated prostheses are used. These are inserted in a tight press-fit fashion and rely on a well-vascularized bone interface to allow porous ingrowth into the component. Osteophytes produced by revascularization of the remaining cartilage and synovial stimulation are located at capsular or tendinous insertions exposed to chronic stretch [42]. Osteophytes are produced in the non–weight-bearing areas of the joint and may be so extensive that movement is restricted. They are relatively softer than the native bone and need to be aggressively trimmed back when the correct size of femoral resurfacing or knee components is being determined. Loose bodies due to fragmentation of the osteochondral surface may also cause painful locking of joints. These can be large, solitary or multiple, free floating or tethered, and are commonly removed at arthroscopy to induce symptomatic relief in the course of arthroplasty.

Osteoarthritis in its advanced stages causes significant destruction and distortion of the capsular ligaments. These in turn lead to deformity, such as flexion contractures seen in the hip and knee, and to malalignment. Correction of this soft tissue imbalance is often the most technically demanding aspect of arthroplasty.

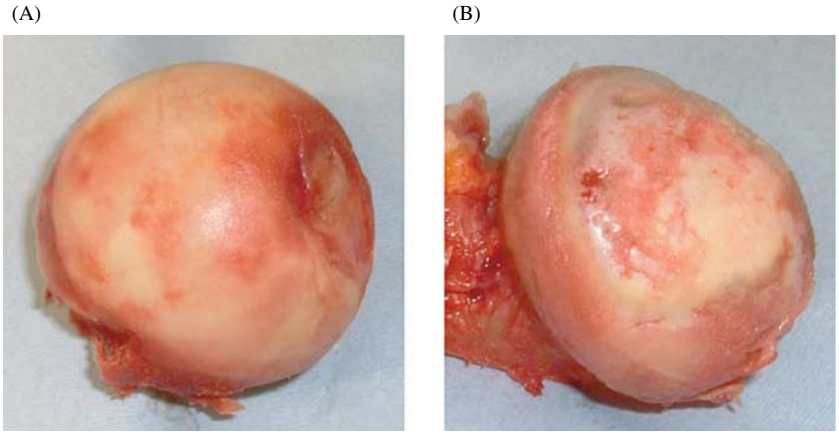

Figure 1.1. Femoral heads, obtained after joint replacement surgery, illustrating the cartilage degradation that occurs during osteoarthritis (OA). (A) Femoral head from a 79-year-old patient with a femoral neck fracture. The cartilage is smooth with no evidence of deterioration. (B) The femoral head from a 69-year-old osteoarthritic patient. Subchondral bone is visible near the weight-bearing regions.

1.4 Diagnostic Procedures for Detecting Osteoarthritis

An accurate history and a thorough examination must precede special tests that are to confirm the diagnosis of OA. Currently there is no single reliable diagnostic test; thus a stepwise approach is employed. In straightforward symptomatic cases, plain radiography is often all that is necessary to confirm the diagnosis. If the process is thought to be linked to crystal deposition disease or secondary to an inflammatory arthropathy, a full workup including urate levels, full blood count, erythrocyte sedimentation rate, C-reactive protein, rheumatoid factor, and autoantibody screen may be indicated.

Table 1.1. Kellgren and Lawrence radiographic scale for osteoarthritis [45]

0	No features of osteoarthritis
1	Doubtful; minute osteophyte of doubtful significance
2	Minimal; definite osteophyte but joint space unimpaired
3	Moderate; moderate diminution of joint space
4	Severe; joint space severely impaired with sclerosis of subchondral bone

Plain radiography may be unremarkable in the early stages, but joints exhibiting classic disease demonstrate characteristic features, as noted by Kellgren and Lawrence [45], who encouraged the classification of OA solely on radiologic grounds (Table 1.1). Alternative grading systems have evolved since the

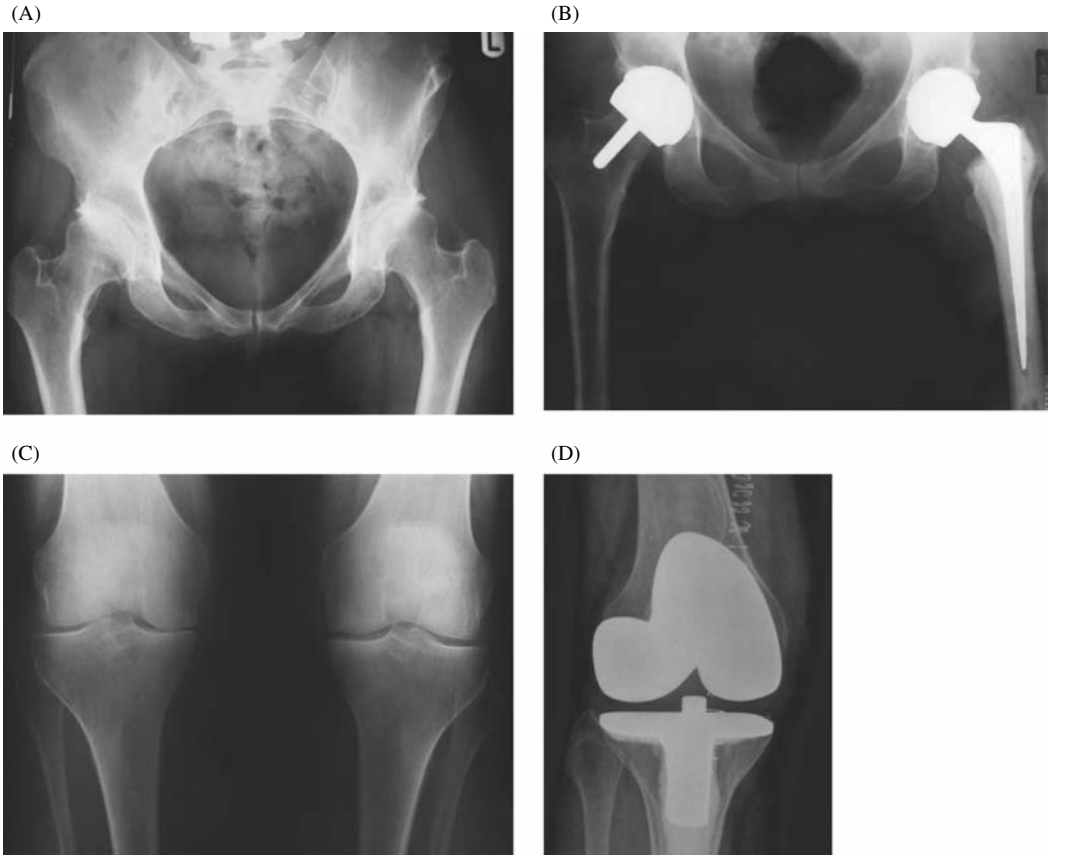

Figure 1.2. (A) Anteroposterior (AP) view of pelvis demonstrating bilateral osteoarthritic changes in both hips. (B) Postoperative radiograph shows a hip resurfacing on the right side and a total hip arthroplasty on the left side. Both acetabular components are uncemented using a hyaluronic acid (HA)-coated shell, and both femoral components are cemented. (C) AP weight-bearing view of patient's knees. The right knee (left on image) has signs of moderate OA with loss of medial joint space and osteophyte formation. (D) The right knee after arthroplasty.

1950s, but physicians and surgeons still regard the radiographic presence of osteophytes, loss of joint space, subchondral sclerosis, and cyst formation, combined with a suggestive history, as diagnostic of OA; see Fig. 1.2 for examples pre- and postoperatively.

Magnetic resonance imaging (MRI) is playing an increasingly important role in the diagnosis of early OA [44,49,60,90]. In addition to quantifying the extent of the disease, MRI is useful in discriminating between OA and non-OA pain in or around joints that cannot be clinically differentiated. For example, the pain of transient osteoporosis in the distal femur/proximal tibia or hip presents like OA of the knee and hip and cannot usually be seen on plain radiographs. Patients with that type of pain do not benefit from arthroscopy or intraarticular joint injection.

Bone scans are of limited value in the diagnosis of OA. Their use tends to be restricted to the elimination of more sinister pathologies, such as primary or secondary malignancy and osteomyelitis. Diagnostic hip injection using a mixture of a local anesthetic and steroid and done under fluoroscopic guidance is commonly performed in patients who complain of nonclassic hip pain or have concurrent back pain. If the patient reports a significant improvement following the injection, the pathology probably has originated in the hip.

Arthroscopy of osteoarthritic joints is a useful diagnostic and increasingly therapeutic aid to the surgeon. Most major joints that are affected by the disease are now within anatomic reach of modern arthroscopic equipment. Chondral changes are graded according to the modified Outerbridge classification (Table 1.2). The original description referred only to radiographic changes [64] of chondromalacia patellae. The modified classifications represent similar grading scales, but rely on arthroscopic observations. They describe the spectrum of change from chondral softening on probing to grade 4 changes, representing OA.

Table 1.2. Modified Outerbridge classification of chondral damage

0	Normal cartilage
1	Articular cartilage softening
2	Chondral fissures or fibrillation $<=$1.25 cm in diameter
3	Chondral fibrillation $>$1.25 cm in diameter (crabmeat changes)
4	Exposed subchondral bone

1.5 Pathobiology of Osteoarthritis

The key functional feature of OA is that the articular cartilage can no longer act as a shock absorber because the extracellular matrix has been destroyed. This does not mean that other joint tissues, such as the subchondral bone, the synovial capsule, and the membrane (Fig. 1.3), are not also involved in the disease process or in causing the pain associated with the disease. Nonetheless, it is the articular cartilage that is central for the initiation and progression of OA, inasmuch as resistance to loading is mostly due to the mechanical properties of the articular cartilage. These properties, in turn, are due to the composition of the extracellular matrix. Degradation of matrix components corresponds to failure of the cartilage to withstand cyclic loading, which, in turn, accelerates further degradation in the load-bearing regions.

1.5.1 The Role of the Subchondral Bone

The subchondral bone plate thickens and changes in OA. The bone becomes sclerotic and brittle and yet is undermineralized, with higher turnover resulting in bone of inferior quality (see recent reviews in [9,14,15,47,48]. It is still a matter for debate, however, whether the subchondral bone changes occur at the same time as changes in articular cartilage, and thus are causative, or are the consequence of cartilage degradation. Because bone adapts to changes in mechanical forces (Wolff's law), subchondral stiffening could be due to normal bone adaptation [20], because loss of articular cartilage would mean that an increased load is transmitted to bone. On the other hand, several researchers [15,47,48] have suggested that bone sclerosis precedes cartilage degradation and that enhanced bone remodeling by abnormal OA osteoblasts is the initiating event that triggers cartilage damage. Evidence in support of that sequence of events is based on in vitro

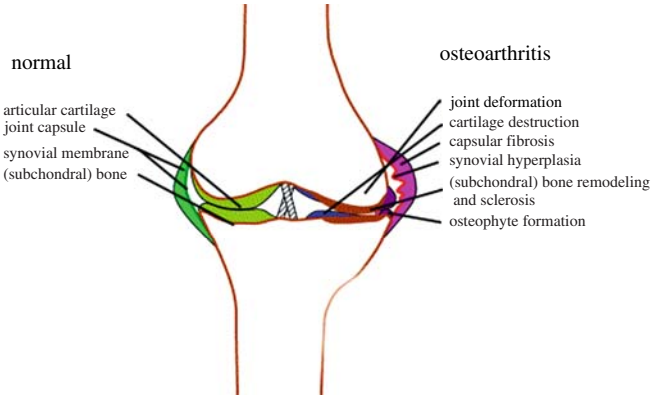

Figure 1.3. Overview of the pathologic changes associated with OA. Apart from cartilage destruction, fibrosis of the synovial capsule, hyperplasia of the synovial membrane, subchondral bone remodeling, and osteophyte formation contribute to the disease. (Adapted from Aigner and Stove [4].)

studies of osteoblasts or of isolated chondrocytes [76]. It is not clear how factors released from osteoblasts can act on chondrocytes in vivo, inasmuch as the mineralized bone matrix constitutes a barrier to diffusion. Microdamage or microfracture could initiate vascular invasion [15,81], but healthy articular cartilage contains antiangiogenic factors [87], is resistant to vascular invasion, and is likely capable of repelling any invasion from the subchondral bone. However, OA cartilage has lost its antiangiogenic factors [39] and its resistance to vascular invasion [30,84]. This suggests that changes in the cartilage itself permit vascular invasion to take place. On balance, although the evidence for an association between OA and changes in osteoblasts and the subchondral matrix is strong, the inference that these are causes of OA is as yet controversial (for further details, see Chapter 2).

1.5.2 The Synovial Membrane and Inflammation

Although OA is commonly described as a noninflammatory disease and, strictly speaking, should be termed "osteoarthrosis," inflammation does play a role. In healthy individuals, the joint space is filled with synovial fluid that contains abundant hyaluronic acid (HA) acting as lubricant. In OA patients, hyaluronan is smaller in size, diminished in concentration, and provides less efficient lubrication, and the joint space narrows. This decrease in joint lubrication can be remedied to some extent by intraarticular viscosupplementation [91]. Synovitis (inflammation of the synovial membrane) can either be the result of an acute inflammatory "flare" or of a chronic, but subclinical inflammation. Synovitis may be the primary event, that is, to initiate or propagate OA, or it may be a secondary result, perhaps due to the accumulation of cartilage breakdown products in the joint space (reviewed in [12,66]). Inflammation contributes to the symptoms of OA in the form of joint swelling, effusion, and stiffness; the release of inflammatory cytokines, in particular interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α), factors known to induce production of proteolytic enzymes by articular chondrocytes [31,36,66].

1.5.3 Structure–Function Relationship in Articular Cartilage

To understand cartilage degradation, one must understand the structure–function relationships of this tissue. Human articular cartilage consists of a hydrated extracellular matrix in which a comparatively small number of chondrocytes is embedded (<3% of total tissue volume; Fig. 1.4). The extracellular matrix is the functional element of the tissue, and all its components are required for the articular cartilage to act as a shock absorber. However, it is the chondrocytes that are the "active players" in cartilage [4], inasmuch as they

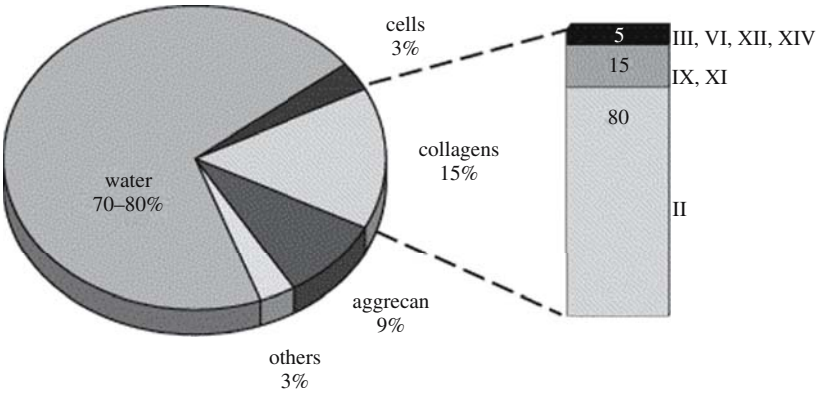

Figure 1.4. The molecular composition of articular cartilage. Water is the main component, while only 3% of the wet weight is chondrocytes. About 50% of the structural components are collagens, with type II being the major collagen. (From Aigner and Stove [4], with permission.)

actively contribute to matrix degradation. The changes that cells undergo in the course of OA are discussed below.

The major constituent (70–80%) of cartilage is water. Major organic components are collagens and proteoglycans, in particular aggrecan (see Fig. 1.3) [4]. The principal collagen is type II, a fibrillar collagen that forms a meshwork of fibers within which aggrecan molecules are contained (Fig. 1.5). Several minor constituents are also important for cartilage function. Collagen XI, located in the core of collagen II fibers, is thought to be involved in fibril formation and in limiting the fiber diameter, while collagen IX, located along the surface of type II, may be involved in cross-linking the collagen network (cf. Chapter 5, [4]). The minor collagens are components of the so-called type II collagen fibrils, as are small proteoglycans (biglycan, decorin, fibromodulin); cartilage matrix protein; perlecan, a heparan-sulfate proteoglycan; and others whose exact function has not yet been determined.

The nonfibrillar aggrecan consists of highly sulfated monomers (chondroitin and keratin sulfate) that are attached to the core protein, which, in turn, is attached to HA via link protein. Large aggregates are interspersed within the fibrillar collagen network (Fig. 1.5). These aggregates are highly negatively charged and thus attract and bind water molecules,

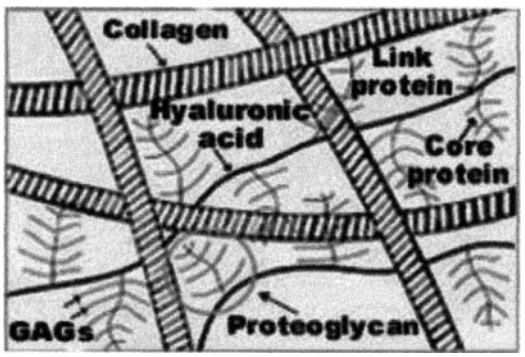

Figure 1.5. The interactions between type II collagen fibrils and aggrecan. The "bottle-brush"–like aggrecan molecules are interspersed and held within the network of collagen II fibrils. Absorption of water causes aggrecan to swell, but swelling is limited by the collagen network. From www.kuvasz.info/cosequinds.htm.

which causes the aggrecan gel to swell. This swelling is constrained by the network of collagen fibrils, which provide tensile strength and compressive stiffness, whereas the aggrecan-hyaluronan-water aggregates provide elasticity. Under loading, the matrix is compressed and water is extruded. However, the matrix rapidly regains its former shape as water molecules are drawn back after unloading. It is this mechanism that provides the shock-absorbing capacity of articular cartilage.

Heparan sulfate proteoglycans, particularly perlecan, also play key roles in chondrogenesis (reviewed in [27]). The primary role of these molecules in cartilage is the binding and release of heparin-binding growth factors, including fibroblast growth factors, heparin binding forms of vascular endothelial growth factor (VEGF), and bone morphogenetic proteins (BMP). In this capacity, they maintain proper gradients of these growth factors through cartilage and bone. Increased degradation of heparan sulfate proteoglycans by glycosidases and matrix metalloproteinases may account for some progressive tissue alterations occurring in OA, including inappropriate vascular invasion and bone formation.

1.5.4 Changes in Matrix Components During Osteoarthritis

Absence of or mutation in any matrix component will result in cartilage that cannot fully resist mechanical loads. Significant defects in type II collagen or aggrecan cause chondrodysplasias and defective skeletal growth [80,85]; as a result, animals do not live long enough to develop OA. However, smaller defects in the type II collagen gene have been linked to early-onset OA in humans [46,96]. In mice created with additional copies of a transgene with a small deletion of type II collagen, the resulting synthesis of a truncated type II collagen, added to the normal gene, leads to osteoarthritic-like lesions as the mice age. The reason is that expression of the transgene causes deposition of a structurally inferior collagen network [75]. Indeed, it is the absence of minor components that is associated with OA, presumably because the small matrix defect will become apparent only with time. Mice lacking alpha 1 (IX) collagen develop noninflammatory degenerative joint disease [28]. Mice deficient in both biglycan and fibromodulin develop severe and premature knee OA [7], even though the cartilage defect may be secondary to tendon defects that cause subluxation of the joints. Genetic defects in the minor matrix components may cause OA in some instances, but not for most OA patients.

1.6 Microscopic Changes in the Articular Cartilage

To characterize cartilage degradation, Mankin et al [53] developed a histologic grading scale that ranges from 0 (normal cartilage) to 14 (severe degradation). Scoring reflects the loss of structural organization (0–6), cellular characteristics (0–3), loss of safranin-O staining (0–4) and tidemark integrity (0–1). More recently, a new histopathologic grading system has been proposed by Osteoarthritis Research Society International (OARSI) [67]. With normal cartilage as grade 0, OA severity is divided into six grades. Grades 1 to 4 involve changes in articular cartilage, and grades 5 to 6 involve the subchondral bone as well. It is important to note that both grading systems are histologic, assessed at a particular location. A patient may have severely degraded cartilage in the weight-bearing regions (Mankin score, Ms >10; OARSI grade >4), yet still have near-normal cartilage in non–weight-bearing regions (Ms <5; OARSI grade 1–2), however severe the patient's OA.

The stages in cartilage degradation can readily be seen in histologic sections, graded according to the Mankin score (53). In control cartilage (Ms = 1, OARSI = 0) obtained from the femoral heads of patients who had fractured the neck of the femur, aggrecan is evenly distributed throughout the cartilage, as indicated by safranin-O (Fig. 1.6A) and Alcian blue (Fig. 1.6E) staining. Loss of aggrecan in the superficial zone, as shown by loss of safranin-O staining (Fig. 1.6B), is the first indication of OA (Ms = 3–4, OARSI = 1). At this stage, the collagen network remains intact, on the basis of positive sirius-red staining (Fig. 1.6F). Aggrecan loss leads to reduced resistance to mechanical loading and to damage to the collagen network. The stage that follows is erosion of

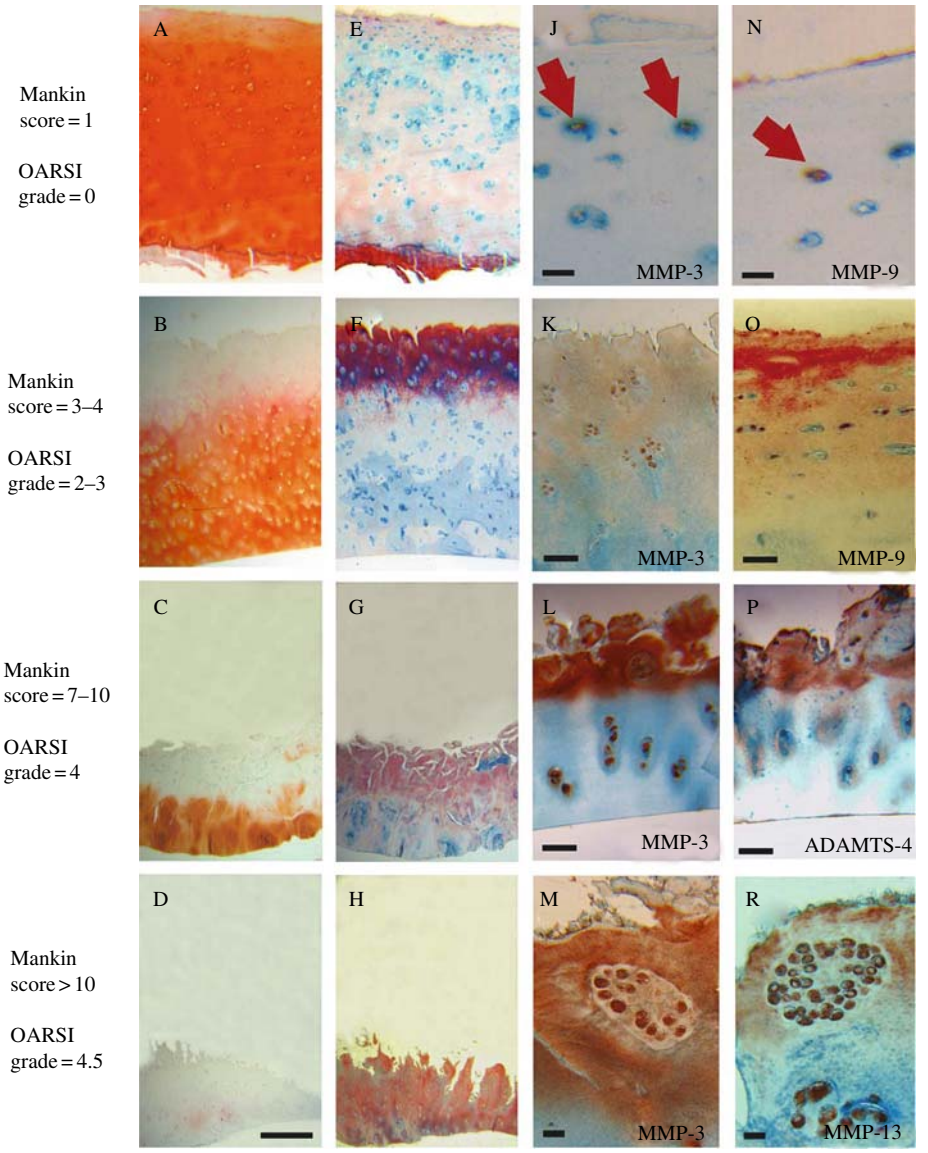

Figure 1.6. The microscopic changes in the cartilage with increasing severity of OA. (A–D) Safranin O staining (orange) demonstrates the loss of proteoglycans from the surface zone as OA progresses. (E–H) Alcian blue/sirius red staining parallels that of safranin O. Sirius red stains collagen, but in normal cartilage there is no sirius red staining, because the binding sites are covered up by proteoglycans. Bone matrix, however, stains red (E). As proteoglycans are lost, the cartilage collagen now stains with sirius red (F–H). All images in A to H are of the same magnification, illustrating the overall loss of cartilage with increasing Mankin/OARSI grade. (J–R) Expression of degradative enzymes by the articular chondrocytes. In control cartilage, only the occasional cell is immunopositive for MMP-3 or MMP-9 (J,N, arrows). In low-grade OA, most chondrocytes located in the region of proteoglycan loss now express the degradative enzymes, which are also present in the matrix (K,O). As the superficial zone has been degraded, chondrocytes in the deep zone now express the enzymes, with strong activity in the surface zone (L,P). Finally, in high-grade OA, only a thin layer of cartilage remains and all the cells in the typical clones of OA cartilage now express the degradative enzymes (M,R).

the superficial zone. In OA cartilage with a Mankin score of 7 to 10 (OARSI = 3), the former superficial zone has completely disappeared and the intermediate zone, now at the surface, shows extensive fibrillations (Fig. 1.6C,G). The thickness of the cartilage is reduced to about half, and safranin-O staining is patchy or absent. In severely degraded cartilage (Ms >10, OARSI = 4–5), most of the cartilage has disappeared. What is left of the matrix is very thin and contains no proteoglycans (Fig. 1.6D,H). The cartilage no longer can withstand mechanical strains, and load is transmitted directly to the underlying bone, making movement impossible.

The process of cartilage degradation thus starts at the surface and proceeds to the deep zone until the whole area is eburnated and the subchondral bone becomes visible macroscopically. There is also a progression with time from the weight-bearing regions to the non–weight-bearing areas of the cartilage.

1.6.1 Degradative Enzymes in Osteoarthritis

Many of the enzymes associated with matrix degradation have been described (cf. Chapter 6). Histologic observations suggest that aggrecan is lost first, probably due to the activities of matrix metalloproteinase-3 (MMP-3 or stromelysin) [32,56,63] and the aggrecanases ADAMTS-4 (a disintegrin and metalloproteinase with thrombospondin motif) and ADAMTS-5 [52,78,88,94]. MMP-13 (collagenase-3) is the prime candidate for collagen degradation [59, 82], followed by the gelatinases MMP-2 and MMP-9 [23,32,55,86,95], which cleave the denatured collagen fibrils, while MMP-7 [62], MMP-8 [16], and MMP-14 (membrane-type I MMP) [41] may also be involved, possibly as activators of the other MMPs.

These enzymes have originated either in the synovium or in the articular chondrocytes and may be elevated in arthritic synovium, particularly in the inflamed synovium [63,98]. Synthetic MMP inhibitors have been shown to be chondroprotective in animal models of OA [57], which are often trauma induced and therefore accompanied by inflammation. However, results on the use of MMP inhibitors in clinical trials have been equivocal [57]. This suggests that synovium-derived enzymes may be relatively unimportant in human OA, which, on the whole, is not an inflammatory disease. The results also suggest that chondrocytes are the main source of the degradative enzymes [92].

1.7 The "Arthritic" Chondrocyte Phenotype

The central role of chondrocytes in the disease process has been recognized for some time [35, 77], and many studies have compared the characteristics of chondrocytes from OA patients with those from non-OA patients. However, chondrocytes from an OA patient are very heterogeneous, ranging from almost normal cells to those in which anabolic genes have been activated, to the clonal cells of late-stage OA that express many of the degradative enzymes. It is thus impossible to define an "OA chondrocyte" without referring to the region (weight bearing vs. non–weight bearing) or zone (superficial vs. deep) or degradation stage (Mankin score). Just as with changes in the matrix, the cellular changes during OA are progressive and can be separated into four broad stages according to the Mankin score or OARSI grading:

1.7.1 Stage 1 (Ms = 1–2, OARSI = 0)

The earliest cellular changes are found in the articular cartilage that is obtained from patients with a femoral neck fracture. These patients have macroscopically normal cartilage and no clinical OA. Histologic sections show a smooth surface and flattened cells that are arranged parallel to the surface (Fig. 1.6I). This cartilage often serves as control in comparison with cartilage from patients with OA. However, a slight loss of safranin-O staining has already occurred in "control" cartilage from the superficial zone (Fig. 1.6A). This indicates disease has started. The superficial zone contains cell doublets, sign of a low level of cell division. At the same time, synthesis of MMP-3 and, to a lesser extent, of other degradative enzymes can be detected by immunocytochemistry in some flattened cells near the surface (Fig. 1.6I,N). However, most cartilage

cells (>95%) from patients with a femoral neck fracture appear to be normal, justifying the use of this cartilage as "control" cartilage.

1.7.2 Stage 2 (Ms = 3–5, OARSI = 2–3)

This cartilage is usually obtained from the non–weight-bearing regions of OA patients. The total thickness is similar to that of control cartilage, but the zone of aggrecan loss has extended to approximately one fourth of the cartilage. Cells within this zone are generally present as doublets or quadruplets; this indicates that some cell division had occurred. The cells from these superficial zones synthesize many of the degradative enzymes, such as MMP-3 and MMP-9, which are released into the matrix (Fig. 1.6K,O). We term these cells "degradative" chondrocytes, to distinguish them from the greater quantity of non–enzyme-producing chondrocytes in the non–weight-bearing regions of OA cartilage. Degradative chondrocytes constitute a paradox, as it is unclear why cells would destroy their matrix. This paradox is central to understanding the pathology of OA and probably offers the greatest potential for therapeutic intervention. Degradative chondrocytes synthesize MMPs 1, 2, 3, 7, 8, 9, and 13 [16,32,62,63,82,83,93], the two major aggrecanases, ADAMTS-4 and -5 [78,89], VEGF [24], pleiotrophin [68], and probably other factors. As already mentioned, degradative chondrocytes are predominantly located in the region of proteoglycan loss. As yet, it is unclear whether changes in the matrix structure are due to enzyme action or whether matrix changes induce changes in the cell phenotype. Whatever the answer, it is the interactions between matrix and cells that ultimately lead to OA. Thus, when cells in the superficial zone synthesize degradative enzymes, anabolic cartilage matrix synthesis may be stimulated in chondrocytes of the middle zone [5]. However, this process is transient and abnormal protein synthesis takes over as OA progresses. Some cells in the deep zone express type X collagen [3,33] as well as osteopontin [69] and osteocalcin [70]. This indicates a phenotypic shift from normal to hypertrophic phenotype. The increased calcification observed around cells of the deep zone is consistent with the notion of a shift. Normal articular chondrocytes are arrested at the prehypertrophic stage and do not respond to factors inducing hypertrophy, such as bone morphogenetic protein-2 (BMP-2) and transforming growth factor-β (TGF-β). The observation that some chondrocytes express genes typical for hypertrophic chondrocytes has led to the hypothesis that the normal constraints, which prevent hypertrophy in articular chondrocytes, are no longer operative in OA (reviewed by Drissi et al [22]).

1.7.3 Stage 3 (Ms = 7–10, OARSI = 4)

The midzone has reached the surface of the cartilage and strong matrix-staining for MMP-3 and ADAMTS-4 can be demonstrated in the surface layer. Almost all remaining chondrocytes in the intermediate and deep zones occur as clusters (clones) of at least four to eight cells, and all cells within a cluster are of the degradative phenotype (Fig. 1.6L,P). The clusters are separated by large acellular or hypocellular regions, an indication that a large proportion of chondrocytes has died.

1.7.4 Stage 4 (Ms ≥10, OARSI = 4.5)

At this stage, most of the cartilage has disappeared. Where cells are present, they are in clusters of up to 64 cells that are immunopositive for the degradative enzymes.

The fact that abnormal degradative cells appear first as individual chondrocytes, later as doublets, and then as quadruplets and clones suggests that, in contrast to the nondegradative chondrocytes, the abnormal cells have proliferated and transmitted the pattern of abnormal gene expression to their daughter cells. In other words, the abnormal phenotype is both stable and transmitted. Somatic heritable changes in gene expression may involve epigenetic mechanisms, a term that refers to heritable changes in gene function that are not the result of mutations of the DNA. A major epigenetic change induces a change in the DNA methylation pattern, a pattern that in turn regulates cell-type specific gene expression [8]. Genes that are not normally expressed in a cell are typically silenced by DNA methylation in the regulatory regions. Hence regulatory regions of the

degradative enzymes would be expected to be highly methylated in normal chondrocytes, but less methylated or unmethylated in degradative chondrocytes. Indeed, recent studies have demonstrated that de-methylation at specific sites in the promoters of MMP-3, -9, -13, and ADAMTS-4 was associated with enzyme expression [73]. For further discussion of epigenetic changes, see Chapter 8.

1.7.5 Progression of Cartilage Damage with Increasing Severity

The progression of cartilage erosion can be seen first at the weight-bearing region, where surface erosion occurs first, leading to progressive eburnation and exposure of subchondral bone. With time, the cartilage in non–weight-bearing areas also becomes eroded. Microscopically, the degradative chondrocytes, originally found only at the surface, now appear in the intermediate and deep zones. This suggests that as a result of a propagation mechanism more chondrocytes become degradative and proliferate until all chondrocytes in regions with a high Mankin/OARSI grade are of the degradative phenotype. Development and propagation of the degradative phenotype are principal reasons for the ongoing catabolism in the cartilage matrix. Interventions directed at preventing the propagation of the degradative phenotype would therefore seem to constitute a promising therapy.

1.8 Cell Death in Osteoarthritis

Cell death can be broadly divided into apoptosis and necrosis. Apoptosis is the mechanism by which surplus, abnormal, or dysfunctional cells are eliminated without inflammation. Necrosis, on the other hand, is the term for cell death due to injury or pathologic damage and is associated with inflammation. Apoptosis is normally beneficial, although aberrant apoptosis occurs in pathologic states or increases with age. Both apoptosis and necrosis occur in OA. Elimination of healthy cartilage cells by cell death is clearly disadvantageous, because articular chondrocytes self-renew poorly, if at all. Cell loss, therefore, becomes permanent and thus may play a role in the pathology of OA. However, estimates of cell death vary widely, from 21% [40] to less than 1% [1]. The role of cell death in OA, therefore, remains controversial. Opinions also vary as to the type of cell death. Most authors classify chondrocyte death as due to apoptosis, even though injury or mechanical damage [19] would result in necrosis. Recently it has been proposed that chondrocytes undergo a variant of classical apoptosis, termed "chondroptosis" [2,72], a mode of cell death that leads to self-elimination and thus obviates the need for phagocytosis of the apoptotic bodies. Consistent with this self-elimination process is the observed increase in empty lacunae in OA samples with a high Mankin score [2] and the presence of hypocellular regions.

Would inhibition of cell death have a beneficial effect on disease progression? This depends on how much cell death contributes to the overall pathology of OA. The authors of this chapter do not believe that cell death itself is a primary factor causing OA pathology, except in trauma-induced OA. Cell death can obviously contribute to matrix loss, because the cells no longer maintain their extracellular matrix. However, loss of matrix cannot account for the entire disease process. A more controversial view is that apoptosis of abnormal chondrocytes, for example the degradative phenotype, should be stimulated, because this would prevent proliferation of the abnormal phenotype.

1.9 What Can We Do About OA?

Neither cause nor cure of OA is as yet known. However, some risk factors can be reduced, symptoms can be treated, pain can be controlled to some extent, and surgical intervention can replace the diseased joint to restore mobility. Disease-modifying drugs, based on better understanding of the pathobiology of OA, may become available [99].

1.9.1 Reduce Risk Factors

Little or nothing can be done about the effects of aging or of genetic predisposition, but other

risk factors can be modified. One example is obesity. Even modest reductions in weight (5 kg) are likely to slow disease progression [61].

1.9.2 Treatment Regimens

Most current treatment modes are directed at alleviating pain and reducing inflammation, thereby improving function and reducing disability [79]. In addition to simple analgesics, such as paracetamol and codeine-based preparations, nonsteroidal antiinflammatory drugs, acting on the cyclooxygenases COX-1 and COX-2, are effective in moderate to severe pain, but may cause adverse gastrointestinal responses. The newer inhibitors that are specific for COX-2 have fewer gastrointestinal side effects, but carry an increased risk of heart disease. A prominent example is the recent withdrawal of Vioxx (rofecoxib) by Merck. Intraarticular injections of corticosteroids may temporarily alleviate pain and stiffness, but are not suitable as maintenance therapy.

Intraarticular injection of HA has become an accepted therapy in many centers for knee OA. Hyaluronic acid is a major component of synovial fluid, cartilage, and connective tissue. A high molecular weight polysaccharide, consisting of a long chain of repeating disaccharides, hyaluronic acid is responsible for some of the viscoelastic properties of synovial fluid. It also plays a role as a trophic factor [26]. Patients with OA have lower concentrations of HA, with reduced molecular weight representing a shortening of chain length. Two categories of commercial intraarticular HA preparations exist—high and low molecular weight—and the former is thought to be superior [43]. These preparations are typically injected once a week, over a 3- to 5-week period. The role of HA in knee OA has been examined in over 40 studies, and there is compelling evidence regarding its superior role in the medium term, as compared to intraarticular steroid injection.

1.9.3 Surgical Intervention

Surgical intervention for OA has traditionally been advocated after medical therapy has failed. Surgeons involved in musculoskeletal trauma must be meticulous with regard to reconstituting the disrupted joint surfaces of intraarticular fractures. Residual joint incongruity has been correlated with early posttraumatic arthritis and a poor clinical outcome. Some articular fractures injure cartilage so severely that the joint will degenerate even with an accurate articular reduction. The late development of OA may be due to chondrocyte apoptosis resulting from the initial impact [97]. Late development OA seems to proceed regardless of the quality of the articular reconstruction. Patient-to-patient variability is wide with respect to the sensitivity of joints to an insult.

In common with many elective surgical interventions, a stepwise approach is often employed in the treatment of OA. The procedures are site specific and in the first instance often involve a combined diagnostic/therapeutic joint injection. In the hip, a radiolucent dye is injected into the hip joint under fluoroscopic guidance to confirm successful needle position. A mixture made up of a local anesthetic and a steroid is then injected. If the pain is truly due to isolated hip disease, the patient will be free of pain in minutes, due to the local anesthetic effect, with the steroid component producing symptomatic relief often over several months.

Other procedures include arthroscopic joint debridement and washout. This procedure is successful in many patients with mechanical symptoms such as locking of the joint or the joint giving way because of the presence of osteophytes and loose bodies. These can be readily removed; the degenerate articular cartilage is then shaved or otherwise ablated to restore a stable surface.

Osteotomy, with correction of bony malalignment, is used as a prophylactic measure in the prevention of OA. In these cases the aim is to restore joint surfaces that are at risk of OA from abnormal mechanics. This procedure is commonly performed in patients with slipped capital femoral epiphyses and developmental dysplasia of the hip. The procedure can also be directed against limited joint damage, where the load is transferred from the degenerate to a less diseased component. In the treatment of medial compartment degeneration of the knee, a proximal tibial valgus osteotomy will correct the varus deformity. Whether for prophylactic or therapeutic reasons, the patients considered for osteotomy are generally young, of normal body mass index, with a relatively good range of motion at the joint, and with only mild symptoms.

Arthrodesis, or joint fusion, is now a rare procedure to treat large joints affected by OA

and has largely been replaced by arthroplasty. Arthrodesis, however, is a reliable, durable, and painless solution to severe arthritis involving joints in the hand, foot, and spine, and, if successfully fused, exhibits surprisingly little limitation of motion.

Recently the experimental treatment of discrete areas of chondral damage in the knee has attracted attention. Traditionally chondral damage has been addressed with debridement, drilling of the subchondral bone, or osteochondral auto- and allografting. In autologous cartilage implantation, grafts of normal cartilage are harvested at arthroscopy from non–weight-bearing sites of the knee, cultered ex vivo to increase the number of cells, and transplanted back into the defect area, which is protected by a periosteal flap or embedded in a collagen matrix. This procedure is successful in well-selected patients with isolated disease in a stable knee. However, regrowth of hyaline cartilage at the defect site is not assured, and the ratios of hyaline to fibrocartilage at biopsy vary greatly. Biocartilage is predominantly composed of type I collagen, which has suboptimal wear characteristics. However, the technique demands two surgical procedures, and the overall outcome may not be significantly superior to that achieved with more conventional techniques [74].

Joint replacements constitute the biggest advance in the treatment of OA. Only 40 years ago there was no cure for the debilitating pain of OA, but today total joint replacements of the hip and knee are one of the triumphs of modern orthopaedic surgery. The earliest developments of total hip replacements by McKee and Farrar in the 1950s were optimized by Sir John Charnley 10 years later who used a stainless-steel stem that fitted into a polyethylene cup, both cemented into the hip and thighbone. With the help of specifically designed operating theaters and novel antibiotics, the success rate of joint replacement surgery has reached an unprecedented high level. The 1970s saw a proliferation of designs similar to the Charnley hip. A high failure rate in young and active patients who placed high demands on hip and knee prostheses posed the greatest challenge to orthopaedic surgeons. Often young and active patients were refused surgery, considerable pain and disability notwithstanding, until they reached an appropriate age for joint replacement. At the other end of the spectrum, many elderly patients were also excluded from surgery as the complication rate was thought to be unacceptably high. Since the early 1990s there has been phenomenal progress in terms of operative techniques, implant design, and orthopaedic anesthesia. There are now many different designs of prostheses that can be safely and reliably utilized in patients of all ages. The new prostheses pose minimal risk and provide maximum improvement in terms of quality of life. New prostheses utilize high-tech materials such as titanium and ceramics, and the surgical procedures now include not only total replacement but also resurfacing of the hip joint and unicondylar or half-knee replacements. It is hoped the new generation of total joint designs will last over 20 years, with complication rates of less than 1%. Patients are now encouraged to walk on the first postoperative day, a far cry from the bed rest of 6 weeks advocated in the not so distant past.

1.9.4 Disease-Modifying Drugs

The development of disease-modifying drugs (DMDs) for OA is still in its early stage. Cytokines, such as IL-1β and TNF-α, are therapeutic targets [36,51], and their inhibition would theoretically reduce the inflammatory component of OA. Inhibitors of MMPs have shown promise in animal models [11, 65], but clinical trials in humans have been disappointing [52]. An alternative approach is osteoarthritis gene therapy, where gene transfer to the synovium would enhance synthesis of the cartilaginous matrix or inhibit its breakdown. Therapeutic effects of IL-1Ra (receptor antagonist) gene transfer have been confirmed in three different experimental models of OA [25].

Perhaps the greatest potential for therapeutic intervention lies in preventing chondrocytes from expressing degradative enzymes. This might involve inhibition of expression or, ideally, preventing the phenotypic change to degradative chondrocytes. Neutraceuticals, such as fish oil, glucosamine, and chondroitin sulfate, are widely used supplements that seem to slow disease progression (reviewed in [17, 34]). Although evidence is still limited, their beneficial effects seem to be due to inhibiting the expression of degradative enzymes in articular chondrocytes. Omega-3 fatty acids (present in fish oil) have been shown to inhibit IL-1β–induced expression of several degradative

enzymes, as well as inhibiting mediators of inflammation in human articular chondrocytes [18]. Chondroitin sulfate inhibits MMP-3 synthesis by chondrocytes [58]. Therapy would be considerably advanced if we understood what causes normal articular chondrocytes to change to degradative chondrocytes. Because this change is transmitted to daughter cells, the changes may be epigenetic, a possibility worth exploring.

1.10 Conclusion

Neither the causes of OA nor its cures are fully known, but there is hope for the future. In severe OA of the hip, knee, or shoulder, mobility can be restored by total joint replacements. As the various causes and steps in disease progression become known, more direct treatment of OA may become possible, as discussed in subsequent chapters of this volume.

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2.

The Role of Bone in the Development of Osteoarthritis

Daniel Lajeunesse and Pascal Reboul

2.1 Introduction

Osteoarthritis (OA) is the most common form of arthritis. It has been regarded as a disease of articular cartilage, with evidence of articular cartilage degeneration followed by erosion, the main identifying characteristic. The etiology is multifactorial, but common features can be observed. The end point of OA is cartilage destruction that impairs joint movement and causes pain. In knee joints, the cartilage destruction is associated with or preceded by subchondral bone sclerosis. Joint destruction is also associated with joint inflammation, with the synovial membrane playing a key role. The chronologic events of these phenomena are still debated in the literature. However, because of the complexity of the disease, it may be initiated via each of these tissues, although inflammation of the synovial membrane is less likely to be a primary cause.

The widely held belief that OA is a disease of cartilage has been a subject of debate, particularly in recent years. Several investigations, including our own, have led to the hypothesis that bone changes may account for joint deterioration and development of OA. Indeed, individuals with OA exhibit striking increases in bone mass at affected sites, such as the knee and hip, as well as at nonsynovial sites like the lumbar spine. Individuals with OA also have a high body mass index (BMI) and exhibit an increase in bone mineral density (BMD). The latter may be the result of an abnormally high rate of metabolism of osteoblasts, observable in vivo and in vitro, and most particularly in subchondral bone. However, stiffness and BMD are not uniform in OA bone, as assessed by quantitative backscattered electron imaging, Fourier transform infrared spectroscopy, and fractal analysis [70,142,228]. Actually, only bone closest to the articular cartilage influences the integrity of cartilage and variations in stiffness and BMD in OA bone tissue may cause more damage to cartilage integrity than to normal bone [43,66]. Moreover, the apparent elevations in BMD in OA may be due to an increase in material density resulting from a more abundant, yet undermineralized osteoid collagen matrix [8, 144,145]. Indeed, the increase in osteoid matrix in OA bone tissue [125,246] is an indication of abnormal mineralization and of general bone metabolic disease [80–82,188]. The altered metabolism of osteoblasts from subchondral bone tissue is not due to changed serum levels of humoral factors or hormones [50], but to an alteration in local signals. Even in the early stages of osteoarthritis, phenotypic differences in subchondral osteoblasts become manifest as a result of a higher rate of metabolism. Indeed, a large number of factors, including inflammatory and growth factors and proteases, are produced in excessive amounts in the diseased tissue [101,135,136,208,241]. These factors may act locally, via autocrine/paracrine

signaling, but increasing evidence indicates that these factors interact with the overlying articular cartilage [92,124,207]. Alterations in the crosstalk between bone and articular tissues may contribute to the pathogenesis of OA.

2.2 Articular Cartilage: Nutrition and the Role of Hypoxia

Articular cartilage is bordered at its base by the subchondral bone plate, a very thin cortical bone structure that possesses an irregular surface to which the articular cartilage is anchored. Many arterial terminal branches are present in the subchondral bone plate and end in irregularly distributed sinusoids of uneven caliber. Because blood flow in subchondral bone is three to ten times higher than in trabecular bone [51], the blood flow to subchondral bone supplies approximately 50% of the glucose, oxygen, and water required by articular cartilage [112,141], whose oxygen consumption rate differs from that of other tissues. Indeed, chondrocytes consume much less oxygen than most other cell types. The oxygen supply to chondrocytes in adult cartilage is limited [21,218], so that these cartilage cells function under mainly hypoxic conditions. Microelectrode studies have shown that the oxygen gradient in cartilage is 7:1 from around 7% in superficial layers to 1% in deep zones [21,218]. However, a mathematical model suggests that the oxygen tension in normal cartilage is not likely to drop to 1%, except under abnormal conditions such as OA and rheumatoid arthritis. In fact, the oxygen supply from subchondral bone may be of particular importance for the deep zone cartilage [245]. Responses to hypoxia include initiation of angiogenesis from vessels within subchondral bone [239], inasmuch as cartilage itself contains no vasculature. Vascular penetration, a characteristic of OA, occurs through the tidemark, with vessels invading the more superficial, noncalcified articular cartilage [31,166]. However, it is uncertain whether this neovascularization brings more oxygen to the cartilage layers, because new vessels at the osteochondral junction are consistently associated with new bone formation in the form of osseous cuffs to the fibrovascular channels. Consequently, as the deep layers of articular cartilage become progressively ossified, the original osteochondral junction is obliterated [141,239]. It is likely this process leads to great variations in oxygen tension and modifies the adaptive responses of the chondrocyte, triggering either repair or degenerating processes.

2.3 Dedifferentiation of Chondrocytes in Osteoarthritis

Articular chondrocytes are subject to phenotypic destabilization during a variety of pathologic conditions [120]. With the onset of OA, chondrocytes undergo metabolic changes that involve new stimuli (proinflammatory cytokines, growth factors) and changes in the composition of the extracellular cartilage matrix (ECM) [155]. As their normal phenotype changes, chondrocytes even express traits of transient growth such as hypertrophy, alkaline phosphatase, type X collagen, and mineralization [151,236]. Even though metabolically active, OA chondrocytes no longer express aggrecan and collagen type II [10,11,189,235]. Clusters of chondrocytes in OA cartilage express collagens type I and III, which are either absent or occur very sparingly in normal articular cartilage [23,171, 183,242]. OA chondrocytes also express type IIA collagen, a marker of prechondrocyte phenotype. This expression is enhanced by transforming growth factor-β 1 (TGF-β1). Bone morphogenetic protein-2 (BMP-2), on the other hand, favors the expression of type IIB collagen isoform, a normal component of articular cartilage [86]. It is therefore evident that high synthetic activity notwithstanding, dedifferentiated chondrocytes do not express cartilage-specific anabolic genes such as aggrecan or type II collagen. In addition to deactivation, phenotypic alteration, therefore, is another mechanism by which OA chondrocytes undergo anabolic failure. Furthermore, a subset of chondrocytes in the superficial and upper middle zone of cartilage express alpha 1 (III), but not alpha 1 (I) messenger RNA (mRNA). These overlap with chondrocytes in the upper and lower middle zones where type II collagen expression is elevated.

Interestingly, a possible reversion to a fetal-like phenotype or postnatal hypertrophic phenotype also occurs in the deepest zones of OA cartilage; here, the cells express type X

collagen [83], a specific marker for hypertrophy of growth-plate chondrocytes [201,212]. Apoptosis of chondrocytes ensues and the cartilage matrix calcifies. All of these events occur in the lowest zone of fetal growth-plate cartilage. Other developmental genes such as annexins II and V [117,164], collagenase-3 [161,200], osteopontin [187], and galectin-3 [91] are also reexpressed in OA cartilage. Changes in proteoglycan expression have been observed in OA cartilage. It is typical for proteoglycans in OA cartilage to be extensively degraded, yet biosynthesis of proteoglycans also occurs during intrinsic repair [44,45,204]. Human OA cartilage shows abnormal expression of versican (Proteoglycan-M) [172], a large chondroitin sulfate proteoglycan that is normally expressed in the prechondrogenic area of limb buds. Finally, at least one transcription factor also shows differential expression between normal and OA cartilage. Wang et al [240] reported that the early growth response protein, Egr-1, is downregulated in OA cartilage compared with normal cartilage. Egr-1, a zinc-finger transcription factor, is important for cell proliferation, differentiation, and apoptosis, and is expressed in epiphyseal cartilage and articular cartilage during development [75,156]. Interestingly, Egr-1 is widely expressed in normal cartilage, but is restricted to the clusters of clonal chondrocytes in OA cartilage [243]. On the basis of these findings, OA chondrocytes seem to constitute a more dedifferentiated phenotype than do normal chondrocytes.

2.4 Bone Tissue Changes in Osteoarthritis

Many studies have explored the structural changes that take place in subchondral bone during the evolution of OA,with symptoms paralleling disease progression. Epidemiologic studies have demonstrated that as subchondral bone becomes sclerotic, the disease worsens [131]. Even though clinical data are largely based on noninvasive technology, such as x-rays, computed tomography (CT) scans, magnetic resonance imaging (MRI), scintigraphy, and biomarkers, Buckland-Wright et al [24–27] have shown with the aid of radiographic studies of the knee and hand that the subchondral bone has thickened. Using quantitative microfocal radiography, they demonstrated that thickening of the subchondral cortical plate is the earliest anatomic change in OA joints. It precedes changes in articular cartilage thickness, evaluated radiographically as joint space narrowing. Using labeled bisphosphonate in a scintigraphic study, Dieppe et al [56] demonstrated elevated bone cell activity in patients who had progressed to severe OA. The same investigators also showed that an increased bone scintigraphic signal at the affected knee was predictive of OA progression in the 5 years to follow. Similar results were reported for OA of the hand [201]. More recently, the same group of investigators has shown that in the OA knee, the scintigraphic abnormalities correlated with osteocalcin concentration in the synovial fluid, osteocalcin being a marker of bone formation [215]. Because increased subchondral bone turnover appears to parallel progression of OA, the level of urinary N-terminal type I collagen telopeptides (NTx) and C-terminal type I collagen telopeptides (CTx), which are markers of bone resorption, was measured at three different time points in a subset of patients from the Chingford study. The findings demonstrate that bone resorption is increased in patients with progressive knee OA, but not in those with nonprogressive knee OA [13]. The increase in bone resorption seen in patients with progressive knee OA is similar to that observed in patients with osteoporosis.

Recent advances in imaging technology provide more precise information about the in situ structural changes of subchondral bone in the course of OA. Moreover, the correlation between those changes in subchondral bone and the development of cartilage lesions has been studied with the aid of MRI, an ideal method to assess structural changes that affect cartilage, soft tissues, and bone. It is now possible to quantify the trabecular structure of subchondral bone and to generate information such as the number and width of trabeculae in the trabecular bone and the distance between the trabeculae. An increase in bone turnover, the presence of edema-like lesions in subchondral bone marrow, and bone attrition are strong indicators of structural deterioration of the knee in OA [68,90,197]. Beuf et al [14], in a cross-sectional study, have demonstrated differences in the trabecular bone structure between healthy and OA patients. In OA, the loss of trabecular bone in the femur was correlated with the severity of the disease as assessed

by the radiography-based Kellgren/Lawrence scale. Blumenkrantz et al [17], in a 2-year longitudinal study of the knee of OA patients with the aid of high-definition MRI, found that the loss of cartilage volume/thickness and the deterioration of the subchondral bone structure were interdependent. In these patients, a positive correlation was established between the loss of cartilage, subchondral bone sclerosis, and osteopenia of the underlying trabecular bone.

Determination of synovial fluid osteocalcin, a marker of bone formation, and serum osteopontin, a bone matrix protein, suggests new bone synthesis exceeds degradation in OA [215]. Since osteopontin increases shortly following trauma, alterations in bone cell activity probably occur quite early in the disease. Gevers and Dequeker [80] have shown that serum osteocalcin levels are elevated in cortical bone explants of women who have OA in their hands. Subsequently, Dequeker and colleagues reported that insulin-like growth factors-1 and -2 (IGF-1 and -2), and TGF-β levels are higher in samples of iliac crest bone of patients with OA [53]. Inasmuch as this bone site is at some distance from weight-bearing joints, the finding suggests a generalized dysfunction of bone metabolism.

2.5 Role of Osteoblasts in Abnormal Bone Tissue Metabolism in OA

The hypothesis that OA may be considered a metabolic disease in which systemic or local factors modify the formation and activity of mesenchymal precursor cells in skeletal tissues is an attractive one [5]. Because OA osteoblasts show increased osteocalcin and alkaline phosphatase levels in vitro [100,101] and in situ [80], the in vivo alterations are likely due to changes in abnormal cellular metabolism and not to changes in systemic regulation. However, this does not mean that local autocrine/paracrine regulation has no role in the cellular changes observed in OA osteoblasts. The OA osteoblasts are partially resistant to parathyroid hormone (PTH) stimulation [101], because of a lower number of PTH receptors [102]. This situation may favor collagen synthesis, inasmuch as PTH inhibits collagen synthesis [12,57]. Whether this is also linked to abnormal degradation of collagen is not known. The activities of two matrix metalloproteases, MMP-2 and MMP-9, are elevated in proximal cancellous bone tissue isolated from the femoral head of OA patients [144], a situation that may be linked to abnormal collagen matrix deposition. The exact mechanism(s) responsible for abnormal osteoblast function in OA individuals remain(s) unknown. However, osteoblasts share a common mesenchymal stem cell precursor with chondrocytes, tenocytes, adipocytes, and myoblasts—all cells affected by OA. Moreover, osteoblast maturation from bone marrow stromal cells in OA patients is enhanced, whereas that of adipocytes and chondrocytes is blunted [168]. This suggests a possible link between lipid and connective tissue metabolism.

Leptin, a key factor in adipocyte maturation [216], may play a role in the abnormal metabolism of subchondral bone osteoblasts [7]. Leptin, the product of the obese (*ob*) gene, is a 16-k protein produced by white adipocytes and the placenta. It influences energy homeostasis through its effects on energy intake and expenditure [34,244]. Leptin levels are increased in OA cartilage [126], and injections of leptin into the joints of normal rats can mimic osteoarthritic features [60]. Indeed, leptin increases by ~40% alkaline phosphatase activity, osteocalcin release, the production of collagen I α1 chains, insulin-like growth factor-1 (IGF-1), and TGF-ß1 levels in normal human osteoblasts [85]; all these parameters are increased over normal levels in OA subchondral osteoblasts [100,102,149]. Leptin is also associated with inflammatory states [33,63,152] and stimulates prostaglandin E_2 (PGE_2) [195] and leukotriene (LT) production [143]. Osteoarthritic subchondral osteoblasts produce high levels of PGE_2 or LTB_4 [149,176]. Lastly, leptin signaling contributes to the mechanism of joint inflammation in antigen-induced arthritis by regulating both humoral and cell-mediated immune responses [33]. Evidence that osteocalcin knockout mice had an increased mineralization pattern without significant effects on bone resorption [59] suggests that the elevated osteocalcin levels in OA may contribute to the abnormal mineralization

pattern in these patients. Moreover, in cancellous bone of the OA hip, the increase in MMP-2 and alkaline phosphatase activity indicates that collagen turnover has increased [144].

2.6 Role of Local Vascularization in Joint Tissue Integrity

The traditional view that subchondral bone is richly vascularized, whereas hyaline cartilage is not, may no longer be true because histochemical studies have shown that the deep layer of hyaline cartilage is vascularized. The articular vasculature, therefore, derives its nutritional supply partly from the vascular bed of subchondral bone, as well as from the synovial fluid. Therefore, any loss of vascular tone in the subchondral bone could affect the cartilage. Early microvascular damages that affect the venous circulation in the bony tissue, therefore, may be considered a plausible cause of altered chondrocyte function [98]. Whether these vascular changes are secondary to bony changes or their primary cause remains unexplored. However, OA and cardiovascular disease risk factors have been shown to be correlated [115,146,220], and abnormal vascularization of OA tissues may be a means to initiate cartilage tissue damage [166]. The hypothesis that OA may be viewed as an atheromatous vascular disease has recently been proposed by Conaghan et al [42]. Abnormal vascular function in OA may also be be linked to elevated leptin levels, because leptin acts on arterial wall thickness, decreases vessel distensibility, and elevates C-reactive protein levels [118].

2.7 Formation of Osteophytes

Thickening of the subchondral plate alters the effects of mechanical loading on articular cartilage. Changes in subchondral blood flow and intraosseous pressure have been implicated in OA disease progression [82,134,217]. As OA develops, new osteochondrophyte structures, named osteophytes, make their appearance. Osteophyte formation occurs commonly at the junction of the periosteum and the activated synovium (synovitis) [79,209]. Osteophytes are found at the margin of cartilage and bone, but the bone content of osteophytes is higher. Bone formation within the cartilaginous matrix of osteophytes appears to recapitulate endochondral ossification in the course of development. Angiogenesis may contribute to osteophyte growth [18,99,239,241]. Osteophytes are considered stabilizing structures that redistribute biomechanical forces during the standup position and when persons walk. Osteophytes, however, limit the natural motion of the joint [185] and have been associated with pain. They thus play a beneficial or harmful role [157]. Scintigraphy is a good tool to identify growing, active osteophytes whose presence may indicate joint disintegration [56,232]. Osteophytes are associated with an inflammation of the synovial membrane. Furthermore, some growth factors such as TGF-β, basic fibroblast growth factor (bFGF), or BMP-2 injected directly in the joint cannot be used to repair an osteochondral defect, because they induce synovial inflammation and the appearance of osteophytes [162,229,230]. In contrast, injection of hepatocyte growth factor (HGF) alone or of bFGF in combination with hyaluronic acid does not activate the synovium, nor does it generate osteophytes [162,237]. Synovial lining macrophages have recently been shown to mediate osteophyte formation during experimental OA, because macrophage depletion dramatically abolished osteophyte formation [16]. Although the mechanism is not understood, one can hypothesize that some macrophages from the synovial membrane differentiate into osteoclasts, releasing growth factors bound to the extracellular matrix of bone, and induce the osteochondral process that leads to osteophyte formation. Indeed, macrophages in the synovial membrane can differentiate into osteoclasts [47].

2.8 Implication of Biomechanical Processes in Osteoarthritis

That OA is due to altered biomechanical function has been widely discussed, but may not be a basic cause. An example is obesity, an

important risk factor for OA, where the impact causing joint overload is associated with radiographically denser bones [67,76,77,123]. It may, however, be the metabolic consequences of obesity that are responsible for disease progression. Joint location and cartilage matrix vulnerability to chemical and mechanical injury are highly correlated with the risk of developing OA [41,62,104,170]. The evolutionary adaptations to upright posture, causing redistribution of loading forces across the hips, knees, bunion joints, and lower back, may also constitute risk factors for OA [109]. Overexercise, macrotrauma, and microtrauma associated with various occupations and sports [28], cartilage cell mechanics [88], and matrix responses to trauma have also been considered as risk factors [213]. Direct injury to joints or injury resulting from abnormalities in growth plate function or bone growth can render joints vulnerable to the development of OA [78,173]. Moreover, because idiopathic degeneration of OA cartilage in underused areas is a function of age, OA should not be considered a result of old age, nor simply a disease of wear and tear [37].

Mechanical stress on weight-bearing joints may increase microfractures in the bone plate and in overlying cartilage. As articular cartilage slowly erodes, sclerosis of the subchondral bone plate also progresses and bone stiffness increases, which, in turn, may contribute to further mechanical disturbances of the cartilage. On the other hand, healing of trabecular microfractures in subchondral bone can result in a stiffer bone that no longer functions as an effective shock absorber [192,193], a situation that may precede cartilage damage. Microdamage due to microcracks and submicroscopic cracks as assessed by confocal microscropy of bone tissue contributes to loss of bone quality [64], a situation that may be more important for the progression of OA than modifications of BMD. Because normal bone, like articular cartilage, is as good a shock absorber as articular cartilage [130,190], subchondral bone stiffening would increase trabecular bone strain in the proximal tibial plateau [22,154] and in the distal tibia [155]. This strain could then lead to subsequent cartilage lesions, especially in individuals with already compromised articular cartilage. A steep stiffness gradient in the underlying subchondral bone, therefore, may initiate OA, inasmuch as the integrity of the overlying articular cartilage depends on the mechanical properties of its bony bed. The sclerosis of subchondral bone in OA may result from an increased stiffness of the tissue and not from an increase in bone mineral density [134]. Indeed, there is no direct relationship between BMD and accumulation of microdamage in bone tissue, whereas the accumulation of microdamage to bone is directly related to OA [65]. Moreover, although subchondral bone sclerosis in OA has been explained as a response to overloading, the morphologic changes observed in underloading are similar and overloading cannot therefore be the cause of subchondral bone sclerosis [112]. The association between osteophytes and femoral bone mineral density also indicates that aspects of bone formation may underlie the pathophysiology of OA [96]. Nonetheless, bone mass of OA patients is better preserved [36,165,205] than that of normal individuals [72]; primary OA and osteoporosis rarely coexist [52,196,233].

2.9 Osteoarthritis-Prevalence and Risk Factors

It is well known that OA differs in men and women. Men younger than 50 tend to be most affected by OA, whereas, in people over 50, two thirds of the OA patients tend to be women [69]. The reason for this is unclear. Osteoarthritis in younger men is considered to be an occupational disease. In contrast, the high incidence in postmenopausal women suggests that the change in hormonal status may play a role in the OA process. However studies dealing with the protective effect of estrogens show conflicting results (reviewed in [62,93,203]). A study in cynomolgus monkeys has shown that long-term estrogen replacement therapy significantly reduces the severity of OA lesions [94]. These data conflict with a common notion that, as noted for many years, there is an inverse relationship between OA and osteoporosis [191]. However, considering the prevalence and characteristics of the two diseases, it would not be surprising if they coexisted on occasion [6]. Moreover, some patients present with nonprogressive OA, whereas others with progressive OA also have increased bone resorption [13]. Conceivably the latter category of patients

respond to estrogen therapy. Interestingly, a recent study suggests that antagonists of cannabinnoid receptors can protect against bone resorption [110]. Magnetic resonance imaging can help obtain a more precise estimate of the effects of estrogens on cartilage and bone. Estrogens may prevent some metabolic changes in subchondral bone osteoblasts and in chondrocytes, thereby causing a slowdown in the onset of OA. The efficacy of estrogen therapy may also be due to genetic susceptibility. Indeed, the estrogen α-receptor gene *(ESR1)* has been identified as a candidate gene that may be involved in OA (reviewed in [137,177]).

Genetic studies have pointed to a recessively inherited gene. Because the cause of OA is likely multifactorial, the "gene" more probably expresses a factor involved in a metabolic pathway rather than a structural protein. Some studies examining a metabolic link between obesity and OA have reported a significant association between hypertension, uric acid, cholesterol, and OA, whereas others failed to substantiate these relationships [48,146,220,225]. A unifying hypothesis could be that OA is a metabolic disease in which systemic or local factors induce changes in skeletal tissues. As tissue homeostasis is disturbed and joint integrity impaired, normal wear and tear could lead to cartilage damage, the hallmark of OA. The loss of cartilage may then be the result of mechanical forces, but this would not be the cause of the disease itself. Furthermore, the OA process may modify the formation and biosynthetic activity of cells derived from mesenchymal stem cells (MSCs) [5]. Osteoblasts derived from mesenchymal precursor cells can also give rise to chondrocytes, myoblasts, adipocytes, and tendon cells [97], all cells affected by OA. However, when bone marrow MSCs from OA patients were cultured, they did not exhibit altered in vitro proliferation, nor did the number of osteogenic precursors change with age [174]. This contrasts with observations on osteoporotic patients. Nonetheless, the osteogenic activity of MSCs of patients with advanced OA is enhanced, whereas their chondrogenic and adipogenic activity is reduced [168]. Because adipocytes share a common mesenchymal stem cell precursor with osteoblasts, chondrocytes, tenocytes, and myoblasts, a link may exist between lipid and connective tissue metabolism in OA [5]. The link may be leptin, a systemic factor that plays a role in body weight regulation, lipid metabolism, and obesity. Indeed leptin may be a key factor in the etiology of OA [60,124,138].

The potential role of bone tissue in OA initiation or progression may also be due to its capacity to serve as a reservoir for MSCs and to provide nutrition for the hyaline cartilage. The role of mesenchymal cells in the appearance or progression of OA is a key issue that has received recent attention. Because there is increasing evidence of the abnormal behavior and phenotypic features of osteoblasts, chondrocytes, myoblasts, and tenocytes in OA joints, MSC development and differentiation are likely to be altered in affected individuals. Evidence indicates that MSC numbers, proliferation rate, population-doubling time and the capacity to differentiate into different lineage cells may be altered in OA [58,168,169]. The response to cytokines and growth factors by MSCs in OA individuals may also be altered [135]. This may indicate that their differentiation into target cells is also altered in vivo, leading to abnormal tissue homeostasis [140,211]. It also suggests that cells not presently residing in the affected tissue may profoundly affect behavior and homeostasis of this tissue. The number of MSCs characterized by in vitro multilineage potential is larger in OA than in rheumatoid arthritis synovial fluid [114]. This indicates that, although related, these two diseases have diverging etiology and progression. Moreover, because the chondrogenic and adipogenic capacity of OA MSCs is impaired [168], OA MSCs either remain undifferentiated or differentiate into limited lineage cells, such as the osteogenic line. This can explain why all joint tissues except bone are impaired or reduced in OA individuals. Indeed, although muscle strength is reduced beyond the normal age-related loss [108], possibly due to muscle cell dysfunction [107], alterations in the differentiation capacity of MSCs to form myocytes may also be altered in these individuals.

Even more importantly, this alteration in MSCs may also affect the important immunoregulatory role played by MSCs in OA tissues [55,119,121]. This regulatory role is modulated by the action of specific signaling molecules such as TGF-β and HGF. In this connection it is important to remember the key role played by cytokines and growth factors in osteophyte formation, and the elevation of TGF-β [149] and HGF levels in OA cartilage, although the

role of HGF is uncertain. Osteophyte formation may be considered a repair response to stabilize the damaged joints. It requires the local recruitment of specific MSCs. This demonstrates the potential therapeutic role stem cells may play in OA joints.

Recent genome-wide scans have revealed an OA susceptibility locus on chromosome 11q, in close proximity to the low-density lipoprotein receptor–related protein 5 (LRP5). This gene product controls bone mass and may be the cause of the abnormal bone tissue mineralization and remodeling observed in OA patients. Although no individual polymorphisms were found in a study of 187 individuals, an altered haplotype of LRP5 was identified that increases the risk of OA 1.6 fold [222]. Therefore, LRP5 and the associated Wnt signaling pathway may constitute significant factors in the pathology of OA.

Felson et al. [68] have provided an interesting new perspective on subchondral bone marrow changes in OA. They found a strong correlation between the marrow edema and pain, but not with the severity of pain. In a longitudinal study, they also found that bone marrow edema was largely related to limb alignment [214,219]. Medial bone marrow lesions were seen mainly in patients with varus limbs, and lateral lesions were seen mostly in patients with valgus limbs. However, even after adjustment for misalignment, bone marrow edema lesions were still strongly associated with radiographic progression.

2.10 Role of Bone Tissue in Osteoarthritis Progression or Initiation

The most important feature of OA is that structural changes in the joint involve the major constituents of articulation including subchondral bone. Whether these changes affect cartilage before reaching subchondral bone or vice versa is still a matter of debate. Resolution of this question is likely to modify knowledge of the disease and potential treatment. There is increasing evidence that crosstalk between cartilage and subchondral bone is an integral part of the disease process [70,129,180]. Morphologic changes of subchondral bone have been the subject of both preclinical and clinical studies, with preclinical studies particularly useful for the study of early disease stages. Radin and Rose [193] were among the first to suggest a possible role for subchondral bone in the initiation and progression of cartilage degeneration. They speculated that the increases in mass and thickness might modify biomechanical properties, favoring the appearance/progression of structural changes in the articular cartilage. A number of studies have reported that subchondral bone undergoes structural changes that vary as the disease progresses and seem to be part of the disease process itself [29,35,49,89,106]. These changes may involve biochemical pathways that not only modulate bone changes but also may contribute to cartilage degradation [19,53,101,128,144,149,178,208,241].

The concept of a role for bone tissue in OA is based on the observation that this tissue is sclerotic and that OA patients show increased bone mineral density (BMD) upon dual x-ray measures. Even though OA patients are said to have higher BMD and increased osteoid matrix, mineralization of the subchondral bone tissue is reduced [144]. This could result from an alteration in bone tissue remodeling or a change in bone turnover [8,17,68,90,145,197] and would also increase bone stiffness [32]. To increase the density of subchondral bone means bone formation exceeds bone resorption, [49,153,217]. On the other hand, studies of changes in structure and metabolism of subchondral bone in the early phases of OA have, in general, indicated that bone resorption has increased more than bone formation [19,20,38,49,106,224,226]. A report by Bettica et al [13] has clearly shown that bone resorption is increased in patients with progressive knee OA. These changes are associated with an increase in the number and size of the remodeling units [20]. Moreover, in experimental dog OA, osteoblasts isolated from subchondral bone have been shown to overproduce biochemical factors that favor osteoblast maturation and activation and the resorption of the bone matrix [128,179]. Subchondral bone remodeling and resorption in dog OA can be reduced by a nonsteroidal antiinflammatory drug (NSAID) that inhibits cyclooxygenase activity [179]. This seems associated with reduced synthesis of urokinase-type plasminogen activator (uPA) and bone remodeling growth factors. These interesting findings are

in accord with a recent report on the key role played by the eicosanoids, PGE_2 and LTB_4, in modulating synthesis by subchondral bone osteoblasts from human OA [178].

An elevated bone turnover rate can result from abnormal systemic regulation, from an altered response to normal signals, or from abnormal cell function. The latter is implied by the finding that osteocalcin, a marker of bone formation, is elevated in synovial fluid of patients whose knee scan showed abnormalities [215]. We have observed a higher than normal metabolic rate in osteoblasts that had been isolated from the bone of tibial plateaus of OA patients. These OA osteoblasts showed elevated levels of osteocalcin [101] and higher IGF-1 and TGF-β levels compared to normal [101,149,150]. Similarly, Gevers and Dequeker [80] have reported elevated serum osteocalcin levels in women with OA of the hand, and elevated osteocalcin in cortical bone explants. The levels of IGF-1, IGF-2, and TGF-β, were also found elevated in samples of iliac crest bone of patients with OA [53]. This is a site distant from weight-bearing joints. High TGF-β and IGF-1 activity in OA bone tissue would promote bone remodeling [101] by altering the balance between bone matrix synthesis [175] and degradation via activation or inhibition of MMPs [206]. In turn, the altered levels of TGF-β and IGF-1 would promote cartilage degradation directly or via a change in mechanical pressure acting on cartilage [54,192,193]. However, stiffness and BMD are not uniform in OA bone [70,142,228]. The bone closest to the articular cartilage has the greatest effect on cartilage integrity, with variations in stiffness and BMD probably causing more damage to cartilage than any of the parameters under normal conditions [43,66]. The rise in BMD in OA may have resulted from an increase in density due to a more abundant osteoid collagen matrix [8,144,145]. This matrix may be undermineralized. Indeed, osteoid matrix is increased in OA bone tissue [144,246], an indication of abnormal mineralization and of generalized metabolic bone disease [80,82,188]. The increase in osteoid matrix is accompanied by an increase in collagen type I in the trabecular bone of the femoral heads of OA patients [144,145], with the ratio of α1 to α2 chains higher in OA than in normal tissue [8]. We have observed a 50% increase in collagen type I production in cultured OA osteoblasts, the result of upregulation of α1 chains. There was no change in the α2 chains. Together with the reduced number of crosslinks in OA bone tissue [144], this could explain the reduction in bone mineralization. In vivo, the increase in bone collagen may have induced the MSCs to differentiate into osteoblasts. Indeed, OA patients exhibit enhanced maturation of osteoblasts from bone marrow stromal cells, while adipocytes and chondrocytes mature more slowly [168]. Of note, leptin stimulates the differentiation of mesenchymal cells into osteoblasts while impeding adipocyte maturation [227]. Leptin also stimulates production of osteoblast and collagen biomarkers [85].

2.11 Possible Interaction of Bone and Cartilage in Osteoarthritis

An earlier concept that targeted repair of the subchondral bone is initiated in response to the formation of microfractures was revived when it was observed that the elastic modulus (stiffness) of the tissue was reduced locally due to an increase in vascularization and in the remodeling rate [40]. These changes would lead to reactivation of the secondary ossification center and a decrease in cartilage thickness [30]. The concept that modified remodeling or bone turnover causes cartilage degradation, as opposed to an effect of subchondral bone plate thickening, is also supported by animal studies that showed the severity of OA lesions was greater and progression of the disease more rapid in guinea pigs, animals that had the highest bone turnover rate, notwithstanding an initial difference in the thickness of the subchondral bone [105]. This suggests that bone tissue homeostasis and turnover are more important for the appearance and progression of OA than the state of the bone tissue at a given time point. It is the mechanical disturbance of the joint tissues that would then induce local responses that lead to the thinning of the articular cartilage. This in turn would contribute to shear stress and lead to complete cartilage loss.

There is still speculation as to whether the abnormal structure and metabolism of subchondral bone are linked to the development/progression of cartilage damage. Some studies have indicated that subchondral bone

changes precede and may be responsible for the evolution of cartilage lesions [35,89,193]. Other studies have indicated that subchondral bone changes proceed simultaneously or even follow changes in cartilage. Subchondral bone changes, therefore, would be only secondary to cartilage degradation [20,29,38]. Although the pathways involved in crosstalk between subchondral bone and cartilage remain largely unknown, several factors synthesized by subchondral bone cells are capable of inducing metabolic changes in the cartilage.

Subchondral bone tissue may, by the production of cytokines and growth factors, induce OA [29,32,112]. Bone does produce a number of proinflammatory cytokines and growth factors that are involved in tissue remodeling and modulate cartilage catabolism. Early pathologic studies have shown that clefts or channels in the tidemark appear early in OA [31,160,166, 223,234]. This indicates a possible way to traffic cytokines and growth factors from the subchondral compartment to the overlying cartilage. Fatigue microcracks are also observed in articular cartilage [167] and may serve as channels for signal molecules from bone to cartilage. This does not rule out the possibility that these molecules diffuse within the bone extracellular matrix to reach the articular cartilage. Indeed, because healthy cartilage is considered avascular and aneural, nutrients must enter articular cartilage either from the surface, via the synovial fluid, or from the underlying subchondral bone.

For a long period of time, synovial fluid was believed to be the only nutrient route, because of the absence of an anatomic barrier and because the cartilage cannot survive when not nourished by the synovial fluid [132]. However, in a number of circumstances, cartilage degenerates even when still in contact with normal synovial fluid. Nutrient supply of articular cartilage by subchondral bone may seem obvious, but the dense calcification in the basal zone of normal articular cartilage has been considered an insurmountable barrier to solute and fluid diffusion [61,112]. However, Malinin and Ouellette [141] have demonstrated that when baboon cartilage is deprived of contact with subchondral bone for any length of time, degenerates as in OA. Therefore, both routes of nutrient entry may coexist. Hence, bone-derived products may indeed drive cartilage metabolism [158,241]. Potential candidates are IGF-1, TGF-β, and interleukin-1β and -6 (IL-1β, IL-6). The IGFs are important growth factors that regulate bone formation [103]. Osteoarthritic subchondral osteoblasts produce variable total IGF-1 levels and less IGF binding proteins compared to normal [101,148,150]. This results in higher levels of free IGF-1 that seem to promote bone remodeling [101,147] and increase bone stiffness, a situation that exacerbates cartilage matrix degradation [54]. Both TGF-β and IGF-1 are involved in matrix deposition and turnover, with TGF-β stimulating matrix synthesis [175] and collagenase activity, whereas IGF-1 inhibits matrix development in bone cells [206]. The increase in growth factors is likely to favor matrix deposition in bone and to limit overall degradation. In this connection it is worth calling attention to a study in murine knee joints that showed that intraarticular injections of TGF-β into the joint induce osteoarthritic-like changes [231]. Blocking endogenous TGF-β production during experimental osteoarthritis prevents osteophyte formation [210]. The localized effect of TGF-β and IGF-1 may be linked to an abnormal response to leptin by OA subchondral osteoblasts. This may be so because the expression of leptin stimulates TGF-β and IGF-1 in joints [60].

Of the cytokines and eicosanoids produced by bone cells, IL-1β, IL-6, PGE_2, and LTB_4 are the major molecules that modulate turnover of the extracellular matrix. Osteoarthritis patients can be characterized as low and high producers of PGE_2 and IL-6 on the basis of the culture of their subchondral osteoblasts [149]. PGE_2 production is inversely correlated with the synthesis of LTB_4 [176]. This is the opposite of what is found in normal subchondral bone [149,176] and may contribute to promoting new matrix deposition and bone formation in OA [73,116]. However, the new matrix is undermineralized. At low concentrations, PGE_2 stimulates bone formation, but appears inhibitory at high concentrations [93,111,194]. PGE_2 stimulates collagen synthesis and promotes osteoblast proliferation. In contrast, leukotrienes stimulate osteoclast differentiation and bone resorption [74].

Therefore, when the ratio of PGE_2/LTB_4 is high in OA, osteoblasts may promote subchondral bone formation. On the other hand, IL-1β and IL-6 promote matrix degradation in both bone and cartilage by their action on specific MMPs [122]. Conceivably these actions are related to the fact that the disease progresses slowly in some patients, but rapidly in others [198]. Because OA patients can be classified by their LTB_4 levels, and because LTs are more potent inflammatory mediators than PGE_2 [84,139,181]), this may explain the protective role of n-3 polyunsaturated fatty acids (PUFAs) in OA chondrocytes [46], particularly because PUFAs modulate the inflammatory process [1,133,184,238].

2.12 Evidence for Crosstalk Between Subchondral Bone and Articular Cartilage

The initiating event responsible for the degradation of cartilage in OA patients is not known, but a contributory role of subchondral bone is gaining support [53,101,144,145, 241]. Factors secreted by osteoblasts may modify chondrocyte differentiation [92,113,207, 208,241]. A plausible explanation is that locally produced cytokines/growth factors/eicosanoids diffuse from subchondral bone tissue through the bone–cartilage interface and stimulate cartilage breakdown. Indeed, there is evidence [39, 159] that channels and fissures between cartilage and bone allow biologic signals to move between the two compartments [112,160,223]. We have reported that hepatocyte growth factor (HGF) may be involved in the crosstalk between the two tissues [92], inasmuch as HGF has been detected in the deeper layer of cartilage, most intensively in fibrocartilage from OA patients [71,182]. Because chondrocytes do not produce HGF [9,92], osteoblasts from the subchondral bone plate are the likely source of HGF. HGF production by cells isolated from trabecular bone has been reported [15,221]. Osteoblasts isolated from OA subchondral bone produce more HGF than normal [92]. Moreover, HGF induces collagenase-3 in chondrocytes, an enzyme that is involved in cartilage degradation in OA [199] and is present in the deep layer of articular cartilage [163], that is, the same site where HGF has been observed. Conceivably, HGF produced by subchondral osteoblasts can reach the deep layer of articular cartilage via local vascularization and/or channels [31,166,234], where it promotes cartilage breakdown or enhances matrix remodeling.

The calcified cartilage layer had been considered impenetrable, but several groups of investigators have reported that many channels exist between the subchondral region (Fig. 2.1) and the uncalcified cartilage [31,39,159,160, 166,223]. In addition, the calcified layer of aging articular cartilage has been shown to possess microcracks [31,223], so that transfer of humoral information from the subchondral bone region to the basal layer of cartilage seems probable.

The sources of angiogenic signals to the subchondral bone are poorly understood. OA chondrocytes within the deeper layers of articular cartilage produce angiogenic factors such as vascular endothelial growth factors (VEGFs) [186]. With disruption of the tidemark, angiogenic factors may also reach the osteochondral junction by mass transport and diffusion from the synovium through synovial fluid and the cartilage matrix [4]. Synovial fluids from patients with OA contain a variety of angiogenic factors that may stimulate endothelial tube formation in vitro [23]. Subchondral bone may contribute or support angiogenic stimuli within the osteoarthritic joint, because osteoblasts express angiogenic factors [5,92, 202].

Vitamin A derivatives promote subchondral bone proliferation. This in turn leads to progressive atrophy of the articular cartilage and initiates ectopic production of collagen type I in cartilage, a feature reminiscent of OA [127]. In fact, when subchondral bone proliferates into the degenerating articular cartilage, the cartilage produces osteoblast-stimulating factor-1, not normally expressed by articular cartilage. This indicates that vitamin A derivatives may promote differentiation of chondrocytes, as observed in OA. The intimate link between articular cartilage and subchondral bone is also illustrated by the fact that cartilage repair by autologous chondrocytes is less effective than osteochondral cylinder transplantation. Chondrocyte implantation produced fibrocartilage, whereas hyaline character was retained in osteochondral cylinder transplantation [87].

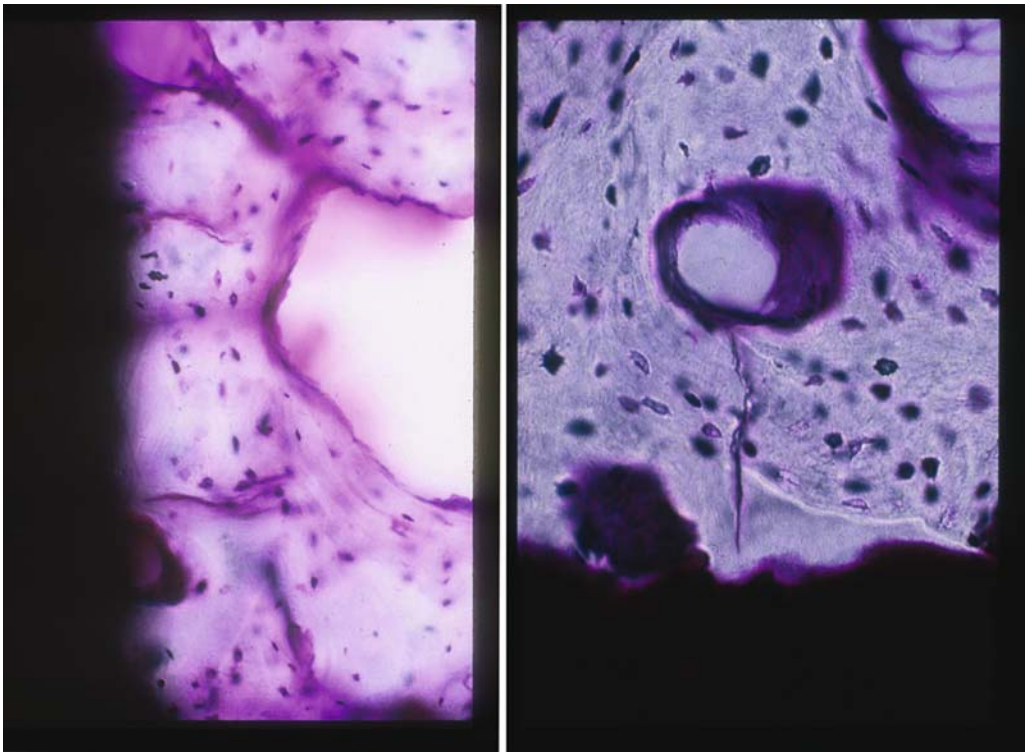

Figure 2.1. A representative histochemical depiction of microcracks in subchondral bone and articular cartilage. Block-stained with fuchsin red. Left: Several microcracks in the subchondral bone plate. Right: A microcrack extending from an osteon in the subchondral bone across the calcified cartilage toward the articular cartilage. Original magnification: 100× (left) and 200× (right). (Courtesy of Dr. David B. Burr, University of Indiana in Indianapolis; studies supported by NIH grant AM27127 to Eric L. Radin and collegues.)

2.13 Conclusion

It is well established that changes in subchondral bone and modifications of osteoblast metabolism are key in the initiation and progression of OA. Both animal and clinical studies support the emerging concept that changes in bone may precede changes in a cartilage. Hence, bone tissue rather than cartilage may be the initiating site of the events that bring about OA.

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3.

Cytokines, Growth Factors, and Bone-Derived Factors in Cartilage

Mary B. Goldring

3.1 Introduction

Osteoarthritis (OA) is a disease of unknown etiology that involves degeneration of articular cartilage, limited intraarticular inflammation manifested by synovitis, and changes in the subchondral bone. Mechanical, biochemical, and genetic factors contribute to the loss of joint integrity and the initiation and progression of cartilage destruction. Chondrocytes, which are the unique cellular component of adult articular cartilage, react to structural changes in the surrounding cartilage matrix by responding to and producing catabolic cytokines and anabolic factors, which act in an autocrine-paracrine manner. Since the initial stages of OA involve increased cell proliferation and synthesis of matrix proteins, proteinases, growth factors, cytokines and other inflammatory mediators by chondrocytes, research has focused on the chondrocyte as the cellular mediator of OA pathogenesis. The adult articular chondrocyte, which normally maintains the cartilage with a low turnover of matrix constituents, has limited capacity to regenerate the normal cartilage matrix architecture, however, and damage becomes irreversible unless the destructive processes are interrupted. Interactions between the cartilage and the other cells and tissues of cartilage damage with the joint, including the synovium and subchondral bone, also contribute to pathogenesis in ways that are largely undefined. Thus, further understanding of the roles of cytokines and growth factors in the initiating events in cartilage destruction and in mediating interactions between the different joint tissues that influence disease progression is needed to develop new approaches that help prevent initiation or progression of OA.

3.2 The Chondrocyte in Adult Articular Cartilage

In physiologic conditions, the adult articular chondrocyte is a resting cell with no detectable mitotic activity that maintains a steady-state intracellular metabolism and low turnover synthesis of matrix constituents [69]. Chondrocytes comprise 2% to 5% of the cartilage volume, which is avascular and lacks innervation. The collagen network of the interterritorial cartilage matrix is composed of types II, IX, and XI collagens, which provide tensile strength and promote the retention of proteoglycans. Type XI collagen is part of the type II collagen fibril and type IX integrates on the surface of the fibril with the outward projecting noncollagen domain to bring about association with other matrix components. The other major component, the large aggregating proteoglycan aggrecan, which is attached to hyaluronic acid (HA) polymers via link protein, bestows compressive resistance [73,172]. Other

noncollagen molecules in the interterritorial matrix include biglycan, decorin, and fibromodulin, the matrilins, and cartilage oligomeric matrix protein (COMP). The chondrocytes are surrounded by a pericellular matrix composed of type VI collagen microfibrils that interact with HA, biglycan, and decorin and maintain chondrocyte attachment. However, the matrix contains little or no fibrillar collagen [173]. In adult articular cartilage, the collagen half-life is more than 100 years and the half-life of aggrecan core protein subfractions ranges from 3 to 24 years. Glycosaminoglycans and other cartilage matrix constituents, including biglycan, decorin, COMP, tenascins, and matrilins, may also be synthesized by chondrocytes under low turnover conditions. However, remodeling of chondrocytes differs in the various regions of cartilage, and the matrix turnover may be more rapid in the immediate pericellular zones [236].

Chondrocytes maintain active membrane transport systems to exchange cations, including Na^+, K^+, Ca^{2+}, and H^+, whose intracellular concentrations fluctuate with load and changes in the composition of the cartilage matrix [234]. In the absence of a vascular supply, the chondrocyte must rely on diffusion from the articular surface or subchondral bone for the supply of nutrients and metabolites. Chondrocytes do not contain abundant mitochondria, and energy requirements may be modulated by normal levels of mechanical stress [115]. Glucose serves both as the major energy source for the chondrocytes and as an essential precursor for glycosaminoglycan synthesis. Facilitated glucose transport in chondrocytes is mediated by several distinct glucose transporter proteins (GLUTs) that are either constitutively expressed (GLUT3 and GLUT8) or cytokine-inducible (GLUT1 and GLUT6) [147].

Chondrocyte metabolism operates at low oxygen tension within the cartilage matrix, ranging from 10% at the surface to less than 1% in the deep zones. When cultured in a range of oxygen tensions between severe hypoxia (0.1% O_2) and normoxia (21% O_2), chondrocytes adapt to low oxygen tensions by upregulating hypoxia inducible factor-1α (HIF-1α). Hypoxia via HIF-1α can stimulate chondrocytes to express GLUTs [147] and angiogenic factors such as vascular endothelial growth factor (VEGF) [119,176], as well as a number of genes associated with cartilage anabolism and chondrocyte differentiation, including Sox9, transforming growth factor-β (TGF-β), and connective tissue growth factor [182]. In the growth plate, hypoxia and HIF-1α are associated with type II collagen production [170]. HIF-1α is expressed in both normal and OA articular cartilage [41]. It maintains tonic activity during physiologic hypoxia in the deeper layers associated with increased proteoglycan synthesis, but unlike what happens in other tissues, it is not completely degraded when normoxic conditions are applied [31]. Long-term systemic hypoxia (13%), on the other hand, may downregulate collagen and aggrecan gene expression in articular cartilage [90]. Thus, by modulating the intracellular expression of survival factors such as HIF-1α, chondrocytes can survive in the avascular cartilage matrix and respond to environmental changes.

3.3 The Chondrocyte in Osteoarthritis

Osteoarthritis is defined largely as a disease of cartilage, since the chondrocytes, which constitute the unique cellular component of adult articular cartilage, are able to respond to mechanical injury, joint instability due to genetic factors, and biologic stimuli such as cytokines and growth and differentiation factors (Fig. 3.1). In young individuals without genetic abnormalities, biomechanical factors due to trauma are strongly implicated in initiating the OA lesion [95,114], and mechanical disruption of the association between chondrocytes and matrix may lead to changed metabolic responses in the chondrocyte [6,82]. The aberrant behavior of OA chondrocytes is reflected in the appearance of fibrillations, matrix depletion, cell clusters, and changes in quantity, distribution, or composition of matrix proteins [175]. In the early stages of OA, a transient increase in chondrocyte proliferation is associated with increased synthesis of cartilage matrix proteins. This is often interpreted as a repair response. At the same time, there is increased synthesis of the catabolic cytokines and matrix-degrading enzymes. Local loss of proteoglycans and cleavage of type II collagen occur initially at the cartilage surface. This results in an increase in water content

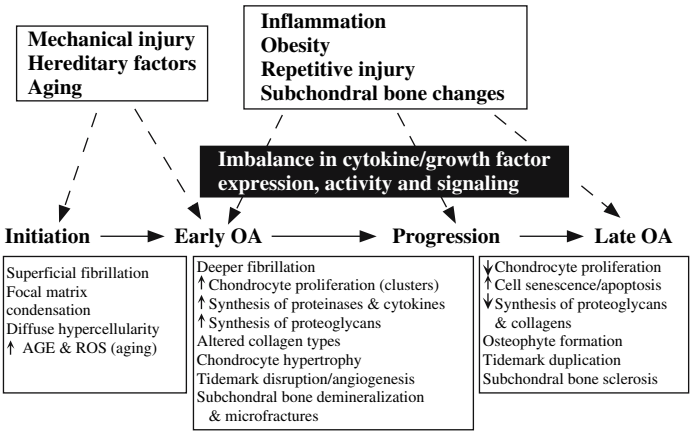

Figure 3.1. Scheme of events involved in the initiation of osteoarthritis (OA) and progression to irreversible cartilage damage. Potential causative factors are listed in the boxes above, and the histologic and biochemical changes are listed in the boxes below.

and a loss of tensile strength in the cartilage matrix as the lesion progresses. At later stages, the degradation of matrix components far exceeds the synthetic capacity of the chondrocyte, which has undergone a loss of phenotypic matrix protein expression. The presence of osteophytes, cartilaginous outgrowths from the periosteum that undergo endochondral ossification, reflects an unsuccessful attempt to repair ongoing tissue destruction.

3.3.1 Cartilage Matrix Degradation in Osteoarthritis

Because the adult chondrocyte in adult articular cartilage cannot maintain the balance between synthesis and degradation of the cartilage matrix, this imbalance assumes significance in OA pathogenesis. In response to traumatic injury or inflammatory processes, global gene expression is activated [60,82,111]. This results in increased proliferative and synthetic activity. Chondrocytes have receptors for responding to mechanical stress, many of which are also receptors for extracellular matrix components [145]. Integrins serve as receptors for fibronectin (FN) fragments [124], annexin V for type II collagen fragments [59], and discoidin domain receptor-2 (DDR-2) for native type II collagen fibrils [238], all of which can stimulate the production of matrix-degrading proteinases. The association *of cartilage damage with* increased production of proteinases, including the matrix metalloproteinases (MMPs), MMP-1, MMP-3, MMP-8, MMP-13, and the aggrecanases, ADAMTS-4 and -5, has been established. Synovial fluids from patients with OA contain both aggrecanase and MMP-generated aggrecan fragments [210]. MMP-13–specific type II collagen cleavage products and MMP-13 itself have been immunolocalized in OA cartilage [220,236], and postnatal overexpression of constitutively active *Mmp13* in cartilage in mice produces OA-like changes in knee joints [160]. Of the proteinases that degrade aggrecan, to date only aggrecanase-2, ADAMTS-5, appears to be associated with increased susceptibility to OA, as shown in *Adamts5*-deficient mice [66,207] (Table 3.1).

3.3.2 Cartilage Matrix Synthesis in Osteoarthritis

In early stages of OA, the synthesis of matrix proteins is altered. Genomic and proteomic analyses of global gene expression have detected an increase in type II collagen gene (*COL2A1*) expression in early OA cartilage [3,16,87], possibly associated with the increased levels of anabolic factors such as bone morphogenetic protein-2 (BMP-2) and inhibin βA/activin [63,87,158]. These and other TGF-β family members may stimulate aggrecan synthesis at the same time as promoting the formation of fibrocartilage and osteophytes,

Table 3.1. Transgenic and knockout mouse models and susceptibility to osteoarthritis

Gene	Gene defect or modification	Features
Extracellular matrix		
Col2a1	Heterozygous knockout or mutation	OA-like changes during aging*
Col9a1	Transgenic truncation	Mild chondrodysplasia; OA*
Col9a1	Knockout	Early onset OA*
Col11a1	*Cho*/+; spontaneous deletion	OA-like changes during aging [237,238]
Aggrecan	Cmd/+; spontaneous deletion	OA-like changes during aging*
Fibromodulin	Knockout	Tendon mineralization; OA [242]
Fibromodulin/biglycan	Double knockout	Ectopic mineralization; early-onset OA [242]
Matrilin-3	Knockout	OA-like changes with aging [226]
Proteinases		
Mmp9	Knockout	Exacerbation of surgically induced OA [65]
Mmp13	Postnatal transgenic	Increased susceptibility to OA [160]
Adamts5	Knockout	Decreased susceptibility to OA [66,207]
Cytokines and related molecules		
IL-1β	Knockout	Exacerbates OA in STR/ORT mice; protects against surgically induced OA [40,65]
IL-6	Knockout	Severe spontaneous OA with subchondral bone sclerosis in aging males but not in females [45]
ICE	Knockout	Exacerbates OA in STR/ORT mice [40]
MK2	Knockout	More severe in surgically induced OA [65]
NOS2	Knockout	Reduced proteoglycan depletion and restoration of IGF-1 responsiveness*
Growth factor signaling		
BmpR1a	Postnatal conditional knockout	OA-like cartilage degeneration [184]
TgfβRII	Transgenic dominant-negative truncation	Enhanced chondrocyte hypertrophy and progressive skeletal degeneration with OA [200]
Smad3	Targeted disruption	Enhanced chondrocyte hypertrophy and OA-like cartilage erosion [240]
Ank	*ank/ank*; homozygous truncation mutation	Early-onset OA associated with crystal deposition [89]
Npp1	Knockout	OA associated with crystal deposition [101]
α1-integrin	Knockout	Accelerated cartilage degradation [245]

OA pathology refers to cartilage damage and, where noted, a subchondral bone sclerosis. According to Glasson and colleagues [65,66], the severity of the OA due to surgical instability induced by destabilization of the medial meniscus depends on the wild-type strain, the 129/SvEv strain developing the most severe OA, followed by C57B10.RIIII, C57BL/6, FVB/N, and DBA. MMP-3, Cox-1, Cox-2, Jak3, MMP-12, cPLA2a, and ADAMTS-4 KO mice displayed no exacerbation of or protection against OA pathology.

*See [86,181,188,223,242].

IGF, insulin-like growth factor; OA, osteoarthritis.

bony structures found at the periphery of the joint surface [196]. Regional differences may exist, however, as some studies of early OA patients show upregulation of matrix synthesis in ankle but not in knee cartilage [11]. Type X collagen, a marker of the hypertrophic chondrocyte that is normally absent in adult articular cartilage, has been detected in certain stages in OA or in atypical sites in OA cartilage [193]. This is also true for type III and type VI collagen, which are normally present at low concentrations, and for the chondroprogenitor splice variant of the type II collagen gene, type IIA. The increase in pericellular type VI collagen microfibrils indicates that the chondrocyte can respond to changes in its microenvironment [204]. In addition to *COL10A1*, which encodes type X collagen, and MMP-13, other genes associated with growth plate development, such as MMP-9 and Indian

hedgehog (Ihh) occur in the vicinity of early OA lesions [219]. Surprisingly, decreased levels of Sox9 messenger RNA (mRNA), the master switch for the *COL2A1*-expressing chondrocyte, are detected near the lesions [219], and the expression of this factor, which is required for activation of *COL2A1* transcription, does not localize with *COL2A1* mRNA in adult articular cartilage [4]. Nevertheless, to enhance cartilage tissue engineering, it has been proposed to transduce Sox9 together with L-Sox5 and Sox6—both of which are required for chondrogenesis during development—into mesenchymal stem cells ex vivo or into joint tissues in vivo [97,221]. At late stages, when catabolic processes dominate, it is more difficult for the chondrocyte to replicate the complex composition of the articular cartilage matrix laid down during development. This is particularly true once there is severe damage to the collagen network. Furthermore, the chondrocyte stress response may result in the loss of viable cells due to apoptosis or senescence [5].

3.3.3 Mouse Models of Osteoarthritis

A number of studies have shown that developmental defects in the cartilage matrix proteins may lead to degenerative changes in adult mice and OA-like pathology (Table 3.1). Heterozygous *Col2a*1+/− mice have a cartilage matrix that has an altered collagen network susceptible to the development of OA-like changes. Mutations in *Col9a1, Col2a1, Col11a1*, and aggrecan also result in osteoarthritic changes in adult mice (for review see [86,181]). In the Del1 mouse, which has a small deletion of the transgene encoding type II collagen, cathepsin K upregulation is associated with early-onset OA [150]. The *cho*/+ mouse, which is *Col11a1* haploinsufficient, develops OA-like pathology by 3 months with increased MMP-3 expression and proteoglycan degradation [237]. Degradation of the collagen network begins by 6 months, because MMP-13 activity has been stimulated by interaction of the discoidin domain receptor 2 (DDR2) with exposed native type II collagen fibrils [238]. Models with deficiency of matrilin-3 [226], α1 integrin [245], or biglycan, together with fibromodulin or decorin [7,243], also show OA-like pathology.

Table 3.2. Genes implicated in hand or hip osteoarthritis

Candidate gene studies	Genome-wide linkage scans
Extracellular matrix molecules	
Type II collagen (COL2A1)	COL9A1
Type IX collagen (COL9A1)	Matrilin-3 (MATN3)
Aggrecan (AGC1)	ADAM12
	Cartilage intermediate layer protein (CILP)
Signaling molecules	
Vitamin D receptor (VDR)	Secreted frizzled related protein-3 (FRZB)
Estrogen receptor α (ESR1)	Bone morphogenetic protein-5 (BMP5)
Interleukin-1 (IL-1) gene cluster	Interleukin-4 receptor (IL-4R)
	Asporin (ASPN)
	Calmodulin 1 (CALM1)
	Bone morphogenetic protein-2 (BMP2)
	Osteoprotegerin (OPG)

In most of these mouse models, the OA-like changes become more prevalent with aging [86]. In some cases, polymorphisms in these genes have been identified in patients with hand and hip OA [128,206] (Table 3.2).

3.4 The Role of Cytokines in Osteoarthritis

Inflammation and accompanying dysregulated cytokine catabolic activities play key roles in OA pathogenesis in addition to the effects of age and excessive mechanical loading (for review, see [70,74,121] (Fig. 3.2). Osteoarthritis is not considered a classical inflammatory arthropathy, because of the absence of neutrophils in the synovial fluid and the lack of systemic manifestations of inflammation. Nevertheless, synovial inflammation is common in both early and late stages of OA [19]. The expression of proinflammatory cytokines is observed in patients with early OA, and synovitis is common in advanced OA, where it involves infiltration of activated B cells and T lymphocytes [156]. There appears to exist a relationship between the levels in OA synovial fluids and joint tissues of catabolic enzymes and inflammatory mediators such as prostaglandins and nitric oxide and the levels of interleukin-1

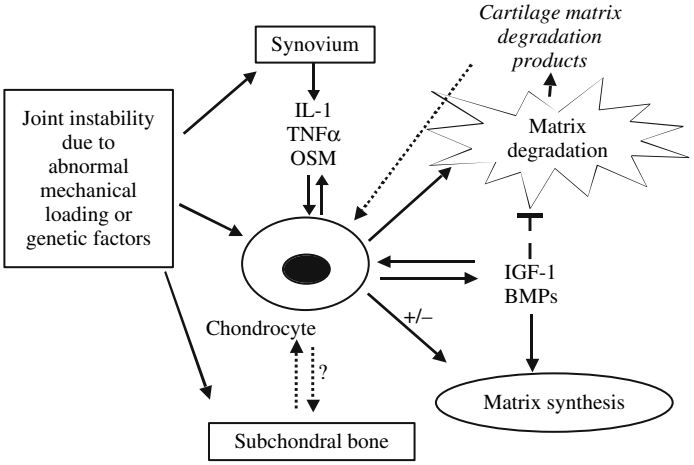

Figure 3.2. The loss of balance between cartilage matrix degradation and synthesis in osteoarthritis. The figure shows potential roles and sources of inflammatory cytokines, such as interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α), and oncostatin M (OSM), and anabolic growth factors, such as insulin-like growth factor-1 (IGF-1) and bone morphogenetic proteins (BMPs), that regulate cartilage metabolism.

(IL-1) and tumor necrosis factor-α (TNF-α). Therefore therapies that interfere with the expression or actions of these cytokines have been explored [68,167].

3.4.1 Interleukin-1 and Tumor Necrosis Factor-α

In vitro and in vivo studies have shown that IL-1 and TNF-α are the predominant catabolic cytokines involved in the destruction of the articular cartilage in OA. The first recognition of IL-1 as a regulator of chondrocyte function stems largely from the early work of Fell and Jubb [55], who identified a soluble factor, termed "catabolin," in supernatants of normal, noninflamed porcine synovial fragment cultures that stimulated chondrocytes to break down the surrounding cartilage matrix. Similar activities were found in culture supernatants from mononuclear cells and synovium [43,142] and later attributed to IL-1 [140,190]. Subsequent studies in vitro and in vivo have established that IL-1 can stimulate the synthesis of most, if not all, proteinases that destroy cartilage.

The major events in OA pathogenesis are localized within the cartilage itself, and chondrocytes participate in this destructive process not only by responding to the cytokines released from other joint tissues, but also by synthesizing them [70,74]. Chondrocytes are therefore continuously exposed to the autocrine and paracrine effects of high local concentrations of IL-1 and other inflammatory mediators. Our understanding of basic cellular mechanisms regulating chondrocyte responses to cytokine mediators has come from numerous studies of cartilage fragment or isolated chondrocyte cultures and from studies with animal models. Chondrocytes in OA cartilage, especially those in clonal clusters, are positive for IL-1 immunostaining [149,220]. Chondrocytes synthesize IL-1 at concentrations that induce the expression of MMPs, aggrecanases, and other catabolic genes, and express IL-1β converting enzyme (caspase-1) and type 1 IL-1 receptor (IL-1RI) [9]. IL-1 colocalizes with TNF-α, MMP-1, -3, -8, and -13, and type II collagen cleavage epitopes in regions of matrix depletion found in OA cartilage [220,236]. The increased sensitivity of OA chondrocytes to IL-1 and TNF-α may be associated with increased levels of IL-1R1 and p55 TNF-R at localized sites [14,233].

Originally known as cachectin, TNF-α, similar to IL-1, acts on chondrocytes in vitro to stimulate the production of matrix-degrading proteinases. On a molar basis, IL-1 is 100- to 1000-fold more potent than TNF-α; however, the two cytokines have strong synergism [224]. For example, intraarticular injection of recombinant IL-1 alone into the joints of rats, mice, or rabbits is sufficient to stimulate the destruction

of the articular cartilage. When TNF-α and IL-1 are injected together, cartilage damage far exceeds the extent of damage observed with injection of either cytokine alone (see, for review, [67]). Work with collagen-induced rheumatoid arthritis (CIA) [224] has led to the concept that TNF-α drives acute inflammation, while IL-1 has a pivotal role in sustaining inflammation and cartilage erosion. Further work is needed to determine whether cytokine synergism also extends to osteoarthritis [223].

In addition to inducing the synthesis of MMPs and other proteinases by chondrocytes, IL-1 and TNF-α increase the synthesis of other proinflammatory cytokines such as IL-6, leukemia inhibitory factor (LIF), IL-17, and IL-18, and chemokines, including IL-8 (see, for review, [70,74]). They also upregulate the production of nitric oxide (NO) via inducible nitric oxide synthetase (iNOS, or NOS2) and prostaglandin E_2 (PGE_2) by stimulating the expression or activities of cyclooxygenase (COX)-2, microsomal PGE synthase-1 (mPGES-1), and soluble phospholipase A_2 ($sPLA_2$). Although PGE_2 and NO have been well characterized as proinflammatory mediators, they may also act protectively in chondrocyte survival and in responses to mechanical stress [57,110]. The mechanisms of crosstalk between prostaglandins and NO that regulate chondrocyte function have been reviewed recently [70]. In the production of prostaglandins, mPGES-1, which is increased in OA cartilage, is a major player [108,118, 136]. In addition to opposing the induction of COX-2, iNOS, and MMPs and suppressing aggrecan synthesis by IL-1, activators of the peroxisome proliferator-activated receptor γ (PPARγ), including the endogenous ligand 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 (PGJ_2), inhibit IL-1–induced expression of mPGES-1 [36,118]. COX-2 is also involved in the chondrocyte response to high shear stress, associated with reduced antioxidant capacity and increased apoptosis [85].

3.4.2 Signaling Pathways Regulated by Cytokines

Interleukin-1 and TNF-α share common signal transduction pathways, and the key signaling molecules involved are targets for therapy [21,132]. The major pathways induced by catabolic cytokines involve signal transduction by the stress-activated protein kinases c-Jun N-terminal kinases (JNKs) and p38 kinases and the nuclear factor (NF)-κB/IκB pathway. In vitro studies in chondrocytes have shown that the p38 and JNK cascades mediate the induction of proteinases and proinflammatory genes by IL-1 and TNF-α. These pathways may also be activated by mechanical stress and cartilage matrix products via integrins and other receptor-mediated events [147,154,160]. Induction of IL-1 and TNFα gene expression by stress-induced pathways suggests a mechanism by which these cytokines may serve as secondary mediators [189].

Small-molecule inhibitors that specifically target cytokine-induced signaling molecules have been explored as potential therapies in models in vitro and in vivo. At least four isoforms of p38 mitogen-activated protein kinase (MAPK) exist [165], with different substrate specificities and differential effects on essential chondrocyte functions [17,174]. The development of specific p38 MAPK inhibitors that target adverse responses to inflammatory mediators has been the subject of intense investigation [48,138,235]. Several p38 MAPK inhibitors have antidestructive effects in animal models of inflammation [161,247]. The JNKs exist in humans as three isoforms, JNK-1, -2, and -3, which have been targeted for the development of small molecule inhibitors [28]. A potent JNK1/2 inhibitor, SP600125, blocks inflammation and joint damage in a model of rheumatoid arthritis [20]. Activated JNK is detected in OA, but not in normal cartilage [39], and JNK inhibition attenuates cytokine-induced chondrocyte responses [2,124,143]. However, the effects of JNK inhibitors in OA animal models have not yet been studied.

While small molecule inhibitors of the NF-κB pathway have efficacy in animal models of rheumatoid arthritis (RA) [183], less information directly relevant to OA is available. Nevertheless, many of the agents developed for treating OA, including diacerhein, *N*-acetylglucosamine, and green tea polyophenols can, to some extent, inhibit IL-1–induced NF-κB activation [21]. Two IκB kinases, IKK-1 and IKK-2, phosphorylate IκB. Inhibitors have been developed that dissociate IκB from NF-κB, thereby permitting translocation of active NF-κB to the nucleus [141,166]. NF-κB mediates the expression of cytokines and chemokines induced

by fibronectin fragments [177]. Inhibition of DNA-binding activity of NF-κB agents that deplete polyamine prevents the induction of IL-8 by TNF-α without promoting chondrocyte apoptosis [53]. A reduction in synovial inflammation often accompanies the reduction of joint damage by the inhibitors of any of the cytokine-induced pathways. When inflammation exists in the OA joint, it therefore becomes a primary therapeutic target [34].

3.4.3 The Role of Cytokines in Animal Models of Osteoarthritis

Animal models have been developed to explore the roles of cytokines in OA pathogenesis [18], (see Chapter 9), and recent studies have examined OA susceptibility in transgenic or knockout mouse models with disrupted cytokine expression or signaling (**Table 3.1**). In the STR/ort mouse that develops spontaneous OA, the catabolic cytokines are expressed in association with lesions [134]. In a number of animal models, delivery of an IL-1 receptor antagonist (IL-1Ra), a naturally occurring antagonist of IL-1, to affected joints by injection or gene transfer, ex vivo or in vivo, inhibits chondrocyte catabolism and cartilage degeneration (for review see [52,58,67,167,223]. In surgically induced models of OA, ex vivo or in vivo gene therapy for an adenoviral vector encoding IL-1Ra resulted in significant inhibition of cartilage degradation [62,245]. Paradoxically, gene deletion of IL-1β or IL-1β–converting enzyme (ICE) seems to accelerate the development of surgically induced knee OA in mice after transection of the medial collateral ligament and partial medial meniscectomy [40]. Clements et al [40] suggested that IL-1β may be essential for maintenance of healthy cartilage, and that overcompensation by other genes with similar activities, such as TNF-α or IL-1α, may increase susceptibility to cartilage degradation. Alternatively, these authors raised the question of whether differential vulnerability of the contralateral joint, which served as an unoperated control, could enhance the spontaneous arthritis [40]. However, Glasson and colleagues [65,66] have shown that IL-1 knockout mice are protected against surgically induced OA. In addition, a study in two murine models of OA has shown that Pralnacasan, an ICE inhibitor, which would also inhibit IL-18 activation, reduces joint damage significantly [187]. In chronic arthritis models, IL-1 knockout mice are protected against cartilage erosion. Moreover, because removal of macrophages from the synovial lining ameliorates OA pathology, synovial inflammation seems to be an important component [24]. No published report is available to date concerning efficacy of a TNF-α inhibitor in an animal model of OA.

3.4.4 The Interleukin-6 Family of Cytokines

Of the cytokines induced by IL-1 and TNF-α, IL-6 appears to play a dual role: it causes an increase in IL-1Ra, sTNFR, and tissue inhibitor of metalloproteins (TIMP), while also enhancing immune cell function and inflammation. Although early studies suggested a potential role for IL-6 as an intermediate, it was found later that the soluble IL-6 receptor (sIL-6R) is required for the full response to IL-6 in chondrocytes in vitro [80]. Interaction of IL-6 with sIL-6R permits synergistic stimulation of MMPs by IL-1 and IL-6 [186]. It also permits upregulation of MMP and ADAMTS and downregulation of COL2A1 and aggrecan in cultured chondrocytes via the JAK/STAT pathway [116,117]. On the other hand, physiologic levels of this cytokine may play a protective role, since IL-6 knockout mice develop OA more readily during aging [45].

Other members of the IL-6 family that act via receptors associated with the gp130 domain may also act as modulators. IL-11 has several effects similar to those due to IL-6, including stimulation of TIMP production without affecting MMP production by chondrocytes [186]. Leukemia inhibitory factor (LIF), which is induced by IL-6, IL-1, or TNF-α, participates in a positive feedback loop by increasing the production of IL-6. Oncostatin M (OSM), another member of the IL-6 family that acts via the JAK/STAT pathway, is a potent stimulator of chondrocyte production of MMPs and aggrecanases in synergism with IL-1 or TNF-α [15,94]. Adenoviral overexpression of OSM alone or together with IL-1 induces synovial inflammation and hyperplasia and severe cartilage damage in mouse knee joints [185]. Since neutralizing OSM antibodies ameliorate cartilage damage in inflammatory arthritis, endogenously produced OSM may have a role distinct from that of IL-1 and TNF-α [171]. Paradoxically, OSM decreases

IL-1β-stimulated production of PGE_2, NO, IL-8, and MIP-1β, but amplifies IL-1β stimulated IL-6 production in OA chondrocytes that are cultured in alginate [191].

3.4.5 Interleukin-17 and Interleukin-18

Interleukin-17 and -18 are two additional cytokines that are potent inducers of catabolic responses in chondrocytes. Interleukin-17 is unique among the proinflammatory cytokines in that it mediates its effects through a receptor that is not related to any known cytokine receptor family. In contrast, the IL-18 receptor shares homology with IL-1RI. Both IL-17 and IL-18 increase the expression of IL-1β by human chondrocytes, as well as stimulating the production of MMPs, IL-6, iNOS, COX-2, and mPGES1 [42, 118, 163]. A role for IL-17 in the promotion of angiogenesis through induction of VEGF in OA chondrocytes and synovial fibroblasts has been proposed [91]. In the CIA model, gene transfer of IL-17 or IL-18 promotes cartilage destruction [102], and blockade with neutralizing antibodies against IL-17 or IL-18 reduces cartilage destruction and joint inflammation [130]. Although IL-17 and IL-18 are proven mediators of the autoimmune processes in inflammatory models [131, 202], the effect of IL-17 or IL-18 blockade has not been tested in OA.

3.4.6 Chemokines

Chemokines are small heparin-binding cytokines that were originally identified as chemotactic factors. Because of the presence of distinct N-terminal cysteine (C) residues, the chemokines are classified as C, CX3C, or CC. The roles played by chemokines in neutrophil activation and chemotaxis in inflammatory arthritis are well established, but their involvement in OA is less clear. When chondrocytes are activated by IL-1, TNF-α, IL-17, IL-18, or OSM, they express several chemokines. They also have receptors that cause responses that are associated with cartilage catabolism (for review see [30, 71]. Borzi et al [29] were the first to report the expression of functional chemokine receptors (CCR1, CCR2, CCR3, CCR5, CXCR1, and CXCR2) on chondrocytes. The receptors, in interacting with their corresponding ligands, MCP-1, Regulated on Activation Normal T-cell Expressed and Secreted (RANTES), and GROα, caused upregulation of MMP-3. Subsequent studies showed that IL-1β and TNF-α increase the expression of the C-C chemokines, MCP-1, MIP-1α, MIP-1β, and RANTES, in normal and OA chondrocytes, and that RANTES increased expression of its own receptor, CCR5. MCP-1 and RANTES, in addition to increasing MMP-3 expression, inhibit proteoglycan synthesis and enhance proteoglycan release from the chondrocytes. The RANTES receptors CCR3 and CCR5, but not CCR1, are expressed in normal cartilage, whereas all three receptors are expressed in OA cartilage or after stimulation of normal chondrocytes by IL-1β. Furthermore, RANTES induces the expression of iNOS, IL-6, and MMP-1. More recent work has demonstrated the expression of an additional chemokine receptor, CXCR4, by chondrocytes, but not by synovial fibroblasts. Its ligand, stromal cell-derived factor 1 (SDF-1), occurs in high concentration in RA and OA synovial fluids [104]. SDF-1 and several other cytokines increase the synthesis of S100A, *N*-acetyl-b-d-glucuronidase, cathepsin B, and several MMPs in chondrocytes and induce DNA synthesis, cell proliferation [139], and PGE_2 production [137]. Osteoarthritis chondrocytes in contact with autologous T lymphocytes produce enhanced levels of MMP-1, -3, and -13 and RANTES [155]. Furthermore, fibronectin fragments increase the expression of several chemokines by a partially NF-κB dependent mechanism [177]. Thus, in addition to recruiting leukocytes to sites of inflammation in arthritic joints and mediating synovial fibroblast responses and actions, chemokines modulate chondrocyte functions associated with cartilage degradation.

3.5 Growth and Differentiation Factors in Osteoarthritis

Growth and differentiation factors are generally positive regulators of structure and function of mature articular cartilage, stimulating chondrocyte anabolic activity, and, in some cases, inhibiting catabolic activity. The best-characterized anabolic factors in articular cartilage include insulin-like growth factor-1 (IGF-1), TGF-β, fibroblast growth factors (FGF), bone

morphogenetic proteins (BMPs), including osteogenic protein-1 (OP-1, or BMP-7), and the cartilage-derived morphogenetic proteins (CDMPs). When synthesis of IGF-1, TGF-β, BMP-2, and BMP-7 is induced by gene transfer, the synthesis of cartilage proteoglycans and collagens in cultured chondrocytes is increased [52,164]. Many of these factors also regulate chondrogenesis and endochondral ossification during skeletal development [72]. In adult cartilage, their expression declines with age, a risk factor for OA, and their activities are downregulated [125] (Fig. 3.2). For example, the capacity of BMP-6 to stimulate proteoglycan synthesis and production of BMP-7 (OP-1) declines with age [25,38]. A reduction in TGF-β signaling in aging chondrocytes may be a factor in the reduced capacity to repair cartilage [22].

3.5.1 Insulin-Like Growth Factor

Insulin-like growth factor-1, also known as somatomedin C, was first discovered as a serum factor controlling sulfate incorporation by articular cartilage in vitro. Later studies showed that IGF-1, by promoting the synthesis of type II collagen and aggrecan, induces and maintains the chondrocyte phenotype in vitro. It also promotes chondrocyte survival, but does not act as a mitogen unless combined with FGF-2 or BMP-7 [123,126]. Chondrocytes from animals with experimental arthritis and from patients with OA are hyporesponsive to IGF-1, notwithstanding normal or increased IGF-1 receptor levels. This has been attributed to increased levels of IGFBPs that may interfere with IGF-1 [99]. Because disturbances in the balance between IGF-1 and IGFBPs in OA joints may contribute to defective chondrocyte responses to IGF-1, small molecule inhibitors to restore IGF-1/IGFBP equilibrium have been proposed for the treatment of OA [44]. An additional mechanism may involve IGF-1–mediated upregulation of the type 2 IL-1 decoy receptor (IL-1RII), which is downregulated in OA chondrocytes and binds to IL-1α or IL-1β, but does not transmit intracellular signals [230]. IGF-1 desensitization may also be due to IL-1–induced suppression of cytokine signaling 3 (SOCS3), which inhibits IGF-1 signaling by reducing phosphorylation of the insulin receptor substrate-1 (IRS-1) [203]. Overproduction of nitric oxide may also contribute to IGF-1 resistance by chondrocytes [122,212].

3.5.2 Transforming Growth Factor-β

Transforming growth factors-β 1, 2, and 3 are generally considered potent stimulators of proteoglycan and type II collagen synthesis in cultured primary chondrocytes and in cartilage explants [78]. Microarray analysis of the chondrocytes indicates that TGF-β counteracts the expression of many IL-1–induced genes that are involved in cartilage injury [213]. Although intraarticular injection or adenovirus-mediated delivery of TGF-β stimulates proteoglycan synthesis and limits cartilage damage in animal models, osteophyte formation, swelling, and synovial hyperplasia may also occur [144,225]. TGF-β also induces expression of ADAMTS-4 and promotes aggrecan degradation in primary human chondrocytes in vitro [151]. Nevertheless, agents that block TGF-β activity, such as the soluble form of TGF-βRII, inhibitory signal transducing acceptor proteins that are related to Drosophila mothers against decapentaplegic (MAD) SMADs, or the latency-associated peptide-1 (LAP-1), increase proteoglycan loss and cartilage damage when administered in experimental OA [195,196]. Decreased TGF-β signaling may be associated with a loss of the protective effect of TGF-β during OA progression [23]. These studies suggest that whether effects are deleterious or protective depends on the balance of TGF-β family members and natural antagonists.

3.5.3 Bone Morphogenetic Proteins

The BMPs constitute a large subclass of the TGF-β superfamily. They are essential for normal development of the appendicular skeleton and for joint development [35,72]. Several BMPs, including BMP-2, BMP-7 (OP-1), and BMP-14, also known as GDF-5 (growth and differentiation factor-5) or CDMP-1 (cartilage-derived morphogenetic protein-1), stimulate differentiation of mesenchymal precursors into chondrocytes and promote the differentiation of hypertrophic chondrocytes. BMP-2, -4, -6, -7, -9 and -13, can increase synthesis of type II collagen and aggrecan by articular chondrocytes in vitro [25,26,37,79,105,209]. In addition, BMP-7 reverses many of the catabolic

responses induced by IL-1β. This includes induction of MMP-1 and MMP-13, downregulation of TIMP expression, and downregulation of proteoglycan synthesis in primary human articular chondrocytes [37].

Because some BMPs stimulate the synthesis of type II collagen and aggrecan by adult articular chondrocytes, they can be used to promote cartilage repair in tissue engineering approaches, such as autologous chondrocyte transplantation, gene transfer, and implantation of scaffolds in cartilage defects [52,127, 197]. Many of these factors promote cartilage repair in various models of focal cartilage defects and induce chondrogenic differentiation of mesenchymal progenitor cells in vitro [93,164]. However, under some circumstances BMPs promote chondrocyte hypertrophy, consistent with its primary role in vivo to promote endochondral ossification. BMP-2, -9, and -13 serve as potent anabolic factors for juvenile cartilage, which contains chondroprogenitors, but this is not true in adult cartilage [88]. BMP-2 placed in resorbable collagen sponges produced appositional cartilage growth from perichondrium more easily in cricoid cartilage from juvenile rabbits than in that from adults, where calcification had already occurred in large areas [218].

3.5.4 Fibroblast Growth Factors

Members of the FGF family, including FGF -2, -4, -8, -9, -10, and -18, together with the FGF receptors FGFR1, 2, and 3, coordinate patterning and cell proliferation during chondrogenesis and endochondral ossification in embryonic and postnatal growth plates [162]. FGF-2, or basic FGF, is the most extensively studied. It is a potent mitogen for adult articular chondrocytes, but its effects on the synthesis of cartilage matrix are variable, as it stimulates, inhibits, or has no effect on proteoglycan synthesis [222]. Early studies had suggested that low concentrations of FGF-2 would stimulate chondrocyte mitogenesis and proteoglycan synthesis, whereas high concentrations might have the opposite effects. FGF-2 stored in the adult cartilage matrix is released by mechanical injury or when the joint is loaded [227,228]. This suggests that excessive release of bFGF from the cartilage matrix during injury or OA progression contributes to the increase in chondrocyte proliferation and to the reduction in anabolic activity. Although FGF-2 and FGF-9 stimulate the expression of Sox9 and increase the activity of the Sox9-dependent chondrocyte-specific enhancer in the type II collagen gene in vitro [153,194], recent evidence indicates that FGF-2 inhibits the anabolic activities of IGF-1 and OP-1 [123]. Both FGF-9 and FGF-18, which promote transition of proliferating chondrocytes to terminal differentiation in the growth plate [162], increase matrix synthesis by mature chondrocytes [10,51,201]. FGF-18 also promotes cartilage repair in a rat meniscal tear model of OA [148].

3.5.5 Signaling Induced by Growth and Differentiation Factors

The major pathways activated by the factors discussed above involve members of the ERK1/2, p38 MAPK, and PI3-kinase/AKT pathways [17]. As in other cell types, FGF family members activate ERK1/2 and p38 kinase in chondrocytes. Specific inhibitors of these pathways block FGF-2– and FGF-18–induced mitogenesis in chondrocytes [201] and prevent FGF-2 induction of Sox9 in primary chondrocytes [153]. The PI3-kinase pathway is required for the stimulation by IGF-1 of proteoglycan synthesis in primary human articular chondrocytes, whereas ERK1/2 inhibitors increase IGF-1–stimulated synthetic activity [208].

Factors of the TGF-β and BMP families transduce signals through the formation of heteromeric complexes of ligand-specific receptors that have serine-threonine kinase activity. Signaling is mediated by the canonical SMAD pathway, through phosphorylation of receptor-activated (R)-SMADs, including BMP-induced Smads 1, 5, and 8, and TGF-β–induced Smads 2 and 3. The R-SMADs form complexes with the common Smad4 and translocate to the nucleus, where they bind to SMAD elements in the promoters of target genes [46,229]. The existence of other BMP-induced transcription factors, including JunB, JunD, and ID and DLX family members, suggests alternate signaling pathways. TGF-β and BMPs can also signal by activating TGF-β–activated kinase 1 (TAK1) [178], which interacts with MEKK1 and activates p38 and JNK cascades, or by activating Ras/ERK1/2 or RhoA/ROCK signaling [46,241]. The differentiation of chondrocytes from mesenchymal precursors is upregulated by p38 kinase and downregulated

by ERK1/2 [27,241]. The p38 and ERK1/2 pathways contribute to BMP-induced signaling during chondrogenesis in vitro [157,198,232], while p38 activity is essential for TGF-β induction of proteoglycan synthesis in primary articular chondrocytes [211].

The inhibitory Smads 6 and 7 prevent phosphorylation of the R-SMADs and also control BMP- and TGF-β–induced activities. An additional SMAD-interacting molecule, Tob1, an antiproliferative protein, downregulates signaling by sequestering R-SMADs. It in turn is downregulated in OA cartilage [64]. BMP antagonists play important roles in spatial and temporal regulation of BMP activities during skeletal development. Among them follistatin, gremlin, chordin, and chordin-like 2 (CHL2) are upregulated in OA cartilage [159,216,217]. Follistatin, which has been linked to inflammatory processes [106], gremlin, which is associated with hypertrophic phenotype [216], and chordin [217] appear at different stages of OA and with differential topographic distribution. Since each antagonist binds preferentially to different BMPs, the differential expression may serve as a feedback mechanism to balance anabolic activities at different stages.

3.5.6 Interactions Among Anabolic and Catabolic Factors that Regulate Matrix Gene Expression in Cartilage

The capacity of IL-1 and TNF-α to suppress cartilage anabolism by inhibiting the synthesis of proteoglycans and type II collagen may contribute to the loss of cartilage matrix and prevent repair [70]. At the same time, these cytokines stimulate production of BMP-2 and PGE_2, both of which act to increase synthesis of cartilage matrix [63,70] (Fig. 3.2). The early BMP-2–induced increase in synthetic activity activates type II collagen and aggrecan gene expression. This permits their interaction with cytokine-induced transcription factors [215]. Thus, the initial events that activate chondrocyte synthetic activity in OA cartilage probably cause activation of the normally inactive *COL2A1* promoter. This molecule in turn is subject to transcriptional repression, which depends on the balance between up- and downregulation. IL-1β also induces the expression of IL-6 that, together with the soluble IL-6 receptor, present in insufficient amounts in cultured cells, downregulates *COL2A1*, aggrecan, and link protein via the JAK/STAT pathway [117]. TGF-β expression is also induced by IL-1β through activation of the bHLH factor AP-4, which binds to a sequence overlapping the AP-β site [8]. NF-κB specifically activates BMP-2 transcription [56], but it also downregulates Sox9 expression by interacting with the Sox9 promoter [154]. On the other hand, NF-κB must be activated for IL-1 to induce expression of transcription factors, such as ESE-1, that upregulate catabolic and inflammatory mediators [75–77], but downregulate *COL2A1* expression [168]. IL-1 differentially regulates inhibitory SMADs, upregulating Smad7 and downregulating Smad6 in chondrocytes. The cytoplasmic localization of these inhibitors in normal and OA cartilage does not correlate with the expression of anabolic genes [103]. The expression of the IL-1 decoy receptor (IL-1RII), which binds to IL-1α or IL-β but does not transmit intracellular signals, is downregulated in OA chondrocytes, but can be upregulated by IGF-1 [230]. On the other hand, IGF-1 desensitization may be due to IL-1–induced suppression of cytokine signaling 3 (SOCS3), which inhibits IGF-1 signaling by reducing phosphorylation of the insulin receptor substrate-1 (IRS-1) [203]. Since the signaling pathways and transcription factors that mediate catabolic and proinflammatory responses in joint tissues also downregulate cartilage anabolism, it may be possible to target specific transcription factors that regulate both processes [21,70].

3.5.7 Mediators of Cartilage Homeostasis

A number of proteins that mediate normal cellular responses to stress, inflammation, and aging have been identified in OA cartilage. Findings that catabolic stress and inflammatory cytokines upregulate HIF-1α suggest that this molecule may serve as a survival factor in OA cartilage [41,133,244]. VEGF and its receptors are expressed in OA cartilage, and the induction of VEGF in chondrocytes or synovial cells by cytokines or mechanical loading indicates that VEGF may play a role in angiogenesis and cartilage destruction [84,92,176]. Recent evidence indicates that peroxisome proliferator-activated receptor γ (PPARγ) agonists protect chondrocytes against IL-1–induced responses by increasing the expression of the IL-1Ra [61]. The

human cartilage glycoprotein 39 (HC-gp39), also known as chitinase 3-like protein 1 (Chi3L1), is induced by inflammatory cytokines and inhibits cytokine-induced cellular responses, thus constituting a feedback mechanism [120,180]. The accumulation of advanced glycation end products (AGEs) in aging cartilage suggests that chondrocyte function may be modulated by changes in the composition/content of the extracellular matrix. The receptor for AGEs (RAGE) in chondrocytes interacts preferentially with S100A4, a member of the S100 family of calcium-binding proteins, and stimulates MMP-13 production via phosphorylation of Pyk2, MAP kinases, and NF-κB [239]. The fibroblast activation protein α (FAPα), a membrane serine proteinase, which is induced by IL-1 and OSM chondrocytes and is elevated in OA, may play a role in collagen degradation [146]. The elevated expression of leptin in OA cartilage and in osteophytes and the capacity of this adipocytokine to stimulate IGF-1 and TGF-β1 synthesis suggest that it may play a regulatory role in anabolic responses by OA chondrocytes [49]. Members of the *CCN* gene family, including connective tissue growth factor (CTGF) and NOV/CCN3, and WISP-3/CCN6 enhance BMP and TGF-β signaling and increase Sox9-dependent *COL2A1* and aggrecan expression [98,112,199]. Together the genes mentioned above seem to play key roles in regulating cartilage matrix homeostasis.

3.6 Factors in Subchondral Bone that May Contribute to Dysfunctional Chondrocyte Responses in Osteoarthritis

As proposed originally by Radin and Rose [179], bone is intimately involved in the initiation and progression of OA, and trauma to the subchondral bone may result in cartilage degeneration [32,33,152; cf. chapter 2]. Increased trabecular bone volume with trabecular sclerosis and increased bone turnover are features of OA pathogenesis. Therefore, therapies that target bone may also be effective in OA. Examples are calcitonin [12,50], bisphosphonates [205], and estrogen [83]. The mechanism of their action may involve subchondral osteoblasts. In a coculture model, osteoblasts isolated from sclerotic subchondral bone stimulate MMP-3 and MMP-13 gene expression and inhibit aggrecan synthesis. These responses are mimicked in nonsclerotic osteoblasts treated with IL-1, IL-6, or OSM [192]. IL-17 is a potent inducer of receptor activation of NF-κB ligand (RANKL), which mediates osteoclast differentiation and activity [131]. Both RANKL and its receptor RANK, a member of the tumor necrosis factor receptor family, are expressed in adult articular chondrocytes, but exogenous RANKL does not activate NF-κB or stimulate the production of collagenase or nitric oxide [109]. However, inhibition of RANKL expression does not block cartilage destruction inflammatory models [169]. Conceivably, RANKL may have indirect effects on cartilage by its protective effect on bone [246].

It is as yet unclear whether OA is the result of changes in subchondral bone [13; cf. chapter 2], but changes in the mineral content and thickness of the calcified cartilage and the associated advancement of the tidemark may play a greater role than previously realized [33] (Fig. 3.1). Recent studies in OA patients have identified polymorphisms in the gene that encodes asporin. Asporin binds to TGF-β, thereby inhibiting cartilage anabolism. Polymorphisms have also been identified in *FRZB,* a gene that encodes secreted frizzled-related protein 3 (sFRP3) [113,129] (Table 3.2). These observations lend support to the concept of increased biologic activity in subchondral bone. Members of the sFRP family, including sFRP3, are glycoproteins that antagonize the signaling of Wnt ligands through frizzled membrane-bound receptors. In OA cartilage, sFRP may have a role in chondrocyte apoptosis [100]. Since activation of β-catenin in mature cartilage cells stimulates hypertrophy, matrix mineralization, and expression of VEGF, ADAMTS5, MMP-13, and of several other MMPs [214], defective inhibition of Wnt signaling due to FRZB polymorphisms may disrupt normal function, leading to abnormal cartilage and bone metabolism.

The localization of COL10A1, MMP-13, and Runx2 in the deep zone of OA cartilage suggests that the chondrocytes attempt a defective repair response by recapitulation of the hypertrophic phenotype [3,231]. In the developing growth plate, Runx2-dependent expression of RANKL is observed in the hypertrophic chondrocytes

that are at the boundary, next to the calcifying cartilage [107]. This suggests that cells in bone and calcified cartilage interact. The growth arrest and DNA inducible protein, GADD45γ, which mediates cell survival and the expression of *Mmp13* and *Col10a1* in hypertrophic chondrocytes in the growth plate, may also be a target in OA cartilage, where it is expressed in chondrocytes, in clusters and in deep zone OA chondrocytes [96]. An imbalance between IGF and IGFBP and the presence of the hepatocyte growth factor may modulate cartilage calcification, affecting interactions between deep zone chondrocyte and the subchondral bone [81,135].

The disruption of cartilage and bone formation during embryonic development also suggests that interactions between these tissues are important for the development of OA (Table 3.1). After birth, mutations in signaling proteins involved in chondrogenesis and in terminal chondrocyte differentiation may enhance susceptibility to OA. Mutation in the *Ank* gene encoding a multipass transmembrane protein and knockout of nucleotide pyrophosphatase phosphodiesterase-1 (*Npp1*), whose gene products control intracellular pyrophosphate levels, cause progressive or early-onset OA, associated with increased chondrocyte hypertrophy and inappropriate mineralization of the cartilage matrix [89,101]. Disruption of BMP signaling by ablation of the *Bmpr1a* gene, which encodes the type I BMP receptor α, results in early embryonic death, but a postnatal conditional knockout results in OA-like cartilage degeneration [184]. Loss of TGF-β signaling also increases OA-like pathology in postnatal articular cartilage, associated with increased chondrocyte hypertrophy and expression of *Col10a1* [200,240].

3.7 Conclusion

Much new information on mechanisms of cartilage destruction and repair has been obtained from studies of chondrocyte responses to catabolic and anabolic factors. Yet much more needs to be learned on how to modify the complex processes involved in OA pathogenesis. Cytokines, acting individually or in networks, can, by modifying catabolic and anabolic processes, profoundly influence chondrocyte responses. The adult chondrocyte maintains cartilage structure and function, but when it undergoes a change in phenotype it may not replicate the original processes that occur in normal cartilage development. Because OA progression cannot be halted if early events are not prevented, it is necessary to develop new strategies for early diagnosis. Recent understanding of how the adult articular chondrocyte functions within its unique environment and the role of catabolic and anabolic factors in the pathogenesis of dysregulated chondrocyte function in OA may lead to new approaches to OA therapy.

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4.

The Synovium and Its Role in Osteoarthritis

Arjen B. Blom and Wim B. van den Berg

4.1 Introduction

Osteoarthritis (OA) has long been considered to be a disease almost exclusively of the cartilage. However, in recent years studies in humans and animals have led to increasing evidence that the synovium also is involved in the OA process, and that its role starts relatively early in the disease. This chapter discusses the involvement of the synovium in OA-related pathology. Although we still are uncertain about the exact mechanism that evokes synovial activation and the mediators that induce and perpetuate its active participation, the role of the synovium should not be underestimated. The first part of this chapter describes the normal function of the synovium in a synovial joint and compares the synovium in two separate diseases: rheumatoid arthritis (RA) and OA. Subsequently, the involvement of synovial macrophages in experimental OA pathology is discussed, followed by an overview of the mediators that may cause or regulate this pathology, the possible mechanisms involved in synovial activation, and the possibility of gene therapy targeting the synovium. Unraveling the mechanisms behind this synovial involvement may help find a suitable therapeutic approach to fight this crippling condition.

4.2 Normal Synovium: Morphology and Function

The synovium in a normal joint is a thin, weak layer of tissue only a few cell layers thick that lines the noncartilaginous surfaces within articular joints. The synovium acts to control the environment within the joint. It does this in two ways: first, it acts as a loose membrane to determine what can pass into the joint space and what stays outside, such as nutrients for chondrocytes on the one hand and pathogens on the other; second, the cells within the synovium produce substances such as hyaluronan and lubricin, important components of joint fluid that give joint fluid its mechanical properties. The synovium can be divided into two compartments—the synovial lining, or intima, and the sublining. The lining layer consists of two different cell types—type A and type B synoviocytes. Intermediate types have been described, but their existence is not generally accepted. The two cell types execute different functions. Type A synoviocytes are macrophage-like cells that function to clear all excess material and potential pathogens from the joint. They can migrate through the synovium and into the synovial cavity and express major histocompatibility complex II molecules and Ia antigen, which

play key roles in the antigen presentation in the initial stages of the immune response. These cells can produce and secrete a number of enzymes and cytokines/chemokines that mediate tissue damage and inflammation. In pathologic conditions, macrophages stimulate B synoviocytes to produce matrix degrading enzymes. Type B synoviocytes are fibroblast-like cells that are thought to produce the main component of synovial fluid—hyaluronan. In addition, they function as a physical barrier that, combined with the water retention by hyaluronan, traps the synovial fluid in the joint capsule. The synovial fluid not only is important for lubrication of the joint, but also functions to transport nutrient and oxygen to the cartilage. In a normal joint, type A and B synoviocytes act to maintain a healthy environment, so that all tissues can function properly. This steady state can be disrupted by stimuli from the outside, for example, microorganisms, immune complexes, components leaking from a damaged cartilage matrix, or by an intrinsic derailment of synoviocytes.

Apart from the production of proinflammatory and matrix degrading mediators, synoviocytes also produce a plethora of protective mediators, including transforming growth factor-β (TGF-β), tissue inhibitors of metalloproteinase (TIMP), and interleukin-10 (IL-10). An elegant approach to restore the steady state would be to make use of this potential. This cytokine balance is further discussed below.

4.3 Comparison of Rheumatoid Arthritis and Osteoarthritis synovium: Lessons from Rheumatoid Arthritis Synovium

In RA, the processes that occur in the synovium have been studied and described extensively, even though the exact mechanisms are not

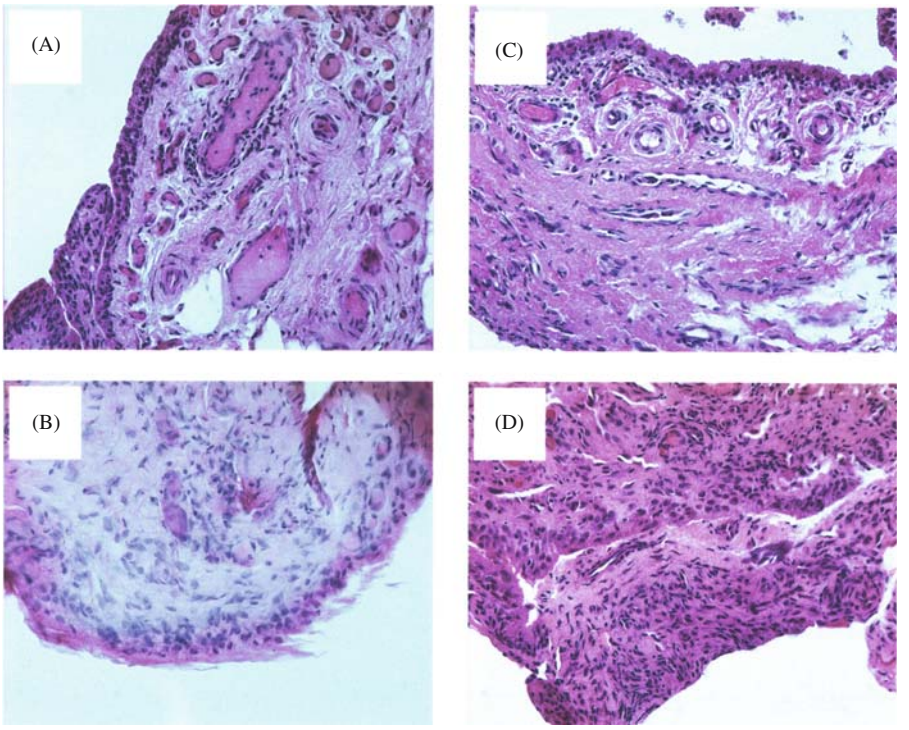

Figure 4.1. Histology of early osteoarthritis (OA) (A,B) and early rheumatoid arthritis (RA) (C,D) samples. Note the thickened synovial lining layer in both conditions. In the deeper layers of some RA synovial samples (D), more inflammatory cells are present. Original magnification 100×.

yet fully understood. Nevertheless, increased insight into the mechanisms behind RA have led to useful therapeutic strategies, such as inhibition of tumor necrosis factor-α (TNF-α) and other cytokines, or T- and B-cell–directed therapies that are in development. In RA the synovium is the target tissue for treatment, and its dysfunction has always been considered the main factor that causes pathology. A wide variety of cell types that occur in varying amounts can be identified, including polymorphonuclear neutrophils (PMNs), monocytes, T cells, B cells, plasma cells, and mast cells. In addition, the number of macrophages in the synovium, especially in the synovial lining, is greatly increased. The presence of these macrophages in the synovium correlates well with disease activity and progression of cartilage damage [12,24]. Lately, a number of studies have reported macrophage accumulation in the OA synovium as well [14]. In many cases early OA synovial biopsies resemble RA biopsies morphologically (Fig. 4.1). It is particularly the lining layer that is thickened in the two synovial conditions. The RA synovium generally contains larger amounts of adaptive immunity-related inflammatory cell types (e.g., T and B cells), although the cells occur also in many OA synovia. The synovial fluid of patients with active RA effusion contains substantial amounts of PMN, which are not found in OA. This indicates differences in the nature of synovitis in the two rheumatic disorders. However, the presence of a thickened synovial lining and a greater number of macrophages in the joints of many OA patients suggest that the OA synovium is activated and may contribute to the symptoms and disease progression.

4.4 Evidence for Involvement of Synovial Macrophages in Osteophyte Formation

One of the main pathologic changes in an osteoarthritic joint is the formation of osteophytes, that is newly formed ectopic bone at the margins of the joint (Fig. 4.2). This may be a side effect of the locally increased levels of growth factor, such as TGF-β, constituting an attempt at cartilage repair. It may also be a reaction of the tissue to changed biomechanics and an attempt to enhance the articular surface. Although the exact function of osteophytes and the role they play in clinical symptoms is not known, one can easily imagine that osteophytes in certain locations will narrow the range of movement of the joint and will cause pain by hindering free movement of soft tissues in the joint. Synovial macrophages are crucial in the induction of osteophytes [5,20]. Macrophages mediate the generation of osteophytes in two models, one induced by a growth factor, TGF-β, and the other via induction of murine OA. In the first study, osteophytes are induced in murine knee joints by intraarticular injection or overexpression of TGF-β [20]. Local TGF-β application strongly induces osteophytes [4], and blocking TGF-β

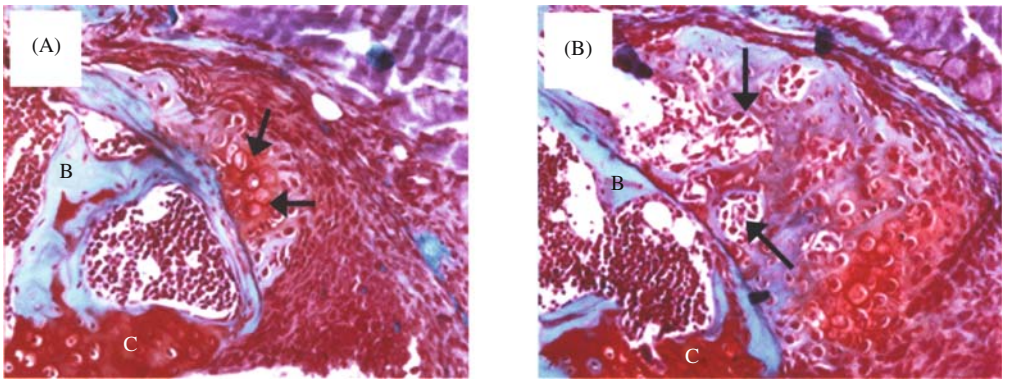

Figure 4.2. Early osteophyte formation. Osteophyte formation (arrows) starts with chondrogenesis (A). The chondroid tissue thereafter is replaced by bony structures (B). The formation of bone marrow cavities is shown by arrows. B, bone, C, cartilage. Original magnification 200×.

using a soluble receptor prevents osteophyte formation [31], thus identifying TGF-β as a key factor. Osteophytes often develop at the cartilage/bone junction, are thought to originate from mesenchymal cells in the periosteum, and start as chondrogenic outgrowths with subsequent transformation to bony structures. Whether TGF-β acts directly on these mesenchymal cells, or whether other, secondary mediators produced by synovial cells are necessary for proliferation and differentiation of these cells was largely unknown until studies of local macrophage depletion with toxic liposomes provided further insight.

After intraarticular injections of clodronate containing liposomes, these vesicles are selectively internalized by macrophages, the clodronate is released in the macrophage, and the intracellular clodronate causes cell death through apoptosis [26]. Apoptotic cells are removed from the synovium with a minimum of inflammation. Free clodronate, if released in the joint, diffuses quickly out of the joint, because the cells cannot take up free clodronate. This treatment results in a complete removal of synovial macrophages within 1 week of intraarticular injection of the liposomes. When synovial macrophages are depleted from the synovial compartment prior to either viral overexpression or injection of TGF-β intraarticularly, the formation of osteophytes is largely prevented (Table 4.1). This is not due to decreased TGF-β levels in macrophage depleted joints, as was demonstrated by enzyme-linked immunosorbent assay (ELISA) of synovial washouts. An earlier study [1] led to similar results using adenoviral overexpression of TGF-β. Immunohistochemical analysis demonstrated a lower expression of bone morphogenetic proteins (BMP-2 and BMP-4) after macrophage depletion. This indicates that macrophage involvement in osteophyte formation may be mediated by secondary growth factors, like BMPs. When injected intraarticularly [4], BMPs induce osteophytes.

Table 4.1. The effect of TGF-β on osteophyte formation by causing depletion of the lining

TGF-β		Surface area (μm^2) Patella	Femur
20 ng	Normal	662 ± 363	435 ± 278
	Depleted	0*	38 ± 72*
200 ng	Normal	2111 ± 932	2235 ± 1159
	Depleted	203 ± 513*	671 ± 627

Note: The mean surface area of osteophytes is measured at four different locations in the joint. Depletion of macrophages on the mean surface area indicates a significant difference between normal and macrophage-depleted joints ($p<0.05$). Adapted from van Lent et al [26].

In addition to these in vivo findings, in vitro data also support the involvement of macrophages in chondrogenesis. As already mentioned, osteophytes are believed to develop from mesenchymal cells in the synovium or periosteum. Mesenchymal cells, like the C3H10T1/2 cell line, undergo chondrogenesis once they are stimulated with TGF-β or certain other growth factors. Chondrogenesis is an early step in the process of osteophyte formation. Interestingly, when these C3H10T1/2 cells are co-cultured in a transwell system with a macrophage cell line (RAW 264.7), substantially less TGF-β is needed to induce chondrogenesis (Fig. 4.3). TGF-β is required to induce chondrogenesis, because co-culture of both cell types without TGF-β did not lead to chondroneogenesis. When, as a control, clodronate liposomes are added in this system, the macrophage effect disappears, even though TGF-β itself can still generate chondrogenesis. This demonstrates the selectivity of the clodronate liposomes for macrophages, without their touching mesenchymal cells. Together these findings show that macrophages produce an additional factor after stimulation with TGF-β that, together with TGF-β itself, induces chondrogenesis. TGF-β levels in the culture supernatant are at a higher concentration than the range of added concentrations. This indicates that TGF-β addition leads to autoinduction of TGF-β. Surprisingly TGF-β levels are the same in cultures with or without macrophages. Therefore, the extra factor produced by macrophages to generate osteophytes is not TGF-β itself.

There can be no doubt that macrophages are involved in TGF-β–induced osteophyte formation. However, this does not mean that macrophages are also involved in osteophyte formation in experimental OA. In follow-up studies macrophages were depleted from the synovium prior to induction of experimental OA by intraarticular injection of collagenase.

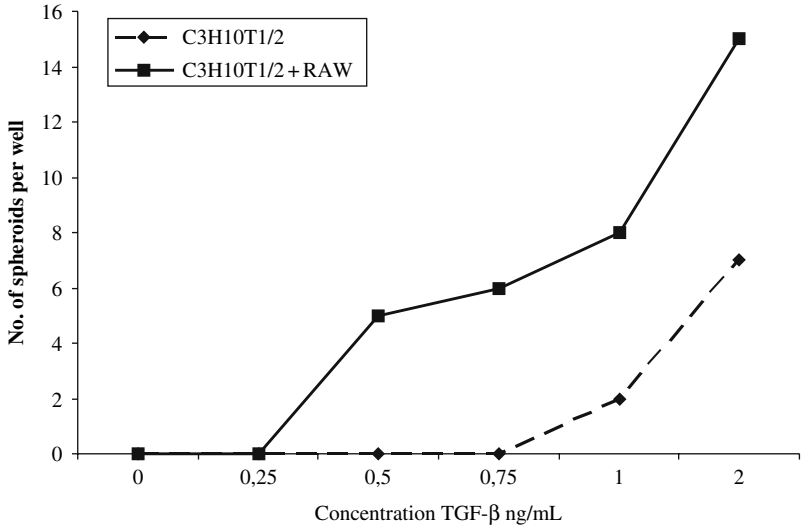

Figure 4.3. The effect of co-culture of macrophages (RAW 264.7) with C3H10T1/2 cells on TGF-β–induced chondrogenesis. As can be seen, the presence of macrophages significantly lowers the level of TGF-β to induce chondrogenesis, as evaluated by the number of spheroids that are formed.

The collagenase damaged the ligaments that normally assure joint stability, with the result that the joint became unstable. The first signs of pathology including osteophyte formation already develop after 7 days. After 6 weeks the knee joint exhibits full blown OA-like pathology. Here macrophage depletion also results in significantly fewer and smaller osteophytes in the early phase [5].

These findings indicate an important role for synoviocytes in osteophyte formation. Apparently synovial macrophages produce an additional growth factor that, combined with TGF-β, is responsible for osteophyte formation. During collagenase induced OA, significant inflammation rarely occurs, apart from the first 3 days. However, macrophage activation is evident, as reflected by the expression of the activation marker myeloid-related protein-14(MRP14) at days 7 and 14 after OA induction. Activation occurs both in the lining and in the deeper layer of the synovium. But because this layer is no longer there, due to the depletion of macrophages, the expression of growth factors such as BMPs in the synovial lining is greatly diminished. BMP expression in the deeper layers of the synovium, however, does not differ between macrophage-depleted and nondepleted animals. This makes it unlikely that the differences in osteophyte formation are due to differential BMP expression. No changes in expression of other growth factors, like platelet-derived growth factor (PDGF) and basic fibroblast growth factor (bFGF), have been reported. So far it is not known what specific macrophage derived factors are involved in osteophyte formation. Nevertheless macrophages are clearly key players in OA-related joint pathology.

4.5 Synovial Macrophages Are Involved in Early Changes in the Cartilage During Experimental Osteoarthritis

Because macrophages are important for osteophyte formation in experimental OA, the question arises whether they are also involved in other OA-related pathology and in irreversible cartilage destruction, in particular. The limitations of the aforementioned technique for synovial macrophage elimination make it difficult to study the long-term involvement of macrophages in an experimental OA model. Normally it takes about 6 weeks for full blown OA cartilage pathology, like cartilage lesions, to develop. Macrophages start to repopulate the lining in a normal joint approximately 3 weeks

after depletion; this may occur even more quickly in a diseased joint [19]. This complicates the study of macrophage involvement in cartilage damage, inasmuch as the erosions typical of experimental OA do not usually arise within this time frame. Instead early cartilage changes were explored by quantifying the appearance of specific neoepitopes with the amino acid sequence VDIPEN, compounds that are indicative of matrix metalloproteinase (MMP) activity in the cartilage matrix. The VDIPEN neoepitope is a specific amino acid sequence that is exposed when certain enzymes (mostly MMPs) cleave aggrecan between the first and second globular domain (Fig. 4.4). Although aggrecan is an essential element of the cartilage, cleavage of this enzyme may not seriously or irreversibly affect cartilage integrity, but may only constitute a sign of enzyme activity. Many enzymes capable of generating the VDIPEN neoepitope also cleave other cartilage proteins.

The VDIPEN neoepitope staining is performed after OA induction in murine knee joints with or without synovial macrophage depletion. Some expression of VDIPEN is already found in the cartilage of mice 7 days after OA induction, but is only present marginally. There is no significant difference in expression in comparison with when macrophages have been depleted prior to induction. Between days 7 and 14 after OA induction, however, VDIPEN expression in macrophage depleted joints does not further increase, whereas VDIPEN expression is about doubled in the joints where macrophages are still present (Fig. 4.5). This indicates that synovial macrophages are involved in the induction of enzyme activity in the cartilage during early OA. The undepleted joints do not exhibit clear signs of inflammation, although synovial macrophage activation is observed when MRP14 staining is used as an activation marker. The question then arises whether the increased induction of MMP activity is caused by enzymes in the synovium, or whether soluble factors from the synovial macrophages induce MMP production by chondrocytes. Analysis of synovial and cartilage samples from synovial tissue and cartilage reveals that MMP-2,-3, and-9 are indeed induced both in synovium and in cartilage when murine OA is induced by collagenase. However, the increased production by chondrocytes is not affected by removal of

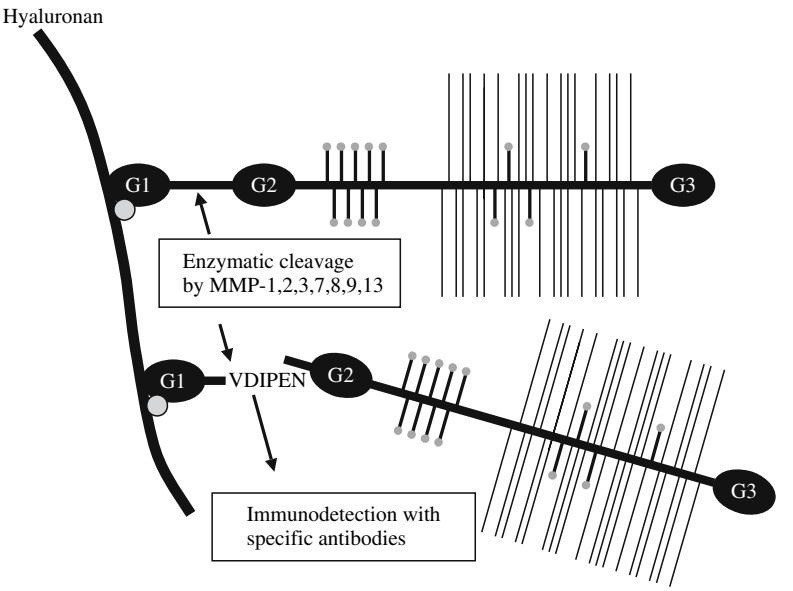

Figure 4.4. Generation of the VDIPEN neoepitope. Some MMPs can cleave the aggrecan molecule between the first and second globular domain. The larger fragment diffuses from the cartilage, whereas the shorter fragment remains in the cartilage, attached to hyaluronan. The latter has an exposed VDIPEN sequence that can be detected with a specific antibody. The amount of VDIPEN staining is a measure of the amount of active MMPs in the cartilage.

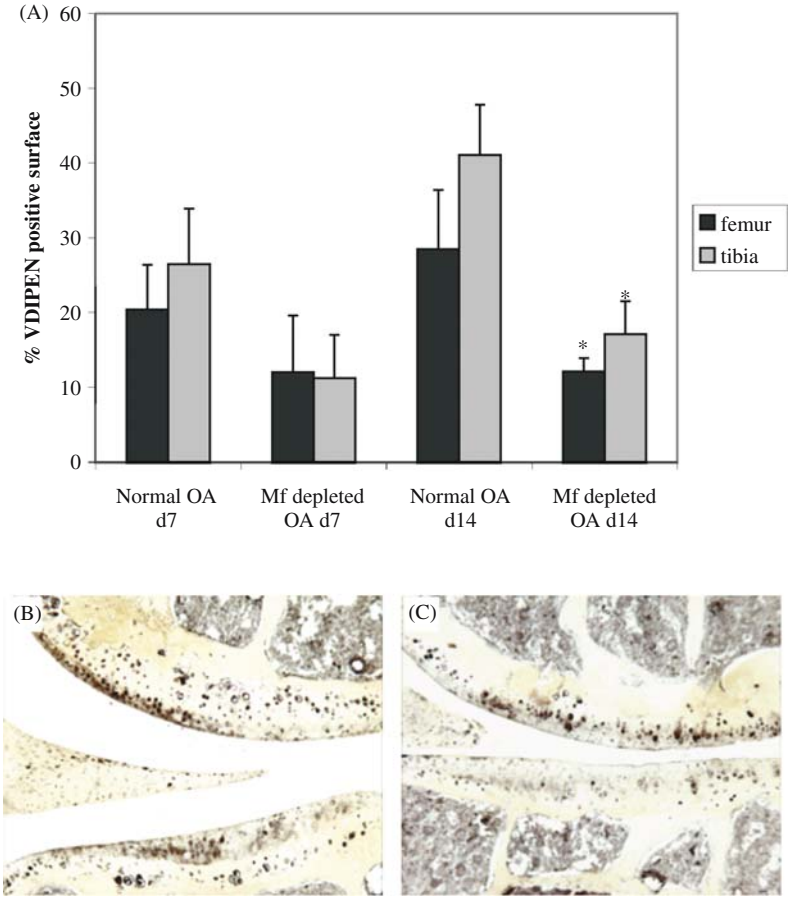

Figure 4.5. The effect of macrophage depletion on VDIPEN neoepitope generation in experimental OA. VDIPEN expression is significantly decreased at day 14 after OA induction in the cartilage of joints from which macrophages are depleted prior to OA induction (A,C), compared to OA joints with an intact synovium (A,B). Mf, *microphage*.

synovial lining macrophages, whereas expression in the synovium is changed. This indicates that macrophages either contribute directly to cartilage damage by the production of MMPs, or do so indirectly by activating chondrocyte-derived MMPs with enzymes that originate in the synovial macrophage. There is no evidence for enzyme induction in chondrocytes by the macrophages. This indicates that the increased expression of MMPs by chondrocytes in experimental or human OA is not under macrophage regulation, but may be caused by matrix fragments that stimulate the chondrocytes. Nevertheless, synovial macrophages are partly responsible for early MMP-mediated cartilage damage by causing direct production of MMPs and their subsequent diffusion to the cartilage matrix. To understand the long-term involvement of macrophages requires further research because, as mentioned earlier, the macrophage depletion technique using clodronate liposomes only allows for studies of up to 2 to 3 weeks.

Macrophages play a major role in OA-related pathology, but that may also require communication between synovial macrophages and fibroblasts as occurs in a normal joint and therefore probably also in the OA joint. Moreover fibroblasts are potent MMP producers and may in fact be the principal producers of MMPs in the synovium, probably regulated by the synovial lining macrophages. It is as yet unclear whether the observed effects are mainly macrophage related, or whether they are the result of crosstalk between macrophages and fibroblasts. Crosstalk may involve direct cell-to-cell contact, or cytokine signaling.

4.6 Expression of Cytokines in the Osteoarthritic Synovium and Implications for Their Role in Pathology

Cytokines that are produced in OA synovium and are implicated in OA pathology can be roughly divided into three categories: (1) destructive cytokines, (2) regulatory cytokines, and (3) anabolic factors. Obviously, the balance between cytokines from these three categories will determine the nature of the synovial and cartilage environment.

4.6.1 Catabolic Cytokines

IL-1 is one of the best known member of the destructive cytokines. It is found in large quantities in OA synovium, especially during early OA [2], and has a pronounced suppressive effect on chondrocyte proteoglycan and collagen type II synthesis. In addition, it stimulates the chondrocytes and synovial cells to release destructive proteases, such as MMPs, which can mediate cartilage breakdown. The net effect of IL-1 is therefore fast depletion of proteoglycans from the cartilage matrix, followed by destruction of the collagen network. Synovial macrophages are major producers of IL-1 in the diseased joint. Other factors in OA joints that in some ways act like IL-1, but with less potency, are TNF-α, IL-17, and IL-18. In both human and experimental OA, IL-1 production in the synovium is increased, as is the production of TNF-α, IL-17, and IL-18. These cytokines also have strong proinflammatory properties in addition to their direct effect on the cartilage. This means that once the synovium is activated to produce cytokines, activation is enhanced by the cytokines it produces. Inhibition of these cytokines is a feasible and potentially interesting approach to treat OA patients, especially in patients with a pronounced inflammatory phenotype. It should be mentioned that apart from higher cytokine levels in the synovium, increased levels of TNF receptors and IL-17 receptors are found in chondrocytes of human OA cartilage. Their very presence acts to enhance sensitivity of OA cartilage to these catabolic cytokines [15]. Of note, IL-1 and IL-18 are both produced in pro-forms and need similar converting enzymes to become active. Recent studies with an IL-1β converting enzyme (ICE) inhibitor in two murine OA models showed significant efficacy [27]. In severe OA, where cartilage damage is significant, patients may develop autoimmune T-cell responses to cartilage breakdown products and this may contribute to the OA pathology [28]. Under these conditions the T-cell–derived cytokine IL-17 is likely to assume pathologic significance.

4.6.2 Regulatory Cytokines

The regulatory cytokines include mediators such as IL-4, IL-6, IL-10, and IL-13. These cytokines inhibit synovial macrophages or chondrocytes from producing destructive cytokines, such as IL-1 and TNF-α. In addition, they upregulate natural inhibitors of those cytokines, such as IL-1ra and soluble receptors of both IL-1 and TNF-α. For a long time, it was believed that IL-4 was produced exclusively by T cells, but more recently this protein has been considered important for the maintenance of normal cartilage integrity. It also inhibits IL-1 and TNF-α production by OA synovium in vitro, and, when locally expressed, also induces inflammation. Whether this occurs in an OA joint is doubtful, because only minimal amounts leak from the cartilage. Interleukin-4 is found in T cells that are encountered in a subgroup of OA patients [17]. Both IL-10 and IL-13 share regulatory properties with IL-4 and are found in the synovial membrane of OA patients [29]. Another interesting regulatory mediator is IL-6, a cytokine that enhances expression of TIMPs, the naturally occurring tissue inhibitors of MMPs. Recent studies of an OA model that simulates aging have called attention to the fact that mice lacking IL-6 develop more severe OA than controls. This suggests that IL-6 acts to prevent of cartilage breakdown during OA. However, IL-6 has a proinflammatory component that excludes direct administration of the cytokine to the affected joint. Regulatory cytokines and their role in OA need further attention.

4.6.3 Anabolic Cytokines

The final category of cytokines involved in OA cartilage pathology is made up of growth factors.

These cytokines do not regulate production or action of destructive cytokines like IL-1 and TNF-α, but counteract their effects. For instance, IL-1 causes depletion of proteoglycans from cartilage by enhanced breakdown and inhibited synthesis, whereas the growth factor TGF-β enhances synthesis of proteoglycans and other cartilage matrix components [3]. It is the balance of these factors that determines whether the net effect is synthesis enhanced or inhibited. Other growth factors that can stimulate chondrocyte synthetic activity include insulin-like growth factor-1 (IGF-1) (32,38), BMPs, bFGF/fibroblast growth factor-2 (FGF-2), and PDGF. Some of these are also involved in catabolic processes in the cartilage [13,39]. In certain subsets of OA patients, decreased levels of IGF-1 are found in the synovium [25]. However, other studies report increased levels of synovial growth factor production in OA patients. This again underlines the heterogeneity of the disease. Many of these factors in addition to their beneficial effects also display unwanted side effects in other tissues, a major concern in planning therapy. When growth factors are administered therapeutically, the synthesis of matrix proteins is enhanced. This indicates possible regeneration of the cartilage matrix. Intriguingly, BMP-7 and TGF-β potently counteract IL-1 and may therefore become tools to induce cartilage repair [7]. However, BMPs have a role in endochondral ossification and stimulate terminal differentiation of chondrocytes. Cartilage calcification can be a serious drawback in the application of BMPs to cartilage repair. Also, TGF-β has many adverse effects. It induces fibroblast proliferation (fibrosis) in the synovium, attracts leukocytes to the synovium, and, as discussed earlier, induces osteophyte formation, phenomena that are also often observed in OA synovium. If these adverse effects can be overcome by maintaining the chondrocyte stimulatory capacity of the cartilage, TGF-β may become a valuable, highly potent therapeutic agent.

Cartilage pathology results from overproduction of destructive cytokines in the synovium, but will also result when anabolic stimulation is insufficient or when cytokines exert insignificant control. Both synovium and cartilage contribute to the total cytokine milieu. The relative balance of the various mediators probably plays a greater role in net cartilage destruction than does the absolute level of destructive mediators. It is tempting to speculate that the course of the illness is determined by the balance of individual cytokines or by defective receptors in the joints of OA patients. The appropriate therapeutic approach depends on which of the two possibilities prevails.

4.7 Expression of Matrix Metalloproteinases in the Osteoarthritis Synovium

An important effect of the catabolic cytokines, like IL-1, is induction of MMP production. As already discussed, MMP-mediated cartilage damage can be overcome partly by MMPs produced by synovial cells, rather than by MMP expression induced in the chondrocytes. Also, as shown above, many cytokines found in OA induce or somehow modulate MMP expression. The MMPs are released from the cell in an inactive pro-form. Once activated, most MMPs directly degrade cartilage matrix proteins, thus causing structural damage to the cartilage. They are also capable of activating other pro-MMPs, thereby increasing the damage. The TIMPs are the naturally occurring inhibitors for MMPs and the balance between MMPs and TIMPs in the joint is likely to be crucial as to whether cartilage is broken down enzymatically.

4.7.1 Synovial Expression of Catabolic Enzymes

A wide array of MMPs is produced in the osteoarthritic synovium, with MMP-13 perhaps the most extensively studied enzyme expressed in OA cartilage. When overexpressed in mouse cartilage, an OA phenotype results. Marini et al [23] reported that MMP-13 expression is enhanced in the synovium of OA patients and that a correlation exists between the synovial MMP-2 and MMP-13 and the arthroscopically observed cartilage damage. We, in turn, have demonstrated increased expression of MMP-13, both in cartilage and synovium, in early as well as late collagen-induced OA (Fig. 4.6). Levels of MMP-13 are upregulated to a larger extent in synovial than in cartilage specimens.

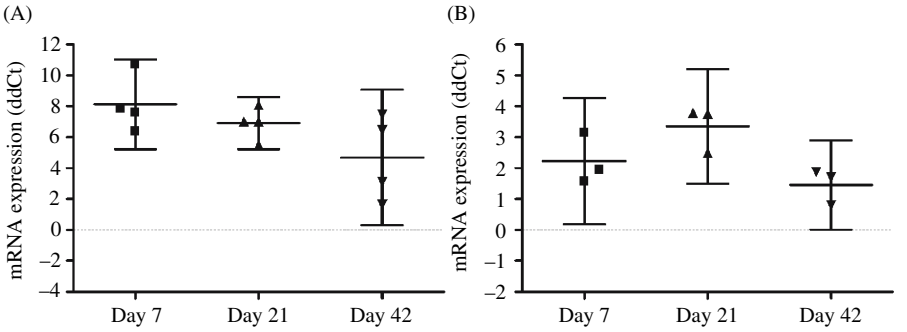

Figure 4.6. MMP-13 messenger RNA (mRNA) levels in synovium and cartilage in experimental OA. MMP-13 expression is significantly elevated in synovium (A) and cartilage (B) of joints in which OA was induced, compared to the naive contralateral joints. MMP-13 expression is already increased 1 week after induction and remains high up to day 42, when full-blown OA pathology is present. Note that expression in the synovium is increased much more (up to 250-fold) than in cartilage (up to eight-fold). Results are expressed, after correction for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and expression in the contralateral knee joint, as ∇, ΔCt with a 95% confidence interval.

Large quantities of pro-MMP-1 and pro-MMP-3 are found and probably produced in the synovial fluid of OA patients [37]. Although there is no increase in enzyme activity in OA synovial fluid, it contains a large quantity of potential matrix-degrading enzymes that, upon local activation, degrade the cartilage matrix and activate other enzymes. Ex vivo synovial cultures also contain many MMPs, for example, MMP-2, MMP-9, and MT1-MMP (MMP-14) [16], both as mRNAs and as proteins. Recently, Dreier et al [8] demonstrated that pro-MMP-9 is produced by synovial OA macrophages and activated by MMPs derived from the cartilage. MMP-9 in turn activates other MMPs. Thus a whole cascade of MMP activation is induced, originating from the cartilage, but strongly enhanced by the synovium. It is apparant the role of the synovium in cartilage degradation is enhanced by the MMPs derived from it.

4.7.2 Enzyme Inhibitors in Osteoarthritis Synovium

So far, four different TIMPs have been described. Increased levels of TIMP1 are found in the synovial fluid of OA patients, but this has not decreased MMP activity in OA synovial fluid [37]. The same report shows that in acute joint injury, which predisposes to OA development, there is a rise in the molar ratio of MMP-3 to MMP-1, on one hand, and TIMP1, on the other. This increase induces a catabolic environment. TIMP-1 and TIMP-2 are found in the synovial membrane in OA patients, but what this means is not clear.

4.8 Synovial Receptors for Cartilage Components

The above findings indicate an active role for synovial cells, especially macrophages, in OA pathology. It is also clear that vast amounts of mediators are produced in the synovium of OA patients. The mechanism that causes macrophages and other synovial cells to function in the OA process is as yet speculative. For synovial cells to get drawn into the OA process, they have to be activated. The activation stimulus probably originates in cartilage, because that is the primary tissue that is damaged, with cartilage protein or cartilage protein fragments leaking to the synovial membrane. These fragments subsequently stimulate synovial cells through as-yet-unknown receptors. In response the macrophages produce mediators that aggravate the process by production of cytokines and matrix-degrading enzymes (Fig. 4.7). Candidate receptors are several of the Toll-like receptors (TLRs), especially TLR4, the receptor for advanced glycation end products (RAGE), and the hyaluronan receptor CD44.

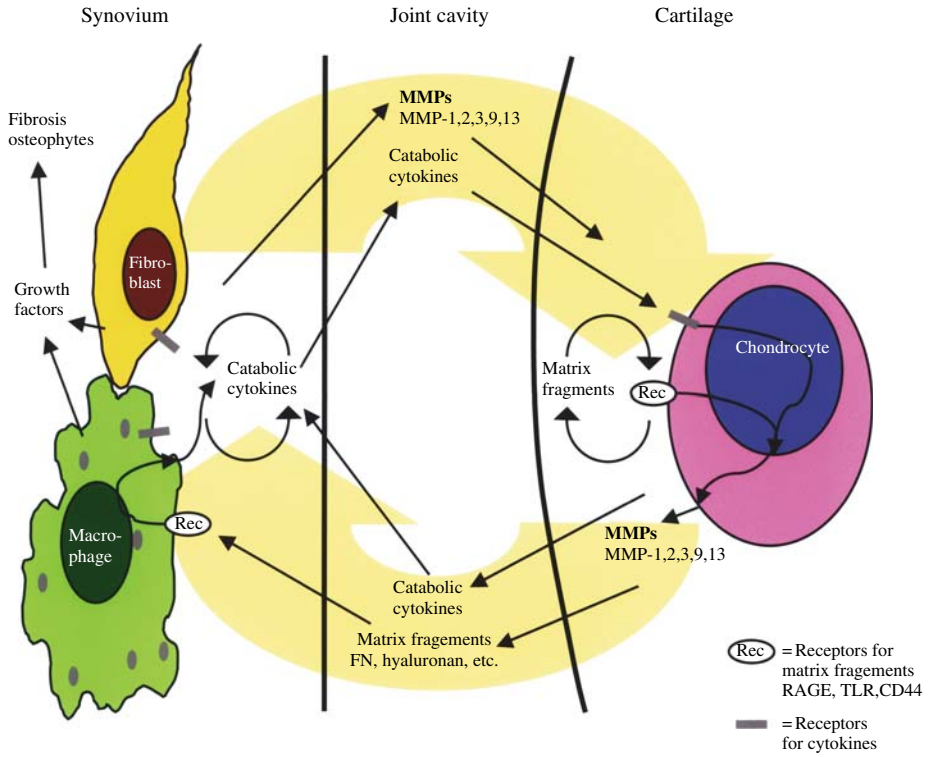

Figure 4.7. A simplified scheme of the vicious circle of macrophage activation in OA. Macrophage activation leads to induction of cartilage damage through the generation and release of MMPs and catabolic cytokines. Cartilage damage results in the release of matrix fragments, such as fibronectin or proteoglycan fragments. The fragments are released from the matrix and stimulate macrophages and other cells in the synovium via, among others, TLRs, RAGE, or CD44 to produce more catabolic cytokines and MMPs; this results in more cartilage damage. Cartilage damage also leads to cytokine production by chondrocytes. Note that self-enhancing loops can occur within the synovium and cartilage. Activation of cytokine receptors leads to more cytokine production. In the cartilage, MMP production and activation cause cartilage degradation. The resulting matrix fragments also stimulate the chondrocyte via receptors like TLRs or RAGE.

4.8.1 Toll-Like Receptors

The TLRs are expressed on many different cell types, including macrophages and fibroblasts. Notwithstanding great interest in the role played by TLRs in RA, their role in OA has not been studied extensively. The TLRs are part of the first line of host defense. They recognize many pathogenic microorganisms. Numerous endogenous ligands can also stimulate these cells via their receptors. Macrophages can be activated by biglycan to produce mediators like TNF-α. TLR2 and TLR4 on macrophages mediate this activation [30].

Biglycan is a leucine-rich proteoglycan that is present in articular cartilage, especially in the pericellular matrix. When structural cartilage damage occurs, biglycan will diffuse from the cartilage and reach the synovial macrophages. Increased levels of biglycan have been found in the cartilage of OA patients. This would suggest that OA cartilage tends to activate synovial cells via biglycan [18]. Other endogenous ligands for TLR2 and TLR4 are heat shock protein (HSP)-70, certain fibronectin (FN) fragments, oligosaccharides of hyaluronic acid, and polysaccharide fragments of heparan sulfate, all likely to be present in an osteoarthritic joint. The FN fragments in particular have been described as causing (experimental) OA. However, so far it has not been possible to assign a specific role to these receptors in human OA. Experiments using specific TLR knockout mice have not provided evidence to substantiate TLR involvement.

4.8.2 Receptor for Advanced Glycation End Products

Advanced glycation end products (AGE) have long been implicated in OA. The presence of these products in cartilage increases with age, and their effect on chondrocytes has been studied. Chondrocytes express the receptor for AGE (RAGE), and evidence is increasing that these receptors play a role in causing cartilage damage [6,21]. However, the expression of RAGE on synoviocytes and the role of this receptor in synovial tissue is less well established. Recently, with the aid of double staining for the CD68 antigen [9], RAGE has been shown to be expressed in synovial tissue of OA patients, with a preference for the synovial lining macrophages. Moreover, activation of RAGE on synoviocytes and chondrocytes in OA patients has led to the activation of these cells [33]. Taniguchi et al [35] have shown that high-mobility group box chromosomal protein 1 (HMGB1) was expressed in OA synovium and induced TNF-α expression via RAGE on macrophages, blocking the signal with soluble RAGE. Although S100A12 (ENRAGE), like some other S-100 proteins, binds to RAGE, it occurs in inflamed RA synovium, but apparently not in OA synovium. The S-100A9 proteins occur in an experimental model for OA [5]. The presence of RAGE in synovium and of its ligands in cartilage, which increase with age, makes this receptor a candidate that helps activate the synovium.

4.8.3 CD44

CD44 is a transmembrane glycoprotein to which several extracellular matrix components such as hyaluronan, fibronectin, and collagens I and IV can bind. It is implicated in processes such as cell-cell adhesion, cell migration, cell adhesion to the extracellular matrix, and lymphocyte activation. It occurs in several splice variants. CD44v6 is increased in OA chondrocytes [36]. Upregulation of CD44 is associated with local loss of hyaluronan. In contrast, CD44 has also been described as a protective receptor, since binding can result in a decreased expression of MMP1 and, if regulated on activation, in normal T expression and secretion (RANTES/CCL5) [34]. Synovial fibroblasts also express CD44, and its expression is increased in OA synovium [11]. Whether this increase in CD44 expression adds to OA pathology is not known. It is difficult to imagine that ubiquitous proteins like hyaluronan, or biglycan in the case of TLR-stimulation, are pathogenic. Alternatively, differential expression of (one of) the less frequently expressed isoforms of CD44 could constitute an additional mechanism, for instance when differences in affinity or effector mechanisms are involved.

There is no conclusive evidence so far that the genes mentioned above are involved in OA; they have been merely mentioned as possible candidates. However, they may mediate synovial activation in OA. More research is needed, and these and other candidates need to be evaluated more extensively to find mechanisms for synovial activation. This knowledge could then be used to develop new therapies to inhibit synovial activation. This, in turn, will affect parameters such as osteophyte formation, cartilage degradation, and pain caused by synovitis. Gene therapy is a promising therapeutic approach to target synovium.

4.9 Gene Therapy Targeting the Osteoarthritis Synovium

Since OA often appears in only one or two joints of OA patients, OA is a disease in which local therapy is indicated. Gene therapy is an obvious approach to achieve high and constant levels of a therapeutic agent with a minimum of systemic side effects. Because there are strong indications that the synovium is an active player in OA pathology, the synovium itself is an obvious therapeutic target. Gene therapy can be used to modulate processes in the synovium or to target the cartilage by soluble factors that diffuse to the cartilage. In the latter case, the synovium is basically a production unit for the therapeutic agent. There is increasing agreement that IL-1 is an important mediator in the etiology of this disease. When inflammation is part of the disease, IL-1 and TNF-α are likely to play an important role, and blocking these mediators may prove beneficial, inasmuch as animal studies have already demonstrated that gene therapy targeting IL-1 is a feasible approach [10,40]. Once it becomes clear which receptors are involved in the activation of the synovium, it may be possible to silence them

by RNA interference (RNAi), as in the case of TLRs and RAGE. In the types of OA that do not involve the synovium and where impaired cartilage anabolism is the major problem, compartmentalized TGF-β supplementation may be a good therapeutic alternative. It is clearly important to discriminate between the several forms of OA before undertaking targeted therapy.

Due to their size, virus particles cannot penetrate cartilage, although recent studies using adeno-associated viruses (AAVs) do claim they can. Targeting chondrocytes directly would not only reduce side effects, but also may bring about a more sustained expression of the transgene, because chondrocytes have a low turnover rate. At present, the cartilage can only be targeted indirectly, through transduction of synovial cells and subsequent diffusion of soluble factors. Another important development will be engineering viral gene constructs that carry promoters for inducible expression. Such a vector would sense local activity and generate IL-1ra production only after synovial activation, as indicated by IL-1 production. We have utilized an inducible IL-1/IL-6 promoter in RA models [22]. To apply this approach in OA requires identifying suitable promoters of mediators or markers of the OA process. Possible promoters are those of certain MMPs, such as MMP-13, which occur in large amounts in OA synovium and cartilage.

4.10 Conclusion

More research is needed to clarify the contribution made by the synovium to OA pathology, a role that has long been underestimated. However, with research focused on the importance of synovium, synovial involvement is clearly seen to be substantial. Synovial macrophages are important in mediating OA-related pathology, such as osteophyte formation and MMP-mediated cartilage matrix breakdown. From a histologic standpoint, synovial OA is a very heterogeneous tissue. In many instances inflammation is substantial; in others the synovial lining appears only thickened, and in others the synovium appears normal. This contrasts with RA synovia that appear more homogeneous with inflammation a common feature. Osteoarthritis, therefore, may not be a single disease, but rather be the outcome of different pathologic processes. In one case, OA may be caused by a process restricted to the cartilage, in another synovial involvement may be more important, with inflammation apparent. However, even when there is no clear synovial inflammation, as in collagenase-induced OA, the synovial cells may still play an important role, as was shown by synovial macrophage depletion in this model. It is therefore important to generate tools that discriminate between the different forms of OA. In some, anti-inflammatory therapy may be satisfactory. In others, therapy may be targeted principally to the cartilage, whereas in others yet, specific targeting and silencing the synovial macrophage may be the optimal approach. However, this kind of customized therapy depends on research that takes into account the many tissue and cell types that together ensure homeostasis of the joint.

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5.

Cartilage Matrix Destruction

Dick Heinegård

Cartilage matrix is made up of a large number of macromolecules in various interacting networks. The cells constitute only some 10% of the tissue, with extracellular matrix making up the remainder. The cells, however, have the key role of building matrix and maintaining the structure in response to altered loads or to material fatigue that results from repeated mechanical stress. To accomplish this, the cell produces a variety of proteinases that degrade specific macromolecules. The need for specialized enzymes to degrade the triple helical structure of collagen is an example of how specific molecules have evolved to accomplish specified tasks. Moreover, every repair process that involves degradation is accompanied by an increase in the synthesis of matrix constituents. Repairs most often are adequate, but not when pathologic changes result.

Matrix degradation may cause release of growth factors that have become bound to and sequestered in the matrix. An example is latent transforming growth factor-β (TGF-β) binding protein (LTBP), which binds to matrix proteins such as fibrillins; TGF-β also binds to members of the small, leucine-rich protein family and is released when these molecules are degraded [31,34]. Similarly, basic fibroblast growth factor (bFGF or FGF-2) is sequestered in the matrix by having become bound to heparan sulfate chains of molecules such as perlecan. Decorin has been reported to bind to and regulate insulin-like growth factor-1 (IGF-1) [76]. The C-terminal part of the cartilage molecule intermediate layer protein (CILP)-precursor (CILP-1-2) may play a role in blocking its activity [43].

These are but some examples of how matrix degradation leads to the release of factors that can trigger cellular responses specific to the structural elements being degraded. Some fragments of the extracellular matrix (ECM) proteins released from the matrix contain active domains that can interact with cell surface receptors, such as integrins, and trigger responses. For example, when certain domains of fibronectin are released from the matrix, they interact with cell surface receptors and trigger an accelerated breakdown response [37, 93]. The response appears to involve integrin binding [36], but may also be mediated by heparin/heparan sulfate binding domains alone [42].

Because a primary role of cartilage is to take up and distribute load, matrix function and integrity play a key role. Interaction and networking between matrix constituents are essential to matrix function. When molecules break down, functionally important domains may become separated. This in turn can lead to tissue malfunction.

Functional properties and interactions of matrix proteins are essential to understand how proteolysis may affect a particular matrix protein and alter tissue function. Proteins with known interactions are described in the following sections.

5.1 Cartilage Matrix Organization and Regulation of Assembly

Figure 5.1 illustrates the three major organizational entities of the extracellular matrix. The fibrillar network with collagen type II as its major constituent predominates. These fibers also contain other matrix molecules, including other collagens. The major role of these fibers is to contribute tensile properties and to maintain tissue volume. Another fibrillar network has collagen type VI as the major constituent in beaded filaments. This network interacts with all other assemblies in the matrix via molecules bound to the filament surfaces. The third entity amounts to some 5% of the wet weight of cartilage and consists of negatively charged aggrecan molecules that contribute fixed, negatively charged groups of extreme density. The counterions in the matrix create an osmotic environment that retains enough water to bring about a large swelling pressure, opposed by the tensile properties of the collagen fibrillar networks.

5.1.1 Aggrecan Aggregates

Aggrecan is a major constituent in cartilage. It has a molecular weight of some three million daltons, contains some 100 chondroitin sulfate chains, each made up of some 50 disaccharides of glucuronic acid and *N*-acetylgalactosamine, with a sulfate group in the 4- or 6-position. Some disaccharides may contain sulfates in both positions. The length of the polysaccharide varies widely, as does the location of the specific sulfates along the chain (Fig. 5.2). The reducing ends of the chains are bound to serine residues, closely spaced along the CS-1 and CS-2 domains. At

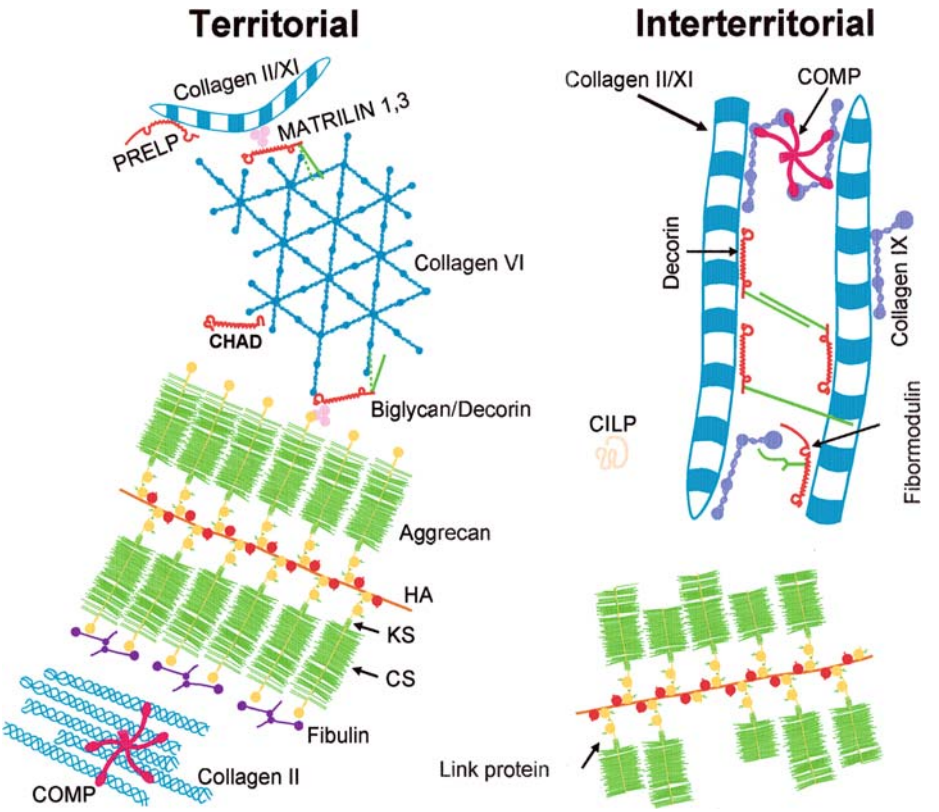

Figure 5.1. Schematic illustration of the molecules in the cartilage extracellular matrix with their assembly into supramolecular networks by the indicated interactions. The two compartments, territorial (close to the cell) and interterritorial (at a distance from the cell), and their partially different compositions are depicted. CS, chondroitin sulfate; HA, hyaluronic acid; KS, keratan sulfate.

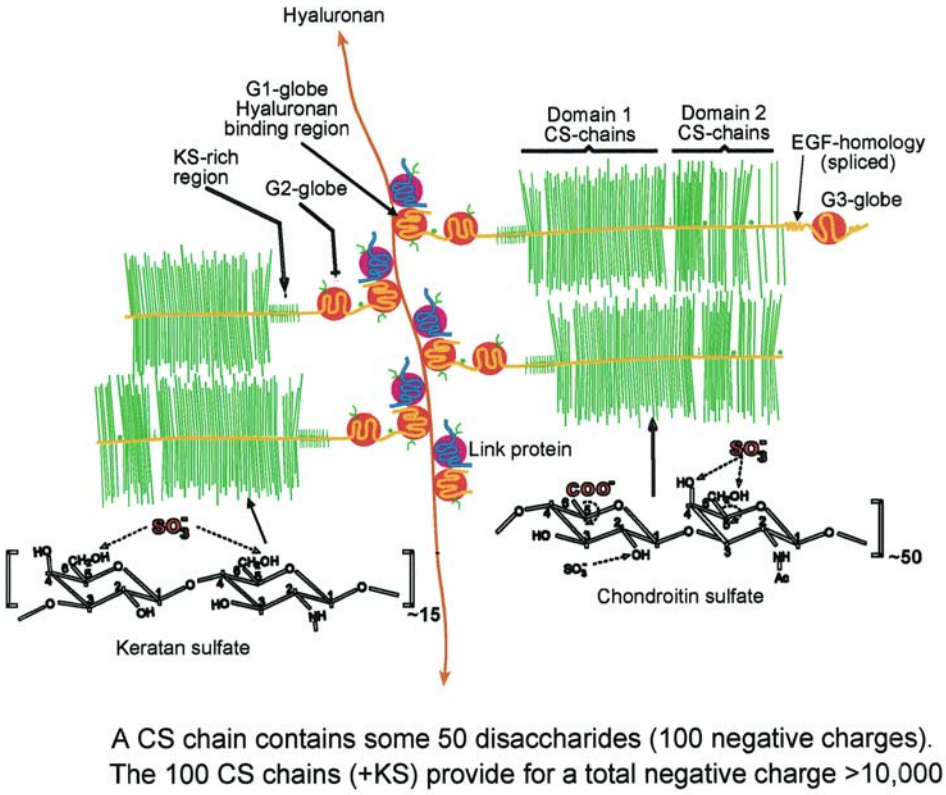

Figure 5.2. A schematic illustration of the large proteoglycan aggrecan and its assembly into higher order aggregates via many molecules binding to the very long glycosaminoglycan hyaluronan via their N-terminal G1 globular domain, an interaction stabilized by link protein. The domain structure of the aggrecan is depicted with globular domains, as well as such linear domains, carrying the glycosaminoglycans chondroitin sulfate (CS) and keratan sulfate (KS). These glycosaminoglycans are built from the two distinct disaccharide repeats, illustrated where the number of such disaccharides in a chain is provided. Note the variability in glycosaminoglycan chain length. Also the native, secreted aggrecan (top left) is modified by partial proteolysis to the shorter variants shown lacking a C-terminal globular domain.

each end there are globular domains, with the C-terminal having a lectin homology domain (G3) and binding tightly to tenascins, fibulins, and fibrillin [13]. These molecules can also form networks. The C-terminal part of aggrecan is subject to partial proteolysis and therefore this domain is lacking in most molecules in the cartilage of older persons (Fig. 5.2). The N-terminal end contains a globular domain (G1) that binds tightly to the glycosaminoglycan hyaluronan, constituted by disaccharide repeats of glucuronic acid and *N*-acetylglucosamine. This extremely long polysaccharide contains in excess of 1000 disaccharides and can bind more than 100 aggrecan molecules. This interaction is stabilized by the formation of a ternary complex among the G1 domain, hyaluronan, and the stabilizing link protein that has a structure homologous to the G1 domain. The result is that the many negative charges along the CS chain are firmly bound to the core protein of aggrecans, which in turn bind to hyaluronan. This causes charges to be fixed and retained in the tissue, to which the entanglement of the very large proteoglycan aggregate contributes. The molecule has a second globular domain (G2). This domain is separated from G1 by a short interglobular domain and shares structural features with G1. It has no known function [33]. A large proportion of the keratan sulfate chains is located between the G2 and the CS-1 domains. These chains are very closely spaced along a sequence rich in proline. Compared to CS, this polysaccharide of galactose and sulfated *N*-acetylglucosamine is short, with unknown function.

5.1.2 Collagen Fiber Network

5.1.2.1 Collagen Molecules

Collagen type II is specific for the fibril forming collagens of cartilage. It has a central triple helix with three α-chains, each about 100,000 kd, with dimensions of 300 × 1.5 nanometers (nm). It is synthesized as a procollagen in which each chain has an N-terminal extension of about 15 kd and a C-terminal extension of about 35 kd. The trimer molecule is covalently linked via disulfide bridges in the C-terminal propeptide. Before assembly into higher order fibrils, the terminal propeptides have to be cleaved off, since they preclude the tight assembly of the triple helical molecules into a fibril. This cleavage is accomplished by specific proteinases, with a member of the A Disintegrin-like And Metalloprotease domain with Thrombo Spondin type I repeats (ADAMTS-2) family cleaving the N-terminal. The enzyme active in the release of the C-terminal propeptide is a member of the tolloid group and was originally named bone morphogenetic protein-1 (BMP-1) [24].

The collagen molecules then associate on a core of two homologous collagen XI [20] and two collagen II molecules, to form an outer shell of 10 collagen II molecules of the microfibril [35]. Interestingly the collagen XI α-1 chain retains its N-terminal propeptide also in the fibril and may actually extend to the fibril surface [28,29]. In addition to these thin fibrils there exist much thicker fibers, but little is known of their assembly mechanisms or detailed composition.

The assembly of the molecules within a fiber occurs by a quarter stagger such that a gap is formed along the long axis of the fiber between consecutive collagen molecules [18]. In addition to collagen type II, fibers contain other collagens, particularly collagen type IX [18]. This molecule has noncollagenous domains (NC1–4) at each end of the three collagenous domains (Col 1–3). The quarter-stagger organization of collagen type II in the fiber creates the gap regions, where the major part of the collagen IX molecule appears to localize between the ends of the collagen II molecules. The Col-3 with the N-terminal NC-4 of the α-1 chain protrudes from the collagen fiber [88]. Because the NC-4 domain is relatively basic, it forms a site for interactions with the abundant, negatively charged molecules in the matrix. It also interacts with other molecules in the matrix, for example, cartilage oligomeric matrix protein (COMP) and the matrilins, discussed below.

An important event in the stabilization of the collagen network is the formation of covalent crosslinks. Lysyl oxidase is a key enzyme that modifies the lysine group to form an aldehyde function that constitutes the Schiff bases, with the α amino groups of neighboring lysine residues part of another collagen α-chain. These crosslinks are rearranged to form structures like pyridinoline that link the collagen II chains. The links are both intra- and intermolecular and between the chains of collagen XI. These crosslinks involve the short telopeptide that remains on the triple helical part after propeptides have been released. Similar crosslinks exist between the collagens, for example, between collagen II and collagen IX [19–21]. Figure 5.1 provides a general view of the collagen fiber.

5.1.2.2 Noncollagenous Collagen-Binding Proteins

Many proteins have a high affinity for collagen (Fig. 5.1). Some of these interactions support fiber formation, while others modify the collagen fiber surface so as to provide sites for interactions with neighboring structures. This includes cross-bridging to neighboring fibers, which enhances tensile properties.

5.1.2.2.1 Thrombospondins

The thrombospondin family is made up of proteins that have a high affinity for collagen. Two thrombospondins 1 and 2, with three identical subunits, bind to a number of extracellular proteins, including those with heparin/heparan sulfate motifs, via the so called thrombospondin type I domain, a motif found also in other extracellular matrix proteins [1]. The other thrombospondins, 3, 4, and 5, have five identical subunits. The subunits in all cases are linked via a coiled-coil domain sited close to the N-terminal. In the case of thrombospondin 5, also referred to as cartilage oligomeric matrix protein (COMP), the site is only a few amino acids away from the N-terminal [1]. The other thrombospondins extend further in the N-terminal direction. All have a unique C-terminal globular domain that is homologous for the various family

members and therefore probably has the same function. This chapter focuses on COMP as typical for this class of molecules. The thrombospondins have a cell-binding arginine-glycine aspartete (RGD) sequence that binds to the integrins $\alpha_5\beta_3$ and $\alpha_5\beta_1$ [11]. However, it is not clear whether this site is actually exposed in the native molecule, because this is not supported by crystallographic evidence [47]. The rat COMP molecule actually does not contain an RGD sequence [60].

Cartilage oligometric matrix protein has also been shown to bind to collagens with high affinity via its C-terminal globular domain [69]. Four binding sites are distributed quite evenly over the collagen triple helical molecule, with one at each end. Because the COMP molecule cannot span two binding sites on the collagen, it will bind to one such molecule via one subunit C-terminal and leave the other units free for other interactions. Preliminary data indicate that COMP may actually function as a catalyst to accelerate the early steps of collagen fibrillogenesis (Halasz, Kassner, Mörgelin, and Heinegård, unpublished). Another interaction of COMP is with the noncollagenous domains of collagen type IX [63,82], which, as discussed above, is bound to the collagen type II fibers, such that the C-terminal NC-4 domain and the following Col-3 domain protrude from the fiber [88]. This domain in particular, therefore, can mediate interactions with COMP and other thrombospondins, and COMP seems to be able to cross-bridge and provide stability to the collagen fiber network. It also interacts with high affinity with fibronectin via its C-terminal domain, but the functional implications of this interaction are not understood [15].

5.1.2.2.2 Leucine-Rich Repeat (LRR) Proteins: Biglycan, Decorin, Fibromodulin, and Lumican

This family of proteins, shown schematically in Figure 5.3, is found in the extracellular matrix. Their common feature is a central repeat region with 10 to 11 repeats of some 25 amino acids. In one class, the repeat region only contains six repeats. The repeat region is surrounded by small disulfide bonded loop structures. Many of the members of this family contain a peptide

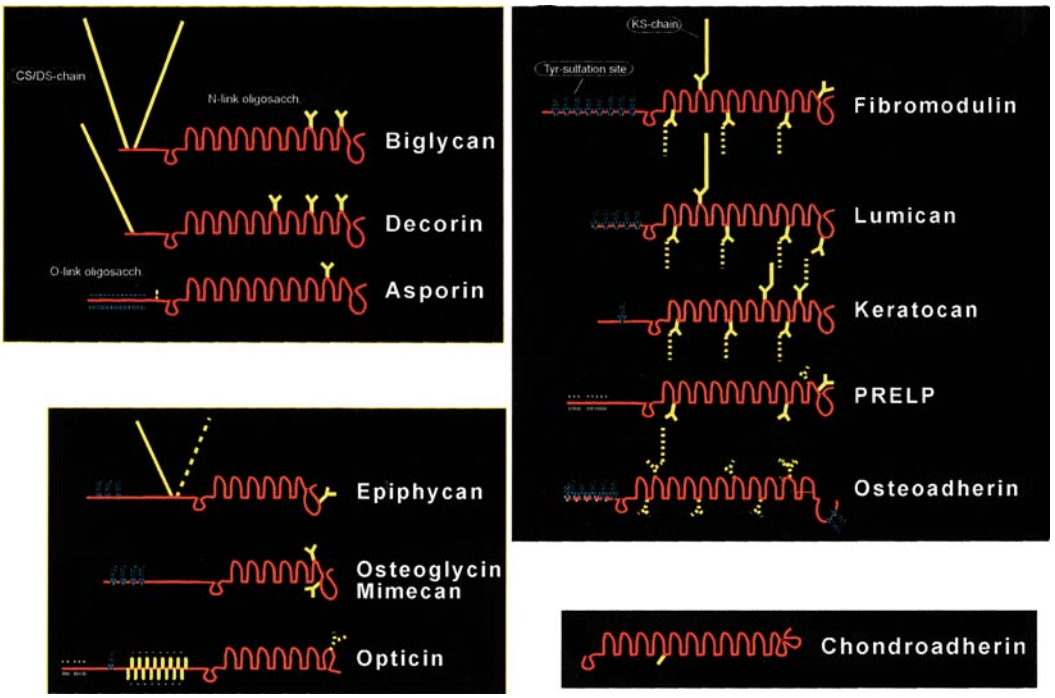

Figure 5.3. Schematic illustration of the leucine-rich repeat proteins in the extracellular matrix. The central repeat region with 6/7 or 10/11 leucine-rich repeats of each around 25 amino acids is surrounded by disulfide loop structures. There is an extension in the N-terminal part of most of the proteins where they carry various modifications varying from glycosaminoglycans, tyrosine sulfate, acidic amino acids, or clusters of basic amino acids.

stretch in the very N-terminal, with structure and posttranslational modification different for each of the proteins.

The three-dimensional (3D) structure has been resolved by x-ray crystallography for two members of this protein family: decorin and biglycan [77,78]. Figure 5.3 shows their structure, where the repeats appear to be curved with a β-sheet structure on the concave side. It is here that many interactions occur. Interestingly, the predominant form appears to be a dimer, with two identical molecules binding to one another, their concave surfaces apposed to each other, and the N-terminal end of one molecule interacting with the middle portion of the other. A dimer such as this exhibits interactions differently from a monomer. At the same time the dimer contains two of every functional domain and therefore represents a suitable form for cross-bridging between matrix constituents. This is important for network formation. Other related LRR proteins may also form dimers, but this has not yet been reported.

A primary function, identified for almost all family members, is collagen binding. This interaction is mediated by the LRR repeat domain. In the case of decorin repeats 4 and 5 have been implicated [79], although other parts of the LRR domain may also contribute to the binding.

Figure 5.3 depicts four groups of molecules that differ by sequence and gene organization. One group has only 6 to 7 repeats, whereas the other LRR proteins typically have 10 to 11 repeats. Family members differ essentially in their terminal domains. Most have highly anionic N-terminals to which one or two glycosaminoglycan chains of chondroitin sulfate or dermatan sulfate (decorin or biglycan, respectively) are bound, depending on the tissue in question. Asporin contains an N-terminal domain with up to 15 continuous aspartates. This domain varies in length and linkage in normal and osteoarthritic tissue [39], but not in all population groups [43,58,68]. Fibromodulin and osteoadherin contain N-terminal domains with repeats of up to 9 tyrosine residues that bind sulfate and thereby contribute negative charges [61]. These highly anionic domains interact with basic domains of other proteins, yet are bound to collagen fibers via their leucine-rich repeat region. They can therefore serve as a cross-bridge between the collagen fiber and surrounding structural entities.

Proline arginine-rich end leucine-rich repeat protein (PRELP), also a member of this family, contains an N-terminal domain with clustered arginine and proline residues that interacts tightly with heparin and heparan sulfate, with the leucine-rich repeat region at the same time binding to collagen [4,5]. PRELP, found at select basement membranes [5], is likely to act as a molecular cross-bridge, connecting the heparan sulfate side chains of perlecan to underlying collagen fibers, thus constituting an anchor of the basement membrane.

Chondroadherin represents a separate class. It has two disulfide bonds that form a double loop in the C-terminal part, whereas the other LRR proteins only have a single bond. Chondroadherin binds to fibrillar collagens I and II at two sites [52], and also binds cells via their $\alpha_2\beta_1$ integrin [8]. Chondroadherin, then, is likely to constitute one of many linkages between the extracellular matrix and chondrocytes and therefore may modulate matrix properties.

5.1.2.2.3 Matrilins

Cartilage contains four matrilins, with matrilins 1 and 3 particularly prominent [14]. The matrilins are composed of three or four subunits that are joined via a coiled-coil domain. They contain one (matrilin 3) or two (matrilin 1) von Willebrand factor A domains that bring about interactions with other proteins. In addition to homo-oligomeric molecules there also exist heteromers that contain subunits of both matrilin-1 and -3 [92]. Interactions involve a number of extracellular matrix proteins, including collagens [7,84], aggrecan [30], and COMP [51], as well as interactions between matrilins that lead to fibril formation [12].

5.1.2.2.4 Fibronectin

Fibronectin is a ubiquitous molecule with two identical subunits joined at the C-terminus. It has several domains that bring about specific interactions [50]. The N-terminal part contains a domain that can bind to heparin and to the heparan sulfate chains of cell surface proteoglycans (i.e., syndecans and glypicans [91]). Toward their C-terminal the fibronectins have a gelatin- and collagen-binding domain. An integrin $\alpha_5\beta_1$ binding RGD sequence provides for cell binding and a second heparin-binding

domain can also bind cell surface proteoglycans. There is a second integrin-binding domain with different specificity for $\alpha_4\beta_1$ and $\alpha_4\beta_7$, as well as a fibrin-binding domain close to the C-terminus. Fibronectin forms fibrils that play an important role in directing cell migration. Fibronectins may also be involved in the assembly of other matrix entities [83].

5.2 Degradation of Cartilage Matrix

In a number of conditions, the structure and function of cartilage matrix are compromised as a result of the synthesis and activation of proteinases. There is abundant literature on upregulation of proteinases in pathology and on their increase in activity as evaluated by zymography and presence by immunoassay. As yet we understand little of how a particular proteinase affects matrix structure. Often studies have focused on the activity of candidate enzymes on isolated proteins, rather than on the more general effects on tissue structure and on what domains may be presented to the enzyme. A given enzyme may act differently on a protein in solution, as compared to what may happen when the same protein resides in the matrix and is tied up in interactions. In vitro, decorin is split by matrix metalloproteinase-3 (MMP-3) and ADAMTS-4 (*A* disintegrin-like *a*nd *m*etalloprotease domain, reprolysin-type, with *t*hrombos*p*ondin type I motifs) between Glu154 and Leu155 [40], whereas, on the basis of a fragment found in skin, the split in vivo has occurred between Phe170 and AsN171 [9]. Fibromodulin is a further example of differences between enzyme activity under different conditions, as the protein is not cleaved by MMP-13 at a specific site, unless bound to collagen in the cartilage matrix [32]. The relationship of proteinases to their established substrates in the extracellular matrix is discussed below.

Over the years the major focus on enzymatic breakdown of cartilage matrix has been on metalloproteinases, notably the MMPs with more than 25 members [57], the ADAMTS [44, 66], and the ADAM [2], with each group made up of many members that have different substrate specificities and whose activities are regulated in different ways.

The serine proteinases are another set of enzymes involved in cartilage breakdown. One of these enzymes involved in joint disease is HtrA1, which is upregulated in osteoarthritis [27]. The enzyme cleaves synovial fluid fibronectin to give rise to fragments [27] that in turn stimulate chondrocytes to produce degradative enzymes leading to tissue breakdown [38].

The many cysteine proteinases play a role in a variety of tissues. Cathepsin K, secreted by the osteoclast in particular, has a central role in bone breakdown. When the enzyme is inhibited or absent, bone mass increases. Studies have shown that when synthetic cathepsin K inhibitors are administered to arthritic animals, inflammation and the concentration of cysteine proteinases are diminished [6]. The activity of cysteine proteinases is counteracted by the cystatins, molecules that are abundant in some tissues. Because rabbit ear cartilage collapses in response to the intravenous injection of papain, a cysteine protease [54,81], it is evident that ear cartilage and possibly other cartilages have a low level of resistance to attacks by this group of proteinases. More recent studies have shown that the calpains, members of an endogenous and largely intracellular cysteine protease family, can be found in the extracellular matrix of cartilage and are involved in tissue breakdown, because aggrecan fragments, typical for the activity of this enzyme, are found in cartilage [62].

Understanding both homeostasis and pathologic breakdown of cartilage requires knowledge of what major functional units and enzymes are involved. The functional units are the aggrecan and collagen networks, while a number of enzymes have been identified that degrade one or the other structure.

5.2.1 Sequence of Events in Cartilage Breakdown

Much of our knowledge of the sequence of events in cartilage degradation is based on in vitro studies with breakdown stimulated by catabolic cytokines, often interleukin-1 (IL-1), sometimes in combination with oncostatin-M. Figure 5.4 depicts the sequence of degradation schematically and is based on current knowledge. The first step in cartilage degradation affects the aggrecan molecules, with the glycosaminoglycan chains released

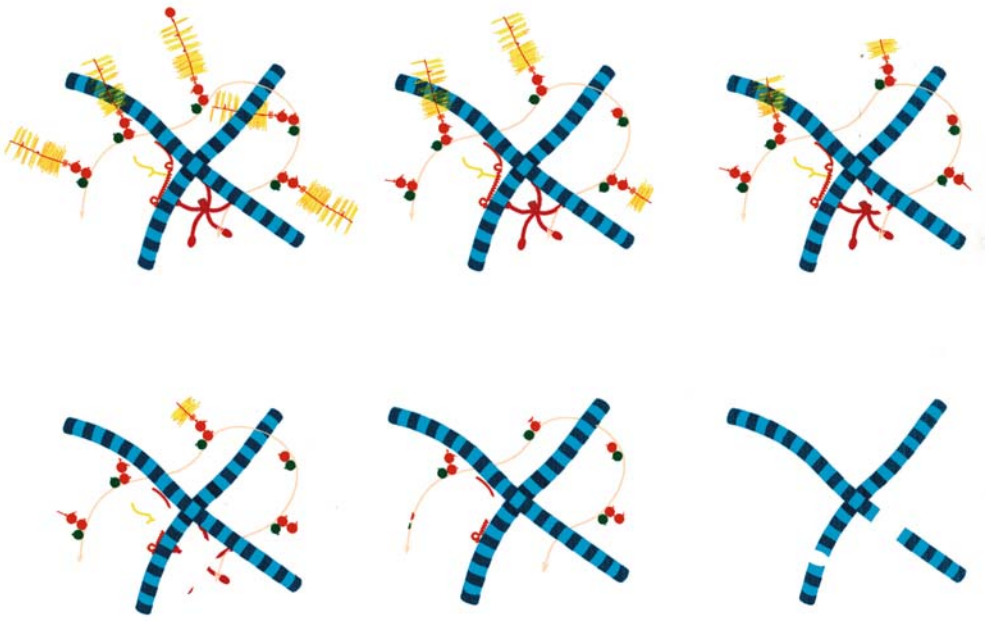

Figure 5.4. Progressive destruction of matrix proteins in cartilage breakdown. Initially the aggrecan (yellow side chains) is cleaved, followed by fragmentation of COMP (red) and fibromodulin, with final destruction of the striated collagen fiber. The hyaluronan binding region plus the link protein are retained in the tissue by binding to the very large hyaluronan.

from the aggrecan domains. The hyaluronan-binding domain, however, remains attached to the very high molecular weight hyaluronan and is released much later. Degradation of aggrecan is followed by proteolysis of noncollagenous molecules that in many instances form part of the collagen network. Ultimately the collagen fibers themselves become degraded [26,32]. Patient studies have identified molecular fragments released from cartilage into the synovial fluid and have shown that the proteins making up the fragments released at different stages of the disease differ distinctly in composition [75].

5.2.1.1 Aggrecan Release

Early studies have shown that almost the entire molecular length of aggrecan is released when cultured cartilage is stimulated with a factor originally referred to as catabolin [71,87], now named interleukin-1. The released fragments cannot bind to hyaluronan [87]. This indicates that the N-terminal hyaluronan-binding domain was either absent, having been retained in the tissue, or is not functional. Subsequently it was shown that the interglobular domain between the N-terminal hyaluronan-binding globule and the G2 second globule was cleaved. Two cleavage sites, shown in Figure 5.5, were identified. One is somewhat closer to the N-terminal G1-domain between asparagine and phenylalanine in the amino acid sequence DIPEN-FFG, and is the result of the enzymatic action of MMPs [22]. The other is closer to the more C-terminal G2 domain between glutamate and arginine in the amino acid sequence NITEGE-ARG, and is due to the action of the so-called aggrecanases [72], members of the ADAMTS family.

Polypeptide fragments with CS chains that are released into the synovial fluid in joint disease have an N-terminal ARG sequence and thus represent an aggrecanase cleavage site [49]. At the same time the fragments that contain the hyaluronan-binding G1 domain are retained in the tissue and expose the expected C-terminal NITEGE and-DIPEN sequence. The latter has been created by an MMP cleavage [73] that appears to follow the aggrecanase cleavage.

At least four additional cleavage sites in the chondroitin sulfate–rich domain of aggrecan are distributed along the core protein [85,86], with cleavage occurring at sites where the

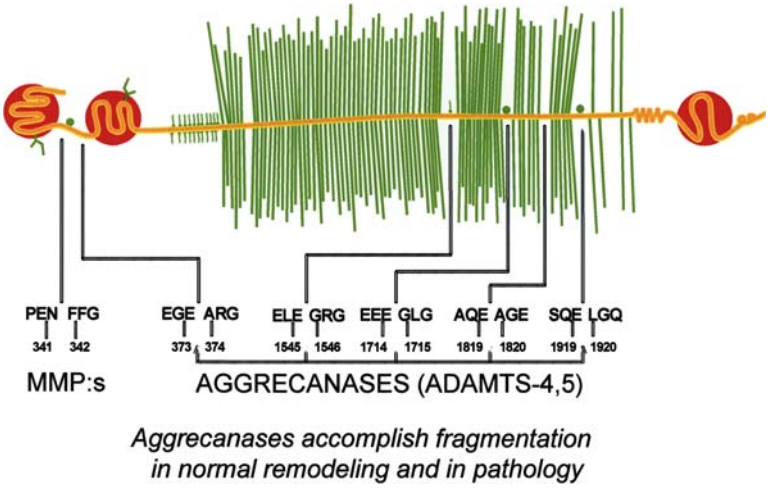

Figure 5.5. Illustration of cleavage of aggrecan by proteolytic enzymes and the specific cleavage sites indicated by the one-letter amino acid code.

distance between the chains is somewhat greater than the amino acid sequence (Fig. 5.5). These sites appear to undergo cleavage before the interglobular domain is cleaved, resulting in tissue retention of fragments that still contain the hyaluronan-binding domain and a variably long CS-rich domain (Figs. 5.1 and 5.2). Interestingly, aggrecanase cleavage and release of major aggrecan fragments appear to occur always before collagen is degraded by collagenase [67]. This is consistent with the sequence of events observed when cultured cartilage explants are treated with IL-1 and with synovial fluid samples.

Further understanding of the relevance of aggrecan degradation for the development of osteoarthritis has come from mice studies in which expression of the gene for ADAMTS-5 was inhibited. Without this enzyme, the null mice did not develop osteoarthritis [25], but whether this enzyme is also required for proteoglycan degradation in the human is not clear. Many other ADAMTS enzymes also show aggrecanase activity, and there may be species differences.

Although ADAMTS proteinases have often been associated with degradation of aggrecan, the enzymes can cleave other substrates, as discussed below. An interesting and important finding is how specificity changes when the enzyme becomes truncated in the C-terminal domain. If such variants are produced by partial proteolysis, aggrecanase activity will decrease and the activities toward molecules like decorin and fibromodulin increase with novel substrate specificity [46].

5.2.1.2 Fragmentation of Noncollagenous Collagen-Binding Molecules

A second set of events in cartilage breakdown is the release of extracellular matrix molecules that in the tissue are bound to the collagen fibers. These molecules provide interactions with surrounding structures, including other collagen fibers (Figs. 5.1 and 5.6). They contain a minimum of two functional domains with at least one binding to collagen, thus effecting cross-bridging via one or more additional binding domains. Several members of these molecules have been shown to be degraded in cartilage breakdown. These include COMP, decorin, biglycan, and fibromodulin, to be discussed below.

5.2.1.2.1 Cartilage Oligometric Matrix Protein Fragmentation

Cartilage oligometric matrix protein can bind to collagen via its five identical C-terminal globular domains [69]. The molecule thus can join together up to five different matrix constituents. Because COMP binds to several different molecular entities, it constitues a key candidate for cross-bridging different structural entities in the matrix.

In IL-1–stimulated cartilage explants, COMP is released after aggrecan in a biphasic manner,

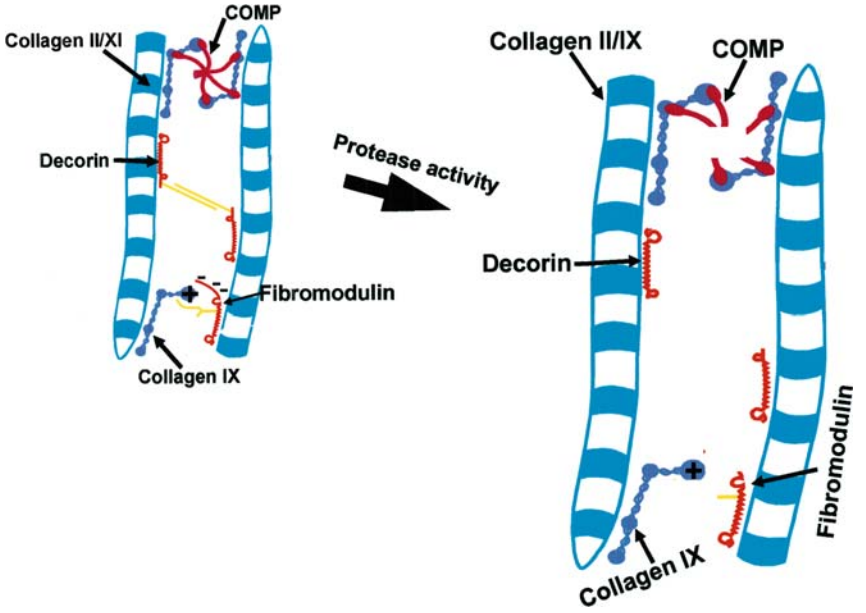

Figure 5.6. Illustration of the collagen type II fiber network with the molecules binding to the surface of the fiber and providing interactions between fibers themselves. Cleavage of the surface-bound molecules disrupts interactions and results in impaired mechanical qualities.

where the initial slow COMP release cannot be inhibited by the addition of a broad-spectrum MMP inhibitor [26]. The phase of the release therefore appears to be mediated by an active member of the aggrecanase family. This inference is supported by the fact that purified COMP can be cleaved by ADAMTS-4, but by neither ADAMTS-5 nor -1. One of the fragments that is generated corresponds in size to almost the entire subunit, but lacks the coiled-coil domain necessary for the formation of the pentamer. Its actual size corresponds to a fragment found in synovial fluid [16,89]. A similar fragment was generated by MMP-9 [16]. Other smaller size COMP fragments are also found in the synovial fluids of patients with joint disease [23,74,89], but little is known about the proteinase that causes their release. Some information has come from experiments in which broad MMP inhibitors have been used to block release of COMP from IL-1–stimulated cartilage biopsies [23].

The bulk of COMP release that occurs in a second phase appears to involve MMPs. What specific MMP is involved is not clear, but, as discussed below, COMP release occurs at the same time as fibromodulin is cleaved by MMP-13. However, as shown by zymography [32], other active MMP molecules may also play a role.

5.2.1.2.2 Fragmentation of Fibromodulin

Studies of molecules cleaved and released when cartilage is treated with IL-1 have shown that fibromodulin is affected after aggrecan and at the same time as COMP [32]. The leucine-rich repeat domain binding to collagen was unaffected initially and retained in the tissue. Primary cleavage was in the N-terminal extension domain and led to the removal of almost the entire tyrosine sulfate domain. This changes the ability of the molecule to cross-bridge to other entities with a cationic positive charge.

Cleavage exposed a new N-terminus in the form of NH2-AYG- (bovine) or NH2-TYG (human) on the major part of the molecule retained in the tissue [32,61]. With the aid of antibodies specific for this new N-terminal neoepitope, it was shown that a number of MMPs, including 2, 3, 8, and 9, did not react with the substrate, whereas MMP-13 efficiently cleaved fibromodulin at the expected site [32]. Cleavage occurred when the substrate consisted of the N-terminal plus the first cysteine knot of fibromodulin. Intact fibromodulin was not cleaved under those conditions [32].

One consequence of this cleavage is that cross-bridging between collagen fibers becomes less efficient (Fig. 5.6). This may be the reason tissue tends to swell in early disease, the result of the impaired tensile properties of the collagen network.

5.2.1.2.3 Fragmentation of Decorin

Decorin, another molecule present in collagen fibers, may contribute to network properties. Interleukin-1 stimulation of cartilage explants did not affect decorin to any extent, with virtually no change taking place during the period when fibromodulin was degraded [80]. Tissues appear to contain a small amount of decorin that lacks the chondroitin sulfate chain [70], presumably a result of proteolytic processing. Isolated decorin is cleaved by several MMPs, including MMP-2, -3 and -7, at several sites, yielding from one site a fragment with an N-terminal L(211)KGLN [40]. One consequence of cleavage of decorin by these MMPs is that TGF-β bound to the molecule will be released and may then activate repair [40]. On the other hand, the relative resistance to MMP cleavage by decorin [80] indicates that when the molecule is bound to native collagen, it may not constitute a substrate. This inference is supported by the fact that a decorin fragment containing the glycosaminoglycan chain had a different C-terminus in the form of VRKVTF170-COOH [10] and that this fragment retained the ability to bind collagen. No information is available on the enzyme that brings about the cleavage.

5.2.1.2.4 Fragmentation of Biglycan

The distribution in cartilage of biglycan differs from the closely related decorin in that biglycan is restricted to the territorial matrix in cartilage, close to the cell, whereas decorin is present throughout the matrix. Interestingly, biglycan colocalizes with the filamentous network of collagen type VI and is bound to the collagen [90], as is also true for decorin. Decorin, however, in addition, is bound to collagen type II fibers. Degradation of biglycan will therefore have different consequences, compared to that of decorin.

Biglycan is cleaved by aggrecanases 1 and 2 (ADAMTS-4 and -5) between Asn149 and Cys150, in the leucine-rich repeat region [55]. Because these fragments have not been detected in normal adult cartilage, biglycan may be bound to structures that are not subject to degradation by these enzymes. Interestingly, the enzymes failed to degrade the closely related decorin. In another study it was shown that MMP-13 will cleave biglycan between Gly177 and Val178, representing a different site in the leucine-rich repeat region [56]. Whether these cleavages occur in vivo is not clear, nor is it known whether enzymatic cleavage affects the binding properties of biglycan.

5.2.1.2.5 Cleavage of Fibrillar Collagen

Collagen type II is a major and collagen type XI is a minor component of the collagen fiber network. The major part of collagen type II is cleaved after release of aggrecan and non-collagenous matrix proteins [26]. Collagen is broken down [57,64,65] primarily by three collagenases, MMP-1, -8, and -13 (collagenases 1, 2, and 3, respectively), all cleaving at the same three-quarters to one-quarter site. The fragments formed will unfold at body temperature and be further broken down to short peptides by a variety of proteinases. The fact that the cell surface MT1-MMP will cleave collagen is important for the migration of cells through collagen-containing matrices [41]. As discussed above, normally collagen type II turns over very little, and its breakdown is considered to be primarily pathologic. Cleavage produces a new C-terminal that corresponds to Gly775 and antibodies that specifically react with this new terminus have been developed and used to assess cartilage breakdown in joint disease [17,53]. The new assays that measure the release of fragments from the telopeptide regions that contain pyridinoline crosslinks are a particularly interesting development in monitoring collagen type II breakdown. An example is the epitope with the amino acid sequence (EKGPDP), which results from the enzymatic activities of MMP-1, -2, -7, and -13. An assay referred to as CTX-II, based on this epitope has been generated and applied to the analysis of collagen II fragments released into the synovial fluid in the course of joint disease [48].

There also exist other types of collagenases. Particularly well characterized is cathepsin K, which plays a key role in bone collagen breakdown [3]. A fragment of the α2(XI) chain released when collagen XI is degraded has been isolated from normal cartilage and termed proline / arginine-rich protein (PARP) [59].

Interestingly, turnover of fibrillar collagen is minimal under conditions of normal tissue adaptation or fatigue. Adaptation may involve cleavage of specific molecules associated with the collagen fiber and play a role in network formation.

5.3 Conclusion

Of the many proteinases involved in turnover and breakdown of cartilage matrix constituents, those most clearly implicated are the matrix metalloproteinases, but other enzyme families may also play a role. Information on enzyme specificity for particular proteins is limited, because most reports are of studies with purified proteins that are not relevant to understanding how substrate sites in the matrix are modulated in vivo. Moreover, the many endogenous enzyme inhibitors ranging from tissue inhibitor of metalloproteinase (TIMP)-1, -2, and -3 for the MMPs to a large family of cystatins and serine proteinase are capable of inhibiting a wide range of enzymes.

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6.

Anabolic Mediators of Cartilage Healing

Naoshi Fukui and Linda J. Sandell

6.1 Introduction

Although osteoarthritis (OA) is essentially a degenerative disease, anabolic activity of chondrocytes is significantly upregulated. Enhanced anabolism in OA chondrocytes was first reported more than 30 years ago [17,41, 47] and has since been confirmed by many studies. Often referred to as OA hyperanabolism, upregulation of synthetic activity is not limited to cartilage, but also affects other tissues inside and around the joint. For example, formation of osteophytes and sclerotic changes of the subchondral bone are pathologic events that symbolize vigorous anabolic activity.

Modern techniques have given us a more comprehensive idea about the metabolic changes in OA. Proteomic analysis has confirmed that synthesis of type II collagen is enhanced in OA cartilage [35], and microarray analysis has provided an overall picture of the metabolic changes that take place in the disease process [7]. To date, the significance of anabolism in the pathology of OA is not well understood.

Due to the dramatic changes in the metabolic activity of the chondrocytes in OA cartilage, the significance of anabolism in the pathology may be underestimated. Just like many other phenomena, anabolism in OA could have two faces. The enhanced synthesis of cartilage matrix components may prevent disease progression by counteracting catabolic events and initiating a repair process. On the other hand, the induction of pathologic proteins and possible disturbance in proper matrix synthesis observed in the disease may promote disease progression. This chapter provides an overview of the anabolism in OA, with emphasis on recent progress.

6.2 Anabolism in Osteoarthritis Cartilage

The primary components of articular cartilage are collagen and protoglycan. In cartilage, types II, IX, and XI collagens build up the fibrillar network that confers tensile strength to the matrix. Among the collagens, type II collagen is by far the most dominant, comprising more than 90% of total collagen [38]. The enhanced expression of type II collagen in OA is well known. The first evidence of an increase in type II collagen synthesis was the report of higher incorporation of [^{3}H]proline into OA cartilage [41]. Later, an antibody against the C-terminal propeptide of type II procollagen (CPII) was used to evaluate collagen synthesis [55]. The propeptide is cleaved and metabolized soon after collagen synthesis. Therefore, the presence of a CPII in OA cartilage indicates that the collagen is synthesized at a rate much higher than normal. We have recently compared the expression of type II collagen messenger RNA (mRNA) in OA and normal cartilage, analyzing regional differences with the aid of laser-captured

microdissection, a technique that permits small regions of cartilage to be isolated. The study revealed that the expression level of type II collagen mRNA differs considerably in different regions, and can be more than 20 times higher in OA than in normal cartilage [27,28] (Fig. 6.1).

Although types IX and XI collagen are critical for the collagen framework, it is not known whether their structure is altered in OA. Their synthesis is elevated [7,9], as is their gene expression [27,28]. Type VI collagen, a minor component in articular cartilage, occurs primarily in the pericellular matrix that surrounds the chondrocytes and may play a role in the maintenance of the pericellular microenvironment [34,61]. Type VI collagen is more highly expressed and constitutes a larger fraction in OA cartilage compared to normal cartilage from age-matched donors [34,64,67]. However, the distribution and organization of type VI collagen is significantly altered in OA. These changes in extracellular matrix may account for the altered chondrocyte metabolism in the disease.

Aggrecan is the predominant proteoglycan in articular cartilage. Aggrecan interacts with hyaluronic acid and link proteins to form large aggregates that endow the cartilage matrix with compressive stiffness. The expression of aggrecan is upregulated as OA progresses. Enhanced mRNA expression was shown by in situ hybridization [3,5], and the presence of new aggrecan molecules was demonstrated by the analysis of OA cartilage extracts [66]. The synthesis of link protein is also enhanced in OA [18], as is the expression of small proteoglycans, such as decorin, biglycan, fibromodulin and lumican, the large proteoglycan, perlecan [5,13,18,19,44,74], and some noncollagenous proteins, such as tenascin, cartilage oligomeric matrix protein (COMP), cartilage intermediate layer protein (CILP), and matrilin-3 [7,44,63].

Enhanced expression is also observed for genes that are expressed in normal cartilage at very low levels or not at all. Gene expression of types I and III collagens results from phenotypic change of chondrocytes to what could be considered a fetal phenotype [2,3]. Type II collagen is synthesized not only as the chondrocyte-characteristic type IIB splice form, but also the noncartilage splice form, type IIA [6]. Fibronectin accumulation in OA cartilage may be a repair response [37]. In OA cartilage, some chondrocytes undergo hypertrophic change as a result of the de-repression of genes for type X collagen, annexin VI, syndecan 3, osteocalcin, osteonectin, and osteopontin [62].

Osteoarthritic chondrocytes present diverse metabolic changes, stemming, at least partly, from considerable regional variations in different sites of OA cartilage [3,5]. Quantitative analysis of regional differences [27,28] has shown that expression of cartilage matrix genes is upregulated in the deeper cartilage zones, but their expression is considerably reduced at the surface of degenerated cartilage. This is especially true for aggrecan. Cells close to the

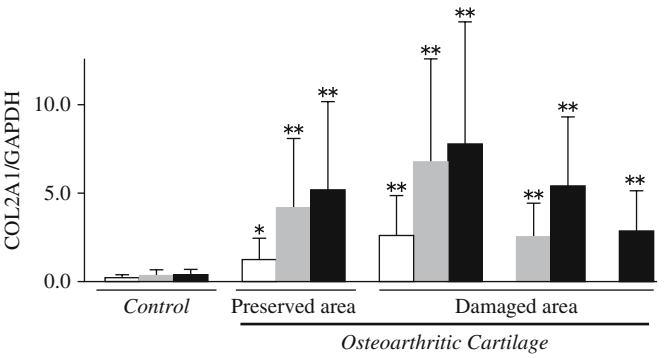

Figure 6.1. Expression of type II collagen mRNA in normal and OA cartilage. Cartilage obtained from normal and end-stage OA knee joints was divided into three cartilage zones by laser microdissection. Total RNA was extracted, cDNA was synthesized, and the expression of COL2A1 was evaluated in respective zones together with that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). In each OA joint, cartilage was harvested from several sites with and without macroscopic degeneration, and the samples from degenerated areas were assigned to three categories according to the number of cartilage zones remaining at the site. In the damaged cartilage category, patients are grouped according to which zones were present. The expression levels of superficial, middle, and deep cartilage zones are shown by open, shaded, and solid bars, respectively. *, $p < .05$, and **, $p < .01$ compared with the corresponding zone in normal cartilage.

degenerated surface, however, tend to express pathologic genes. Type X collagen is expressed mostly at the fibrillated area of OA cartilage, with induction of type III collagen more obvious in the degenerated regions. Evaluation of the metabolic changes in OA, therefore, must consider regional differences, particularly because chondrocytes in the degenerated regions seem more susceptible to phenotypic alteration.

6.3 Significance of Osteoarthritis Hyperanabolism in Disease Progression

As a result of the higher metabolic rate of OA chondrocytes, newly synthesized matrix may constitute a significant proportion of cartilage matrix. This is evidenced by elevated quantities of CPII, with the amount of CPII directly related to the content of total type II collagen [55]. A recent study based on an evaluation of racemization has provided direct evidence for the replacement of type II collagen in OA cartilage. In articular cartilage, the turnover of matrix molecules is exceptionally low, and for type II collagen, the half-life in cartilage is estimated to be over 100 years [48]. During its long sojourn in the matrix, the collagen molecule undergoes changes and modifications, with racemization; conversion of the L-amino acid to the D-enantiomer is one such change. Because racemization is fastest in the case of aspartic acid, residence time is often estimated by the accumulation of D-aspartic acid. In articular cartilage, the ratio of L- to D-aspartic acid in type II collagen molecule increases with age [80], but this process is slowed or stopped altogether in OA-affected cartilage [59]. Consequently, a substantial fraction of type II collagen molecules in OA cartilage would have to be synthesized after the onset of the disease.

Osteoarthritic cartilage also contains newly synthesized proteoglycan molecules as evidenced by the uptake of [^{35}S]sulfate into cartilage matrix [75]. Subsequent studies have revealed the details of aggrecan turnover. In normal cartilage, the molecular size of aggrecan decreases with age due to the accumulation of truncated molecules [11]. In OA, the size of aggrecan molecules increases as the disease progresses [66]. The change is accompanied by an increase in 846 epitope, characteristic of fetal tissue aggrecan, and considered to be a marker for newly synthesized aggrecan molecules [19,32,60,66]. In fact, the epitope was found on the largest aggrecan molecule in the tissue [66]. Osteoarthritis cartilage must therefore contain a large quantity of new aggrecan molecules, an amount that will increase further as the disease progresses. Small proteoglycans are also synthesized and incorporated into OA cartilage matrix. Other molecules that occur in OA cartilage in increased amounts include decorin, biglycan, fibromodulin, and lumican [18,19].

It seems certain that OA cartilage contains many matrix molecules that have been synthesized in the course of disease progression. The question then arises as to whether the newly synthesized cartilage has functional properties equal to those of normal cartilage. If not, the new molecules may facilitate rather than hinder the disease process. Several studies have suggested that the matrix synthesized in OA cartilage is indeed different from that of normal tissue [2, 62] and that the presence of these proteins may change the quality of matrix because its assembly has been altered. One report called attention to the possibility that the induced type III collagen may copolymerize with type II collagen and thereby impair fibril assembly [4].

The composition of the collagen framework, comprised of type II collagen, together with types IX and XI collagen, may be altered in OA cartilage. To evaluate the repair capacity of OA chondrocytes, we have compared the expression of six genes that encode the three types of collagen in OA and normal cartilage with the aid of laser-captured microdissection [27,28]. The expression of all six genes was found to be enhanced in OA cartilage, whereas the expression of types IX and XI collagen was reduced in OA cartilage, when compared to age-matched normal cartilage.

In the degenerated regions of OA cartilage, the ratio of Col9a1 to Col2a1 was less than one-third that in control cartilage (Fig. 6.2). Analysis of human hereditary diseases and gene-mutated mice has shown what is the proper level of type IX and type XI collagen expression necessary to maintain the integrity of articular cartilage [56]. Our observation indicates that the

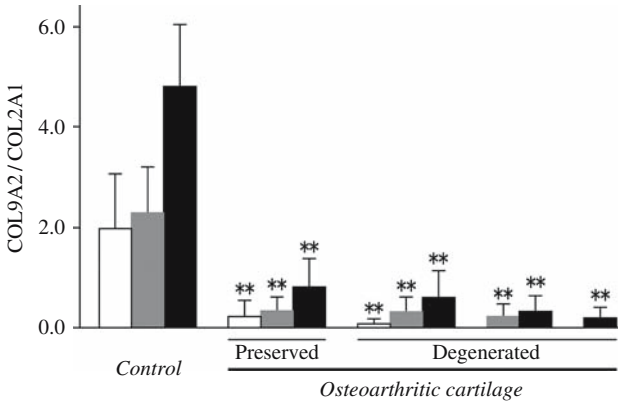

Figure 6.2. Expression ratio of type IX and type XI collagen relative to type II collagen in normal and OA cartilage. Cartilage was obtained by cartilage layers from normal and OA knee joints as described for Figure 6.1 and the expression of COL9A2 and COL11A1 was evaluated by real-time polymerase chain reaction (PCR) together with that of COL2A1. The expression ratio was obtained between type IX or type XI collagen and type II collagen, and compared between normal and OA cartilage in respective cartilage zones. The expression levels of superficial, middle, and deep cartilage zones are shown by open, shaded, and solid bars, respectively. **, $p < .01$ compared with the corresponding zone in normal cartilage.

collagen composition synthesized in OA cartilage may not have normal functional properties due to the paucity of minor collagens.

Collagen synthesized in OA cartilage may be altered by still another mechanism. Previous work on human cartilage revealed that the ratio of the pepsin-soluble fraction of collagen is significantly increased in OA cartilage, compared with normal tissue [67]. To test the hypothesis that this could be due to a reduction in crosslinking, we examined the expression of lysyl oxidase, a primary enzyme for crosslink formation. Our preliminary data have shown that the expression level of this enzyme is reduced in OA cartilage, notwithstanding the obvious upregulation of other collagen genes (N. Fukui, unpublished observation). Reduced crosslinking, therefore, may be another cause of the fragility of the collagen framework.

Changes in proteoglycan structure have been observed in OA. The aggrecan molecule synthesized in OA tends to have more chondroitin-4-sulfate [19,47,66], although the chain lengths of the chondroitin sulfate are unchanged [66]. Changes are also found in other proteoglycan molecules. The core proteins of small proteoglycans are often found without glycosaminoglycan [19], a change, however, that may not be the result of altered synthesis, but rather of proteolytic degradation. Even though there is a general increase in expression in OA cartilage, the degree of upregulation of the various proteoglycans differs considerably [7,18]. The imbalance of small proteoglycan expression may have some influence on the quality of the cartilage matrix. It therefore seems clear that matrix synthesis in OA cartilage differs considerably from that in normal tissue. Why OA progresses in spite of vigorous anabolism in the affected cartilage is not known; it could be due to matrix failure.

6.4 Disease Progression, Anabolism, and the Difference in Anabolic Response Between Joints

Notwithstanding the various animal models of OA, the disease is still best studied in human samples, to which there is, unfortunately, limited accessibility. Samples of end-stage OA are relatively easily obtained in the course of total joint replacement, but it is rare that one can obtain samples of early or progressing stages. Yet, in the early phase of the disease, metabolic activity of the chondrocytes may be considerably different from that in the late stage. Microarray analysis has revealed that type II and type VI collagens are not upregulated until the late stage of the

disease [7]. Lorenzo and colleagues [44] evaluated procollagen synthesis and showed it to be moderately increased in early-stage OA cartilage, at a time when cartilage degeneration was initiated. In another study, in which collagen synthesis was evaluated by its content of CPII, it was reported that synthesis in early OA knee cartilage at or around the degenerated areas had increased, but the total increase in CPII was still small [55]. Thus, collagen synthesis can be seen to be slightly upregulated in early phases of OA when cartilage degeneration is initiated, but CPII increases significantly only in the late stages of the disease.

The time course of proteoglycan synthesis in early OA cartilage is basically similar to that of type II collagen. When aggrecan synthesis was evaluated by the antibody for the 846 epitope, a putative marker of aggrecan turnover, synthesis appeared to be elevated in the early-stage OA cartilage, at and around the areas that exhibited cartilage fibrillation [71]. Again, the initial increase may be minimal because the reported increase had not been normalized by the wet weight of the cartilage [10]. In another study [44], the synthesis of sulfated proteoglycan in early OA cartilage seemed to be increased in the regions next to the fibrillated cartilage. It seems likely, therefore, that proteoglycan synthesis is slightly enhanced around cartilage lesions in early-stage OA joints, with significant increase occurring later.

Although collagen and proteoglycan synthesis are not obvious in early OA, total protein synthesis seems to be elevated in early OA at the fibrillated cartilage areas [44]. In fact, measurement by enzyme-linked immunosorbent assay (ELISA) has shown that the concentrations of fibronectin, COMP, and CILP have also increased in early OA joints at and around the fibrillated cartilage. DNA microarray analysis confirmed this for fibronectin [7]. This increase may play a role in disease progression, because fibronectin fragments are known to induce a catabolic response in articular chondrocytes [36]. Thus, the anabolic activity of the chondrocytes in early-stage OA appears to differ from that in late-stage cartilage, further studys needed to understand the significance of these changes for OA pathology.

Recently, another attempt to clarify the mechanism of OA progression was made by analyzing cartilage lesions in ankle joints. Both knee and ankle joints sustain body weight, but OA rarely develops in ankle joints, whereas knees are highly susceptible to the disease [53]. In a recent comparison of cartilage matrix turnover in ankle and knee joints [10], ankle cartilage in early OA was shown to have a higher rate of anabolism than did knee cartilage. Interestingly, anabolism was enhanced in the entire ankle joint cartilage, whereas in the knee it was limited to the fibrillated region. Conceivably this vigorous anabolic response may be the reason why ankle joints are less susceptible to OA, with perhaps a proper balance between catabolism and anabolism maintained in the ankle, leading to repair. This could be the basis for a novel strategy to prevent disease progression.

6.5 What Are the Causes of Hyperanabolism?

Although the mechanisms that cause OA hyperanabolism are not known, it is widely believed that the enhanced anabolism is due to a reparative response by the chondrocytes. In fact, the anabolic activity of the chondrocytes is enhanced when cartilage matrix is damaged [79]. A specific factor that may be involved in the anabolic response is transforming growth factor-β (TGF-β) [8], which induces anabolism in articular chondrocytes in vitro [30] and in vivo [77]. However, the response to TFG-β differs in different experimental conditions [14]. The response of OA tissue to TGF-β is greater than that of normal cartilage [39,40], and the response decreases with age [33]. TGF-β plays an important role in the maintenance of chondrocyte metabolism in both normal and OA cartilage, with a decrease in TGF-β activity thought to lead to early-onset OA [70]. Inhibition of TGF-β activity in mouse OA facilitates disease progression [69]. Therefore, TGF-β may be important in slowing disease progression through its stimulation of chondrocyte metabolism. However, the effect of TGF-ß on chondro-osteophyte may be positive and will be discussed later.

Another candidate promoter for OA hyperanabolism is insulin-like growth factor-1 (IGF-1), which has potent effects on the articular chondrocytes that are critical in normal cartilage [43,50]. In OA joints, the concentration of IGF-1 in synovial fluid is increased [23]. In OA

cartilage IGF-1 expression is upregulated, especially at the site of cartilage degeneration [52]. It could be an active metabolic process, provided expression and function of the receptor are preserved [51].

Bone morphogenetic proteins (BMPs) may also be important in the pathology of OA. They stimulate anabolic activity of chondrocytes [12, 25], and are important for the maintenance of normal cartilage integrity [68]. Expression of BMP-2, -6, and -7 is increased in OA [12,15,29, 54]. (Fig. 6.3).

Other anabolic factors induced in OA cartilage include cartilage-derived morphogenetic proteins 1 and 2 (CDMPs-1 and -2) and connective tissue growth factor (CTGF), all of which stimulate chondrocytes [20,57], but whose role in OA is not known.

It should be pointed out that factors that stimulate normal chondrocyte metabolism may not have the same effect in OA cartilage. For example, the response of chondrocytes to IGF-1 is considerably reduced in OA because of the increased concentration in OA of IGF-binding proteins, especially IGF-BP3 [23]. Moreover, the expression of the binding proteins increases as the severity of the disease increases [22]. Chondrocytes in aged subjects, on the other hand, exhibit a reduced response to the growth factor [42], a fact that could further compromise the significance of IGF-1 in OA hyperanabolism. The activity of BMPs in OA cartilage may be diminished, because BMP antagonists are upregulated in OA cartilage. The induction in OA cartilage of follistatin, gremlin, chordin, and of a novel BMP antagonist CHL2 has been reported [72,73].

Recent studies have reported the seemingly paradoxical finding that pro-inflammatory cytokines may be responsible for the induction of anabolic factors (Fig 6.5). Interleukin-1β (IL-1ß) and tumor necrosis factor-α (TNF-α) are known to induce BMP-2 in human articular chondrocytes [27,28,29] (Fig. 6.4) and BMP-2 and BMP-6 in synovial cells [45]. Both transcriptional and posttranscriptional mechanisms seem to be involved in the induction of BMP-2 in chondrocytes (Fig. 6.5). Interestingly, the expression of TGF-β is also upregulated by the proinflammatory cytokine. IL-1B induces the expression of TGF-β1 in primary cultured articular chondrocytes through transcriptional upregulation [8]. This response may play a significant role in the pathology of OA, because an in vivo study showed that the anabolic response following the exposure to IL-1 was diminished when TGF-β signaling was blocked. Also, the mice that lack functional genes for IL-1B and the IL-1B converting enzyme have higher susceptibility to OA than the

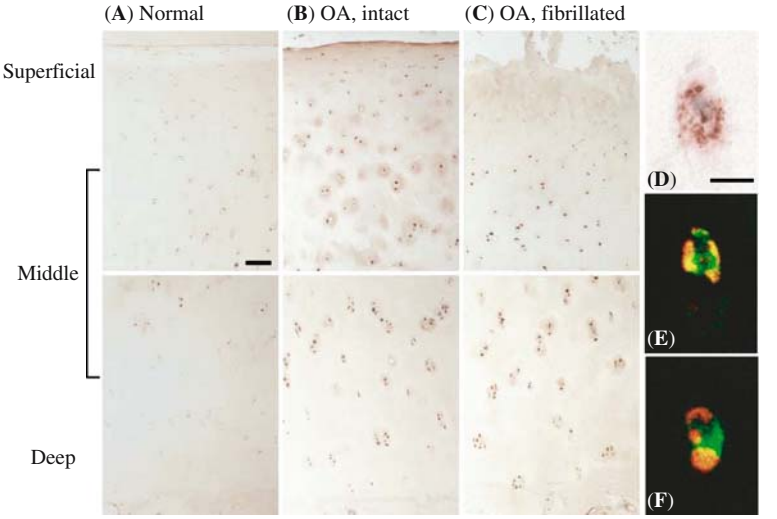

Figure 6.3. Expression of BMP-2 in normal and OA cartilage. (A–C) Immunostaining for BMP-2 in normal cartilage (A) and macroscopically intact (B) and fibrillated areas (C) of OA cartilage. (D) High magnification image of a positively stained chondrocyte. (E,F) Double immunofluorescent staining with use of anti BMP-2 (red) and anti-Golgi 58K (E, green) or anti-Hsp47 (endoplasmic reticulum; F, green) antibodies on OA cartilage from macroscopically intact areas. For A–C, cartilage zones are indicated on the left. Scale bars, 100 μm in A–C and 10 μm in D–F.

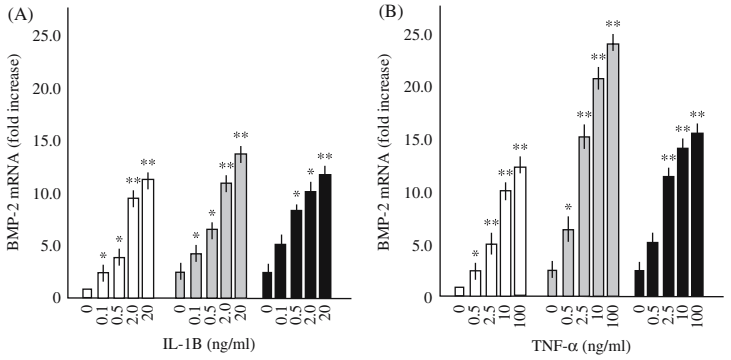

Figure 6.4. The effect of proinflammatory cytokines on the expression of BMP-2 in primary cultured adult human articular chondrocytes. Chondrocytes obtained from normal (blank bars) and macroscopically intact (shaded bars) and fibrillated areas (solid bars) of end-stage OA knee joints were treated with IL-1β or TNF-α at indicated concentrations. After 48 hours, the expression of BMP-2 mRNA was evaluated by real-time PCR. The data are shown by the ratios against GAPDH.

wild type [16]. Conceivably, pro-inflammatory cytokines play a protective role, at least in the early stages of OA.

The anabolic response in OA may also be induced by other mechanism(s). Leptin may play a role in the enhanced anabolism in OA. In vivo and in vitro experiments have shown that leptin is induced in OA chondrocytes in proportion to the degree of cartilage degradation [21] and can stimulate anabolic actions in articular chondrocytes [21,24]. Dumond and colleagues [21] have produced evidence to the effect that the peptide exerts an anabolic action on chondrocytes directly or through the induction of IGF-1 and TGF-β1. The expression of IGF-1 and TGF-β colocalized with that of leptin in OA cartilage; this suggests that the adipocyte hormone may be responsible for OA hyperanabolism by promoting anabolic factor expression or by direct stimulation of chondrocytes.

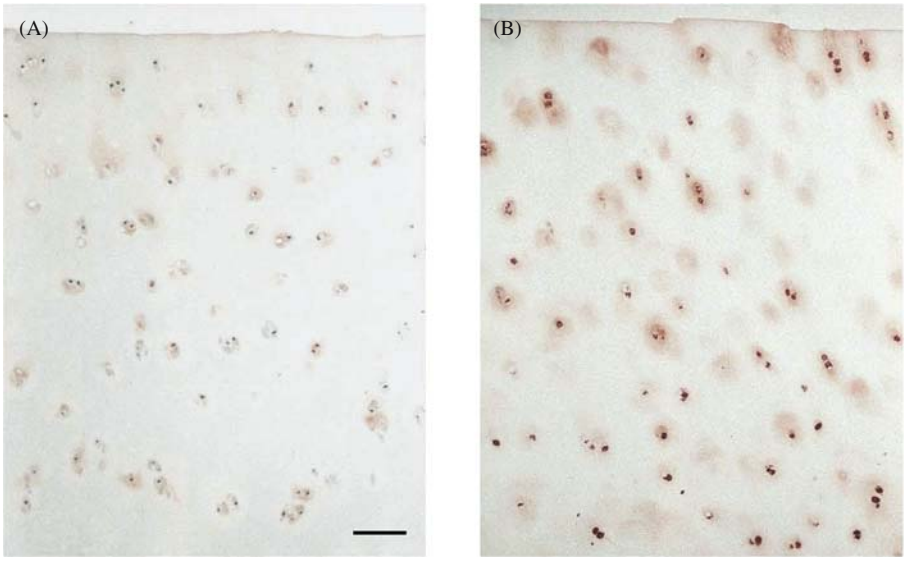

Figure 6.5. Induction of BMP-2 in cartilage explants. Explants were obtained from macroscopically intact areas of OA cartilage and cultured for 3 days in the absence (A) or presence (B) of 2.5 ng/mL of TNF-α. The expression of BMP-2 was assessed by immunohistochemistry. In the explants treated with TNF-α, intense staining for BMP-2 was observed in both the cytoplasm and extracellular matrix around cells, while the staining in untreated explants was weak and primarily observed in the matrix; this suggests the lack of BMP-2 synthesis by the cells. Scale bar, 100 μm.

6.6 Osteophytes

In the course of OA progression, dysregulated chondrogenesis is observed along the margins of articular cartilage. Osteophytes, sometimes called chondro-osteophytes, are bony projections that are formed primarily around the periphery of the synovial joint. Osteophytes are thought to constitute a repair response by joint tissues in an attempt at stabilizing the degenerating joint. Osteophytes result from differentiation of chondrogenic cells that reside in the periosteum (periosteal cells) as they mature from prechondrocytic cells into hypertrophic chondrocytes [26,49]. Cartilage formation is followed by cartilage maturation. Its removal and its replacement by bone is analogous to endochondral bone formation [49].

Factors that induce mesenchymal cells to differentiate along the chondrocytic lineage in the direction of cartilage and bone formation include cytokines and growth factors, extracellular matrix proteins, and specific transcription factors that regulate chondrocyte differentiation. The TGF-β family of growth factors is important in the induction of osteoblast activity in vitro. These growth factors also are potent mitogens for human osteoblast precursors [58]. Intraarticular injection of TGF-ß into murine knee joints induces large osteophytes [76], while inhibition of endogenous TGF-ß prevents formation of osteophytes [69]. In addition to TGF-ß, BMPs and other members of the TGF-ß superfamily also play an important role in osteoinduction [81] and promote mesenchymal stem cell differentiation along the osteoblastic lineage. Injection of BMP-2 into murine knee joints leads to the formation of large osteophytes [77].

Recently, van den Berg and colleagues [78] reported that synovial lining macrophages have a role in promoting TGF-ß–mediated osteophytogenesis, in addition to the cytokines and growth factors originating from macrophages [65] and the resident macrophage-like (type A) cells that cover the inside of diarthrodial joints. In the course of experimental OA, macrophage-like cells become activated and may be a substantial source of growth factors. To identify the effect of synovial macrophages in vivo, chondrogenesis was induced by injecting TGF-ß into the joint cavity of murine knees. When macrophages lining the synovium were selectively removed from the synovium prior to injection of TGF-ß, osteophyte formation was reduced by 78%. After TGF-ß stimulation, synovial lining cells produced BMP-2 and BMP-4, molecules that are absent in the synovial tissue after macrophage depletion. In vitro experiments confirmed that macrophages were responsible for secreting the growth factors that induced chondrogenesis, but the exact factors that stimulated BMP synthesis are not known. Interestingly, TGF-ß1 stimulation of macrophages induced almost no upregulation of BMP-2, or BMP-4, mRNA. This suggests the involvement of still unknown factors that control the production of BMPs. In light of our findings that the proinflammatory cytokines, IL-1ß and TNF-α, stimulate BMP-2 production [29], proinflammatory cytokines may also be responsible for macrophage activation.

6.7 Conclusion

The pathogenesis of OA is unknown. What we do know is that OA chondrocytes that reside in the cartilage become hyperactive and synthesize various molecules, including extracellular matrix. Emerging evidence demonstrates that this hyperanabolism may be quite specific to different stages of disease and varies with the depth of the cartilage. Consequently, this response is much more complex than originally thought. Attempts have been made to utilize the neosynthesis of secreted molecules as serum markers for the metabolic state of the chondrocytes. However, it is difficult to distinguish newly synthesized molecules from older ones, once the molecules or fragments derived from catabolism of the molecule leave the matrix. The propeptides of type II procollagen have been measured—both the C-propeptide (55) and the N-propeptide of type IIA procollagen. The type IIA procollagen is synthesized in very small amounts, but is unique to the disease state. It is stable in the serum, and there is a good antibody available to detect the collagen fragment (31). For aggrecan, the monoclonal 846 has been used as a serum marker, as it is more prevalent on newly synthesized molecules, but the exact epitope is not known [46]. Other molecules that have been used to measure cartilage degradation include COMP, but, except for the propeptides of type II collagen, no specific

marker is available to differentiate anabolic from catabolic activity.

Because the hyperanabolic state of the cartilage cells results in increased synthesis of the matrix and of degradative enzymes, degradation may be the result of an increase in cellular metabolism. After all, in all repair situations, the removal of damaged tissue must precede laying down of new matrix. When, in OA, does this balance change from normal repair to the generation of degraded cartilage?

Understanding the mechanism(s) that underlie(s) hyperanabolism may lead to strategies that harness the repair capacity of cartilage. Future studies, therefore, should aim at identifying anabolic markers that indicate the metabolic state of OA cells. Questions that need to be answered include the origin of the anabolic response, whether hyperanabolism constitutes a response to injury, whether mechanical events are the stimulus, whether a specifc growth factor or cytokine initiates the disease process, and whether and how metabolic events change as the disease progresses.

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7.

Chondrocyte Hypertrophy and Apoptosis at the Cartilage–Bone Interface

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7.1 Chapter Summary

Osteoarthritis (OA) is a complex multifactorial disease confounded by age, genetic factors, and mechanical forces. It is characterized by focal changes in the volume of some cells in the superficial as well as the deeper zones of the cartilage. Ongoing studies suggest that the cell volume increases in response to degradation of the extracellular matrix and the concomitant change in the osmotic pressure of the cartilage matrix fluid. The osmotic properties of the intracellular fluid alter the "shape" of the cytosol, and modify many critical responses; a change in osmotic pressure may also influence integrin expression and change the activity of cytoskeletal proteins. When cells have assumed a hypertrophic state, they become sensitized to cytokines and local apoptogens. Once the apoptosis cascade has been initiated, cell killing is achieved through activation of both the extrinsic and intrinsic pathways. One common feature of both pathways is the induction of inducible nitric oxide synthase (iNOS) and the decrease in the mitochondrial transmembrane potential ($\Delta\psi_m$). These events result in the formation of oxygen and nitrogen radicals, as well as activation of calpains and downstream effector enzymes (caspases). The radicals individually compromise the activity of membrane pumps by inhibiting adenosine triphosphate (ATP) generation; cell killing is achieved through the oxidation and cleavage of critical macromolecules, as well as activating calpains and other proteases.

This chapter puts forward two new ideas concerning the terminal activities of chondrocytes in the progression of the osteoarthritic lesion. The first, based on the appearance of late-stage chondrocytes, is that these cells are maintained in a catabolic state, autophagy; since death signals are postponed, autophagy maintains the cellularity of the cartilage. At the termination of this stage, the induction of apoptosis decreases the number of chondrocytes in the cartilage zones. Autophagy is regulated by mammalian target of rapamycin (mTOR), a signaling molecule that serves as a sensor of the nutritional state of the cell and, surprisingly, the osmotic pressure of the cytosol. Whether the cells undergo autophagy or not, it is highly likely that this protein plays a critical role, not just in mediating cell number, but also in controlling the hypertrophic status of cells in the arthritic lesion. The second new idea concerns the role of vascularity in the pathogenesis of OA. We review evidence that a variant of ischemia reperfusion injury generates conditions that promote OA. If valid, this mechanism provides a totally new insight into the interaction between the two tissues and emphasizes the importance of the generation of radicals in directing cartilage breakdown and cell death.

7.2 Introduction

Osteoarthritis is one of the most common diseases of connective tissues, affecting almost all joints of the body. Known since prehistory, the debilitating pain of the disease and the reduced mobility of the hands and legs hampered tool construction, prevented sowing and harvesting, thwarted foraging, and limited military prowess. It is likely that many of the readers of this chapter, now or in later life,[a] will endure the excruciating pain of osteoarthritic cartilage. As a last resort, they will request the surgical removal of the femoral or tibial head and its replacement with a high-tech metal or ceramic prosthesis. Yet, despite the presence of a growing armamentarium of new surgical techniques and drugs designed to provide pain-free locomotion, the disease remains a source of distress and suffering, and the societal costs continue to be enormous.

The slow progress in prevention and treatment is due to limited understanding of the etiology and pathogenesis of the disease and the confounding effects of age, genetic factors, and mechanical forces. One further complication is the elevated secretion of cytokines and chemokines; together they contribute an inflammatory component to the osteoarthritic condition. In this chapter, rather than delineate the contributions of each of the etiologic factors, we focus on events that characterize development of the disease process—chondrocyte hypertrophy and apoptosis. Understanding how changes in chondrocyte physiology impact on the disease state is critical for the design of new therapeutic agents intended to maintain the health of the cartilage cells.

Articular cartilage is a highly specialized tissue, exquisitely adapted to serve its function as a load-bearing surface. The matrix of the cartilage, which accounts for more than 95% of the total tissue volume, has been the subject of an extraordinarily large number of chemical investigations. They have shown that the matrix is composed of a fibrillar protein network embedded in a hydrated proteoglycan matrix. While the proteoglycans are space-filling molecules, the direction, density, and orientation of the fibrillated collagens (especially collagen types II, IX, and XI) provide nanoscale architecture to the cartilage. In the superficial zone of the cartilage (zone 1), fibril direction is parallel to the surface and closely packed, whereas in the deep zone (zone 3), tightly packed sheets of collagen fibrils form the territorial matrix. The interterritorial matrix contains isotropically arranged fibrils that are perpendicular to the surface. This orientation provides the cartilage with resistance to pressure as well as to shear forces [42]. At the base of the articular cartilage (zone 3), the matrix is mineralized and contains hydroxyapatite crystallites that nucleate around and in matrix vesicles [57]. The thickness of the calcified cartilage is variable, usually about 0.1 mm, occupying about 5% of the total tissue volume. It is in this cartilage zone that hypertrophic and apoptotic cells are often located. Between calcified cartilage and the deep zone, Fawns and Landells [24] described one or more deeply staining undulating lines that have been termed the "tidemark."[b] The calcified cartilage serves as a boundary tissue between the underlying bone and the nonmineralized deep articular cartilage and provides the chondro-osseous junction with mechanical stability. In all zones, the network of orientated collagen fibers endows the tissue with much of its mechanical (tensile) strength; the aggrecan molecules serve to cushion the effects of loading by rapidly changing the cartilage hydration state. Hydration of the proteoglycan network swelling is constrained by the cartilage collagens, as well as by the other noncollagenous matrix proteins.

Surprisingly, notwithstanding its high water content, the cartilage is not fully hydrated. If immersed in a physiologic fluid, further imbibition of water causes the tissue to swell [5]. The hydrated matrix also enfolds the sparse cellular elements of cartilage, which, unlike most other tissues, comprise one cell type, chondrocytes. Often in pairs, these cells occupy domains of matrix (chondrons), and while the dimensions are variable, they present the pathologist with a characteristic morphology: a large basophilic nucleus, evidence of an extensive endoplasmic

[a] Also known as the "golden years," possibly related to the treatment of a similar disease, rheumatoid arthritis, with sodium aurothiomalate.

[b] Inappropriate, as the tidemark is seen when water (the tide) leaves the sandy beach; in cartilage, at the boundary between the deep zone and the mineralized cartilage, there is no evidence of water depletion.

reticulum and Golgi apparatus, and a significant number of individual mitochondria.

Nearly 50 years ago it was reported that chondrocytes possess a cilium. Almost half a century later, the importance of these organelles has been recognized. Poole et al [94] proposed that the primary cilium serves as a "cybernetic probe" that transmits microenvironmental information to the centrosome and Golgi apparatus. In a subsequent study, Jensen et al [52] suggested that the organelle was intimately intertwined with matrix macromolecules, where it serves as a mechanosensor of shear stress. If these roles are confirmed experimentally, then this information will add one more facet to our understanding of articular cartilage biology, and possibly the pathogenesis of OA.

In terms of microstructure, unlike most other tissues, the nonmineralized zones of articular cartilage are not penetrated by blood vessels. In contrast, a considerable number of vascular channels traverses the deep calcified cartilage zone [45]. Even this generalization may not be completely accurate, as a study by Milz and Putz [87] has indicated that channels exist in the unmineralized region of articular cartilage that are responsive to compressive forces. Nevertheless, in the relatively unstressed state, the tissue is essentially avascular in that most chondrocytes are at a distance from penetrating blood vessels. Cells survive in this graded or even low oxygen environment by generating energy, mainly through anaerobic glycolysis, and secreting lactic acid [86] as a metabolic end product. Whether lactate can be used to generate substituted glycan chains of proteoglycans by a truncated gluconeogenic pathway is not known. Related to this anaerobic state, there is reliance on diffusion of synovial fluid components for both nutrient delivery and the removal of products of metabolism. The combination of limited nutrient diffusion and the generalized hypoxic state of the tissue impose metabolic limitations on chondrocytes that may serve to govern the fate of these cells.

Progression of the osteoarthritic disease state is characterized by disturbances in the metabolic activity of resident chondrocytes. There is a net loss of matrix macromolecules (in particular, the proteoglycan aggrecan), disruption of tissue architecture, and a concomitant limitation in joint function [105]. Once the disease has been initiated, the cellular reaction is twofold [101]. There is an initial *biosynthetic phase*, during which the chondrocytes respond to etiologic factors invoking reparative activities that are directed at limiting further tissue destruction. If the lesions cannot be repaired, the *degradative phase* begins. It is characterized by a rise in the secretion of matrix metalloproteinases, ADAM-10, and aggrecanases 1 and 2; these are also known as ADAMTS (a disintegrin and metalloprotease with thrombospondin-like repeat) (for discussion, see below and [101]). These enzymes promote matrix degradation by hydrolysis of the collagenous matrix and proteoglycans. It is in the degradative phase that, enigmatically, endochondral ossification is activated, frequently leading to development of small bone spurs, osteophytes, which are formed at the bone–cartilage junction. Relevant to subsequent considerations of the chondrocytic death process, the vascularity of the underlying bone may be increased with blood vessel redistribution. Frequently, these subchondral vascular changes predate frank cartilage pathology [53]. Some workers believe that the pathogenesis of the disease results from changes in bone vascularity; degradation of the actual cartilage is seen as a secondary manifestation of the disease.

The cellular response to the disease process is complex. Many authors have focused on the overt cellular changes that occur during the biosynthetic phase when chondrocytes proliferate and cells enlarge. During this period, cells can occupy a single lacuna; these chondrocytes are frequently hypertrophic. Unlike the growth plate, hypertrophic chondrocytes can be seen in all zones of the osteoarthritic lesion. Volume changes may not always be equally dramatic across all zones, but large, engorged multinucleated cells are often observed within the deep zone. The presence of these enlarged cells has prompted some clinicians to label the disease *hypertrophic OA*. As will be discussed shortly, within the calcified cartilage zone there is evidence of a modest number of dying and apoptotic cells. Because hypertrophy and apoptosis are characteristic of endochondral ossification, it has been proposed that articular chondrocytes initiate hypertrophy and often undergo apoptosis, changes in the disease state that are not dissimilar to those of terminally differentiating cells of the epiphyseal growth plate.

At this stage, it would be useful to comment on the changes that take place as growth

plate cells undergo terminal differentiation. Chondrocyte maturation has been extensively studied, and a plethora of proteins has been shown to be expressed by these cells. These include, collagen types X, II, and I, aggrecan, alkaline phosphatase, matrix metalloproteinases (MMPs), ADAMTS, osteopontin, and annexins. In an excellent study of osteoarthritic tissue, Kirsch and colleagues [57] have shown that osteoarthritic cells express alkaline phosphatase and annexins II and V in the upper zone of early and late human tissue. They noted the appearance of collagen type X and commented that its presence lends direct support to the idea that chondrocytes redifferentiate and take on a terminal phenotype in the disease state.

There is an ongoing debate concerning the relationship between cells of the growth plate and articular chondrocytes. One school of thought views articular chondrocytes as protected from the type of terminal differentiation program exhibited by cells of endochondral cartilage. Part of that protective status may stem from modifications, such as promoter methylation (silencing), due to the expression of specific genes of articular chondrocytes. In support of this idea, Roach et al [100] considered abnormal expression of MMPs 3, 9, and 13 and ADAMTS-4 in human osteoarthritic chondrocytes to be epigenetic "unsilencing," mediated by the methylation status of specific genes. From this perspective, OA is seen to develop when the silencer is removed, thereby permitting these chondrocytes to differentiate. In the growth plate, such terminal differentiation ends with the induction of apoptosis [36].

It is important to note that apoptosis of epiphyseal chondrocytes is matched by concomitant repopulation of cells from the proliferative zone. In articular cartilage, on the other hand, there is little physiologic repopulation; as a result, the induction of apoptosis causes chondrocyte depletion and a decrease in net biosynthetic activity. Moreover, the effects of hypertrophy are singularly different in the two tissues. In growth cartilage, the induction of hypertrophy is functional, as bone elongation is dependent on the increase in cell volume. In articular cartilage, a similar process leads to development of an extracellular matrix that is chemically and mechanically flawed. Together, both changes, hypertrophy and apoptosis, lead to a tissue that is cell depleted and suffers from a loss of elasticity due to modifications in fiber (collagen) and water (proteoglycan) content.

As with any discussion of OA, analysis is confounded by a number of contributing conditions that include inflammation and aging, abnormal mechanical forces, and genetic factors. Kraus [63] has noted that biomechanical and structural imbalances, in particular trauma, chronic overuse, and joint loading, profoundly influence the development of the disease state. After the age of 50, there are significant age-dependent increases in OA. Nonetheless, as hypertrophy and apoptosis are pathognomic of OA, the focus of this chapter is (1) to examine the causes and mechanism of swelling in hypertrophic chondrocytes of osteoarthritic cartilage, (2) to review possible relationships between hypertrophy and cell death, and (3) to consider the role of the subchondral bone in the induction of OA. We advance the notion that cell death seen in OA may be a variant of apoptosis in which the tissue microenvironment delays the actual death process.

7.3 Hypertrophy: Mechanisms of Cell and Tissue Swelling

The term *hypertrophy* usually refers to an increase in tissue size due to an expansion in cell volume. This definition is totally appropriate for the growth plate, where the cartilage undergoes a substantial increase in thickness, due to cell enlargement and matrix synthesis. In articular cartilage, possibly due to the small number of chondrocytes, no such growth is seen, although, clinically, cartilage swelling is frequently observed. Since cells of large volume are commonly identified within the swollen cartilage, it is necessary to consider in detail how changes in cell size can occur and how this type of swelling influences cell function.

Chondrocytes in all zones of the articular cartilage can vary considerably in volume and shape (Fig. 7.1). Bush and Hall [14,15] have defined the morphologic characteristics of the cells in the living tissue with the aid of confocal laser microscopy. This technique obviates the need to utilize fixation and staining, thereby avoiding artifacts due to matrix swelling. In very well designed studies of normal and osteoarthritic cartilage,

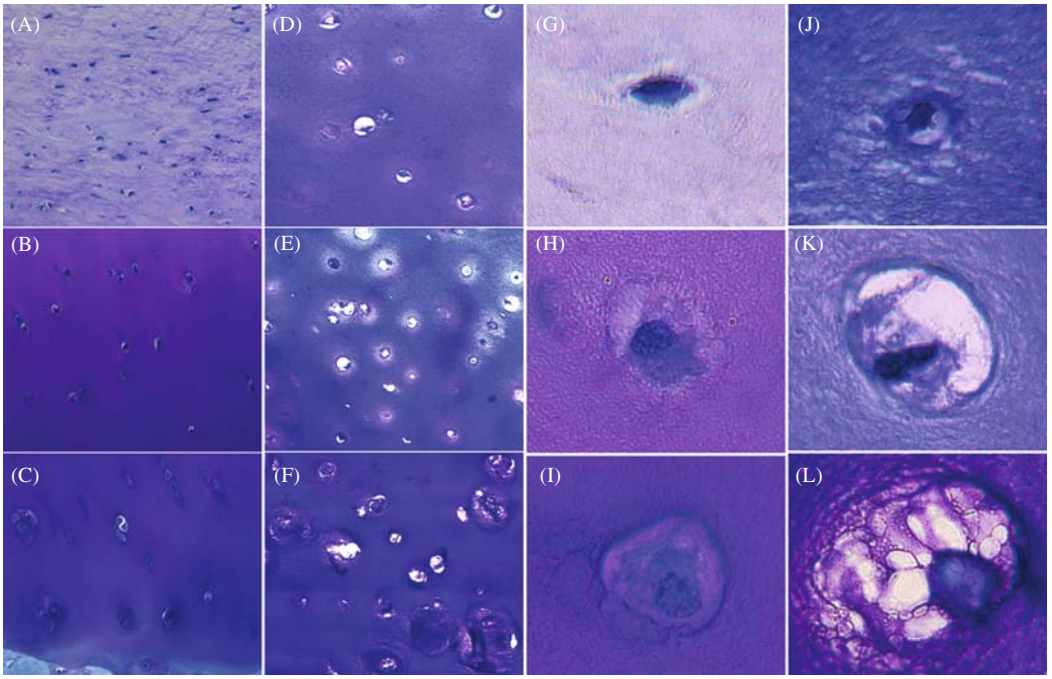

Figure 7.1. Toluidine blue–stained longitudinal sections through normal and osteoarthritic human cartilage showing characteristic changes in matrix staining and cellular morphology. (A,D,G,J) show sections from the superficial cartilage (zone 1); (B,E,H,K) are representative of middle zone (zone 2); (C,F,I,L); are from the deep cartilage (zone 3). A–C (10×) and G–I (40×) show normal and D–F (10×) and J–L (40×) show osteoarthritic tissue. The cells in all three zones of the osteoarthritic cartilage are swollen when compared to chondrocytes in normal tissue. Around the swollen cells, the loss of staining corresponds to changes in the matrix proteoglycan composition. The swollen cell (L) shows vacuole inclusions and may be autophagic.

Bush and Hall evaluated chondrocyte volume in the superficial, middle, and deep zones. They showed that cell shape changed progressively. In the superficial zone, the cells were flat and ovoid and their direction was parallel to the surface of the cartilage; in the middle zone, the cells were in pairs and had a semi-ellipsoid shape; in the deep zone, the cells were round and aggregated in columns perpendicular to the cartilage surface. With respect to size, the volume varied from 396 to 590 μm^3, being 50% greater in the deep than in the superficial zone. Compared with cells in the superficial zone, chondrocytes in the middle zone exhibited a large increase in volume (92%). The authors noted that the volume of individual chondrocytes in osteoarthritic cartilage was greater than might be due to tissue degeneration, thereby implicating possible changes in the concentrations of cellular osmolytes.

The morphology of chondrocytes within normal and osteoarthritic articular cartilage is shown in Fig. 7.1. In normal cartilage, flat ovoid cells populate the superficial zone (Fig. 7.1A). Within the middle zone, the orientation of the cells has changed and many chondrocytes are present in pairs (Fig. 7.1B). The deep zone contains round aggregated chondrocytes, and some of the cells are organized in columns, similar to the growth plate (Fig. 7.1C). In contrast, in the osteoarthritic lesion the chondrocytes within all zones are swollen. Characteristically, the matrix around the swollen cells has lost some of its metachromatic properties; this indicates some depletion of proteoglycans (Fig. 7.1D–F).

7.3.1 Influence of Matrix Macromolecules on Cell Hypertrophy

In OA, cell swelling is accompanied by major changes in the composition and hydration status of the extracellular matrix, changes that may directly influence the size and volume

of the articular chondrocyte. Moreover, the changes in the fibrillar architecture of the tissue may serve to release mechanical restraints and allow the cell to occupy a larger tissue volume. Indeed, studies of chondrocytes within early osteoarthritic lesions indicate changes in focal adhesion plaques, reorganization of actin microfibrils, and modification of a number of related proteins [44].

The crosstalk between chondrocytes and extracellular matrix proteins is mediated through families of integrins and transmembrane heterodimeric proteins. The proteins stud the plasma membrane of the cell and bind to ligands, usually RGD(S) (-arginyl-glycyl-aspartyl-(seryl)) motifs typical of many extracellular molecules [70]. Attachment of these motifs to integrins generates outside-in signals (usually mediated by phosphorylation-dephosphorylation reactions) that regulate growth, differentiation, and maintenance of cell function [102]. In OA, numerous studies have shown that changes in matrix proteins modulate the expression of specific receptors. For example, an increase in fibronectin in osteoarthritic cartilage causes a corresponding elevation in integrin-fibronectin receptors ($\alpha_v\beta_1$ and $\alpha_v\beta_3$) [95]. How the change in integrin receptor profile is related to an accelerated development of the osteoarthritic lesions is not understood. However, integrin-mediated adhesion to matrix macromolecules modulates synthesis of growth factors and matrix metalloproteinases [39], molecules concerned with promoting anabolic and catabolic events that are linked to the pathogenesis of OA [119].

Related to activation or engagement of the integrin receptor are concurrent changes in cytoskeletal proteins that function to maintain cell shape. This relationship was used by Ingber [46] to develop the theory of *cellular tensegrity*. Accordingly, the cell is fastened to the extracellular matrix by actin filaments and thus can resist forces applied through the microtubular system. Ingber proposed that any change in cell volume is sensed by the cytoskeleton, with cytoskeletal elements then activating signals that promote and adapt the cells to the new environment [47,48]. This concept, of particular relevance to soft tissues, is probably of minor importance for cells in the articular cartilage or the growth plate. As already stated, hypertrophic chondrocytes in the growth plate generate forces responsible for elongating long bones; these forces, therefore, must be greater than the body weight; likewise, cells in the deep zone of articular cartilage must contend with high and rapidly changing fluxes in hydrostatic and osmotic forces. From this perspective, it is probable that the tensegrity system itself plays only a minor role in maintaining the swollen state of chondrocytes in normal or osteoarthritic cartilage.

7.3.2 Cell Hypertrophy and the Osmotic Pressure of the Extracellular Matrix

Early in the development of the osteoarthritic lesion, there is a marked change in aggrecan distribution. When stained with toluidine blue, dye uptake is decreased around the cells, although in the matrix itself, between chondrons, there is an increase in stain intensity (Fig. 7.1). There is also some evidence to indicate that aggrecan fragments are released into the synovial fluid [111]. As noted earlier, aggrecan core protein degradation in OA is mediated by enzymes of the ADAMTS family, in particular ADAMTS-4 and ADAMTS-5 (aggrecanase-1 and aggrecanase-2). Using a mouse model, Stanton et al [108] showed that ADAMST-5 was the most active aggrecanase, while Glasson et al [29] demonstrated that deletion of the gene for ADAMST-5 prevented development of OA.

Although a considerable number of water-binding molecules exist in cartilage, we focus on aggrecan for three reasons. First, based on the studies described above, aggrecanase expression and, by inference, aggrecan degradation are directly linked to the initiation of OA. Second, aggrecan is a major constituent of the extracellular matrix of cartilage. This large proteoglycan contains approximately 100 chains of chondroitin sulfate, 40 to 50 keratan sulfate chains, and 60 to 70 O-linked and 6 to 8 *N*-glycosidically linked oligosaccharide chains [71]. Third, the large numbers of charged groups in chondroitin and keratan sulfate serve a critical function: regulation of the osmotic pressure of the matrix. The charged COO^- and SO_4^{2-} groups of *N*-acetylgalactosamine, glucuronic acid, and other substituted sugars bind hydrated Na^+ ions. Associated with these

fixed bound ions are mobile free ions that are in equilibrium with the bound ions, and, in dilute solution, act as charged particles. These water-bound molecules oppose compressive forces applied to the cartilage; accordingly, the outward pressure exerted by the bound water is resisted by tension within the collagenous fiber network. When the collagen matrix is disturbed, it markedly influences the water-binding characteristics of aggrecan molecules of the articular cartilage [20,81]. Hence, the actual osmotic pressure is integrated with the mechanical restraint function discussed above.

In disease, ADAMTS-5 and other aggrecanases mediate profound changes in aggrecan fixed charge density, thereby altering the hydration status of the cartilage. Maroudas [80] and Urban and Hall [114] measured the osmotic pressure of cartilage and showed it to be higher than that of other tissues, including synovial fluid. For example, Na^+ levels range from 240 to 350 mM, and in the deep zone the Ca^{2+} concentration is 14 to 20 mM. The concentrations of Na^+ are twofold higher, and of Ca^{2+} tenfold higher than in synovial fluid. In normal cartilage, the cells are thought to be iso-osmotic with respect to the extracellular milieu; however, in disease, the change in the structure of the extracellular matrix, for example a loss of fixed charge (proteoglycan degradation), would disturb this relationship and lead to changes in bound ion and water content in the tissue. Bush and Hall [16] recently confirmed that there was a significant difference in cartilage hydration status between degenerative and nondegenerative cartilage. These authors also showed that maximal swelling of cells of degenerative cartilage and isolated chondrocytes was greater than that of nondegenerative cartilage and normal chondrocytes. Finally, using magnetic resonance imaging (MRI) data from osteoarthritic patients, Lammi et al [63] showed that there was a loss of proteoglycans and an accumulation of tissue fluid. The MRI data and water measurements indicated that even if there was no change in charged groups, there was a shift in the level of water in the arthritic tissue. Based on the measurements described above, and the measured shifts in water status, it is the hydrolysis of aggrecan and other proteoglycans that seems to induce focal shifts in osmotic pressure. In all cases, cells respond to these shifts by swelling.

7.3.3 Chondrocyte Adaptation to Changes in Osmotic Pressure

In cartilage, the osmotic strength is high and variable. Variability is caused by changes in physiologic pressures within the tissue, which serves to redistribute (squeeze out) resident hydrated ions and to disturb the local iso-osmotic state. Unlike most other cells, the chondrocytes are capable of adapting to large variations in osmotic forces. Remarkably, in accord with Boyle–Van't Hoff's equation, chondrocytes can act as osmometers, displaying almost perfect osmotic behavior in the range of 250 to 600 mOsm [14]. This fact is well known to investigators who try to disrupt the plasma membrane of chondrocytes using hypotonic solutions. Frustratingly, instead of bursting, these cells simply swell in shape and rapidly reach equilibrium with the surrounding medium.

In the cell, the osmotic pressure is generated by ions such as Na^+, Cl^-, and PO_4^{3-} (Ca^{2+} contributes little to the overall pressure, as its concentration is low micromolar). Other weakly ionized groups include glucose phosphates, nucleotides, citric acid cycle intermediates, and possibly some phospholipids. Many of the proteins of the cytosol are charged (usually phosphorylated), and they also contribute toward the (colloid) osmotic pressure. Because the cell membrane is impermeable to charged proteins, a disequilibrium (the Gibbs-Donnan relationship[c]) exists between the charged particles within the cell and the ionized hydrated ions of the extracellular fluid. The Gibbs-Donnan relationship is a steady-state disequilibrium, which, unless balanced, results in a continuous influx of water, and eventual death through cell swelling and bursting. The cell responds to this disequilibrium in a number of ways. If the cell (cytosol) particle pressure (osmotic strength) is higher than that of the matrix, the cell will swell; swelling will lower the osmotic pressure of the intracellular compartment and the cell will become iso-osmotic with its environment. The swelling process is regulated by a Na^+ pump-leak balance mechanism involving a Na-K–adenosine triphosphatase (ATPase) membrane enzyme system.

[c] This critically important relationship was deduced by Josiah Willard Gibbs and was published in 1870 in the *Transactions of the Connecticut Journal of Arts and Science*, a very low impact journal!

Even if the extracellular matrix osmolarity is constant, a continuous cationic leak across the plasma membrane causes influx of ions and serves as a driving force toward equilibrium conditions.

The chondrocyte can also regulate its osmotic pressure by synthesizing a number of charged low molecular weight molecules (osmolytes) that include taurine, glycine, glutamine, betaine, and inositol phosphate. Synthesis or degradation of these molecules serves to maintain the osmotic pressure of the cell [23,32]. A final mechanism that causes (Donnan) swelling is generation of metabolic acid. In cartilage, lactic acid is the final product of anaerobic glycolysis. Lactic acidosis donates anions to the cell. If the anions are not extruded or reutilized, they accumulate and cause an increase in the osmotic pressure.

The mechanism by which osmotic pressure directly affects cell function is difficult to understand, especially if the nucleated cell is viewed as a distensible container of organelles suspended in a cytosolic "soup" of enzymes. In the soup, the myriads of regulated chemical reactions, characteristic of each of the innumerable metabolic pathways, are thought to operate stochastically. Undoubtedly the ion concentration of this aqueous environment will affect chemical reactions, but unless there are massive ion fluxes, the impact, due to dilution, would be slight. While this explanation may apply to events that take place at or inside membranes, it is difficult to reconcile the efficiency of anabolic and catabolic reactions with the low level of organization implied if the cytosol were a mere bag of enzymes!

A more current approach to understanding these events is based on thermodynamic analyses that support the notion that enzymatic complexes form and cycle from a soluble to an insoluble form. These molecular complexes interact with each other to form macromolecular structures that express highly reactive surfaces for chemical activities. These surfaces channel ions, substrates, and cofactors, and, at the same time, release metabolic end products. Alberts [4] commented that in the cytoplasm enzymes worked together as a "collection of molecular machines." Addressing this theme, Spitzer and Poolman [107] viewed these molecular machines as a "bustling metropolitan city." "The molecular machines are supramolecular complexes of different proteins, proteins and DNA and proteins and RNA which emerge and disappear into the cytoplasm in a well orchestrated and predictable manner to enhance catalytically critical anabolic and catabolic reactions."

The molecular machines are bathed in electrolytes that contain ionized species such a adenine nucleotides [adenosine tri-, di-, and monophosphate (ATP, ADP, AMP)], as well as phosphorylated low molecular weight catabolites such as sugar phosphates, tricarboxylic acid intermediates (succinate, oxaloacetate, citrate), and simple K^+ ions. While the functional relationship between ionized species and the machines (enzymes) is not in dispute, the next stage of the Spitzer and Poolman argument is more difficult to understand. Accordingly, these complexes "shape" the cytosol into pools of electrolytes that then serve as Maxwellian[d] switches or semiconductor pathways to modulate macromolecule complex formation, dissociation and function [107]. Because these complexes channel intermediates and concentrate them at enzyme surfaces, any change in "shaped" cell water or ionized species content will cause rapid changes in cell function and even structure. Molecular crowding of these complexes occurs when there is a cell volume change [21]. From this perspective, it is easy to see why a cell volume change would interfere with normal rates of stochastic interactions and even modify the rate of protein folding in a confined space. All in all, whether there is crowding or, as in the case of the hypertrophic chondrocyte, an increase in the cell volume, the cell metabolism, function, and architecture are likely to undergo significant changes.

Returning to the disease state, the development of the osteoarthritic condition is accompanied by chondrocyte swelling. This changes the "shape" of the cytosol, which in turn degrades normal control of macromolecular complex formation, activity, and architecture. Molecular

[d] "About 130 years ago James Clerk Maxwell imagined a 'finite being' that could reverse an otherwise favorable diffusive process by willfully controlling the passage of different molecules between two compartments. . . . This little gatekeeper came to be known as Maxwell's Demon, undoubtedly because of its devilish designs to violate the Second Law of Thermodynamics. We now know that living systems are full of Maxwell's Demons, working hard to perform seemingly impossible tasks like moving molecules up electrochemical gradients, or magically transmuting one kind of cell or organism into another. The archetypical Maxwellian Demons of biology are the Na+/K+- and Ca2+-ATPases that produce electrochemical gradients across animal cell membranes" (Stock and Da Re [109]).

crowding can thus modulate metabolic reactions so as to cause profound alterations in carbohydrate metabolism that involve gluconeogenesis, glycolysis, glucose uptake and phosphorylation, and, ultimately, protein synthesis and ATP turnover [51]. Additionally, the cytosol "shape" can influence inside-out signaling mediated by integrin activation or even tensegrity-dependent changes in the cytoskeleton. If there are no regulatory volume changes and if the cells maintain the swollen state, they will assume a phenotype that is consistent with that of the osteoarthritic chondrocyte. Furthermore, these same changes, resulting from a cytosolic "shape" change, would alter the ability of the osteoarthritic chondrocyte to synthesize the water-binding glycosoaminoglycan chains that characterize the extracellular matrix.

In summary, OA is characterized by changes in the volume of some cells in the deeper zones of the cartilage. How swelling takes place is not clear, but current investigations suggest that it is a response to a change in the osmotic pressure of the fluid of the cartilage extracellular matrix. The osmotic pressure of the intracellular fluid alters the "shape" of the cytosol, and modifies many critical responses; it may also influence integrin expression and modify the activity of cytoskeletal proteins. The modified state of the cell generates conditions that favor the expression of the osteoarthritic phenotype (collagen type X expression, matrix vesicle formation, etc.). It is important to note that these changes may also influence the activities of many signal transduction pathways. One such protein, mTOR, a member of the phosphatidylinositol-3 (PI_3) kinase signal transduction pathway, is exceedingly sensitive to the osmotic status of the cell. When activated, this protein dissipates the proton gradient across the mitochondrial membrane, while at the same time initiating responses that influence cell size. The importance of this protein is discussed in more detail below.

7.4 Chondrocyte Apoptosis in the Osteoarthritic State

The induction of the death program kills chondrocytes embedded in the articular cartilage matrix, especially those buried in the environmentally *hostile* deep zone. The mechanism of death is probably mediated by apoptosis,[e] although, in the following section, we raise the possibility that autophagy may be involved. Programmed cell death or apoptosis is a biologic *blueprint* conserved throughout evolution. It serves a myriad of biologic functions, all of which are designed to remove unwanted or damaged cells from tissues [55]. Biologically speaking, it does not seem surprising that the apoptotic process is part of the pathogenesis of OA. Since considerable space will be devoted to cell killing, it is worthwhile to summarize here some key aspects of this terminal system.

The apoptotic regulatory mechanism is complex and may be mediated through activation of cell surface receptors by cytokines such as (TNF-α, IL-1, and FasL)—(the *extrinsic* pathway of apoptosis). More commonly, apoptosis is activated through the mitochondrion, or in some cases it can be stress mediated via the endoplasmic reticulum; these organelles trigger the *intrinsic* apoptotic pathway. Common to both the intrinsic and extrinsic pathways is a group of downstream effector enzymes—caspases. In mammals, about a dozen of these protein-splitting enzymes exist as inactive zymogens. Once activated, caspases cleave a large number of proteins within the cell and elicit changes that include cell shrinkage, nuclear condensation, and eventually fragmentation of organelles and membranes. Products of caspase hydrolysis can be engulfed by cells; as a result, problems linked to activation of the inflammatory response are minimized. Caspase 3 is categorized as an executioner enzyme, meaning that it directly causes cell death, whereas caspase 8, 9, and 12 recruit and activate other caspases. Cell death can also be activated without involving these enzymes [41].

Much effort has been expended to learn if the mitochondrion is involved in deleting chondrocytes from the articular cartilage [112]. Mitochondria are the site of aerobic

[e] The Greek term *apoptosis* is derived from apo (away) and ptosis (to fall over or to droop), and most texts refers to falling off of petals from a flower or of leaves from a tree. However, further etymologic study suggests that the term was "falling off from bone." While our Greek forebears undoubtedly suffered from osteoarthritis, they were 2000 years ahead in their thinking about the pathogenesis of the disease.

energy generation mediated by multienzyme complexes (complex I–IV) that utilize the energy gradient across the inner membrane to generate reducing equivalents and ATP. Loss of membrane potential ($\Delta\psi m$) and the consequent induction of a transitory permeability at the mitochondrial membrane reduces ATP generation through oxidative phosphorylation. This in turn may induce apoptosis.

The Bcl-2 family of proteins closely controls the apoptotic process at the mitochondrial level. Bcl-2 and Bcl_{xL} protect the cell from apoptosis by preserving the voltage difference across the inner membrane of the mitochondrion, but other family members promote cell death. For example, Bax and Bak bind to the mitochondrion and cause a decrease in the membrane potential. At an ill-defined stage, a process—the mitochondrial inner transmembrane transition—is initiated that leads to the release of mitochondrial proteins, including cytochrome c and apoptosis inducing factor (AIF). Together with pro-caspase-9, they form the *apoptosome* complex. Once assembled, this multimeric complex activates caspase 3, the enzyme that executes apoptosis. Changes in mitochondrial function promote the formation of reactive oxygen species (ROS), while the activation of nitric oxide synthase enzymes generates reactive nitrogen species (RNS), that is, radicals that advance cell killing.

7.4.1 Evidence for Apoptosis in Osteoarthritic Cartilage

One of the most striking age-related changes in articular cartilage is the decrease in cell number [6,75,83,84,89,96,97,110,113,115], a decrease that is common to both osteoarthritic and aging cartilage [3,38,49,66,76]. Moreover, cell loss is correlated with an increase in the degradation of matrix components [67]. In a much cited study, Adams and Horton [1] showed that with age apoptosis of the articular chondrocytes increased and that most dead cells were localized in the calcified cartilage. Adams and Horton [1] also suggested that the age-dependent increase in apoptosis is closely related to the initiation of OA. Furthermore, human articular chondrocytes isolated from older arthritic patients were more sensitive to apoptosis than those isolated from younger patients [17].

Surprisingly, notwithstanding the loss of cells with age, questions remain concerning the degree to which apoptosis is linked to OA. A number of workers have noted that apoptosis is increased in osteoarthritic cartilage [2,8,33,35,37,54,56,57,61,82,103,118]. Thus Blanco et al [8] reported 51% apoptotic cells in osteoarthritic cartilage and 11% in normal tissue. Hashimoto et al [33] showed 22% apoptotic cells in diseased cartilage compared to nearly 5% in normal tissue. Heraud et al [37] indicated that, in arthritic cartilage, 18% to 21% were apoptotic (assessed by Terminal deoxynucleotidyl Transferase Biotin-dUTP Nick End Labeling (TUNEL) staining and annexin-V fluorescence) compared to 2% to 5% in normal cartilage. A more recent investigation by Sharif and coworkers [103] of TUNEL-positive cells in human articular cartilage provided very different values: 3.1% apoptotic cells in the knee and 1.8% in hip osteoarthritic cartilage, compared with 0.4% cells in normal cartilage.

Increased numbers of TUNEL-positive cells were also seen in animal models of OA, including transgenic mice expressing bovine growth hormone [27], and the STR/ort mouse [88]. While the number of apoptotic cells varies widely, and Sharif's work [103] may well represent half a decade's improvement in the detection of apoptosis, it is clear that in all cases, apoptotic cells can be identified in articular cartilage. Furthermore, when compared to healthy tissue, there is a four- to fivefold increase in apoptosis.

The location of the dead and dying chondrocytes in osteoarthritic cartilage has received considerable attention. A survey of the literature finds no unanimity on the disposition of these cells. For example, while Kirsch et al [57] found apoptotic cells in the upper zone and the mineralized region of the lesion, TUNEL-positive cells have been observed in superficial proliferating chondrocytes, clustering chondrocytes, and deep-layer chondrocytes of osteoarthritic cartilage. Only a few positive cells have been seen in proliferating chondrocytes of the middle zone. It is probably not unreasonable to assume that the variability in distribution from investigator to investigator reflects the age of the sample and the extent of the disease. Figure 7.2 shows TUNEL-positive cells in the different zones of human osteoarthritic cartilage. Note the presence of apoptotic cells in all cartilage zones.

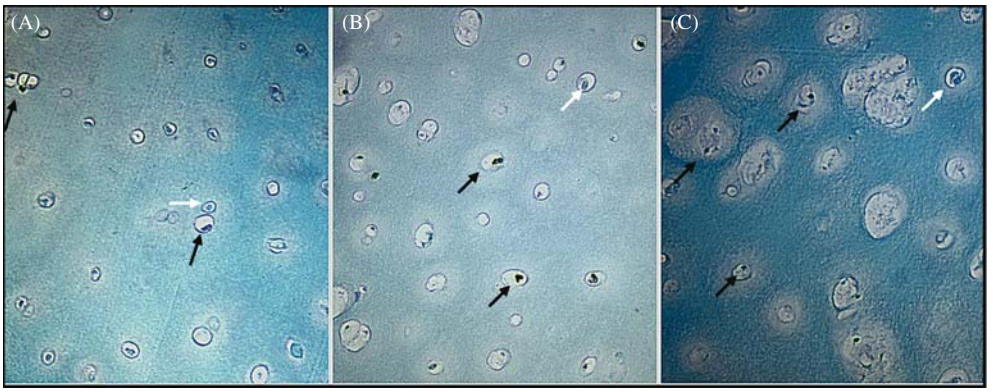

Figure 7.2. TUNEL-stained longitudinal sections through osteoarthritic cartilage. (A) A section though the superficial cartilage (zone 1) (B) Representative of the middle zone (zone 2) (C) From the deep cartilage (zone 3). The dark brown TUNEL-positive apoptotic cells (black arrows) can be seen in the osteoarthritic tissue section. Normal nuclei are light green in color (white arrows) (methyl green counterstain). Note the presence of apoptotic cells in all three zones. (Magnification × 20.)

7.4.2 Regulation of Apoptosis in Osteoarthritic Chondrocytes

Inasmuch as the rate of apoptosis is correlated with the pathogenesis of OA, it is necessary to delineate how the process is initiated. Using mainly in vitro systems, investigators have determined the efficacy of selected reagents in activating chondrocyte killing. Possible chondrocyte apoptogens include serum withdrawal [26], growth factors [28], inorganic ions [77–79], small RGD-containing peptides [92], H_2O_2 [69], Fas ligand [34], nitric oxide [9], and Tumor Necrosis Factor (TNF)-related apoptosis-inducing ligand (TRAIL) [93]. Even though these studies have generated insights into the etiology of OA, they were conducted at 20% oxygen, whereas cells in articular cartilage exist at a low (2–5%) oxygen tension. In addition, the studies did not take account of the complexity of the chondrocyte microenvironment. It is therefore difficult to identify with certainty those apoptogens that trigger cell death in osteoarthritic cartilage.

A number of investigators have studied the role of the antiapoptotic protein Bcl-2. Because Bcl-2 is the major survival protein, it is plausible that expression of this factor may be altered in OA. Kim et al [56] showed Bcl-2 expression was significantly elevated in lesional tissue as compared to non-lesional cartilage. On the other hand, Iannone et al [43] reported that Bcl-2 was elevated in diseased cartilage. The only other protein of the Bcl-2 family that has been carefully evaluated is the proapoptotic protein Bax. Iannone et al showed that there was no significant difference in Bax expression among normal, lesional, and nonlesional cartilage.

To summarize these findings, it is probable that Bcl-2 is expressed in disease, but at variable and probably lower levels than in normal cartilage. It is unlikely, therefore, that this protein family is a major determinant of chondrocyte fate. Bcl-2 may play a limited role in protecting chondrocytes from apoptosis; however, it does appear to be involved in the regulation of proteoglycan synthesis. Feng et al [25] reported that under conditions of serum withdrawal, constitutive expression of Bcl-2 maintained aggrecan levels. Treatment of chondrocytes with antisense Bcl-2 resulted in decreased aggrecan mRNA expression.

In their excellent review of mitochondrial function in OA, Terkeltaub et al [112] pointed out that a prominent feature of cells in the osteoarthritic lesion was their lowered energy state, possibly due to de-energization of mitochondrial complexes, and the linked failure to generate adequate energy (ATP) reserves. This energy deficit alone can degrade chondrocyte function. For example, a decrease in the energy charge would lower the activity of membrane pumps and downregulate proteoglycan biosynthesis, especially sulfation reactions. Once the mitochondria have been de-energized, release of mitochondrial proteins and apoptosome formation would serve to activate caspase 3. However, few studies have

reported an apoptosome formation in OA. Sharif et al [103] noted elevated caspases-3 activity, while Matsuo et al [82] showed that both caspase-3 and caspase-9 were activated in apoptotic (TUNEL-positive) chondrocytes.

Nutritional deprivation and loss of oxidative reserve can also cause a stress response in the endoplasmic reticulum. This response is usually linked to metabolic and cell cycle arrest and activation of apoptosis. In a recent study, Yang and coworkers [117] examined the role of the endoplasmic reticulum in mediating chondrocyte apoptosis and reported that induction of the endoplasmic reticulum stress response, GADD 153, led to an increase in caspase 12 levels. Since caspase 12 can activate caspase 9, changes at the level of the endoplasmic reticulum can advance chondrocyte killing independently of mitochondrial dysfunction [90]. Another direct link to the initiation of the disease process was the observation that the stress response altered proteoglycans levels and downregulated the synthesis of collagen type II.

In summary, there is good evidence that the number of apoptotic cells is elevated in osteoarthritic cartilage. Although the specifics of the apoptotic process are unclear, it likely involves both intrinsic and extrinsic pathways, as well as the stress response mediated by the endoplasmic reticulum. Notwithstanding that Bcl-2 proteins appear to play a minimal role in protecting the mitochondrion, there is definite evidence that this organelle undergoes a transitional change in membrane permeability, along with some interference with normal oxidative phosphorylation. This in turn causes a decrease in ATP levels and generation of oxygen radicals. The interrelationship between radical generation, mitochondrial function and energy metabolism in chondrocytes undergoing hypertrophy is shown schematically in Figure 7.3. The importance of these radicals in relationship to apoptosis is discussed in more detail in the following section.

7.4.3 Reactive Oxygen and Nitrogen Species in the Pathogenesis of Osteoarthritis

A second effect of deregulated mitochondrial metabolism is the generation of ROS. It may be recalled that the major use of oxygen in the cell is in the process of oxidative phosphorylation that serves as a final electron acceptor, converting protons to water. When electron transport is disturbed, much of the oxygen is converted to ROS. In chondrocytes a second radical, nitric oxide (NO), is generated in addition to ROS. Formation of this radical is catalyzed by inducible nitric oxide synthase (iNOS), an enzyme that is expressed when TNF-α or FasL is bound to the cognate receptor. A number of workers have shown that NOS transcripts and proteins are present in arthritic chondrocytes, and studies with antibodies to nitrotyrosine and nitrosocysteine indicate that NO has attacked the cells and the immediate pericellular matrix [35,59]. Commenting on the importance of NO in the etiology of OA, Lotz [72] noted:

> Production of NO in arthritis-affected cartilage and synovium is a consistent feature of human and experimentally induced arthritis. The production of NO is associated with matrix degradation and chondrocyte apoptosis. The administration of NO synthase inhibitors in experimentally induced arthritis has resulted in reduction of synovial inflammation and destruction of cartilage and bone.

It is probable that NO and ROS form the cytotoxic reactive nitrogen species peroxynitrite or $ONOO^-$. $ONOO^-$ and possibly other reactive species can oxidize a large number of cellular molecules, including nucleic acids, lipids, and many proteins. Lipid peroxidation products, nitrite and nitrated collagen peptides, are all present in arthritic joints.

There is little doubt that NO, ROS, and $ONOO^-$ play a key role in lesion development, yet, from the perspective of NO metabolism (enzyme expression level, levels of nitrosylation), the nonapoptotic cells are not substantially different from apoptotic cells [31]. In a recent study, Whiteman et al [116] addressed this issue by noting that, in chondrocytes, $ONOO^-$ can activate calpains, a group of Ca^{2+} activated proteases. These investigators show that $ONOO^-$ caused collapse of the mitochondrial transmembrane potential and release of Ca^{2+} from mitochondria. It is important to note that stress on the endoplasmic reticulum can also raise the cytosolic Ca^{2+} concentration. Ca^{2+} activation of calpains leads to caspase-independent cell death. This novel pathway offers a new approach to probing apoptosis in chondrocytes and explains why NO itself is not

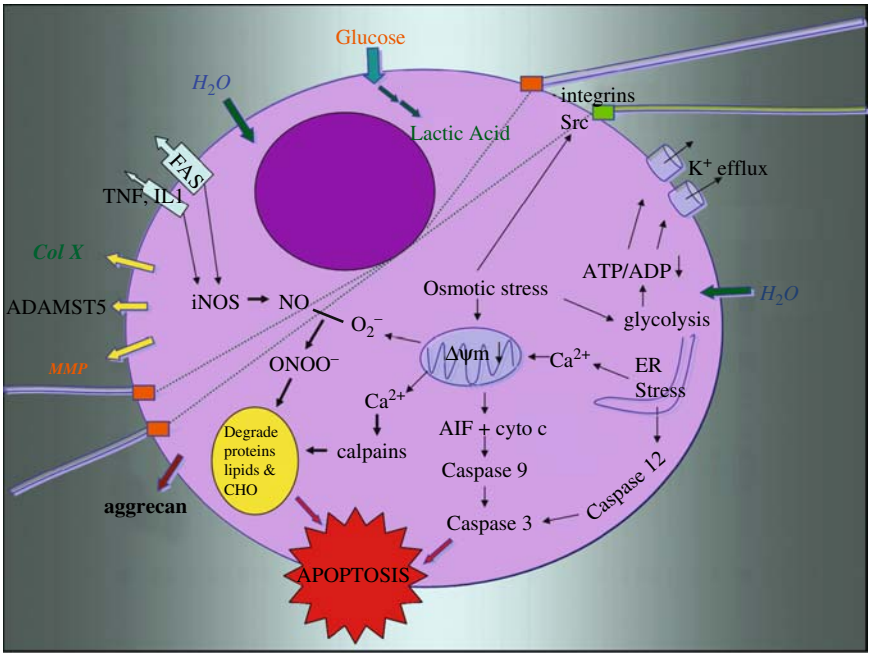

Figure 7.3. Overview of metabolic changes linked to the induction of hypertrophy and apoptosis in chondrocytes in osteoarthritic cartilage. Binding of TNF-α, IL-1, and FasL to cognate receptors activates iNOS, leading to the generation of NO. While NO does not cause apoptosis, when combined with ROS (O_2^-) it generates the powerful peroxynitrite radical ONOO$^-$. ONOO$^-$ can further degrade mitochondrial function, release Ca^{2+} and cause a membrane transition ($\Delta\psi_m$). When this occurs, there is release of cytochrome c and AIF from mitochondria and the formation of an apoptosome, which recruits and activates pro-caspase 9 and proteolytically converts pro-caspase 3 to caspase 3, the executioner enzyme. Caspase 3 can also be activated by caspase 12, an enzyme that is released, along with Ca^{2+}, from the endoplasmic reticulum (ER). The rise in cytoplasmic Ca^{2+} from the ER and mitochondria activates calpains, a family of proteolytic enzymes. Calpains, caspases, and ONOO$^-$ all participate in cell killing. On swelling, volume regulatory KCl efflux is induced due to activation of K^+–Cl^- channels and cotransporters such as the K^+–Cl^- symport. All of these activities are degraded when ATP levels fall, due to mitochondrial dysfunction and osmotic stress induced glycolysis. Moreover, the low-energy charge lowers the activity of the pump leak balance mechanism. Finally, osmotic swelling activates outside-inside signaling through integrin receptors that can influence both apoptotic and survival pathways. Integrins can also influence the remodeling of the actin cytoskeleton, possibly through Src binding to the plasma membrane and activating small (rho) GTPases.

pathognomic of OA. However, it is still necessary to assess whether the calpain system is active in osteoarthritic chondrocytes.

In summary, a major event in the pathogenesis of OA is a decrease in cell number. Because OA is a multifactorial disease, it is reasonable to assume that cell loss is triggered by factors that within the confines of a changing microenvironment generate stress conditions. It is also likely that when the cells have assumed a hypertrophic state, they become sensitized to cytokines and local apoptogens. Once the apoptotic cascade has been initiated, both extrinsic and intrinsic pathways contribute to cell killing. One common feature of both pathways is the induction of iNOS and the decrease in the $\Delta\psi m$; these events result in the formation of oxygen and nitrogen radicals as well as activation of calpains and of downstream effector enzymes (caspases). The radicals individually compromise the activity of membrane pumps and inhibit ATP generation; cell killing is achieved through oxidation and the cleavage of critical macromolecules, and by activating calpains and other proteases (Fig. 7.3). Because death is a multistep process, agents can be utilized to block specific reactions. Indeed, Lo and Kim [68] have shown that caspase inhibitors such as Z-VAD and Z-DEVD also inhibit chondrocyte apoptosis. These peptide inhibitors, by blocking chondrocyte death, may therefore provide a mechanism to maintain the viability and cellularity of the articular cartilage.

7.5 Bone Vascularization and Induction of the Osteoarthritic State: The Autophagic Response

Throughout this chapter, there have been passing references to the potential role of the subchondral blood supply in the pathogenesis of OA. A small number of investigators have recognized this relationship and pointed out that the osteoarthritic state is characterized by increased activity of the subchondral bone in relationship to fibrillation (fissuring and degeneration) of the superficial and middle zones of the articular cartilage (see also Chapter 2). In fact, Brandt et al [11] labeled OA a *whole organ disease* because of abnormalities in bone, ligaments, and synovium of the diseased tissue. If there is tissue connectivity, it would imply that there is crosstalk between the overlying cartilage and the subchondral bone A chronic disruption of the metabolic state of subchondral osteoblasts, therefore, could have a profound impact on the biosynthetic activities of the overlying chondrocytes. That contiguous tissues communicate is likely. Indeed, while the superficial cells of the cartilage accept nutrients from the synovium, cells buried in the deep zone of the cartilage receive nutrients and oxygen from the blood capillaries and vascular channels of subchondral bone.

Microscopic analysis of the chondro-osseous junction has revealed that vascular channels from subchondral bone pass into the deep zone of the articular cartilage [7,10,18,106]. Therefore, any disruption in the vascular supply of bone would affect the viability and health of cartilage cells and could exacerbate development of OA. Biomechanical studies of the subchondral cortical plate show that in disease there is an apparent change in bone density, accompanied by a loss of elastic modulus [120]. These changes are accompanied by thinning of the overlying articular cartilage. Whether serving as a primary cause of disease or resulting from changes in the cartilage function, these events could serve to increase the shear force on the deep zone of the cartilage. As a result, there would be damage to both the subchondral plate and the interfacial calcified cartilage. It is not known if the bone changes are an initiating event or a consequence of OA; in either case, it is clear that pathogenesis of the disease state is linked to the metabolic state of the subchondral tissues.

The tidemark may hold some clues to the role of the chondro-osseous junction in the progression of OA. In most sections of the osteoarthritic cartilage, the calcified zone contains multiple tidemarks. The presence of these bands suggests that as the disease develops there were discontinuous periods of mineralization, possibly synchronized to vascular invasion and to resorption of the calcified cartilage matrix [74]. If this is so, then cells contained within the calcified matrix would be subject to transient changes in microenvironmental conditions. It can be argued that these changes would perturb the metabolic status of cells that normally exist under hypoxic conditions. These metabolic interruptions are not unlike those documented in a condition described as ischemia-reperfusion injury in which the re-oxygenation event generates ROS, activates apoptosis, and causes a loss of tissue function [40]. Based on this paradigm, the loss of chondrocytes, a key event in the pathogenesis of OA, is analogous to a skeletal form of ischemia-reperfusion injury of the deep cartilage zone. If such an injury is part of the pathogenesis of the disease, then cell death would be increased. Over time, the cartilage would become depleted of chondrocytes.

7.5.1 Chondrocyte Autophagy in Osteoarthritic Cartilage

Examining the ultramicroscopic structure of the hypertrophic chondrocytes in the growth plate, Roach and Erenpreisa [22,99] described cells that exhibited unusual, albeit characteristic, features. While condensed chromatin was present, suggestive of apoptosis, the morphology was unlike necrosis or apoptosis. They further noted that there was an initial increase in the amount of endoplasmic reticulum and Golgi apparatus in the terminal hypertrophic chondrocyte [98]. In describing the ultrastructural characteristics of osteoarthritic chondrocytes, Kouri-Flores et al [62] reported that the cells contained a prominent and sometimes disrupted Golgi apparatus and rough endoplasmic reticulum and an increased cytosolic osmiophilic reaction. In addition, phagocytosed organelles and membranes were engulfed inside vacuoles.

These morphologic changes lend support to the concept that cell death in osteoarthritic cartilage may be a variant of classical apoptosis. One such variant is the process of autophagy (type II programmed cell death) [30].

Autophagy differs from conventional apoptosis (type 1 programmed cell death) in that the cells exhibit a characteristic morphologic feature: cytosolic components and organelles engulfed in vacuoles (autophagosomes). In this double-membrane vacuole, fused lysosomes degrade the enclosed macromolecules. Studies with yeast indicate that as many as 20 genes (ATG genes) are involved with autophagolysosome formation [58]. The autophagic response is seen during stress conditions such as nutrient depletion, hypoxia, and aging, and may even be implicated in the pathogenesis of diabetes, neurodegenerative diseases, and cancer. While the autophagic response has not been reported in chondrocytes of articular cartilage, there are reasons to consider that this form of the death response may be operative in OA. For example, hypertrophic degenerate chondrocytes contain large lipid droplets and phagosomes; the nuclear architecture and plasma membrane remain intact, and there is a decrease in the $\Delta\psi_m$. Figure 7.3 shows a hypertrophic cell in osteoarthritic cartilage. Remarkably, while these cells are dying, many of the pathognomic signs of death are absent. Cell membranes are patent and the cells remain swollen (rather than condensed) in their lacunae. These morphologic changes are consistent with those described for autophagy [85].

Lum et al [73] investigated the relationship between nutrient requirements and the autophagic response. When the response is triggered, the cell cannibalizes itself, using its own protein and lipid-building blocks as nutrients. In autophagosomes, these macromolecules are catabolized to generate energy for the nutritionally challenged cell. The energy released from these molecules can be used to extend the longevity of the cell. Whether autophagy does delay cell death is still debated and it may be species-specific [64]. However, *to eat or not to eat oneself* is obviously a very complex activity. It is not surprising, therefore, to learn that this process is very carefully controlled. Factors involved with controlling the autophagic response include regulators of mitochondrial function (Bcl-2), the energetic state of the cell (AMP kinase), and survival pathway activity (PI3kinase/Akt). All of these functions are regulated by the protein, the mammalian target of rapamycin, or mTOR.

7.5.2 Control of Autophagy by mTOR

Shintani and Klionsky [104] have reported that mTOR is an inhibitor of autophagy. When treated with rapamycin, TOR inhibition was relieved and autophagy took place. Signals integrated by mTOR include nutrients (amino acid availability), cyclic adenosine monophosphate (cAMP) levels, Bcl-2, and, surprisingly, osmotic stress [13,19,50,91]. How mTOR integrates and senses the microenvironmental status of the cell is not fully understood. It is known that a hypoxia-mediated fall in ATP levels and a rise in AMP-dependent protein kinase activity serve as negative regulators of mTOR [60,73]. Once mTOR is inhibited, apoptosis is blocked, while autophagy is activated. As for the function of articular cartilage, it would not be unreasonable to consider that mTOR could respond to changes in chondrocyte metabolism. Thus, if there are diffusion problems across the tidemark, then changes in the cartilage microenvironment such as low protein, glucose, and oxygen concentrations would increase anaerobic glycolysis and further lower ATP generation. In this case, mTOR would be inhibited and autophagy would be induced. Another factor that impacts on the induction of autophagy is the ATP status of the cell [12]. Aside from the reported decrease in $\Delta\psi_m$, the osmotic pressure of the cytosol can dissipate proton gradients across the inner mitochondrial membrane and further deplete the cells of energy-rich compounds. Depletion of nutrients leads to increased glycolysis and, according to the Van't Hoff equation, the subsequent production of lactate causes colloid pressure increase. The elevated osmotic pressure would cause an additional fall in the $\Delta\psi_m$ and could generate a mitochondrial membrane transition. Again mTOR would be blocked and autophagy induced. Seen against this background, and from what is already known about the physiology of the swollen chondrocyte, it would not be a stretch to consider that the engorged swollen cell, shown in Figure 7.1L, is autophagic. If that were the case, it might be possible to regulate mTOR

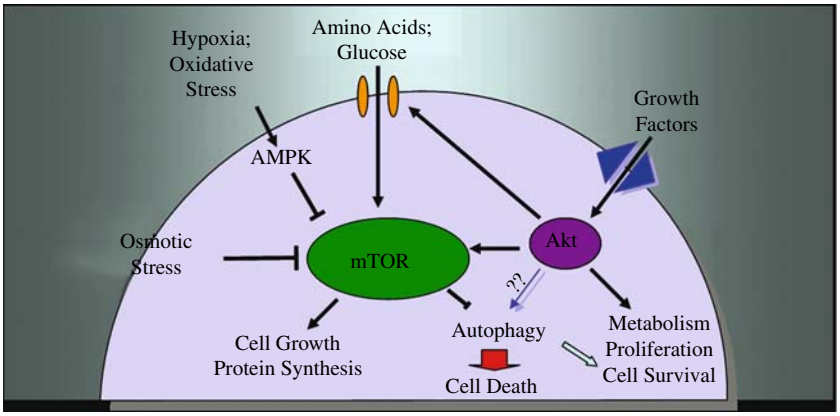

Figure 7.4. The role of mTOR in the regulation of autophagy. When nutrients are abundant, mTOR is activated; this results in enhanced protein synthesis and cell growth. Furthermore, in response to growth factor signaling, Akt is activated. This in turn activates mTOR. It also results in enhanced nutrient uptake and cell proliferation and cell survival. Under conditions of hypoxic or oxidative stress, a fall in the cellular energy charge activates AMP kinase (AMPK), resulting in mTOR inactivation. It is also inactivated under conditions of osmotic stress or growth factor depletion. mTOR is the negative regulator of autophagy, a process that can result in cell survival under conditions of nutritional distress. The autophagic cell may eventually die through the induction of apoptosis.

signaling with the aid of therapeutic agents and thereby protect chondrocytes of osteoarthritic cartilage from undergoing apoptosis (Fig. 7.4).

In summary, in this section we put forward two novel ideas concerning the terminal activities of chondrocytes in the development of the osteoarthritic lesion. The first, based on the appearance of late-stage chondrocytes, is that these cells are maintained in a catabolic state, that is, autophagy; because death signals are postponed, autophagy maintains the cellularity of the cartilage. At the termination of this stage, the induction of apoptosis decreases the number of chondrocytes in the cartilage zones. Autophagy is regulated by mTOR, a signaling molecule that serves as a sensor of the nutritive state of the cell and surprisingly, of the osmotic pressure of the cytosol. Whether the cells undergo autophagy or not, it is highly likely that this protein plays a critical role, not just in mediating cell number, but also in controlling the hypertrophic status of cells in the arthritic lesion.

The second area of discussion concerns the role of vascularity in the pathogenesis of OA. While there has been some interest in the relationship between subchondral bone and the cartilage lesion, we suggested that a variant of ischemia reperfusion injury generates conditions that promote OA. If this novel concept is valid, this mechanism provides totally new insight into the interaction between the two tissues and emphasizes the importance of ROS generation in directing cell death and cartilage fibrillation.

7.6 Conclusion

This chapter discussed factors that lead to and control late-stage events in the pathogenesis of OA—hypertrophy and apoptosis. In relationship to these events, two more points need to be briefly considered. The first is whether terminal differentiation of chondrocytes in the growth plate can serve as a useful model for understanding changes that determine chondrocyte function in OA. The second is whether knowledge of the mechanism of hypertrophy and apoptosis can be used to develop drugs targeted to control disease progression.

It may be recalled that ADAMTS and other enzymes mediate changes in the extracellular matrix proteins of articular cartilage that result in a decrease in the fixed charge density. The decrease in fluid osmotic pressure and overall change in the architecture of the matrix may cause cell spreading. Responding to this local change, membrane pumps are activated to establish a new iso-osmotic state. Because these pump proteins respond to many pharmacologic agents, it may be possible to identify some that limit hypertrophy and maintain the chondrocyte phenotype. That in turn may make possible

halting or even reversing the process of the disease.

Once hypertrophy has been initiated, the cartilage is depleted of cells, probably by way of apoptosis. We have suggested that the swollen, sick chondrocytes initiate an autophagic response that serves to maintain cellular viability until the cells' own macromolecules have been consumed to provide energy. This process is regulated by mTOR, and therefore should be sensitive to many of the agents currently under investigation to regulate the autophagic response in other tissues. It may be useful to determine whether these drugs prevent autophagy by chondrocytes of the articular cartilage. Moreover, relevant to the notion that the response is akin to ischemia reperfusion injury, approaches similar to those being developed for control of stroke, myocardial infarction, and cerebral ischemia may also control chondrocyte hypertrophy and apoptosis.

The last cellular change is death. Cell death may be deferred by autophagy, but when cells die, they do so by apoptosis. The development of agents to control the apoptotic response is a burgeoning field of pharmacologic research. An example is in cancer therapy, where neoplasia may be prevented or minimized by the induction of apoptosis. Indeed, Lo and Kim [68] are currently using caspase-3 inhibitors to regulate chondrocyte apoptosis in an animal model. Because apoptosis comprises several sequential steps, blocking agents other than caspase inhibitors should also be examined for their ability to prevent chondrocyte death.

All along, we have compared and contrasted events seen in osteoarthritic lesions with processes that occur in the growth plate. In comparison with articular cartilage, the growth plate is a highly cellular tissue in which proliferative cells undergo controlled cycles of division that are followed by a maturation phase in which the chondrocytes undergo swelling, not unlike that seen in osteoarthritic cartilage. The swollen cells are deleted from the growth plate by apoptosis. Before this occurs, the terminally differentiated hypertrophic chondrocytes alter the structure of the extracellular matrix in two ways: by secreting proteins like collagen type X and by initiating matrix mineralization. The hypertrophic cells in osteoarthritic tissue follow a similar developmental trajectory. The cells undergo hypertrophy and generate a matrix that is remarkably similar to that of the growth plate. They then commit to apoptosis. Although the time spans may be very different (in juveniles, the growth plate cells complete this process in hours; in OA, the process might take years), the similarity between the two processes is remarkable. The question then arises: Does osteoarthritic cartilage recapitulate the maturation events seen in endochondral tissues? If yes, as the authors of this chapter believe, then it may be wise to consider the growth plate as representing a pattern for the development of OA. Substantive information is available on how maturation of the growth plate is regulated. It would therefore seem advantageous to use normal tissue for understanding the regulation of cell function and to probe the relationship between the terminal chondrocyte and the underlying bone. Finally, growth plate cells, which are easy to manipulate and have a defined, stage-specific phenotype, may be a useful model to evaluate agents that prevent or limit the loss of cells in the osteoarthritic lesion.

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8.

Genetic and Epigenetic Aspects of Osteoarthritis

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8.1 Introduction

For many years primary osteoarthritis (OA) was thought to have no genetic component and to be merely the inevitable consequence of aging. Primary OA is a common, late-onset condition (first radiographic signs and symptoms do not usually appear before the fifth decade) that has no identifiable cause. It concentrates in families, but does not follow a clear-cut pattern of inheritance. Secondary OA, in contrast, appears as a response to clearly identifiable causes such as trauma and congenital or developmental abnormalities. In cases where it is due to developmental abnormalities (a type of skeletal abnormality known as osteochondrodysplasia), the OA is severe and usually presents during the third or fourth decade. These rare developmental diseases have for many years been known to segregate within families in a Mendelian manner. Linkage and positional cloning studies have identified a number of the single gene mutations that are involved. However, this chapter discusses the primary form of OA with its considerable economic impact in the Western world.

Over the last 10 years, much effort has been expended in an effort to explain the genetic aspects of OA. Like many other common diseases such as diabetes, asthma, and hypertension, OA is a multifactorial disease subject to the effect of different genes and environmental factors. Genes that predispose to OA are discussed in the first part of this chapter. In addition, regulation of gene expression and changes in chromatin structure that underpin normal development may also play a role in the incidence and progression of the disease. These heritable changes, which do not alter the DNA sequence, are termed "epigenetic" and may profoundly influence gene activity. DNA demethylation is a major type of epigenetic modification that may play a role in OA and is discussed in the final section of this chapter.

8.2 Genetic Factors in Osteoarthritis

Comprehensive epidemiologic studies of family history and clustering, along with twin studies, have made it clear that genetics plays a major role in idiopathic OA. Other risk factors include obesity, repetitive joint injury, nutrition, occupation, race, joint deformity, hormonal status, and knee meniscectomy [7].

8.2.1 Family Studies

Familial clustering was first reported more than half a century ago by Stretcher [93], who studied Heberden's nodes. This observation was then

extended to include knee OA and confirmed in studies in the United States and the United Kingdom [21,29,39]. Two studies demonstrated that siblings of probands who had undergone total hip replacement (THR) had a two- to three-fold increased risk of OA, compared with controls [12,45]. This finding was also confirmed in an Icelandic study [33]. Another U.K. sibling risk study showed similar findings for knee OA [68]. Interestingly, a later study from the Netherlands showed that siblings had an increased risk for hand and hip OA, but not for knee OA [77]. However an Australian study found a major genetic component for knee OA, as measured by magnetic resonance imaging (MRI) [16]. These studies demonstrate the clustering of hand, knee, and hip OA within affected families, but do not differentiate between shared environmental and genetic factors.

8.2.2 Twin Studies

Twin studies compare disease occurrence in monozygotic (MZ) and dizygotic (DZ) twins. Thus they differentiate the effects of shared environmental and genetic factors, because variation between MZ twins must be attributed to the environment, and variation between DZ twins can be attributed to both environmental and genetic factors. Twin studies have demonstrated that genetic factors account for between 39% and 65% of hand and knee OA in women, approximately 60% of hip OA, and 70% in OA of the spine [49,50,88].

Overall, these studies clearly demonstrate that idiopathic OA has a major genetic component, but that the genetic risk varies between the sexes and among skeletal sites, thus highlighting a significant genetic heterogeneity in the disease. It is therefore likely that the disease has a polygenic basis, with genetic predisposition reflecting the cumulative (and maybe epistatic) effect of multiple genes at different loci, each with a small effect on the phenotype. To investigate the genetic components and to search for genes that confer susceptibility for OA, researchers have used a combination of approaches, including candidate gene studies, gene expression studies, and genome-wide linkage and association scans. Each of these is discussed below.

8.3 Candidate Gene Studies

Many researchers have used their knowledge of the biologic aspects of OA to select candidate genes for an association study that uses case-control cohorts. The advantages of this approach are clear in terms of cost and time, but so far it has yielded only a limited amount of convincing data. The initial attempts focused on genes that code for cartilage extracellular matrix (ECM) structural proteins. This approach was chosen because these genes are mutated in the osteochondrodysplasia class of skeletal dysplasias, with secondary OA a part of their phenotype. These genes may therefore also predispose to primary OA. Targeted genes have included the genes encoding cartilage collagens, such as *COL2A1* (chromosome 12q13.11), which encodes the α1 polypeptide chain of type II collagen, the main collagenous component of articular cartilage; and the type XI collagen genes *COL11A1* (1p21.1) and *COL11A2* (6p21.32). Genes coding for noncollagenous components of the ECM have also been studied. These include *COMP* (19p13.11), the cartilage oligomatrix protein gene, and the aggrecan gene *AGC1* (15q16).

A second strategy has targeted those genes that encode proteins that regulate bone density and mass. This approach was based on reports that subchondral sclerosis and increased bone density are encountered during the early stages of OA. It was felt that sclerosis may damage the cartilage by hindering the joint's ability to transmit a mechanical load. Several groups have thus looked at both the vitamin D receptor gene *VDR* (12q13.11), which is physically close to *COL2A1* (less than 300 kb apart), and the estrogen receptor α gene *ESR1* (6q25.1).

A third approach has been to examine those genes that encode proteins involved in the equilibrium between cartilage breakdown and cartilage maintenance, because deterioration of the ECM is a fundamental property of OA. Even though OA is not an inflammatory arthritis, a number of cytokines are synthesized by the chondrocytes of articular cartilage and synovial cells [72]. Interleukin (IL)-1 is the main catabolic cytokine of the OA joint and can stimulate synthesis of proteinases that cause breakdown of cartilage ECM proteins. This has led some investigators to look for dysregulation in cytokine activity by examining

Table 8.1. Genes identified from genome-wide linkage scans showing evidence of association to osteoarthritis

Protein	Gene	Chromosome	OA type	LOD	Country	Reference
Matrilin 3	*MATN3*	2p24.1	Hand (CMC1, DIP[a])	4.97	Iceland	33
			Spine, hand (CMC1)	–[d]	Netherlands	51
Interleukin-1 gene cluster	*IL1*	2q11.2-q13	Hand (DIP), knee, hip	2.3	Finland[e]	30
			Hip	–	Netherlands	29,55
			Knee, hip	–	U.K.	28,54
Secreted frizzled-related protein-3	*FRZB*	2q32.1	Hip, female hip[b]	1.6	U.K.	39,56
			Generalized	–	Netherlands	57
			Hip	–	U.S.	58
Bone morphogenetic protein-5	*BMP5*	6p12.1	Female hip	4.8	U.K.	42,43
Low-density lipoprotein receptor-related protein-5	*LRP5*	11q13.2	Female hip, knee[c]	2.4	U.K.	40,65
Interleukin-4 receptor-α	*IL4R*	16p12.1	Female hip	1.7	U.K.	41,68

[a] DIP, distal interphalangeal joint; CMC1, first carpometacarpal joint.

[b] Linkage was found in hips; the association was confirmed in female hips.

[c] Association was reported for knee OA by an independent group that reported the linkage in female hips.

[d] Lack of a LOD score indicates that these were follow-up studies investigating the previously reported association.

[e] Evidence of association was detected for a region encompassing the IL-1 gene cluster and not a single locus.

the catabolic cytokine IL-1β (2q13), its inhibitor IL-1RN (2q13), and the anabolic cytokine transforming growth factor (TGF)-β1 (19q13.1-13.3) [28,108].

A clear picture has yet to emerge from these many studies [49,50]. Work on the ECM structural protein genes has been most disappointing, even though it was very logical to target them in light of the role they play in some early-onset monogenic forms of OA. A potentially interesting finding [61], an association of a rare haplotype of *COL2A1*, has not been confirmed except for the report by Uitterlinden et al [99] that two genes located in the short region between *COL2A1* and the *VDR* gene appear to encode for different features of knee OA susceptibility.

Much attention has been paid to the *VDR* gene. Two early reports found an association with knee OA [38,98]. Many groups have failed to replicate this finding [57], yet recently a London-based group reported a modest effect for knee OA in males but not in females [102].

Results for *ESR1* look promising. Ushiyama et al [100] reported an association in females that has now been independently confirmed three times [5,35,102]. The effects are modest, but in light of the probable polygenic nature of OA, it is unlikely that a single locus will constitute a major risk factor.

Most current studies target linkage regions that have been uncovered by the genome-wide linkage scans. As will be discussed in detail below, this approach is clearly yielding positive results (Table 8.1). This is certainly the case for the *IL1* gene family on chromosome 2q, where follow-up investigations are proving to be consistently positive [62,86,92]. The cluster encompasses at least 12 cM with approximately 11 family members. Major interest has focused on *IL1A, IL1B, IL1RN*, and *IL1R1* (which encode the IL-1α, IL-1β, and the IL-1 receptor antagonists, respectively). Although the effects are small, there is a consistent association with the gene cluster in primary OA.

Many studies have used cohorts of such small size that it has not proved possible to demonstrate a statistically convincing association with primary OA. At present, more groups are working together, employing a more systematic approach and embarking upon a genome-wide scan employing anonymous, evenly spaced markers. This approach overcomes the problem of selecting gene regions at random, but is expensive and time consuming. Ultimately, advances in genotyping technology and bioinformatics will overcome these drawbacks.

8.4 Genome-Wide Scans

To date there have been six genome-wide searches for predisposing loci of primary OA. Four of these have been linkage studies based on a considerable number of small families

(generally including affected siblings) collected in the U.K. [10,55], Iceland [91], Finland [48], and the U.S. [14,31]. The remaining two are association-based scans employing a large cohort of case and control individuals collected in Japan [66] or in the U.K. and in Newfoundland [89]. Overall the model-free approach to the search has been fruitful and has revealed several regions that contain novel loci.

8.5 Linkage Scans

The U.K. study (1999) was the first genome-wide linkage scan to be published. Based at the University of Oxford, the group employed a total of 481 pedigrees from individuals who had a hip and/or knee replacement. Each family contained at least one pair of affected sibling (ASP) whose joint replacement was due to idiopathic OA only. In addition, the researchers utilized additional siblings to help infer the missing parental haplotypes. As with all late-onset diseases such as Alzheimer's, recruitment of parents is rare. The LOD (logarithm of the odds to the base 10) scores represent statistical estimates of whether two loci are likely to be proximal on a chromosome and are therefore likely to be inherited together. A LOD score of 3 (odds a thousand to one in favor of linkage) is generally considered to mean that two gene loci are close to each other on the chromosome. Linkages were reported on chromosomes 2q and 11q with LOD scores of 1.2 and 3.1, respectively. When these linkages were investigated further, it was found that the linkage to chromosome 2q was predominantly accounted for by those ASPs with hip OA only, with a LOD score of 2.2 at 2q12-q13. In contrast, the 11q linkage was primarily restricted to female ASPs concordant for hip OA with a LOD score of 3.0 at 11q12-q13 [9]. The investigators then stratified their data by sex and joint, and revealed additional linkages on chromosome 4q (LOD = 3.9) and chromosome 16 (LOD = 2.1) in female ASPs with hip OA, and chromosome 6 (LOD = 2.9) in ASPs concordant for hip OA only [55]. The findings confirmed the conclusions from epidemiologic studies that OA is significantly heterogeneous.

The linkage intervals were relatively large, owing to the medium density of the markers (15 cM interval). Thus, each highlighted region of interest was subjected to finer linkage mapping in an expanded cohort of 571 families. The subsequent marker interval of 5 cM was successful in refining the linkage region, confirming or refining the group to which the linkage was restricted and sometimes increasing the evidence for linkage [9,23,53]. For example, the linkage on chromosome 6 was refined from a 50-cM to a 12-cM interval with an increased LOD score of 4.8. [54,87]. This region has been followed up by two research groups seeking to replicate the findings—one from the Netherlands [3] and one from Ireland [60]. The Netherlands group looked at radiographic OA, using the Kellgren/Lawrence (K/L) scale in 83 probands and 221 siblings belonging to 100 pedigrees. They concentrated on just two of the markers utilized by the Oxford group (two polymorphisms from within the *COL9A1* gene) and confirmed the linkage on chromosome 6 for hip OA, but not for OA of the knee, hand, or spine. The Irish group, however, found no evidence for linkage on chromosome 6 in 109 small pedigrees ascertained by hip replacement. However, their study did not have the power to replicate the original linkage [8].

The remaining three genome-wide linkage scans recruited families ascertained for hand OA. The first of these to be published, genotype 302 microsatellite markers in 27 Finnish pedigrees, each composed of at least one ASP ascertained for severe radiographic distal interphalangeal (DIP) OA [48]. Initially, nine linkage regions were identified with LOD scores >1.0. The typing of additional markers and family members confirmed the linkage of just four of these regions, namely chromosomes 2q12-q21, 4q26-q27, 7p15-p21, and Xcen. The authors noted that the linkage on chromosome 2 harbored the *IL1* receptor and ligand gene cluster (*IL1A* on 2q12, *IL1B* on 2q13, *IL1R1* and *IL1R2* on 2q12-22, and *IL1RN* on 2q14.2). Previous to their study, the *IL1* gene cluster had been identified as a strong candidate gene for rheumatoid arthritis [59]. The presence of a strong candidate gene in their strongest linkage region persuaded the group to perform an association analysis of both single and multiple loci that revealed evidence of an association when four markers, including *IL1R1*, were analyzed jointly ($p = .012$).

The second genome-wide linkage scan for hand OA was the U.S. study published in 2002 [14]. The survey evaluated radiographic

hand OA in the cohort and their offspring (U.S. Framingham Study). The sample consisted of 684 parents and 793 offspring in 296 families. Each group was studied at adult age and measurements were taken for three radiographic OA features. These were the K/L score, the frequency of osteophytes, and joint space narrowing (JSN). From these, four quantitative phenotypes were separately tested for linkages. Eight regions were found to have LOD scores >1.5, with the highest multipoint LOD score (MLS) being 2.96 on chromosome 1p for JSN. Generally the higher LOD scores were seen for the JSN phenotype. The other regions identified were on chromosomes 2p, 7p, 9q, 11q, 12q, 13q, and 19q. One region at 7p14.1-p12.3 was approximately 10 to 30 cM proximal to the 7p linkage reported in the Finnish study [48]. Also the region they analyzed on chromosome 11q13.2-q14.2 overlaps with the chromosome 11 linkage [10]. The fact that no LOD score for overall hand OA exceeded 1.9 prompted the authors to reconsider their phenotype definition by stratifying their data according to joint-specific OA status. They separately tested 10 phenotypes: the DIP, proximal interphalangeal (PIP), and thumb interphalangeal (IP) joints; the metacarpophalangeal (MCP) joints 1 to 5; the first carpometacarpal (CMC1) joint; and the wrist joint [31]. The results highlighted the heterogeneous nature of OA, inasmuch as 16 sites yielded LOD scores >1.5. Only the linkage on chromosome 12q for OA of the DIP joint was confirmed. Four of these new regions had LOD scores >3.0, which included chromosome 7q for OA of the DIP joint (LOD = 3.06) and chromosome 15q for OA of CMC1 (LOD = 6.25). Further stratification by sex and joint revealed two further loci on chromosomes 1 (LOD = 3.03) and 20 (LOD = 3.74).

A genome-wide scan for hand OA [91] utilized a panel of 1000 microsatellites(stretches of DNA that consist of tandem repeats of a simple sequence of nucleotides with a high tendency to mutate) on 1143 affected individuals (along with 939 of their relatives) from 329 families. Each family was composed of patients with idiopathic hand OA of either or both of the DIP and the CMC1 joints. Assignment of an idiopathic hand OA phenotype required the presence of a minimum of two nodes at the DIP joints on each hand and/or a squaring or dislocation of the CMC1 thumb joint. The study identified five linkage regions with LOD scores above 1.0. The most significant were on chromosomes 4q, 3p, and 2p, with LOD scores of 2.61, 1.79, and 1.48, respectively. Stratifying the families into those who were DIP only (274 families), who were CMC1 only (204 families), and who had both (142 families), revealed a number of regions with LOD scores >1. However, only regions on chromosomes 2, 3, and 4 achieved a LOD score >2.0 in at least one of the strata. The addition of extra markers to the three linkage regions on chromosomes 4, 3, and 2 increased the evidence for linkage on chromosomes 4 and 2 alone. The LOD score for chromosome 4 increased to 3.39 for the DIP cohort. The most striking result came from chromosome 2, where the LOD score for the CMC1 cohort increased from 2.23 to 4.97; it was also significant (LOD = 4.44) for the cohort with both CMC1 and DIP. Stefansson et al [91] noted that the region (2p24.1) was very close to a peak identified by Demissie et al [14]. They targeted a gene, *MATN3*, located within 100 kb of the LOD score peak, that encodes the noncollagenous ECM protein matrilin-3. This protein is widely expressed in developing cartilage and bone tissue [42] and highly upregulated in human osteoarthritic cartilage [76]. Mutations in this gene had already been implicated in a class of dysplasias of large joints with early-onset OA [11]. On screening the exons and promoter region of the gene the authors identified six common variants with frequencies >0.05. These were tested for association in a large cohort of 745 patients and 368 controls. One nonsynonymous variant in the third exon of the gene, with threonine changed to methionine in the first epidermal growth factor domain of the protein, occurred in the patients with greater frequency. This association was reaffirmed in a subsequent cohort of 2162 cases and 873 controls. The authors noted that although the mutation cosegregates with hand OA in several families (30 of the 45 carriers were from the families with linkage), its frequency was only 2% in patients with hand OA. They therefore performed a linkage analysis to assess the effect of the mutation on the original linkage result. The LOD score dropped to 3.8. This demonstrates that the mutation has an impact on the linkage, but that one or more variants coding for OA susceptibility occur in this region. The variant may be either within

MATN3 itself (noncoding regions or regulatory elements of the gene) or in one or more adjacent genes.

8.6 Findings from the Genome-Wide Linkage Scans

A number of linkage intervals have been identified from four genome-wide scans, many of which overlap. The regions have provoked a lot of interest from research groups who have targeted them for association analysis. Some potentially exciting results are listed in Table 8.1.

8.6.1 Chromosome 2

It is interesting to note that all of the above scans reported a linkage interval on chromosome 2 and the chromosome has so far yielded three OA susceptibility loci (Table 8.1). Although it is a large chromosome, two of the scans for hand OA (Iceland and U.S.) are very close together, on the p-arm of chromosome 2. The U.S. report may seem on the face of it to be an independent confirmation of the *MATN3* locus reported by the Iceland researchers. However, this locus did not account for all the linkage in the region; the cohort from the U.S. may perhaps uncover further variants that confer susceptibility to OA from the 2p arm of the chromosome. Recently, the Netherlands group confirmed the *MATN3* association with spinal disk degeneration and OA of the CMC1 joint of the hand [65]. The original finding was associated with OA of the DIP and CMC1 joints [91]. An objective of the Netherlands group was not only to replicate the Icelandic findings, but also to investigate whether the variants of the *MATN3* gene increased risk in other OA phenotypes. They tested three of the single-nucleotide polymorphisms (SNPs) identified by Stefansson et al [91] in a population-based cohort scored for radiographic OA in the hip, knee, hand, and spine ($n = 809$), as well as in a population of affected siblings with symptomatic OA ($n = 382$). Stefansson et al did not observe association with the hand OA phenotype, but did find association with spinal disk degeneration ($p = .02$). In addition, one of the other variants tested was found to be associated with the CMC1 cohort. This polymorphism has no known function; possibly it is in linkage disequilibrium (LD) with the causal variant. The authors therefore did a haplotype analysis of the three polymorphisms, along with two haplotype tag SNPs (htSNPs) selected from the International HapMap project (http://www.hapmap.org/), and found an significant increase in risk for a common haplotype, containing the risk allele, in sibpairs with CMC1 OA of [95% confidence interval (CI), 1.1–2.7, $p = .02$]. When they stratified for women only, the risk increased to 2.1 (95% CI, 1.3–3.5, $p = .004$). This result, demonstraties only a moderate risk due to the gene, but is important because it confirms that the gene is relevant for more than one OA phenotype. At the same time it confirms the heterogeneous nature of OA.

A second region on chromosome 2 of great interest is the *IL1* gene cluster located within the pericentromeric region on chromosome 2 (2q11.2-q13). It is associated with a number of OA phenotypes (Table 8.1; studies from Oxford and Bristol [52,86] and the Netherlands [62]). The study from Oxford has reported an association with knee OA, but the sample of individuals with knee OA was very small and the evidence marginal ($p = .01$). The 2004 study from the Netherlands used a population-based cohort of 886 subjects, radiographically assessed for OA of the hips, knees, hands, and spine. They genotyped two of the SNPs located within *IL1B* (3953C>T and −511C>T) and the variable number of tandem repeats (VNTRs) within *IL1RN* (these SNPs were also genotyped in the Oxford study, but were not associated with knee OA). The investigators reported association for the four minor alleles of the VNTR. Of the four alleles, two are so rare they had to be recoded to the common allele, which was then considered biallelic. They also reported association with −511 SNP, with increased risk for hip OA ($p = .001$ and .004, respectively). In contrast, the minor allele of 3953 was associated with a decreased risk for hip OA ($p = .003$). Subsequent analysis identified two haplotypes that were strongly associated with risk for hip OA ($p = .0002$ and .0008) and a protective haplotype ($p = .012$).

The Bristol group [86] employed two cohorts collected from London ($n = 163$) and Bristol ($n = 141$) that had been assessed radiographically for knee OA. The cases and controls

($n = 195$) were genotyped for nine polymorphisms within the *IL1R1* promotor region, two variants within *IL1A*, three variants within *IL1B*, and three variants within *IL1RN*. The study included the variants employed in the Netherlands study. A weak LD between *IL1R1* and the *IL1A-IL1B-IL1RN* cluster was found, although LD within the two groups was high. No haplotypes from the nine *IL1R1* promoter variants showed association with disease, and no single polymorphic marker revealed significant association with knee OA. However, an analysis of the eight variants within the *IL1A-IL1B-IL1RN* complex provided evidence for one haplotype in the Bristol cohort that conferred fourfold increase in risk for knee OA ($p = .00043$). This was confirmed in the London cohort ($p = .02$), where the haplotype conferred a twofold increase in risk of OA. Once again a protective haplotype was also identified in both the Bristol (fourfold reduced risk) and London (fivefold reduced risk) cohorts ($p = .004$ and 8.0×10^{-7}, respectively).

Both groups have expanded their initial studies. The U.K. group examined the IL-1 haplotypes in individuals with severe hip OA who had undergone surgery [84]. They identified an eight-variant haplotype in the patient population ($n = 44$) that was very rare in controls ($p = 3 \times 10^{-7}$). Also the Netherlands group increased the number of variants they genotyped to include those used by the U.K. group. This enhanced the accuracy of the haplotype analysis, with the extended haplotype conferring an increase in risk for hip OA ($p = 8 \times 10^{-5}$).

Finally, there is the frequently reported association to the frizzled-related protein *(FRZB)* gene with hip OA in females [51]. The biologic basis for the genes for FRZB to be involved in OA is strong, inasmuch as the protein plays a key role in the development and maintenance of bones and joints. The U.K. (Oxford) group published a linkage to chromosome 2q24.3-q31.1 for hip OA in both males and females [53]. Using microsatellites on 378 probands (from the hip families that produced the original linkage) and age-matched controls ($n = 760$), they targeted eight candidate genes within the linkage interval and found a significant association ($p < .05$) for the tumor necrosis factor-α (TNF-α)-induced protein 6 gene in male and female probands; the integrin α6 gene and *FRZB* were found in female probands only. The same report also demonstrated an association of female hip OA with a functional SNP in *FRZB* ($p = .04$). The two *FRZB* SNPs probably encode for the substitution of highly conserved arginine residues (Arg200Trp in exon 4 and Arg324Gly in exon 6). The association of the SNP from within exon 6 was confirmed in an independent cohort of female hip cases ($n = 338; p = .04$). Analysis revealed that a haplotype coding for the substitution of both arginines was a strong risk factor for hip OA in females, with an odds ratio (OR) of 4.1 ($p = .004$).

In a follow-up study a Dutch group genotyped the two variants in 1369 individuals from a population-based cohort scored for radiographic OA of the hip, hand, spine, and knee [64]. There was no association with hip OA, but there was an association ($p < .05$) of the G-allele of the Arg324Gly SNP with the generalized OA phenotype (possession of OA in at least two of the four sites). In a recent report, Lane et al [44] examined the two variants in a community-based cohort of elderly Caucasian women scored for radiographic hip OA. Genotypes were obtained for 569 patients and 1317 controls for the Arg200Trp variant and for 4136 controls for the Arg324Gly variant. Association between female hip OA and the Arg324Gly SNP ($p < .05$) was confirmed, as was the possession of a rare haplotype. The minor alleles of both SNPs were found to be a risk factor for developing hip OA ($p < .01$).

The three studies above provide convincing evidence on the contribution of *FRZB* to OA susceptibility. The Dutch study confirmed only the association in generalized OA. It could be argued that because *FRZB* encodes for a glycoprotein that inhibits signaling for the wingless-type (Wnt) ligands, an essential pathway for skeletal development, a functional variant would not be limited to increased risk in hip OA in women. Alternatively, the variant may only accelerate disease progression and therefore be associated with a severe (not radiographic) phenotype. The initial U.K. report had joint replacement as the criterion, whereas Lane et al [44] found that only a severe JSN showed the association. The frequency of susceptibility loci in a complex trait may well vary in different cohorts. They may also interact with other loci (or nongenetic factors; see below) in a population-specific manner.

In conclusion, not all investigations on chromosome 2 have proved fruitful. A linkage scan

on 2q by Wright et al [106], which initially looked promising, was probably a false positive, inasmuch as a subsequent analysis on a larger sample (with additional markers) yielded negative results [27]. Also, Stankovich et al [90] examined 2q for linkage in a Tasmanian population and found negative results, although their highest region did encompass the *IL1* gene cluster.

8.6.2 Chromosome 6

The linkage reported by the U.K. (Oxford) group to this chromosome was centered on 6p12.3-q13 [54]. The finding was restricted to those families with hip OA and female sibling pairs. This is a multigenic region and there are many strong candidate genes within the linkage interval. Two of the strongest are *COL9A1* and *BMP5*. A scan of these two genes for nonsynonymous polymorphisms revealed a number of unassociated variants [87]. The Oxford group therefore decided to employ microsatellite markers at the relatively high density interval of 0.36 cM. This generated a particularly high LOD score of 4.8. An association analysis highlighted three markers in this region, one within intron 1 of *BMP5* and two located immediately downstream of the gene [87]. *BMP5* encodes bone morphogenetic protein 5, a regulator of articular chondrocyte development. An earlier report [15] had shown no common coding polymorphisms within *BMP5* that were associated with OA. Mouse studies have revealed that the expression of *BMP5* is highly complex and regulated by a number of *cis* elements that may reside some distance from the gene. Variation in the *cis* regulatory elements may contribute to the linkage, since the nonsynonymous polymorphisms are not significant.

8.6.3 Chromosome 11

The linkage to chromosome 11 has also been reported by a study from Israel [37] that employed 295 pedigrees of Chuvashians from southern Russia with a phenotype of hand OA (K/L scale). The region was well known to harbor genes controlling bone mass [43]. One of these is *LRP5*, which encodes for the low-density lipoprotein receptor-related protein 5, a co-receptor for Wnt/β-catenin signaling. Its role in osteoblast proliferation and postnatal bone development makes it a strong candidate within this linkage interval. It is a large gene and contains 23 exons and spans 160 kb. When the *LRP5* gene was targeted by genotyping five common variants (including two htSNPs from the HapMap project) in two cohorts of 158 (London-based) and 110 individuals (Bristol-based) with knee OA, with 187 controls [85], no single SNP gave evidence of association. Haplotype analysis revealed a common five-marker haplotype that yielded a 1.6-fold increased risk of OA ($p = .02$) in the London cohort. This was confirmed in the Bristol cohort. However, because of the limited size, the result is necessarily preliminary.

8.6.4 Chromosome 16

One of the linkage intervals on chromosome 16 is centered at 16p12.3-p12.1 [23] and was in the families with female siblings who had hip OA. Similarly in an Icelandic family with early-onset hip OA, there was linkage to chromosome 16 at an interval of 28 to 47 cM, located at 16p13.13-p12.1 [32]. This overlaps with the Oxford region. A particularly strong candidate in this interval is the IL-4 receptor α-chain gene *IL4R*, located at 16p12.1. This is because of the essential role played by IL-4 and its receptor in the response of articular chondrocytes to mechanical stimulation [63]. Nine common SNPs from within *IL4R* (including six coding SNPs) were genotyped [22]. Two nonsynonymous SNPs were found to be associated ($p < .05$). This result was confirmed for both SNPs in a separate cohort of 310 females with hip OA ($p = .004$). Linkage disequilibrium analysis revealed that the two SNPs defined two distinct groups, the members of each group being in strong LD with each other. Possession of an associated allele from both groups conferred a risk for hip OA in females with an odds ratio of 2.4 ($p = .0008$).

8.7 Association Scans

Major advances in bioinformatics and genotyping technology along with recent improvements in our knowledge of human genetic variation have made it possible to carry out genome-wide association studies aimed to identify susceptibility genes for common diseases.

Two such studies have involved OA. In the first of these, from Japan [48], the investigators recruited 428 individuals (mean age 54 years) and 1008 controls (mean age 47 years), of whom 94% were female. The cases with hip OA were symptomatic and radiologically screened for JSN or osteophytes. Of the 75,253 gene-based SNPs and 71,880 that satisfied the Hardy-Weinberg equilibrium, 2219 of the SNPs showed evidence of association ($p < .01$). The positive SNPs were genotyped in independent cohorts of patients and controls and several SNPs were identified to be highly associated with hip OA.

In the second study from the U.K. [89], 25,494 SNPS were genotyped; 82 were found positive at a level of $p < .05$. Two independent collections from the U.K. and Newfoundland confirmed the findings from the initial screen.

8.8 Findings from the Genome-Wide Association Scans

The Japanese study has yielded two loci with evidence of association (Table 8.2), and has demonstrated the functional importance of these genes for OA development and progression. The first locus is the asporin gene *ASPN* [41]. Asporin is expressed in adult articular cartilage along with a number of other tissues. The gene is located on chromosome 9q22.31. This is a region that does not contain any of the linkage scans outlined above; it includes eight exons. Eight common polymorphisms were identified: six SNPs, one deletion, and a triplet repeat within exon 2 encoding for aspartic acid (D)—the D-repeat. These were genotyped in a cohort with radiographic knee OA and in unaffected controls. Only the D-repeat polymorphism yielded a positive association ($p = .0013$). The variant has seven alleles; the most common (D13) occurred significantly more frequently in the control group, whereas the D14 allele occurred more frequently in the patient group. This suggests the susceptibility allele (D14) is acting with a protective allele (D13), an inference that was confirmed in a study with another group of patients with knee OA and their controls ($p = .018$). A third cohort of patients with hip OA also provided evidence of association for the D14 allele ($p = .0078$). Functional studies established the role that asporin plays in inhibiting the expression of *AGC1* (coding for aggrecan) and *COL2A1* (coding for type II collagen) genes.

These findings need to be replicated in a European population, inasmuch as studies in the U.K. and Greece have reported negative results for *ASPN* [36,67]. The calmodulin 1 gene, *CALM1* (14q24-q31), has a positive association with hip OA [41] but not with knee OA. A SNP within intron 3 of the gene is significantly associated with inheritance of two copies of the risk allele giving an OR of 2.4 ($p = .00065$). A subsequent analysis of all other common polymorphisms in the gene revealed that the associated SNP was in strong LD with four other SNPs (in the promoter region, intron 1, and 3′UTR), all of which had a strong association with hip OA. The variant in the core promoter region, which is likely to have a functional effect on calmodulin 1, led to reduced transcriptional activity, whereas calmodulin 1 increased expression of *AGC1* and *COL2A1*.

Given that calmodulin 1 and asporin both regulate expression of *AGC1* and *COL2A1*, analysis for a possible epistatic effect showed that possession of two copies of the risk allele of *CALM1* and at least one copy of the D14 allele conferred a particularly high risk of hip OA with an OR of 13.16 (95% CI, 1.66–104.06). It should be noted that, although this finding is fascinating, the confidence interval of the statistic is very broad, and Loughlin et al [56] were unable to replicate the result in a European cohort.

The final gene identified from a genome-wide association scan is the *LRCH1* (leucine-rich repeats and calponin homology containing 1) gene from the U.K. study [89]. The gene resides on chromosome 13q14 and encodes a novel protein of unknown function. The leucine-rich repeats are 20-to29-residue sequence motifs that contribute protein interactions, whereas the calponin domain is an actin-binding domain present in the cytoskeleton and in the signal transduction proteins. Of the 31 SNPs, one

Table 8.2. Genes identified from genome-wide association scans

Protein	Gene	Chromosome	OA type	Country	Ref
Asporin	*ASPN*	9q22.31	Hip, knee	Japan	69
Calmodulin-1	*CALM1*	14q24-q31	Hip	Japan	36
Unknown	*LRCH1*	13q14	Knee	U.K.	37

variant reached significance within intron 1 of *LRCH1* ($p = .0078$) and demonstrated the strongest and most consistent effect in both U.K. and Newfoundland cohorts. No difference between males and females was noted. However, stratification of the Newfoundland cohort for knee OA revealed slightly increased evidence for association ($p = .0234$). The investigators selected 10 additional SNPs from the HapMap project in order to define the region of association more clearly. No SNP exhibited stronger association with knee OA than the original variant, and haplotype analysis failed to implicate any other region than the 5′ end of intron 1. Although the role played by this gene in the development of OA is not known, its discovery is a promising new finding.

8.9 Epigenetics of Osteoarthritis

As the preceding discussion has shown, the genetics of OA is complex, and inheritance appears to be both Mendelian and non-Mendelian, as is the case in a number of other diseases, for example, schizophrenia, type 2 diabetes, Alzheimer's, and osteoporosis. These diseases all have a heritable component, but with a high degree of discordance between monozygotic twins. Heredity clearly influences disease susceptibility, but the discordance indicates that DNA sequence alone cannot completely account for the disease. So we need to look beyond genetics [73,105], to epigenetics, defined as the study of mitotically and meiotically heritable changes in gene function that are not the result of changes in DNA sequence. Epigenetic events can result from changes in DNA methylation, histone acetylation, or methylation, and may be part of intrinsic mechanisms whereby environmental changes interact with genes [34]. The most studied epigenetic mechanism is DNA methylation, which refers to the addition of a methyl group to the 5-position of deoxycytosine bases to form deoxy-5-methylcytosine. Almost all methylated cytosines (^{m}C) in vertebrate DNA are found at the dinucleotides cytosine-guanine, the so-called CpG sites, where "p" represents the phosphate connecting the two nucleotides. Methylation is carried out by *DN*A *m*ethyl*t*ransferases (Dnmts). Dnmt1 is the maintenance methyl-transferase and is responsible for maintaining methylation patterns during replication, whereas Dnmt3a and Dnmt3b are involved in de novo methylation of primarily unmethylated DNA [71,75].

The genetic sequence of DNA bases is identical for every cell in the body and for cells of monozygotic twins, but epigenetic modifications are cell-type specific and can vary significantly between monozygotic twins. As shown in Figure 8.1, the environment, certain hormones, or simply random events can lead to loss of DNA methylation (red circles) with time. If this loss is taking place at a regulatory region in the promoter of specific genes, critical levels can be reached at some point and result in expression of previously silent genes that may then contribute to the disease [105]. It is important to note that epigenetic changes are heritable at a cellular level. When a cell with altered methylation pattern divides, the new methylation status will be transmitted to the daughter cells, because Dnmt1 will reproduce the methylation pattern, irrespective of whether or not this pattern is appropriate for the cell. In contrast to the DNA mismatch repair, there is no "memory" of the appropriate methylation pattern for a particular cell, nor is there a mechanism for "proofreading" or repairing defective methylation status. Epigenetics may thus explain the observed discordance between monozygotic twins.

CpG sites occur throughout the genome, and 70% to 80% of human CpG sites are usually methylated. Regions of DNA that contain many closely spaced CpG sites are termed "CpG islands." Such islands are frequently located within the promoter regions of housekeeping genes or tumor suppressor genes and are kept methylation-free by binding of the transcription factor Sp1 [6]. Tissue-specific genes may have CpG islands or sparse CpG promoters.

Methylation may have evolved as a protective mechanism to limit expression of foreign DNA, such as transposons, intragenomic parasites, and proviral DNA, which threaten the orderly expression of the genome. Around 40% of human DNA consists of such silent or "junk" DNA, of obvious importance for host defense. However, DNA methylation is also essential for normal cellular functions, in particular for the imprinting of specific genes, for X chromosome inactivation in the female and, in the present context, for silencing all genes not expressed in

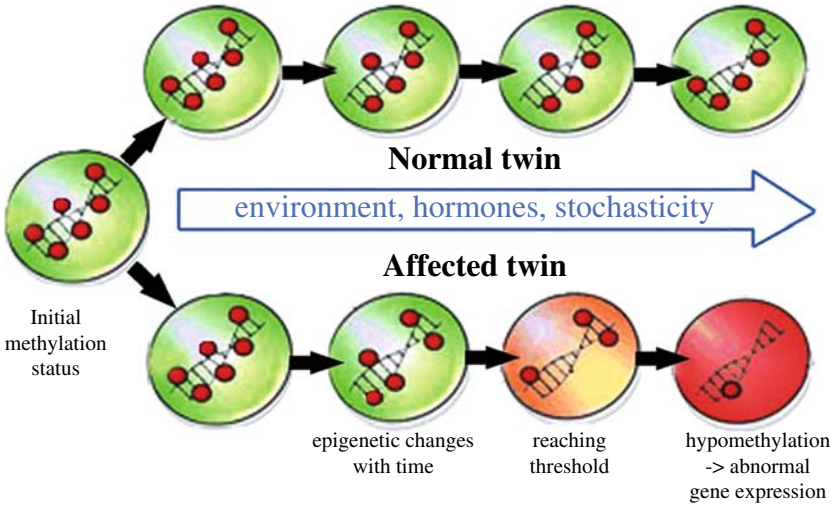

Figure 8.1. Epigenetic variability between monozygotic twins. Progressive loss of methylation due to a variety of factors can lead to hypomethylation and expression of abnormal genes that contribute to the disease. (Adapted from Wong et al [105], with permission.)

a particular somatic cell. For examples of genes whose tissue-specific expression is controlled by DNA methylation, see Ehrlich [18].

Apart from developmental changes in DNA methylation, aberrant DNA methylation has been identified as causing or at least contributing to a number of diseases. This has led to the suggestion that epigenetically caused expression of genes seems more consistent with the features of complex diseases than DNA mutations [73]. The most telling examples are found in cancer. Cancerous cells may arise not only following a mutation, but also following changes in methylation status if these changes affect regulatory regions of key gene promoters. These may involve two types of changes: increased methylation (hypermethylation) with associated silencing of the gene, or loss of methyl groups (hypomethylation), possibly leading to expression of previously silenced genes. The first case is frequently found in tumor cells, where the island promoters of tumor-repressor genes become hypermethylated and silenced—providing the obvious advantage of growth for the tumor cell. There are also many examples for various types of cancer in which loss of methylation at specific CpG sites is associated with expression of abnormal cancer-inducing genes [4,13,19,25,47,58,69,80,94,95,97]. Interestingly, hypermethylation of tumor suppressor genes can occur at the same time as hypomethylation of tissue-specific genes.

What features of OA might be due to underlying epigenetic changes? There is wide evidence that during the disease process gene expression in articular chondrocytes undergoes change (for reviews see [1,26,28,81]. Typical chondrocytic genes, such as aggrecan or collagen II, tend to be downregulated or switched off (albeit after an interim activation during the repair response), while degradative enzymes, which are normally silenced in articular chondrocytes, are upregulated or switched on, at least in some articular chondrocytes. These changes may be heterogeneous and only partially coordinated. The question then arises as to whether these alterations in gene transcription are primarily dependent on specific transcription factors or whether DNA methylation also plays a role. Theoretically, hypermethylation can lead to permanent silencing of the anabolic genes known to be switched off in OA chondrocytes (analogous to the hypermethylation of tumor suppressor genes), while de-methylation can induce "unsilencing" of the abnormally expressed catabolic genes. A decisive piece of evidence supporting the role of methylation is that the changes in the expression of degradative enzymes appear to be transmitted to daughter cells, as illustrated in Figure 8.2.

If methylation plays a role, one would predict that in normal articular chondrocytes the promoters for aggrecan and collagen II (plus all other chondrocyte-specific genes) are

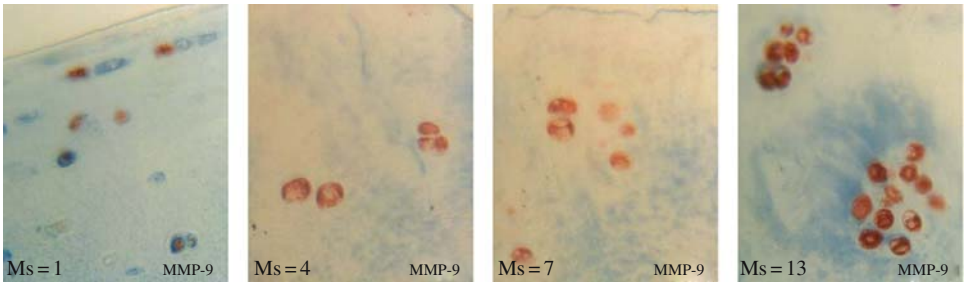

Figure 8.2. Transmission of aberrant gene expression to daughter cells. The Mankin score (Ms) is a histologic grading system for severity of matrix erosion in osteoarthritis. MMP-9 immunopositive cells are initially present as single cells in the superficial zone, then as doublets and quadruplets, until the typical clones of OA are seen. Note that all the clones express MMP-9 protein, consistent with the notion that the aberrant gene expression has been transmitted to the daughter cells during clonal formation. (from Roach et al [79], with permission.)

hypomethylated, but that the CpG sites in the promoters of all genes that are not part of the repertoire of articular chondrocytes are hypermethylated. Because articular chondrocytes do not differentiate to the hypertrophic state, one would also expect that hypertrophic genes, for example, type X collagen, alkaline phosphatase, and matrix metalloproteinase-13 (MMP-13), are silenced in normal articular chondrocytes. To date, very few studies have attempted to determine the normal methylation status of genes relevant to articular chondrocytes. Kim et al [40] found that inflammatory arthritis was associated with overall DNA hypomethylation in peripheral blood mononuclear cells, but they did not investigate chondrocytes or specific genes.

8.10 DNA Hypermethylation and Silencing of Chondrocytic Genes

As already mentioned, some promoters contain CpG islands, consisting of many closely spaced CpGs. Examples are the aggrecan and type II collagen (α 1), the two main chondrocytic genes. Poschl et al [74] investigated whether the downregulation of aggrecan is due to hypermethylation of the CpG-rich promoter regions. The methylation status of CpGs within a 340-bp region of the aggrecan promoter was unchanged in normal aged or osteoarthritic chondrocytes. But this study examined only the 340-bp region in detail, and not the many CpG sites of the CpG island. The possibility that some of these sites were hypermethylated, therefore, cannot be excluded.

8.11 Loss of DNA Methylation and Aberrant Expression of Degradative Enzymes

Osteoarthritic chondrocytes express de novo many genes that are involved in cartilage catabolism. Examples are matrix degrading enzymes such as MMP-1, -2, -3, -9, -13 and ADAMTS-4 and -5 [2,17,20,24,46,70,82,83,104]. Immunocytochemistry has demonstrated that these enzymes are expressed predominantly in the superficial region of OA chondrocytes, particularly those that are proliferating and forming clones [70,79,96,103,107].

Could this de novo expression be due to a loss of methylation at specific CpG promoter sites? The first gene examined for changes in methylation status was MMP-9, which contains just six CpGs in the 670-bp promoter sequence [30]. Using methylation-sensitive restriction enzymes followed by polymerase chain reaction (PCR) of selected regions, Roach et al [78] demonstrated that AciI digestible sites were methylated in chondrocytes that did not express MMP-9, but were unmethylated in OA chondrocytes that expressed MMP-9. Further studies investigated the methylation status of the promoters of MMP-3, -13 and of ADAMTS-4, all degradative enzymes that are typically expressed de novo in OA chondrocytes [79]. All of these promoters contain relatively few CpG sites (seven

in 2000 bp for MMP-3, eight in 600 bp for MMP-13, and 13 in 900 bp for ADAMTS-4), a situation that favors pathological de-methylation. Figure 8.3 shows typical results, obtained with the aid of methylation-sensitive restriction enzymes, followed by PCR. When the CpG site is methylated, the enzymes cannot cut at their recognition sequence and PCR amplification can proceed as in nonenzyme controls. However, if there is no methylation, the DNA will be cut and PCR amplification cannot proceed. The presence of a band, therefore, indicates a methylated CpG and band absence means that the CpG is not methylated. Genomic DNA was extracted from the superficial zone of osteoarthritic samples and from control samples (either from the cartilaginous bones of 8- to 10-week-old aborted fetuses or from the cartilage of patients with a femoral neck fracture). Figure 8.3 shows considerable variation in the DNA methylation pattern. Initially it had been expected that all CpG sites in the promoters would be methylated in control, but not in OA samples. This was not the case. Individual CpG sites varied in their methylation status, ranging from sites that were methylated in both control and OA samples (most of the CpG sites in the MMP-13 promoter) to sites that were largely unmethylated in both groups (the *Ava*I site at −721 in the MMP-9 promoter; Fig. 8.3). However, for each enzyme, there were one or two CpG

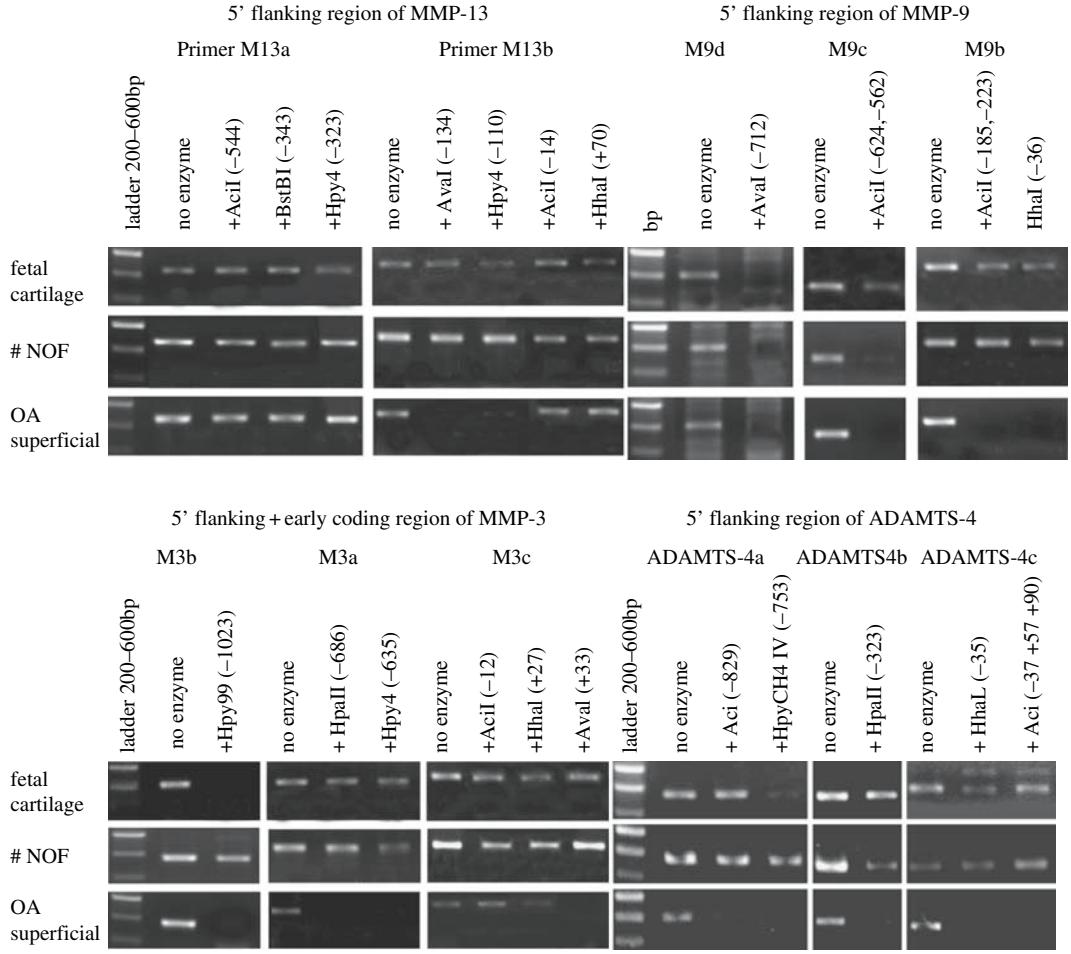

Figure 8.3. Loss of methylation at specific CpG sites in the promoters of four degradative enzymes in OA. Each sample was subjected to the polymerase chain reaction (PCR) in the presence or absence of the indicated methylation-sensitive restriction enzymes. Typical reaction patterns are shown for DNA extracted from fetal cartilage, from the deep zone of a patient with femoral neck fracture (#NOF), and from the surface zone around the weight-bearing area of an OA patient. A band indicates methylation and absence of a band means no methylation. (From Roach et al [79], with permission.)

sites at which a loss of methylation had occurred in most OA patients. Overall, the percentage of nonmethylated CpG sites increased from 20% to 48% in OA samples [179]. It has not yet been established whether methylation loss caused the degradative enzymes to be expressed. However, the data, together with the heritable nature of abnormal enzyme expression, are consistent with the possibility that demethylation is one factor that is responsible for the focal gene activation pattern typical of osteoarthritic cartilage cells in vivo.

8.12 Conclusion

Recent breakthroughs have shed some light on the nature of OA susceptibility. The disease is multifactorial and is brought about by genetic and epigenetic factors and their interactions. Genetic inheritance seems nonmendelian and involves combinations of genes that may differ in different OA types. Many candidate genes have had their roles confirmed. Examples are the *IL1* gene cluster and the estrogen α-receptor gene *ESR1*. Several genes coding for proteins that regulate cartilage homeostasis such as the interleukin gene cluster are now reported as having reasonably powerful linkage and association, but much larger scale studies are necessary to identify genes that confer risk.

Genetic analysis alone may not suffice to identify the causes of complex human diseases such as OA. An alternative strategy is to identify genes that exhibit significant up- or down-expression changes in diseased and normal cartilage and to test them for association. This approach has yielded the gene *ADAM12* on chromosome 10q26.2, which encodes a metalloproteinase that is associated with disease prevalence and progression [101]. An alternative approach is to determine whether the altered gene expression could have resulted from epigenetic silencing or "unsilencing." Four proteases (MMP-3, -9, -13, and ADAMTS-4), known to be associated with disease progression have resulted in "unsilencing" [179]. Future studies combining genetic with epigenetic analyses and genome scans may lead to better understanding of the pathogenesis of OA.

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9.

Animal Models

Alison M. Bendele

Animal models of osteoarthritis (OA) that are commonly used to study the pathogenesis of cartilage degeneration and potential therapy can be natural or induced. Induction involves surgical manipulation or injection of matrix modifying agents into the joint. The knee joint is the most common joint that is affected (spontaneous OA) or utilized (induced). Spontaneous OA occurs reliably only in certain animal species, but some form of surgical or chemical OA can be induced in any animal species. On the assumption that the disease is induced in mature animals (tidemark well formed) and if species-specific load-bearing characteristics are taken into account, the location of the lesion and the structural changes due to it are generally similar in animals of the same species. By using different animals one can achieve diversity in the lesion characteristics. Alternatively, diverse lesions of varying severity can be obtained by inducing injuries in animals of the same species. This approach makes possible investigating different types of therapeutic interventions. Comparison of profiles of cartilage degradation in various models with those in human or spontaneous animal disease indicate that most OA models have more similarities than differences [11,36, 49,61]. Model selection for a particular purpose generally comes down to selecting the appropriate animal species and to making sure the selected species exhibits the desired morphologic and biochemical changes needed to evaluate the efficacy of a potential therapeutic agent in a reasonable period of time.

In general, scoring systems for microscopic evaluation of cartilage degeneration should take into account depth and area affected by the changes (chondrocyte death, proteoglycan loss, collagen fibrillation), and should include as many measured dimensions as possible (μm), rather than subjectively scored parameters. The area of viable cartilage from surface to tidemark, excluding peripheral marginal zone changes and osteophytes, can be measured by image analysis if sectioning is done in a consistent manner. Similarly, cartilage thicknesses and depth of lesions relative to thickness can be determined, as can the width of lesions across surfaces. Osteophytes can be measured and thickness of subchondral bone can be determined. The scoring and evaluation methods should be tailored to the model, its morphologic features, and the type of agent under evaluation. A critical feature of any scoring system is that it be simple to understand and remember. This avoids the need constantly to consult notes about the meaning of the various numbers used. The result is less variability in the collected data and greater likelihood that different scientists will arrive at the same conclusion.

9.1 Animal Model Selection

Numerous models involving many different animal species and various manipulations have been described [11,14,28]. The diversity of choices often leaves the investigator confused about the choice of an appropriate model, yet,

as a result of preliminary work, the investigator may already have chosen a particular animal species. A further important consideration relates to the morphologic features of the lesion and to knowledge about mediators involved in the pathogenesis, particularly when a pharmaceutic agent is being tested. For example, inhibitors of collagenase should be tested in models where collagen degradation is of sufficient severity to generate an observable morphologic change. Aggrecanase inhibitors, on the other hand, should be tested in models where the primary morphologic change is proteoglycan loss, so that collagen loss does not obscure the proteoglycan evaluation. Another way to address this issue within most instability models is by individual evaluation of load-bearing zones of articular cartilage. In virtually all surgical models, there are areas of mechanical abrasion adjacent to areas of milder, enzymatic degradation. Histopathologic evaluation that takes into account these zonal variabilities will often be as useful as utilizing multiple models of different severity. However, just as criteria for efficacy are set at the onset of a human clinical trial, animal studies must also have predefined parameters. Criteria for success depend on the limits of the agent under investigation.

The descriptions below of animal models of OA that are commonly used for research and pharmacologic testing will accordingly be classified by species, and their morphologic features and suggested applications will be detailed.

9.1.1 Dog Models of Osteoarthritis

Although spontaneous OA occurs in dogs in veterinary practice and in certain colonies with hip dysplasia or other conformational abnormalities, the only two dog models of OA in routine use are the partial medial meniscectomy and the anterior cruciate ligament (ACL) transection models.

9.1.1.1 Dog Partial Medial Meniscectomy Model of Osteoarthritis

Use of the beagle dog can generate efficacy data in a model that requires a 1-month study in a species that is commonly used in toxicology studies. A unilateral, partial medial meniscectomy is performed in retired breeder females [11]. Generally, readily available 2- to 5-year-old grade B dogs weighing 6 to 12 kg are used. Because these dogs rarely have spontaneous OA lesions, they can be studied over a wide age range. Minor, bilaterally symmetrical superficial areas of spontaneous degeneration can be observed in the area not protected by meniscus on the medial tibia.

Dogs need to be housed in large runs or enclosures that allow plenty of exercise, as individual housing in stainless steel cages with intermittent exercise will result in mild, highly variable lesions. It is important to minimize trauma during the operation, as the animals need to resume weight bearing immediately postsurgery. Following removal of approximately one half of the anterior portion of the medial meniscus, the animals need to be treated for 3 days with an analgesic, on the assumption they will resume weight bearing the morning after surgery. Operated knees develop moderate degenerative changes in the tibial and, to a lesser extent, femoral cartilage (Fig. 9.1). Even though the animal attempts to regenerate the meniscus by proliferation of fibrous tissue, the experimental lesions are reasonably consistent with respect to location and severity. Disarticulation of the joints allows gross evaluation (scoring, lesion measurement) and photography of the morphologic changes. The ultimate evaluation, however, must be histologic, as lesion depth and morphology have to be taken into account in addition to

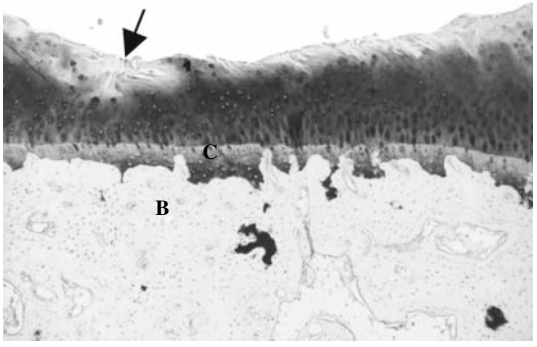

Figure 9.1. Tibial cartilage from beagle dog with meniscectomy shows areas of moderate degeneration characterized by chondrocyte and proteoglycan loss with fibrillation (large arrow). Subchondral bone (B) is thickened under the area of most severe damage. Calcified cartilage (C) and tidemark are normal.

the area of lesion. Sectioning (division of each tibia and femur into three anterior to posterior frontal slabs of approximately 0.4 cm) is important in order to maintain zonal relationships, because lesions at different locations differ in severity. The microscopic evaluation takes into account both the anterior to posterior as well as axial to abaxial variations in lesion severity. The morphologies of the various zones differ distinctly, ranging from areas of collagen fibrillation with severe proteoglycan loss in the central load-bearing areas to areas of proteoglycan loss with intact collagen matrix in the peripheral areas. Attention to these zonal differences facilitates detection of both collagenase inhibitors and agents that decrease the loss of proteoglycan alone. In addition to focal chondrocyte/proteoglycan loss and fibrillation, subjacent and surrounding chondrocytes often exhibit striking clonal proliferation and obvious changes in the matrix staining color (orthochromatic to metachromatic with toluidine blue). Marginal zone proliferative changes are present on the medial side of the knee at 1 month postsurgery, but osteophytes are rarely seen. Subchondral femoral bone thickening subjacent to areas of greatest lesion severity is prominent and is an indication of the important role of load-bearing in the pathogenesis and the response of the bone to changes in load-bearing. Synovial membrane changes remain relatively mild and generally consist of fibrous proliferation in the area of the surgical wound, with minimal to mild mononuclear inflammatory cell infiltrate (mainly macrophages). Synovium should always be evaluated, so as to be able to exclude animals that have developed postsurgical joint infection, which gives rise to excessive cartilage pathology.

Besides the obvious advantage of generating data in a species in which toxicology testing is also likely, there are other advantages to using beagle dogs. Beagle cartilage is relatively thick (as compared to that of rodents) and therefore permits greater discrimination with respect to the depth of lesions. Moreover, the lesions induced by meniscectomy seem to progress much more slowly than those in rats (medial) or rabbits (lateral). While all operated animals generally have a focal, moderately severe, degenerative change in their tibial cartilage, most femoval lesions are minimal or mild, with proteoglycan loss as their main feature. This makes it possible to evaluate not only agents intended to protect against collagen degradation but also agents that only inhibit proteoglycan loss.

The lesions in the beagle dog model (assuming good surgical technique) are consistent enough to allow testing to be restricted to 12 to 15 animals per treatment group. Also, the 1-month duration is acceptable for screening/testing purposes. The beagle dog model is also suitable for testing cartilage repair strategies. To this purpose the operation is followed by 1 month of observation before initiation of intra-articular treatments of chondrogenic agents. Since there is never eburnation to calcified cartilage or bone, repair strategies can be evaluated without the complication involved in exposure to pluripotential cells from the marrow. The different zonal severities allow evaluation of repair strategies on cartilage with moderate to minimal degenerative changes. Although I have used this model extensively over the last 5 years, there is only one published report on the efficacy of a chondroprotective agent in this relatively new model [23].

Complete removal of the medial meniscus in beagle dogs resulting in OA lesions has been described [39], with the lesions similar to those resulting from partial meniscectomy.

9.1.1.2 Dog Anterior Cruciate Ligament Transection Model of Osteoarthritis

Transection of the ACL results in a true instability-induced OA lesion that mimics the OA that occurs naturally in large dogs. The operation is performed in large, hound-type dogs (Walker hounds) or in mongrels with hound-type conformation [40,48,67]. In most dogs, the cartilage lesions progress to classic severe OA morphology only after 3 to 4 years [13,25], and the initial phase of the model (which occurs over the first 3 years) generally consists of hypertrophic changes [1] with some focal, more classic degenerative lesions. Structural changes include severe osteophytosis and marginal zone proliferation, especially in the patellar groove, and cartilage hypertrophy, characterized by focal or diffuse cloning, with matrix expansion resulting in up to a 70 % increase in cartilage thickness (Fig. 9.2A). Zonal evaluation of lesions is extremely important in these animals, as instability induced by the ACL transection results in excursion of the proximal femoral condyles (near trochlear

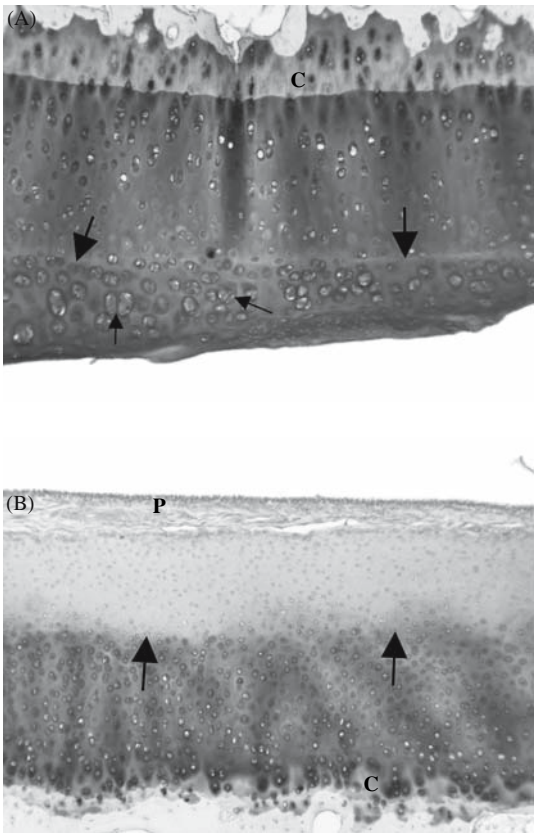

Figure 9.2. (A) Femoral cartilage (central area) from Walker hound dog with anterior cruciate ligament (ACL) transection (4 months) shows marked focal cartilage hypertrophy with numerous clones (small arrows) and junction of hypertrophic cartilage with subjacent, more normal-appearing cartilage (large arrows) Calcified cartilage (C) and tidemark are normal. (B) Tibial cartilage (anterior area) from Walker hound dog with ACL transection (4 months) shows proliferation of pannus-like tissue (P) at the cartilage surface with loss of proteoglycan in subjacent cartilage (arrows).

ridge) onto the anterior fibrocartilage of the tibia in an area that is normally not load bearing. Several distinctive lesions result from this process. The trochlear ridge may have a focal, full-thickness (to tidemark) lesion of cartilage degeneration. In addition, the anterior tibia (both medial and lateral) develops a pannus-like change that appears to be both anabolic and catabolic (Fig. 9.2B). This area is not normally load bearing, and in nonoperated dogs is fibrocartilaginous, rather than hyaline. Subchondral bone thickening occurs early and is easily detectable by imaging at 18 months postsurgery [24]. Disadvantages of the model include the time required to achieve classic OA pathology, the variability in the location and severity of the lesion, and the need to interpret complex structural changes (anabolic and catabolic in the same joint). Forced exercise may enhance the tendency of the animals to develop classic OA pathology more quickly, but testing for anticatabolic effects of agents is still likely to involve periods of at least 2 to 4 months. Lesions in this model are more likely to occur in the trochlear ridge and anterior tibia, but can occur anywhere in the knee joint, necessitating thorough sectioning of both tibia and femur. Because these are large dogs, medial and lateral surfaces must also be sectioned separately in order to fit into the standard paraffin blocks, resulting in four slabs of tissue of approximately equal thickness from each surface, for a total of 16 for each joint. Tibias are sectioned in the frontal plane, while femurs are sectioned in the sagittal plane. Dogs with ACL transection display a wide range of responses to the instability, ranging from severe meniscal destruction and fibrous repair and striking cartilage changes to near-normal menisci and minimal cartilage pathology [60].

Because this model has been used extensively, the effects of various agents in modifying disease have been widely described. Studies documenting the presence of cytokines such as tumor necrosis factor (TNF) or other mediators in diseased cartilage have utilized this model [37]. In addition, doxycycline and diacerhein, agents that exert chondroprotective action in human clinical trials for OA, have shown activity in this hound-dog model [56,77].

The obvious advantage of this model is that it provides an opportunity to study OA in a situation where pathogenesis mimics the naturally occurring disease. It also makes possible the evaluation of potential treatments on anabolic and catabolic processes in the same joint.

9.1.2 Rat Models of Osteoarthritis

Spontaneous OA is extremely uncommon in rats of all strains. In rare cases, minimal focal areas of tibial degeneration may be seen in the area not protected by the meniscus [55], and cartilage cysts may be observed, especially in the lateral tibial cartilage. However, neither of these changes occurs often enough to constitute a problem for the interpretation of the surgically or chemically induced OA in rats. The

most commonly used rat model is the medial meniscal tear; the next most common is the iodoacetate injection model, followed by the ACL transection model.

9.1.2.1 Rat Medial Meniscal Tear Model of Osteoarthritis

Unilateral medial meniscal tear in 275- to 300-g rats will result in rapidly progressive cartilage degenerative changes characterized by chondrocyte and proteoglycan loss, fibrillation, osteophyte formation, and chondrocyte cloning [11,35]. Utilization of younger rats (without tidemark) results in extremely large osteophytes and lesions more typical of those seen in osteochondrosis. As with most surgical models, evaluation of lesions with attention to zonal distribution is important. In the meniscal tear model the medial collateral ligament is transected just below its attachment to the meniscus, so that when the joint space opens, the meniscus is reflected toward the femur. The meniscus is cut in half at its narrowest point (away from the ossicles), and care must be taken not to damage the tibial surface and to ensure the resulting transection yields meniscal fragments that are freely movable both anteriorly and posteriorly. Because the lesion is mainly tibial, iatrogenic damage to the femur during surgery is of little consequence. Aseptic techniques should be used, but infection, to which rats are very resistant, is seldom a postoperative problem unless steroids or TNF inhibitors are being tested.

Cartilage degenerative changes (Fig. 9.3) develop progressively, and by 3 to 6 weeks postsurgery, tibial cartilage degeneration may be focally severe on the outer third of the tibia, with degenerative changes of lesser severity in the middle and inner thirds. Osteophytes are ultimately quite large (medial tibia) and progressively increase in size. The model is progressive and results in total cartilage loss (to eburnated bone) in 12 months in virtually all rats. Evaluation of antidegenerative therapies requires at least 1 to 3 weeks. A group of 15 to 20 animals is generally sufficient to account for variable lesion severity. Because cartilage degenerates rapidly in rats, this model constitutes an extremely high hurdle for the detection of protective effects. This is especially true for the outer third of the tibial cartilage, where collagen disruption and matrix loss are often quite severe. However, zonal analysis may result in detection of treatment effects in the middle and inner thirds of the tibia, areas in which mechanical trauma is not an important cause of pathogenesis. Substantial subchondral and epiphyseal bone changes occur in the medial tibia subjacent to the areas of greatest lesion severity. These changes range in magnitude and type from increased basophilia of the calcified cartilage with small fractures into subchondral bone to overt collapse of articular cartilage into areas of bone resorption in the epiphysis, surrounded by sclerosis of bone (Fig. 9.3). This model, therefore, facilitates evaluation not only of chondroprotective effects but also of bone preserving activities.

One advantage of using rats is that they are commonly used in toxicology testing. Efficacy in this species in combination with toxicology evaluation facilitates the generation of a therapeutic index for compounds under evaluation. Results can be obtained in relatively short periods, and the response of the animals to surgery is very consistent. Inhibitors of matrix metalloproteinases-13 are consistently active in the rat model [35]. This model has also been used to evaluate cartilage repair strategies [43]. Repeated injections of fibroblast growth factor-18 (FGF-18) into rats 3 weeks after the OA lesions were established led to chondrogenesis and the filling of defects in areas of severe degeneration. Evaluation of repair strategies in rodent models must always take into account the marked tendency for rodent marginal zones and pluripotential marrow cells to proliferate in response to irritant stimuli.

9.1.2.2 Rat Intraarticular Iodoacetate Injection Model of Osteoarthritis

Cartilage degeneration can be induced in virtually any species by the intra-articular injection of iodoacetic acid (IA), an inhibitor of aerobic glycolysis that kills chondrocytes [73]. Lesion severity depends on the concentration and frequency of injection. In rats, a single 25- to 50-μL injection of 10 mg/mL sodium iodoacetate is sufficient to kill most of the chondrocytes [36]. The chondrocytes at the far outer margins of the joint (marginal zone), in the area where chondrophytes/osteophytes form, usually survive this insult and ultimately proliferate to form these structures. The insult in

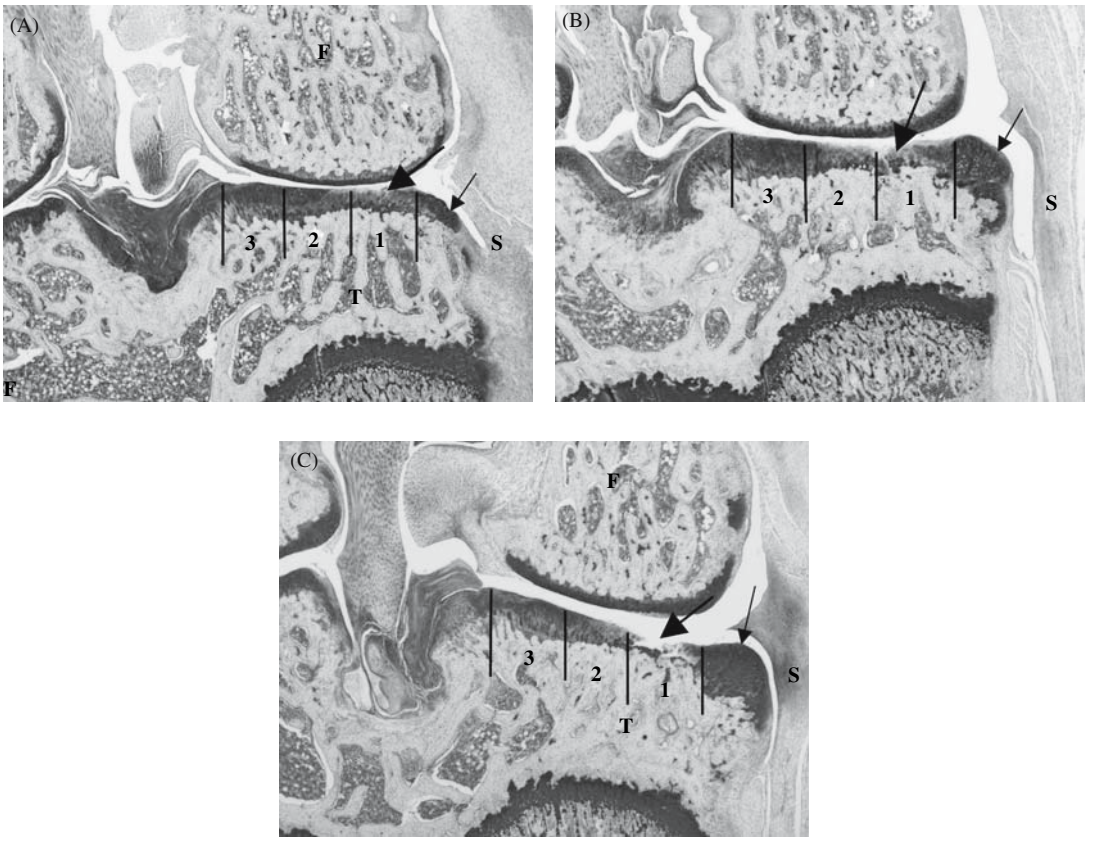

Figure 9.3. (A) Medial knee of rat with meniscal tear (1 week) shows minimal to mild degeneration in the outer third of the tibial (T) articular cartilage (large arrow) and minor marginal zone proliferative changes (small arrow). Bars divide the cartilage into zones 1, 2, and 3, and illustrate that the most severe changes are in zone 1 adjacent to the synovium (S). (B) Medial knee of rat with meniscal tear (3 week) shows moderate to marked degeneration in the outer two thirds of the articular cartilage, with the most severe changes being in zone 1 (large arrow) and minimal superficial changes in the inner third. Bars divide the cartilage into zones 1, 2, and 3, and illustrate that the most severe changes are in zone 1. A large osteophyte is present on the tibia (small arrow), and subchondral bone fractures as well as subchondral bone sclerosis are evident below the large arrow in the area of most severe cartilage degeneration. (C) Medial knee of rat with meniscal tear (6 week) shows marked to severe degeneration in the outer two thirds of the articular cartilage, with the most severe changes being in zone 1 (large arrow) and minimal superficial changes in the inner third. Bars divide the cartilage into zones 1, 2, and 3, and illustrate that the most severe changes are in zone 1. A large osteophyte is present on the tibia (small arrow), and subchondral bone fractures as well as marked subchondral bone sclerosis are evident below the large arrow in the area of most severe cartilage degeneration.

the presence of normal load-bearing results in a progressive loss of proteoglycan (days 3 to 14 postinjection), as evidenced by decreased toluidine blue matrix staining and atrophy of the remaining collagenous portion of the matrix. Fibrillation is a late change (days 15 to 21), as is the collapse of the remaining collagenous matrix into partially resorbed and degraded subchondral bone (Fig. 9.4). The bone changes in this model are quite striking and form the basis for cartilage lesions observed macroscopically. Ultimately, large osteophytes are formed and bone sclerosis occurs [31]. Characterization of the time course of aggrecan and type II collagen degradation has revealed an increase in both aggrecanase-and MMP-generated epitopes, with the NITEGE aggrecanase neoepitope being significantly elevated on days 7, 14, and 21. The DIPEN MMP neoepitope is elevated on days 7 and 14 [36]. The type II collagen neoepitope recognized by Col2-3/4Cshort does not increase until days 14 and 21, coinciding with the period of observable collagen degradation. Potential uses for this rat

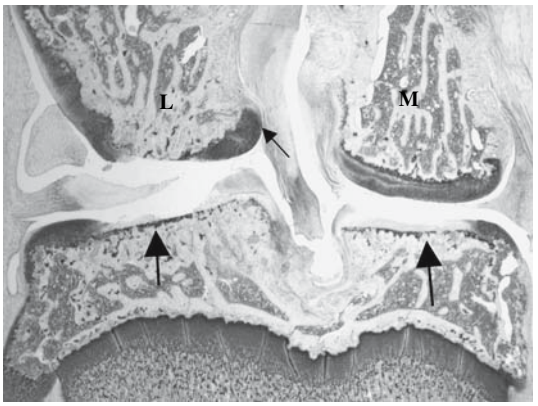

Figure 9.4. Medial knee of rat injected with iodoacetate (3 weeks) shows typical alterations characterized by cartilage loss (large arrows, both tibias) and resorption of subchondral bone and calcified cartilage. Note that sclerosis of subchondral bone (lateral-L femur) is also present in areas where resorption has occurred. Marginal zone chondrogenesis is especially evident on the inside aspect of the lateral femur (small arrow). Medial (M) femoral cartilage has areas of chondrocyte and proteoglycan loss, but collagen matrix is retained.

model include evaluation of agents designed to inhibit acute proteoglycan loss mediated through aggrecanase or MMPs (days 7 to 14 termination), or collagen matrix degeneration via collagenase (day 21 termination). The model can also be used to evaluate effects on gait alterations. Since osteophyte formation is a prominent feature, the model can be used to study induction or inhibition of osteophyte formation.

9.1.2.3 Rat Anterior Cruciate Ligament Transection Model of Osteoarthritis

Another rat model that is gaining in popularity is the ACL transection model. Mature (tidemark well formed) rats at least 10 weeks of age undergo surgical transection of the ACL [32,58]. Animals develop progressive changes, mainly on the medial side of the joint. In general, joints exhibit proteoglycan loss by 2 weeks postsurgery and areas of fibrillation by 4 weeks. This is therefore a more slowly progressing, milder OA model than the meniscal tear model. Interestingly, many of the same alterations described in dogs with ACL transection occur in rats (pannus on anterior tibia, cartilage hypertrophy, large osteophytes, and marginal zone proliferative changes); ACL transection in immature rats leads to osteochondrosis, with the most striking lesions in the area of the articular-epiphyseal junction. The lesions consist of cartilage hypertrophy and separation from subchondral bone. Advantages of this model include more slowly progressive lesions that may be more amenable to therapeutic intervention by noncollagenase inhibitor therapies. Disadvantages are similar to those described for the dogs with respect to variability in lesion severity and location and the need to evaluate anabolic as well as catabolic changes.

9.1.3 Mouse Models of Osteoarthritis

Spontaneous OA occurs in the knee joints of various strains of mice [15,44,45,57,69,70,72,76], and transgenic and mutant mouse models of OA have been developed and characterized [27, 29,33,41,51,52,59,74,75]. Naturally occurring or transgenically induced disease often results in slowly progressive disease, so the period for drug testing or pathogenesis study is long. However, pathology and pathogenesis (especially in the case of spontaneous models) are similar to what occurs in the most common forms of slowly progressive human disease. Disease progresses more rapidly and consistently in several induced mice models (see below). These models have also been used to study mediators involved in matrix destruction or to evaluate potential drugs [12].

9.1.3.1 Spontaneous or Mutational Osteoarthritis in Mice

Various factors including patellar luxation [57,69,70], varus or valgus conformational abnormalities [72], and other genetic defects including mutations in the type II collagen gene [33,51,52] have been implicated in causing knee OA in some strains of mice. The STR/ort model of arthritis is the most extensively characterized with respect to morphologic and biochemical changes in cartilage [49]. The model has also been used to evaluate inhibitors of matrix metalloproteinase (collagenase) [15]. Approximately 85 % of the male mice of this strain develop some evidence of OA in their knee joints (medial tibial plateau) by 35 weeks of age; the disease becomes progressively more severe with time. Both aggrecanase and collagenase activity have been demonstrated in the developing cartilage lesions. Studies using

this model have generally demonstrated that collagenase activity can be detected only in lesions and not in regions where the cartilage is morphologically normal. Testing of potential antiarthritic agents in strains of mice with spontaneous OA or where OA was due to genetic manipulations is somewhat uncommon due to issues associated with animal availability and the extended time frame required. Nonetheless, these studies have provided valuable insight into OA pathogenesis and comparison with the disease in humans and other animals.

9.1.3.2 Surgically or Chemically Induced Osteoarthritis in Mice

As with virtually all other animal species, lesions of knee OA in mice can be induced by creating surgical instabilities. Medial collateral ligament transection with partial medial meniscectomy [21] results in moderate to severe degeneration of medial cartilage, with the lesion developing in about the same time as in rats. Anterior cruciate ligament transection and medial meniscal ligament transection have also been described, with the ACL model resulting in severe lesions and the ligament transection model generally resulting in proteoglycan loss with much less collagen destruction. These models have been utilized to investigate the importance of various mediators of cartilage destruction (especially ADAMTS-4 and 5, aggrecanases) in severe versus milder disease [30].

Intraarticular injection of bacterial collagenase (type VII, *Clostridium histolyticum*) into the knee joints of mice induces lesions of OA that vary in incidence and severity [62–66]. In studies designed to investigate the potential utility of this model for drug testing, we determined that trauma to the ACL induced by intraarticular injection of saline could consistently induce the development of OA lesions in a 1- to 3-week period. Subsequently we also determined that simple trauma to the ACL by simulating an injection (insertion of a 28-gauge needle on an insulin syringe, 1 to 2 times) would destabilize approximately 95 % of joints. This OA induced in the knee by ACL instability has morphologic features that are similar to lesions observed in other models of OA. The model, therefore, may be useful for pharmacologic testing [12]. Although successful destabilization is not always achieved, knees that have been destabilized can easily be identified by proliferative changes in the medial aspect of the joint, and joints without evidence of successful destabilization can be eliminated from the data set (Fig. 9.5). As in the rat and dog ACL transection models, there are areas of cartilage degeneration that result from mechanical trauma, as well as areas that result from enzymatic degradation. Another similarity is the presence of profound anabolic effects that result from attempts by the mouse to restabilize the knee. Anabolic activity in the mouse is marked in the medial tibial and femoral marginal zones and in the collateral ligament. Because the cartilage lesions are distributed over all four cartilage surfaces of the knee, all four must be included in any histopathologic evaluation. In addition, dividing the cartilage into three zones, as in the rat meniscal tear model, reveals distinctive regions of varying severity. In general, the most severe, mechanically abrasive changes occur on the medial femoral condyle and the outer third of the medial tibial plateau. Milder lesions, potentially more receptive to pharmacologic intervention, occur on the inner two thirds of the medial tibia, the lateral femoral condyle, and the lateral tibial plateau. Extreme changes in subchondral and epiphyseal bone occur in locations where mechanical forces have been altered. These range from mild sclerosis to eburnation of cartilage and extreme sclerosis.

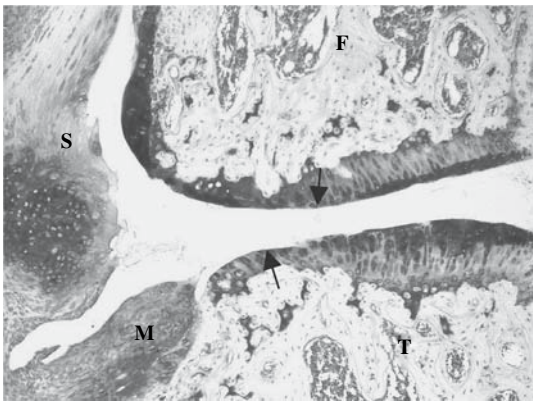

Figure 9.5. Medial knee from mouse with ACL trauma (3 weeks) has marked proliferative changes in the medial collateral ligament and synovium (S), as well as in the tibial (T) marginal zone (M) with areas of severe cartilage loss on the medial tibia and femur (small arrows) and milder changes adjacent to these more severe alterations.

This model is probably the best example of the need to match the expectation of what may be achievable with where the lesion is located before testing pharmacologic agents. This will avoid anticipating a benefit to the areas of mechanical abrasion. Different mouse strains also exhibit different lesion severity when subjected to ACL trauma, probably as a result of different activity levels (author's unpublished observation). This situation can then be utilized to advantage. The potential utility of this model for pharmacologic evaluation of antiarthritic agents is yet to be determined. However, it is a simple model that can be utilized in relatively inexpensive strains of mice or in mice also used for transgenic and knockout studies. Intraarticular injections in mice should be done very cautiously, as there is a high probability of damaging the cruciate ligaments and inducing OA lesions when this is not the experimental goal.

9.1.4 Guinea Pig Models of Osteoarthritis

Spontaneous OA in guinea pigs represents a well-characterized, important animal model of OA [2–6]. However, as with all spontaneous models, time to disease development is often a factor limiting the model use, so surgical models of OA in guinea pigs have also been developed [7,10,11]

9.1.4.1 Naturally Occurring Osteoarthritis in Guinea Pigs

Spontaneous OA occurs in the medial compartment of the knee joint of male and female Hartley albino guinea pigs [2–6] and of some other strains [53]. Although OA occurs spontaneously in both males and females, males tend to grow faster, reaching greater body weights, and therefore tend to have more consistent pathologic alterations. The disease is generally bilaterally symmetrical with respect to incidence and severity, and the earliest changes can be seen when animals are approximately 3 months old and weigh about 700 g [5]. The lesions are initially present on the medial tibial plateau in the area not protected by the meniscus, and consist of focal chondrocyte death, proteoglycan loss, and fibrillation. Usually about 50 % of the animals of this age and weight will have minimal focal changes. The underlying chondrocytes do not exhibit cloning at this stage, nor are there morphologic changes in subchondral bone, menisci, or synovial membranes.

When animals are 6 months old and weigh approximately 900 g, minimal to moderate lesions (extending into middle zone) are present in 90 to 100 % of the medial tibial plateaus. Lesions are generally bilaterally symmetrical. Histopathologic features include chondrocyte death/loss extending into the upper middle zone, fibrillation, and proteoglycan loss. In addition, cloning extends well into the middle and sometimes into the deep zones, and shifts in toluidine blue orthochromatic (blue) to metachromatic (purple) staining of matrix occur. This indicates changes in proteoglycan synthesis in areas that do not undergo the severe changes that lead to fibrillation/cartilage loss. Small osteophytes are often present at the outer aspect of the medial tibial plateau. There are generally no obvious subchondral bone changes, meniscal degenerative changes, femoral cartilage degeneration, or any synovial inflammation at this stage.

Nine-month-old animals have mild to moderate medial tibial cartilage degeneration, mild femoral condylar degeneration, and tibial osteophytes. Mild degenerative changes may be present in the menisci, and synovial membranes may be minimally thickened as a result of synoviocyte proliferation. Early sclerosis of medial tibial subchondral bone may also be apparent. Femoral cartilage degeneration lags behind tibial degeneration in development, and small focal degenerative changes that are minimal to mild become apparent at 8 to 9 months.

By the time the animals are 1 year old, cartilage degenerative changes are usually quite profound and involve all aspects of the medial compartment of the knee. Chondrocyte and proteoglycan loss with fibrillation may extend into the deep zone, and cloning is prominent. Subchondral sclerosis is often extensive, and subchondral bone cysts are present with severe meniscal degenerative changes. Synovial hypercellularity increases and papillary proliferation is seen. Osteophytes, usually with significant cartilaginous matrix remaining, may be very large and contribute to the marked recontouring of the shape of the medial tibial plateau and medial femoral condyles. Clinical abnormalities in gait and ability to extend the knee joint are detectable at this stage.

Severe medial compartment degenerative changes (tibial and femoral) are present in animals that are 18 months to 2 years and older. Their medial surfaces are dramatically contoured, and they have bone sclerosis, bone cyst formation, and large osteophytes that have undergone near-complete endochondral ossification. The synovium is thickened as a result of papillary proliferation and mild mononuclear inflammatory cell infiltration. Mild to marked degenerative changes may be present on the lateral side of the joint of some animals six months or older.

The pathogenesis of naturally occurring knee OA in guinea pigs is not completely understood. However, as is the case in human disease [24], body mass is an important factor. When guinea pigs are placed on restricted diets that are designed to decrease overall food consumption and thus to prevent the animals from becoming sedentary and obese, the incidence and severity of knee OA are greatly reduced [8]. In a study in which guinea pigs were exercised on treadmills from an early age (approximately 2 months to 5 months), OA lesions, instead of being enhanced, as might be expected, were decreased along with their body weights (author's unpublished observation).

Guinea pigs seem to load the medial aspect of the knee joint preferentially. This is brought out by the fact that medial meniscectomy results in severe lesions and lateral meniscectomy results in mild to no lesions (author's unpublished observation). This is similar to the situation in humans, where approximately 75 % of the load (normal conformation) passes through the medial aspect of the knee [16]. Therefore, any additional stress, such as increased body mass, would tend to load this area further, and possibly contribute to adverse matrix/cellular changes that lead to degeneration.

Other theories that have been proposed to explain the increased incidence of instability and disease include the presence of bone cysts in the area where the cruciate ligaments attach [71]. Although these cysts may contribute to the pathogenesis, they also occur routinely in aging rats, a species that has virtually no spontaneous knee OA.

Recent advances in the development of matrix metalloproteinase (MMP) inhibitors have raised concerns about the expression in rodents of the various MMPs, a family of highly homologous zinc endopeptidases that include the collagenases, stromelysins, and gelatinases. Both collagenase 1 (MMP-1) and collagenase 3 (MMP-13) have been implicated in the pathogenesis of arthritis in humans [42,50]. Guinea pigs, unlike rats and mice, express both collagenase 1 and 3 at the site where OA lesions develop [34]. Mechanical forces increase expression of collagenase 1 messenger RNA (mRNA) [68]. The high levels present on the medial aspect of the guinea pig knee may therefore be in response to increased loading, relative to the lateral side. Guinea pigs with OA also express patterns of an early biochemical neoepitope marker of OA, called 3-B-3(−) [18]. This is due to a change in the termini of the chondroitin sulfate (glycosaminoglycan) chains of aggrecan [19,54] and is also found in human OA cartilage.

Because of the very predictable manner in which guinea pigs develop spontaneous knee OA and the obvious similarities to human disease, the model can be used for a variety of studies, such as pathogenesis and potential therapeutic intervention. Diacerhein has been effective in this model [9] and taken through clinical trials designed to determine safety and disease-modifying activity [26,47].

9.1.4.2 Guinea Pig Medial Meniscal Tear-Induced Osteoarthritis

Surgical instability from medial meniscal tear in guinea pigs, similar to that described for rats, results in cartilage degeneration in the knee joints [10,11]. Since guinea pigs preferentially load the medial aspect of the knee joint, the meniscal tear procedure must be done on the medial side in order to induce consistent pathologic alterations. Similar surgery on the lateral meniscus results in highly variable, if any, cartilage degenerative changes. Three-month-old male guinea pigs (with well-formed tibial tidemark) are utilized. If surgery is performed on immature animals, the histologic appearance of the lesions will resemble that of osteochondrosis rather than OA. The medial collateral ligament is transected and the medial meniscus is transected at the approximate midpoint. Guinea pigs killed 3 days post–meniscal tear exhibit loss of medial tibial chondrocytes and proteoglycan (as evidenced by decreased toluidine blue staining) in only the superficial and upper middle zones of the articular cartilage. Mild collagen disruption of the superficial layer

is also evident. Moderate acute inflammation, edema, and fibroblast proliferation are evident in the transected synovium. At 3 weeks postsurgery, cartilage degeneration in guinea pigs extends through one third of the medial tibial articular cartilage, and large chondrophytes are present on the tibia. Smaller chondrophytes are evident on the opposing femoral condyle. Inflammation is absent from the synovium, which is markedly thickened as a result of fibrous tissue proliferation. Animals killed at 6 weeks postsurgery have cartilage degeneration that extends into the middle zone of the medial tibial plateau, and cloning of chondrocytes is evident in the subjacent cartilage. Large tibial chondrophytes undergoing endochondral ossification are present. By 12 weeks postsurgery, degeneration extends into the deep zone of the cartilage, and clones are prominent. Chondrophytes exhibit extensive but incomplete ossification. The synovium is still thickened as a result of fibrous tissue proliferation. The importance of load bearing in the generation of these lesions has been made evident by studies in which sciatic nerve transection was done in conjunction with meniscal damage [7]. In short-term studies, animals failed to develop typical OA-like lesions of cartilage degeneration in the absence of loading.

So far no findings have been published in this model that describe activity of agents now in human clinical trials.

9.1.5 Rabbit Models of Osteoarthritis

Because there is minimal evidence of spontaneous cartilage degeneration in rabbit knee joints, all rabbit models of OA are induced. Although ACL transection can induce OA lesions, the most commonly used models employ medial or lateral meniscectomy.

9.1.5.1 Rabbit Meniscectomy-Induced Osteoarthritis

Partial meniscectomy surgery in New Zealand white rabbits (body weight $\simeq$4 kg) results in lesions that resemble those occurring in human OA. Rabbits, unlike rats, mice, and guinea pigs, preferentially load the lateral aspect of the knee joint. Partial meniscectomy on the medial aspect of the joint generally results in relatively mild to moderate degenerative changes. This model has been used extensively to test potential chondroprotective agents [44]. Partial lateral meniscectomy induces a very consistent focal degenerative change that involves approximately half of the lateral tibial plateau and femoral condyle [22]. If the surgeon is consistent in removing from each meniscus tissue of the same size and from the same lateral location, and if the histotechnologist is consistent in the sectioning, animals at 6 weeks postsurgery will have lesions that are remarkably consistent. The surgical procedure involves transection of the fibular collateral ligament prior to entry into the joint space.

Because of the larger size of the rabbit joint, it is preferable to disarticulate the tibia and femur and visualize the gross lesions on both surfaces. Both tibia and femur (after decalcification) can be trimmed into two to three slabs of approximately equal size (from anterior to posterior) and embedded with the posterior surface down. This will result in two to three sections that reliably represent the extent of lesions on the various surfaces. Unlike the situation in rodents, where the scoring is generally the average for the various sections, it is best to sum the scores for the tibial and femoral sections so as to arrive at a total joint score that reflects the area of the lesion.

Although partial lateral meniscectomy offers a model with very consistent lesion development with respect to location and severity, the lesions progress fairly rapidly, thus offering a challenge for therapeutic intervention. The same zonal approach to evaluation already described for all other species must be applied in order to separate areas of mechanical trauma from areas of enzymatic matrix degradation. Termination of the study ($N = 10$/group) 3 to 6 weeks postsurgery is adequate to evaluate compounds for their effect on focal chondrocyte loss, proteoglycan loss, and fibrillation. Osteophyte formation will be fairly striking and subchondral bone on the lateral side will have obviously thickened trabeculae at 6 weeks postsurgery. The presence of subchondral bone alterations will indicate a shift in the loading patterns as a result of the instability created at surgery. Ideally, when a compound is being tested, the bone changes should be present,

thus confirming the surgical procedure, but the cartilage matrix would be less damaged as a result of protection by the compound. If the matrix is seen to be protected in the absence of bone changes, provided the compound did not act on bone remodeling. It is important to make sure the surgical procedure was successful.

Rabbits have tremendous capacity to regenerate the transected meniscus with fibrous tissue. Therefore, when the joint is opened 6 weeks postsurgery, it is often unclear whether the meniscus was actually transected. The presence of subchondral sclerosis makes it likely that the surgery was done correctly.

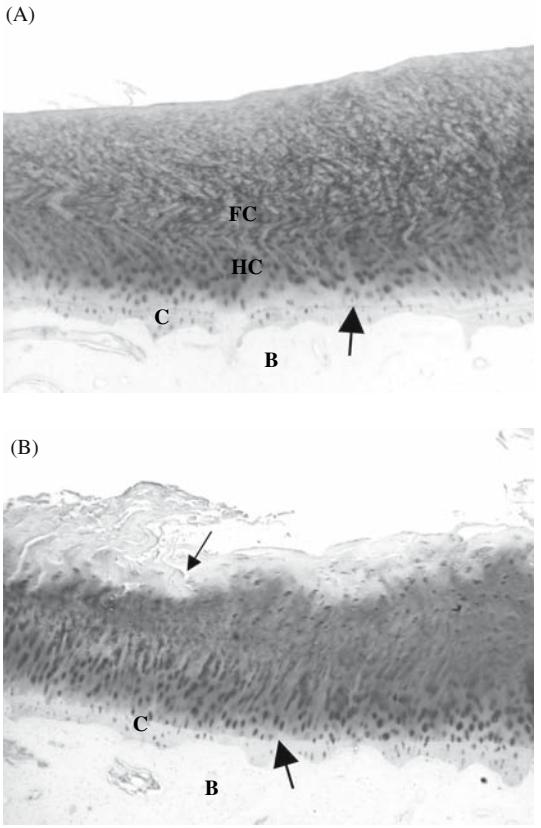

Figure 9.6. (A) Normal medial tibial cartilage of cynomolgus monkey shows fibrocartilage (FC) morphology from surface to about two thirds of the depth, with deep zone having a slightly more hyaline appearance (HC). Calcified cartilage (C), tidemark (arrow), and subchondral bone (B) are normal. (B) Osteoarthritic medial tibial cartilage of cynomolgus monkey shows similar fibrocartilage morphology in intact areas with typical lesions (arrow) extending into the mid zone). Calcified cartilage (C), tidemark (arrow), and subcondral bone (B) are normal.

9.1.6 Primate Models of Osteoarthritis

Spontaneous OA has been described in knee joints of nonhuman primates [17,20]. Variability in lesion severity and difficulties associated with obtaining adequate numbers of primates for meaningful studies probably preclude general use of this interesting model. The tibial cartilage of cynomolgus monkeys is largely fibrocartilaginous, whereas the femoral cartilage appears hyaline (Fig. 9.6). This is an important morphologic feature that must be recognized when studies of anti-degenerative agents or anabolic therapies are contemplated in this monkey species.

9.2 Discussion

Virtually all spontaneous or surgical animal models of OA ultimately result in morphologic changes that resemble those occurring in some stage of human OA. Clearly, the spontaneous models offer the best opportunity to study the slowly progressive OA that is most characteristic of human disease.

Surgically induced models of OA, especially in rodents and rabbits, usually have rapid and severe cartilage degeneration after the instability has been created. Generally, greater instability produces a more severe lesion. However, the load-bearing tendencies of the particular species can be factored in, and regions of cartilage more prone to mechanical (abrasive) injury can be separated by zonal analysis from regions with more enzymatic damage.

Surgically induced instability models of OA have been described in most animal species utilized in OA research. Because traumatic OA occurs in humans, the models must mimic at least some aspects of OA pathogenesis and pathology. One important difference, however, is that humans with a traumatic injury generally decrease use of the affected limb until restabilization has occurred. In the author's experience, animals (especially rodents) in the same situation generally do not decrease use of the affected limb. Disease progression, therefore, tends to be much more rapid in animal models, making the experimental situation less amenable to therapeutic intervention. Since

most surgical models use knee joints, an important consideration in the use of surgical instability models is the load-bearing (medial vs. lateral) pattern of the species being used. Animals that predominantly load the medial aspect of the joint will develop more severe lesions on the medial side after a medial meniscectomy than on the lateral side after lateral meniscectomy, and vice versa. These features can be utilized to create different types of matrix degeneration.

Animal models of OA have been used fairly extensively to test potential antiarthritic agents and disease-modifying effects have been reported [9,26,38,46,47,56,61] for agents that are currently being used to treat OA patients. Documentation of efficacy in human clinical trials (other than symptomatic relief) is lacking, largely due to the difficulties in monitoring OA disease progression and the long duration of clinical trials. Therefore, unlike the situation in rheumatoid arthritis, where several well-characterized animal models have been shown to predict human efficacy, there currently is no "gold standard" animal model for osteoarthritis.

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10.

Biomechanical Aspects: Joint Injury and Osteoarthritis

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10.1 Introduction

Osteoarthritis (OA) is a degenerative joint disease of mechanical wear-and-tear, often localized in weight-bearing joints such as the knees and hips [55]. Age is the most important risk factor for OA, but joint trauma, repetitive joint use, obesity, and gender are also risk factors for the disease [55]. Arthritis is associated with a progressive wearing away of the articular cartilage of the joint surface caused by mechanical injury, joint instability, or an inappropriate response to normal mechanical stimuli [3]. The molecular pathogenesis of the disease is associated with loss of aggrecan, damage to the collagen network, and, ultimately, loss of normal chondrocyte phenotype and a limited degree of chondrocyte cell death [3]. Chondrocyte dedifferentiation, marked by the decrease in type II collagen and aggrecan and an increase in collagen type I and type X, occurs as the disease progresses and marks the beginning of the inevitable end as dedifferentiated cells can no longer synthesize useful matrix material [3]. Although the mechanism of matrix loss and the driving force for the disease progression are not completely understood, acute mechanical injury and prolonged inflammatory insult increase the risk of developing osteoarthritis [73].

Cartilage degeneration is driven by the entire synovial joint; however, the chondrocyte, by virtue of its location within cartilage, plays a primary degenerative role by producing matrix-degrading proteases, altering the synthesis of matrix molecules, and producing inflammatory cytokines and inappropriate levels of morphogenetic or growth factors [3,40]. In response to joint injury or cytokine stimulation, latent matrix proteases or newly secreted proteases may rapidly degrade aggrecan [1,85,117,118], with loss of aggrecan significantly altering the mechanical properties of the tissue [120,121]. Chondrocytes, however, may synthesize and replace lost aggrecan, causing the tissue moduli and function to return to normal, without long-term damage [8, 93,121]. Collagen network damage, on the other hand, seems to be an irreversible step in the pathogenesis of osteoarthritis [3]. Protease-induced collagen degradation often occurs after aggrecan depletion; this suggests that aggrecan may protect collagen fibrils from proteolytic degradation [95].

Joint injury can lead to loss of chondrocyte viability and to damage of cartilage matrix and other joint tissues, for example, ligaments, tendons, and synovium. The resulting change in cartilage matrix composition and mechanical properties may be partly responsible for increasing the risk of arthritis, inasmuch as chondrocytes can rarely repair the damage [14]. Thus, human knee injuries including anterior cruciate ligament (ACL) or meniscal tears may significantly increase the relative risk of developing OA, a risk that increases with age at the time of injury and with the time

that has elapsed since the injury [39,104,122]. Joint instability may contribute to secondary disease development, yet ACL correction does not seem to decrease the risk of OA development [15,35,76,103]. The acute traumatic event, therefore, may have been sufficient to trigger a cascade of irreversible effects that initiate arthritis and cause its progression.

Osteoarthritis can have an inflammatory component, which involves the production of cytokines, continued local, low-level inflammation without inflammatory cell migration, and accompanying systemic immune responses. In vivo, mechanical joint injury occurs with concomitant inflammation, characterized by an increase in proinflammatory cytokines, tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) [17,18,49], as well as by an increase in matrix metallo proteinase-3 (MMP-3), cartilage oligomeric matrix protein (COMP) fragments, collagen II crosslinks, and aggrecan fragments [72,75,77,78,115]. Proinflammatory cytokines IL-1β and, to a lesser extent, TNF-α cause extracellular matrix breakdown in cartilage and may be present in OA.

10.2 Joint Loading and Cartilage Biomechanics: Changes with Osteoarthritis

Articular cartilage functions as a weight-bearing and lubricating layer in joints that absorb and distribute loads to underlying bone. Cartilage is exposed to a variety of mechanical forces during normal joint loading in vivo [9,44,46]. Loading across joint surfaces can be highly nonuniform [9,46]. Whereas local stresses in the hip may reach 10 to 20 MPa during activities such as stair-climbing [9,44,46], forces of two to five times body weight in the hip during walking [5] cause peak stresses on the cartilage surface of 2 to 3 MPa [46]. These forces cause changes in cartilage thickness in vivo as high as 20%, though long-term (static) loading causes even greater compressive deformation [9,44,46].

The structure of the extracellular matrix of cartilage is designed to withstand the dynamic range of forces. The main structural components are densely packed, negatively charged aggregating proteoglycans (aggrecan), water (~80%), and a network of collagen fibrils. Aggrecan contributes to the compressive stiffness of the tissue, while collagen provides tensile and shear resistance. Tissue biomechanical properties vary with depth and are strain-dependent [12,69,120]. The equilibrium compressive stiffness measured in uniaxial confined or unconfined compression is on the order of 1 MPa, and the frequency-dependent dynamic compressive stiffness is approximately 5 to 10 times greater (reviewed in [65]). Alterations in the matrix structure, due to mechanical injury or pathologic degradation, can greatly affect the mechanical properties and therefore the deformations experienced by the tissue.

In osteoarthritis, changes in matrix content and structure weaken the tissue and lead to changes in the subchondral bone [37,53,108]. Loss of collagen integrity, increased water content, decreased aggrecan content, and alterations in glycosaminoglycan (GAG) sulfation (charge) patterns contribute to decreases in mechanical stiffness of the tissue [7,13,53]. Kleeman et al [57] found that equilibrium stiffness decreases significantly with disease progression from 0.5 MPa to 0.28 MPa. This decrease is reflected in the grade assigned to the tissue when the International Cartilage Repair Society (ICRS) grade scale is used. The ICRS grade scale is based on gross examination of fissures, cracks, lesion extent and depth, and the Mankin score.

10.3 Clinical Findings

Injurious joint loading results in peak stresses and deformations that can be significantly higher than the normal ranges quoted above and can lead to significant tissue and joint damage. Insights concerning the molecular mechanisms of cartilage degeneration in vivo have come from analyses of synovial fluid samples taken from Swedish patients after an ACL or meniscal tear [71,73,74]. The concentration of proteoglycan fragments in the synovial fluid was elevated two- to three fold after injury, and these levels were similar to those found in patients with primary OA [71]. Enzyme-linked immunosorbent assay (ELISA) analysis of the synovial fluid revealed the presence of matrix metalloproteinase-3 (MMP-3), a protease linked to matrix degradation; MMP-3 levels in synovial fluid were markedly increased at presentation

and remained elevated for many years [73]. Joint fluid also showed an initial and persistent elevation of the neoepitope Col2CTx in the C-telopeptide crosslinking domain of type II collagen. This indicates digestion of mature, crosslinked collagen by a matrix metalloproteinase. Taken together, these clinical studies suggest that proteoglycan and collagen degradation rates are significantly altered within days of the injury and remain altered for years. It thus seems that the acute response by the joint tissue to the original mechanical insult initiates an unbalanced degradative process that can significantly increase the risk of OA.

10.4 In Vitro Models of Acute Mechanical Injury to Cartilage

10.4.1 Biomechanical Parameters

For more than a decade, in vitro model systems have been developed and specialized to study the effects of acute mechanical trauma on articular cartilage. For example, the incubator-housed instrument shown in Figure 10.1A can apply compressive loads or displacements to individual or multiple geometrically defined cartilage explant disks held in specially designed autoclavable loading chambers that are mounted within the instrument [36]. Direct mechanical compression of tissue can be performed by applying a known force ("load control") or a known displacement ("displacement control") to one surface of the specimen via a solid platen, while the opposite platen is held fixed. One-dimensional ("uniaxial") *unconfined* compression [24,70] typically employs a nonporous compression platen, and the sample is allowed to bulge slightly and exude fluid in the radial direction (Fig. 10.2 A [66]). In radially *confined* compression, a barrier is placed around the circumference of the sample and a porous compression platen is used; the sample is not allowed to bulge radially, and fluid flow occurs in the axial direction emulating an articular surface geometry [67]. Joint loading in vivo produces within the tissue a complex, nonuniform three-dimensional distribution of stresses and strains that has attributes of the idealized confined and unconfined loading configurations.

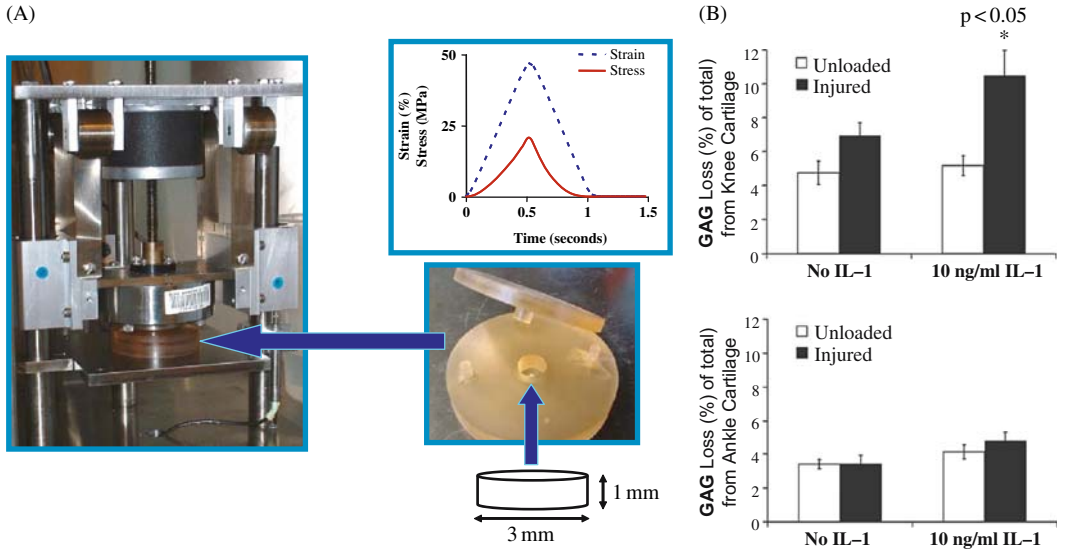

Figure 10.1. (A) Example of an apparatus for applying injurious mechanical compression to individual cartilage explant disks. A triangle wave of displacement reaching 50% compression in 0.5 sec is applied, resulting in a peak stress of ~20 MPa. (B) Glycosaminoglycan (GAG) loss 3 days after IL-1 treatment of normal and mechanically injured human knee and ankle cartilage. Unloaded and injured cartilage from adult human donors was incubated with 0 or 10 ng/mL IL-1α, and the GAG content of the medium was measured after 3 days of culture. In the knee tissue, incubation of injured tissue with IL-1 caused a synergistic increase in loss of GAG ($n = 8$, $p < .05$ for interaction). In ankle tissue from the talar dome, the interaction between injury and IL-1 treatment was not significant ($n = 6$, $p = .50$ by two-way analysis of variance). (A) from [64]; (B) from [88].

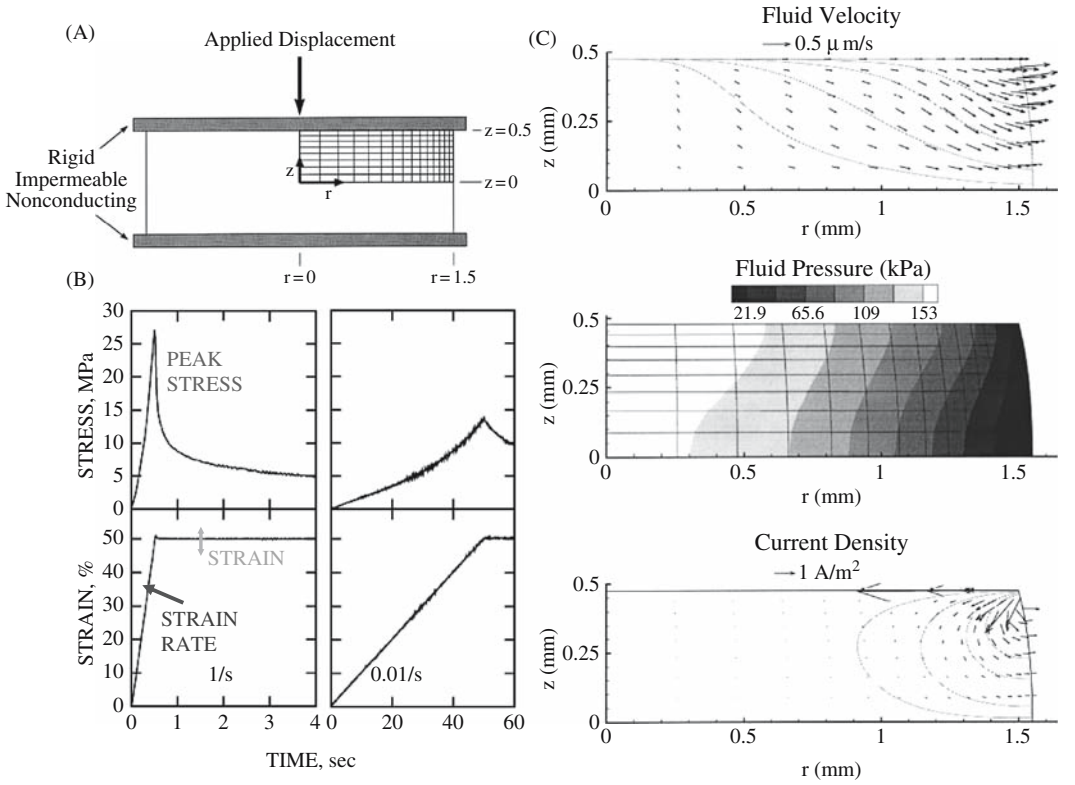

Figure 10.2. (A) Schematic of radially unconfined axial compression geometry to apply injurious compression to a cylindrical disk cartilage specimen. (B) Application of a "ramp-and-hold" compression in the configuration of A to 50% strain at strain rates of 1% /sec and 100% /sec; higher strain rates produce higher peak stress. (C) Spatial profiles of intratissue fluid velocity, fluid pressure, and current density in the top quadrant of A induced at the end of a 60-sec ramp, similar to that in B, but employing only a 5% (noninjurious) compression. The fluid flow is predominantly radial with maximal flow near the radial edge. Radial and axial pressure gradients develop, with maximal pressure near the center of the disk beneath the loading platens. Interestingly, an electrical streaming current is also induced with relatively high magnitude and inwardly directed beneath the loading platens, along with lower, more dispersed, outwardly directed currents toward the midplane. In principle, each of these physical forces affects cell behavior. (B) Adapted from [58]; (A,C) adapted from [66].

The choice between confined and unconfined loading for in vitro studies may depend on assumptions underlying the experimental hypotheses, as well as on practical considerations concerning maintenance of the organ culture with an adequate nutrient supply. Whether loading is confined or unconfined, it is essential to understand the spatial distribution of physical forces and flows within the explant (e.g., intratissue strain, fluid flow, fluid pressure gradients, etc.). These fields and flows can be highly nonuniform in either configuration, and the resulting cellular responses, therefore, are equally nonuniform. For example, a compressive strain applied rapidly (at a high "strain rate") to a cylindrical cartilage disk results in high peak stresses that may damage the tissue matrix (Fig. 10.2B). Chondrocyte biosynthesis changes and glycosoaminoglycan (GAG) is lost in response to such injurious compression. However, quantitative autoradiography at the tissue and cell levels has shown that cellular biosynthesis [16,99] and GAG loss can vary markedly across the tissue cross section. Corresponding theoretical analyses of the biomechanical and biophysical forces and flows have also shown that intratissue fluid flow, hydrostatic pressure, and even electrical streaming currents induced by compression will vary within the explant disk with radius and height (Fig. 10.2C[66]). It therefore may be possible to correlate the resulting spatially nonuniform matrix and cellular responses with the spatially varying biomechanical stimuli, thereby enabling the investigator to understand better which

biomechanical parameters are responsible for the biologic response [98].

In general, the *rate of loading*, the *peak stress*, and the *final strain* must be clearly specified to define an injurious loading scheme (e.g., Fig. 10.2B). Any two of these parameters can be imposed experimentally; the third must then be measured. Without knowledge of all three parameters, it is difficult to compare results to those in the literature. For example, two investigators loaded cartilage explants at different loading rates to a prespecified peak stress (in load control) [23,30]. Comparisons of their data show the counterintuitive result that cell death was higher in more slowly loaded cartilage. However, compressing cartilage more quickly in these circumstances means that the prespecified peak stress was reached more quickly, and less total compression (strain) was produced. Thus, studies in which two different loading parameters are varied independently in a parametric fashion are most helpful in understanding the fundamental mechanical variables that cause injury to cartilage cells and matrix.

For example, Morel and Quinn [84] subjected young adult bovine cartilage disks to uniaxial unconfined compression at five different strain rates (over five orders of magnitude) and three different peak stresses (between 3.5 and 14 MPa). The strain rate was defined relative to the intrinsic gel swelling [114] or poroelastic relaxation time, $\tau \sim [\delta^2/(Hk)]$, where τ is related to the swelling (or de-swelling) time for a disk having equilibrium compressive modulus H, hydraulic permeability k, and characteristic distance δ through which fluid flows (e.g., the disk radius for the case of unconfined compression with fluid-impermeable platens [6]). This relaxation time is also related to the characteristic mechanical stress relaxation or creep time to within a numerical constant. The constant in turn depends on the mechanical boundary conditions for each configuration. At the lowest strain rates (at or below the relaxation time) that lead to the highest final strains, no cracks in the matrix occurred, but cells died throughout the depth of the tissue. In contrast, the highest strain rates resulted in high intratissue pressurization, causing impact-like surface cracking with cell death occurring only near the superficial zone. Kurz et al [58] used slices of immature middle one bovine tissue and found increased cell death and decreased mechanical stiffness with increasing strain rate, above the poroelastic relaxation rate, applied at constant total strain.

It has been proposed that threshold levels of either strain rate or peak stress determine the threshold of tissue damage. Tissue age and species also appear to be strong determinants of the ability of cartilage to withstand overload. Torzilli et al [119] compressed mature bovine occipital cartilage in a load-controlled apparatus at a constant stress rate of 35 MPa/s to reach final stress values in a range of 0.5 to 65 MPa. Cell death was significant at a stress of ~17.5 MPa; cartilage damage, therefore, may occur when a threshold is reached at peak stress. Similarly, cell death increased with peak stress in immature bovine knee cartilage [70]. Patwari et al [87] studied the relation between peak stress and GAG loss after injury to disks of normal human knee and ankle cartilages. Compression was applied to 65% final strain at 400% /s strain rate. At these loading conditions, knee cartilage suffered significantly more damage than ankle cartilage, but peak stress was not an important correlate of GAG loss.

10.4.2 In Vitro Models of Acute Mechanical Injury to Cartilage: Damage to Matrix and Cells

Using a variety of instruments as in Figure 10.1A, investigators have demonstrated a range of events that can occur immediately following cartilage traumatic injury. These include loss of proteoglycan constituents to the culture medium [30,51,70,99,119], increased tissue swelling [70,119], and increased levels of denatured collagen neoepitopes [22,116], indicative of damage to the collagen network. At the same time, the biomechanical properties of the tissue become degraded [59]. Loening et al [70] observed decreased equilibrium and dynamic stiffness associated with increased tissue swelling in hypotonic saline, suggestive of collagen network damage. Mechanical injury can also cause cell death by apoptosis [21,26,70]. Apoptosis can be induced at levels of mechanical loading below those that cause macroscopic damage [70], as assessed by tissue swelling and GAG loss. This suggests that apoptosis can be triggered by direct loading injury to the chondrocytes.

Chen et al [20,22] and Thibault et al [116] tested the effects of mechanical injury on the production of collagenase-generated neoepitope of type II collagen. In both confined and

unconfined compression injury models, injury caused an increase in collagenase-generated collagen fragments. Thibault et al [116] postulate that this is the result of mechanical denaturation of the collagen fibril, making the fibril susceptible to cleavage by metalloproteinases. A study focusing on repair secondary to mechanical compression injury indicated that synthesis of fibronectin and proteoglycan increased over control levels in the 10 days that followed the injury. This situation is similar to that seen in early OA [22]. DiMicco et al [28] analyzed the release of sulfated glycosoaminoglycan (sGAG) into the medium over a period of 7 days following injury. A small, but statistically significant, increase in sGAG release occurred during the first 24 hours after injury. This was attributed to mechanical disruption of the matrix, inasmuch as it was not reversed by inhibitors of biosynthesis (cyclohexamide) or degradative enzymes. However, a broad-spectrum hydroxamate MMP inhibitor reduced the cumulative GAG loss from injured disks in the course of the 7-day postinjury period [28].

Studies using a drop-tower loading system to apply impact loads on cartilage–bone cylinders have documented decreases in cell viability in a canine model [102], in the porcine patella [29], and in bovine articular cartilage [50]. These studies have shown that bone plays a major role in mediating the effects of an impact load on cartilage [50]. With bone attached to cartilage, the cartilage was damaged much less [50]; higher-energy impacts damaged the bone rather than the cartilage. This finding is consistent with the importance of subchondral fracture in animal impact models [100; cf. also chapter 2] and with the early work of Radin et al [101], who emphasized the involvement of subchondral bone, having found that impact trauma to the patellofemoral joint led to OA in animal models. The response of bone to impact and subsequent changes in joint stresses are clearly important in OA pathology. For this reason, impact models using cartilage that has been removed from underlying bone do not simulate in vivo loading magnitude and distribution. Nevertheless, injury to the cartilage alone may suffice to initiate OA processes in vivo [86,94].

Moreover, using cartilage explants without underlying bone makes it possible to impose controlled mechanical loading, allowing specific loading parameters to be more easily quantified and correlated with cellular and matrix changes. Thus, studies regarding the mechanism of response to injurious loads by cells and matrix in cartilage explants are meaningful.

10.4.3 Apoptosis Versus Necrosis

Recently, it has been proposed that cell death by apoptosis may be an important event in osteoarthritic cartilage. Most compression injury models suggest that chondrocytes may undergo apoptosis in response to injury [24,25, 70,90]. High-stress repetitive loading can also cause chondrocyte necrosis, which occurs soon after the injury as visualized by transmission electron microscopy (TEM) [79] (see also [59] for a recent review). We and others have used TUNEL (Termunal deoxydeotidyl transferase Brothi-dutp Nick end labeling) staining, along with light microscopy of nuclear morphology, to demonstrate induction of apoptosis by mechanical injury [24,70]. Investigators have since emphasized that false-positive staining by the TUNEL assay can be a major limitation [2,41]. Chen et al [21] found that cells in cartilage subjected to freeze-thaw cycles were 90% TUNEL positive after 3 days of culture; this suggests that TUNEL staining does not reliably distinguish apoptotic from necrotic cell death. In addition, there clearly exist modes of cell death with features of both necrosis and apoptosis.

To investigate further, Patwari et al [90] subjected cartilage disks from newborn bovines to compression injury (50% strain, 100%/s), performed a quantitative analysis of cell morphology by electron microscopy (EM) and compared results to those with the TUNEL assay. By TUNEL, the cell apoptosis rate increased significantly from $7\% \pm 2\%$ in unloaded controls to $33\% \pm 6\%$ after injury ($n = 8$ animals) and, by EM, the apoptosis rate increased from $5\% \pm 1\%$ in unloaded controls to $62\% \pm 10\%$ in injured cartilage. Analysis by EM also indicated that 97% of the dead cells in these injured disks were apoptotic by morphology. These results confirm that cell death increases significantly after injurious compression and suggest that in the injury protocol used in these experiments most of the observed cell death involved an apoptotic process.

As a further example of the relationship of the apoptotic response to the biomechanical parameters of injurious compression, we note that high strain rates cause tissue pressurization in the center of the explants (Figure 10.2C), with the periphery experiencing more strain and fluid flow [28,34]. Most reports have focused on the central pressurized region of the explant to avoid cutting artifacts [70], which cause apoptosis independent of injury. Patwari et al [90] suggested that compression injury may alter cell-matrix interactions sufficiently to initiate apoptosis. While injury-induced cell death and accompanying damage to the extracellular matrix are clearly demonstrable, the contribution of cell death to arthritis is still controversial [3].

10.4.4 Effects of Cartilage Injury on Chondrocyte Gene Expression: Recent Discoveries

Several studies have provided evidence of marked changes in chondrocyte expression of MMPs and other selected genes following mechanical injury to cartilage in vitro. Techniques used for these studies include Northern analysis, reverse transcriptase polymerase chain reaction (RT-PCR), and in situ hybridization [59]. Recent technologic advances involving real-time quantitative PCR and gene array technologies have made it possible to use systems-level genomic approaches to study changes in chondrocyte transcription in response to mechanical injury of the cartilage. Chan et al [19] used a bovine complementary DNA (cDNA) microarray and real-time PCR to characterize changes in transcription 3 hours after unconfined compression injury to metacarpophalangeal cartilage explants from 18- to 24-month-old steers. They loaded to a peak stress of 30 MPa at a stress rate of 600 MPa/s, and found 19 genes that were differentially expressed. Upregulated genes included chemokine (CCR10, HMGB2, neurogranin, and ezrin) and cytokine receptors, enzymes, and molecules involved in signal transduction. In contrast, intercellular adhesion molecule-3 (ICAM-3), nerve cell adhesion molecule (NCAM), N-cadherin, vascular cell adhesion molecule-1 (VCAM-1), and insulin-like growth factor-1 (IGF-1) were downregulated [19].

In complementary studies using immature bovine cartilage explants, Lee et al [64] used real-time qPCR to measure levels of mRNA encoding selected matrix molecules, proteases, their natural inhibitors, transcription factors, growth factors and cytokines. Expression levels were assessed in free swelling culture (4 and 24 hours) and at 1, 2, 3, 6, 12, and 24 hours after application of a single injurious compression to 50% strain at 100%/s strain rate. Expression levels measured in noninjured free-swelling cartilage varied over five orders of magnitude, with matrix molecules being the most highly expressed, and cytokines, MMPs, aggrecanases (ADAMTSs), and transcription factors showing lower levels of expression. Changes in expression levels after mechanical injury were gene specific and time dependent. While the matrix molecules showed little change in expression after injury, MMP-3 increased ~250-fold, ADAMTS-5 increased ~40-fold, and TIMP-1 increased ~12-fold over free-swelling levels by 12 hours after injury. Genes typically used as internal controls, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and β-actin, increased expression levels ~4-fold after injury; this makes them unsuitable for use as normalization genes in this and similar studies. TNF-α and IL-1β did not change expression levels in the chondrocytes after injury. Group expression profiles, using principal component analysis (k-means clustering techniques), showed the main temporal gene expression patterns that were induced by injurious compression of the cartilage explants (Fig. 10.3). Interestingly, one of the group profiles was associated exclusively with the immediate response genes c-*fos* and c-*jun*, which showed increased transcription within the first hour of injury. This suggests that the AP-1 pathway may be an important response pathway in injury [64]. The authors concluded that changes in expression may alter the quantity of specific proteins that lead to degradation of the tissue structure and function [81].

10.4.5 Mechanical Injury Compromises Chondrocyte Biosynthesis and Mechanoresponsiveness

Several studies have shown that chondrocyte biosynthesis in cartilage explants decreases after an injury [22,32,51,111,119]. Quinn et al [97] examined changes in biosynthesis and increased

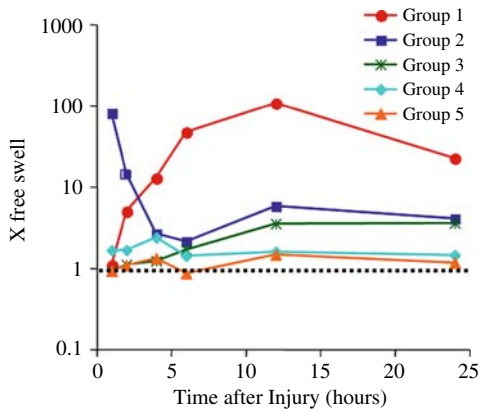

Figure 10.3. Group expression profiles generated by k-means clustering, showing the principal gene-expression patterns induced initially by injury of cartilage explants. Group profiles were calculated by averaging the expression profiles of genes within each group. Results are the mean change from free swelling levels, with a value of 1 (broken line) indicating similar expression after injury to the level measured in free swelling conditions. Group 1: MMP-3, ADAMTS-5, TGF-β; Group 2: c-*fos*, c-*jun*; Group 3: MMP-1, 9, 13, collagen 1, TIMP-1, TIMP-2, fibronectin, sox 9, GAPDH, β-actin, TNF-α; Group 4: IGF-1, IGF-2, ADAMTS-4; Group 5: collagen II aggrecan, fibromodulin, link protein, IL-1β. From [64].

cell death in osteochondral explants from 18-month-old steer shoulder joints subjected by unconfined compression to peak stresses between 3.5 and 14 MPa, at strain rates between 3×10^{-5}/s to 0.7/s. With higher strain rates, these authors observed that matrix damage occurred primarily in the superficial zone. Furthermore, proteoglycan synthesis was suppressed at low strain rates throughout the cartilage depth in a radially dependent manner. Kurz et al [58] showed that increasing the strain rate of injurious mechanical compression significantly decreased recovery from injury in chondrocytes. Recovery was defined as the cell's ability to respond after injury to subsequent stimulation by low-amplitude cyclic compression. The latter was considered an anabolic stimulus in normal cartilage explants. At higher strain rate injury (i.e., 1/s in Fig. 10.2B, but not 0.01/s), there was a dramatic decrease in ^{35}S-sulfate and ^{3}H-proline incorporation three days after the injury. Even more striking, chondrocytes in tissues subjected to high strain rate injury lost their the ability to be stimulated by dynamic compression in a dose-dependent manner [58]. The results were normalized to the surviving viable cells and therefore are not simply due to loss of cell viability. These studies had focused only on early time points following injury, but the findings suggest that mechanical injury causes a decrease in extracellular matrix production by chondrocytes. This in turn may contribute to further degeneration. A critically important unanswered question is whether the remaining cells can increase biosynthetic activity in an attempt to repair the cartilage matrix. It would be of great therapeutic interest if mammalian chondrocytes responded to biologic and mechanical injury anabolically.

10.5 Osteoarthritic Changes in Mechanoresponsiveness of Chondrocytes

Mechanical forces are widely thought to play a major role in regulating chondrocyte behavior (see recent reviews [60,123]). However, the mechanotransduction pathways by which mechanical injury alters long-term activity of the surviving chondrocytes are not understood. There is increasing focus on the effect of injury on intracellular signaling and regulation of gene expression. Microtubules and comparable connections between the cell surface and the ECM transmit deformations of the pericellular matrix to the cell membrane and from there to intracellular organelles via cytoskeletal elements [42,54,61]. Within the cell, changes in nuclear structure can lead to compaction of chromatin and altered molecular transport through nuclear pore complexes, processes important to cellular metabolism. Deformation of the rough endoplasmic reticulum and the Golgi apparatus can affect proteoglycan synthesis, GAG chain length, and sulfation observed during compression [56]. Deformations of the pericellular matrix can also change the physicochemical environment of cells, altering transport of soluble factors to cell-surface receptors [43, 81,98]. Mechanical activation of chondrocyte surface receptors, such as the $\alpha_5\beta_1$ fibronectin-binding integrin, induces multiple intracellular signaling pathways that involve tyrosine protein kinases, cytoskeletal proteins, ion channels, and second-messenger signaling cascades [106]. Mitogen-activated protein kinase signaling that involves ERK-1 and -2, JNK, and p38, is activated in immature bovine cartilage explants

that have been subjected to static, dynamic, and shear loading [31,52,68], and by fluid shear across isolated chondrocytes [47].

Osteoarthritic cartilage is characterized by reduced mechanical properties, increased matrix degradation, and altered responses to mechanical stimuli. Increased force transmission to cells and changes in mechanoresponsiveness may play a role in the development and progression of OA [109]. The ratios of collagen II to collagen I, and of aggrecan to versican, are defined as measures of chondrocyte dedifferentiation and of the decrease in human OA cartilage [80,82]. Expression of matrix proteins and cell surface receptors is altered with OA progression [4,11,82,124,125]. Integrin receptors known to be involved in mechanotransduction have altered expression patterns in OA cartilage, with increased $\alpha 1$ and decreased $\beta 1$ expression [125]. These changes can result in altered responses to applied forces. Enhanced expression of fibronectin and osteopontin, two matrix components that bind and signal through integrin receptors, has also been observed [11]. In addition, isolated OA chondrocytes respond differently from normal chondrocytes to direct mechanical stimulation [83]. While cyclic stretching of normal chondrocytes increases aggrecan and decreases MMP-3 transcription, mRNA levels in OA chondrocytes remain unaltered. In addition, normal chondrocyte membranes hyperpolarize in response to cyclic stretch, whereas OA chondrocytes depolarize. These altered responses to mechanical stimulation may represent adaptive responses to the altered mechanical environment that prevails in OA tissue [83]. OA chondrocytes also respond to intermittent hydrostatic pressure with increased aggrecan and type II collagen mRNA levels, and to fluid shear stress with increased nitric oxide release and decreased expression of matrix proteins [110].

10.6 In Vitro Models Emulating Injury to the Joint

10.6.1 Injury Plus Cytokine Treatment

Certain aspects of OA have led to the development of new in vitro models of the mechanically injured joint. It is widely accepted that OA is a disease of the whole joint [33,92] that, in addition to cartilage, also involves the synovium, bone, muscles, and the nervous system. Inflammatory processes associated with cytokine-induced activity are increasingly acknowledged to play a role in the pathogenesis of OA [10,92]. As a result, research using in vitro injury models has broadened to account for interactions with other tissues, such as factors secreted by the joint capsule [89]. These novel models include (1) incubation of normal or injured cartilage in the presence of exogenous cytokines, and (2) co-culture of normal or injured cartilage in the presence of intact joint capsule tissue (Fig. 10.4 [62]). The direct use of an exogenous cytokine allows focus on the role played by that specific cytokine following injury and subsequent effects on mechanically injured cartilage.

In a study involving exogenous cytokines, Patwari et al [88,89] examined proteoglycan (PG) loss from mechanically injured bovine knee cartilage explants that had been cultured in the presence of IL-1α or TNF-α. Similar experiments were performed with injured knee and ankle cartilage obtained from the same human donor whose injured tissue had been cultured with IL-1. In the case of the bovine tissue, the PG loss was significantly increased, after mechanical injury alone, but loss amounted to only 2% of the total PG content and occurred only in the first 3 days following injury. The addition of 1 and 10 ng/mL IL-1α and 100 ng/mL TNF respectively, increased the PG loss over that due to injury or cytokine treatment alone. This interaction between cytokine treatment and injury was statistically significant. In human knee cartilage, the interaction was significant for both IL-1α (Fig. 10.1B) and TNF-α, but the relative increase in PG loss was less than in bovine cartilage. Importantly, there was no significant interaction between injury and IL-1α in human ankle cartilage (Fig. 10.1B). This study suggests that mechanical injury and cytokine-induced chondrocyte-mediated degradation cause changes in tissue behavior and properties in complementary and synergistic ways, even though the human ankle cartilage was relatively impervious to either injury or cytokine treatment.

10.6.2 In Vitro Models of Joint Injury: Co-Culture of Joint Capsule Tissue with Injured Cartilage

The role played in cartilage matrix degradation and remodeling by synovial tissues and the release of cytokines and other mediators can be studied in vitro. Jubb and Fell [126] have studied how joint capsule tissue, when co-cultured with normal cartilage, affects chondrocyte biosynthesis. Ilic et al [48] have shown that bovine joint capsule and its fibroblasts express soluble aggrecanase activity. Patwari et al [88,89,91] in this culture system (Fig. 10.4) observed that co-incubation of human joint capsule tissue with normal human knee cartilage explants inhibited chondrocyte biosynthesis, as was also observed in animal models. Blockade of IL-1 in a newborn bovine model had no palliative effect on the inhibition of ^{35}S-sulfate incorporation and it was therefore apparent that the effect was mediated by an interleukin-1–independent signaling pathway. Using this model, Lee et al [63] have reported that co-culture of bovine joint capsule tissue with normal or injured immature bovine cartilage caused dramatic upregulation of chondrocyte gene expression (via qPCR) of certain proteolytic enzymes including ADAMTS-5, but not of ADAMTS-4. The mediating factors, presumably released from the joint capsule tissue, have not yet been identified. This model system may lead to identifying soluble factors that may contribute to the initiation and progression of OA and may therefore become therapeutic targets.

10.7 New Directions: Proteomic Approaches to Biomarkers of Joint Injury and Osteoarthritis

Biomarkers of OA typically target indicators of inflammation or indicators of matrix degradation. Markers of interest include hyaluronic acid, type II collagen N-propeptide, type II collagen C-propeptide (CTX-II), crosslinked collagen II peptides from the C-telopeptide, collagen III N-terminal propeptide (PIIINP), COMP, osteocalcin, pyridinoline, AgKS (keratan sulfate containing aggrecan fragment), and ykl-40 [96]. Recent publications have demonstrated the utility of proteomics in the study of normal versus OA human cartilage. An example is two-dimensional gel electrophoresis followed by mass spectroscopy [45,105]. Proteomics facilitates following groups of proteins, as well as specific target biomarkers. Stevens et al [112] used mass spectrometry applied to an entire in vitro system to quantify the effect of mechanical compressive injury of cartilage and

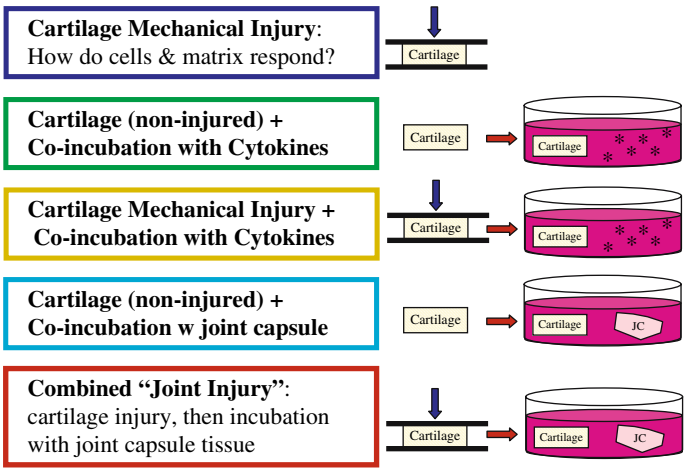

Figure 10.4. Schematic of in vitro models of joint injury, including injurious compression of cartilage alone, treatment of compressed and noncompressed cartilage with cytokines, and co-culture of compressed and noncompressed cartilage with explants of joint capsule tissue (synovium). (Adapted from [62], © Massachusetts Institute of Technology.)

how treatment with TNF-α and IL-1β affected the joint injury (Fig. 10.1A). Protein profiling together with clustering analyses revealed distinguishing treatment features that may be specific markers for a given degradative process.

10.8 New Engineering Directions: Molecular Nanomechanics and Chondrocyte Response

The functional properties of normal and injured cartilage tissue are determined, in part, by the mechanical properties of the various ECM molecules that are synthesized by chondrocytes. Functionally inferior matrix macromolecules that cannot properly contribute to or assemble into a functional ECM may be a hallmark of the progression of post-traumatic cartilage degradation. Using the newer techniques of optical tweezers, atomic force microscopy, and high-resolution force spectroscopy, recent studies using isolated matrix molecules have focused on the tensile properties of hyaluronan and collagen [38,113] and the repulsion between chondroitin sulfate GAG chains [107] and aggrecan [27]. This is an exciting new area that emphasizes the connection between tissue-level mechanical properties, and molecular mechanical properties of the matrix PGs, GAGs, and collagens. It also points to the importance of chondrocyte mechanotransduction as the glue between the tissue, cell, and molecular constituents in cartilage remodeling.

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11.

Novel Osteoarthritis Therapeutics

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Therapies for osteoarthritis (OA) have been directed mostly toward the alleviation of signs and symptoms of disease, predominantly manifested by pain. Evaluating therapeutic efficacy has largely focused on improvements in pain and joint function. The mainstay of treatment has included nonsteroidal antiinflammatory drugs (NSAID), cyclooxygenase II (COX-2) selective inhibitors, and other analgesics including acetaminophen/paracetamol, tramadol, and opiates. Other approaches to pain control have included topical application of NSAID creams and patches. Intraarticular delivery of hyaluronic acid has shown some improvement in pain in some patients with OA [1–6]. Interest in nutriceuticals including oral glucosamine and chondroitin sulfate has grown, and many patients with OA take these compounds [7]. Alternative therapies have also been reported to be effective in pain relief, including acupuncture [8]. Studies of nonpharmacologic interventions including physical therapy and weight loss have demonstrated effects that approach or exceed those seen in clinical trials of analgesics [9–11].

Some controversy remains as to the mechanisms of absorption and action of glucosamine and chondroitin [3–6,12]. Some clinical trials of glucosamine sulfate have shown improvements in knee OA on the basis of symptomatic end points; however, a recent meta-analysis has shown negative results. Both positive and negative studies have been reported for chondroitin sulfate [13,14]. The recently reported glucose arthritis intervention trial (GAIT) study was funded by the National Institutes of Health (NIH) to address the question of efficacy of glucosamine hydrochloride, chondroitin sulfate, alone or in combination, compared to placebo using a celecoxib comparator as a positive control [15]. In the overall study for the primary symptomatic end point, there was no improvement due to either supplement, as compared to placebo. The active comparator, celecoxib, on the other hand, was shown to provide more symptomatic benefit. The placebo benefit seen in the study was substantial, with more than 60% of placebo-treated patients meeting the primary outcome criterion of a 20% reduction in pain at 6 months. Only a subset of patients with moderate levels of pain had a benefit from the combination of chondroitin and glucosamine, but this was not the case with either agent alone. Interestingly, in this subset celecoxib had no statistically significant effect, thus raising questions as to the internal validity of these results.

Pain is an important component of OA, but its cause, likely to be multifactorial, remains unknown. Cartilage is an aneural structure. This is not true, however, of the joint capsule, the synovium, supporting ligaments, and periosteum, which have nociceptive fibers and bone marrow neurons that extend to near the subchondral plate. Trabecular microfractures, periosteal elevation due to osteophytes, pressure on subchondral bone, and hypertension in bone marrow have all been proposed as mechanisms of bone-related pain in OA. Central mechanisms may also play a role in pain perception.

The understanding of the pathophysiology of OA has increased in the last few years. It is now thought of as a dynamic process that involves the many components of the joint organ, including cartilage, synovium, and bone, under the influence of cytokines, growth factors, and mediators. Increasingly attention has focused on mechanism-based pharmacotherapies directed at the disease process, with the aim of slowing progression or preventing further disease destruction. These therapies are termed disease-modifying OA drugs (DMOADs) or structure-modifying OA drugs (SMOADs) [16,17]. Ultimately these therapies may improve signs and symptoms of the disease to the extent of making joint replacement unnecessary.

This chapter addresses therapeutics that have been or are currently in development for the treatment of OA, with a focus on agents that have a rationale for potential SMOAD or DMOAD activity. This chapter concentrates on compounds tested in clinical trials that act on bone. Potential therapies are also discussed.

11.1 Disease Modification in Osteoarthritis

Symptoms of OA become more pronounced with time. The clinical sequelae of the disease include persistent pain, loss of function, diminished quality of life, and, ultimately, "joint failure," necessitating surgical joint replacement. The efficacy of analgesics is limited, with many patients continuing to suffer pain. This is especially true for those with advanced disease. While arthroplasty relieves symptoms in many patients, the attendant costs, morbidity, and even mortality are significant [18]. Therefore, it is extremely important to develop drugs that retard or delay disease progression.

The concept of "progression" of articular disease has been increasingly incorporated into clinical trials and is an outcome that is assessed in rheumatoid arthritis [19–21]. Several agents, including methotrexate, leflunomide, tumor necrosis factor (TNF) antagonists, etanercept, infliximab, adalimumab, and the interleukin-1 (IL-1)-receptor antagonist, anakinra, have been approved as disease modifying antirheumatoid arthritis (RA) drugs (DMARDS) and shown to slow radiographic progression over 1 year. With the aid of quantitative scoring systems, progression in RA has been evaluated by assessing bone erosions and joint space narrowing, the latter reflecting cartilage loss. Differences have been seen at a group level, but the small number of patients in a progression "tail" tends to drive these changes, with most patients showing no worsening [20,22]. Some patients show improvements in erosions, possibly indicative of repair, but these also occur in placebo-treated groups. By including only patients with one or more risk factors for progression, for example, rheumatoid factor positivity, elevated acute phase reactants, or damage at baseline, mean group changes reflecting slowing of disease progression can be demonstrated. That a DMARD can prevent damage and progression is an accepted concept in RA. Many lessons have been learned in patient selection and study design to evaluate such outcomes. Furthermore, the increased sensitivity of other imaging techniques [e.g., magnetic resonance imaging (MRI)] to assess change over shorter time periods has been demonstrated in RA, as well as in ankylosing spondylitis [23,24]. MRI changes are not yet acceptable to claim disease modification from a regulatory perspective.

Disease progression has not been well defined for OA [24–26]. Some studies have used plain radiographic worsening of Kellgren and Lawrence scoring, while others have evaluated mean joint space narrowing. Still others have looked at a dichotomous definition of progression that involves predefined amounts of joint space loss. No method for radiographic assessment is agreed on or standardized by regulatory agencies or clinical trials. Imaging modalities such as MRI offer additional discrimination of articular structures, based on composite indices, cartilage volume, cartilage thickness, or bone lesions, but no uniform imaging technique or analysis has been established [24]. Even with these caveats, it is not clear that progression in OA is linear. Patients with OA may "progress" in a stepwise fashion or do so rapidly, exhibiting significant narrowing of the joint in a very short period. According to recent studies, only a small percentage of patients has exhibited a predefined degree of significant progression [27]. To define risk factors more precisely in clinical, biochemical, or imaging terms and to develop predictors of rapid progression are goals of active research. One reason why disease agents

like DMOADs are difficult to evaluate is because of the limited relationship between signs and symptoms, on the one hand, and the radiographic evaluation of disease severity, on the other, even though some MRI features, such as lesions of subchondral bone, have been correlated with pain and disease progression [28,29].

11.2 Bisphosphonates

11.2.1 Mechanism of Action

Bisphosphonates have been approved for the treatment of osteoporosis and Paget's disease of bone, conditions with increased rates of bone turnover. The approved bisphosphonates differ in structure and activity, but all modulate the bone remodeling unit. They inhibit osteoclastic bone resorption by causing the osteoclast to internalize the bisphosphonate and inducing apoptosis by inhibition of intermediate enzymes in the mevanolate pathway. In addition bisphosphonates are adsorbed to freshly exposed bone matrix and are bound to hydroxyapatite at sites of active bone remodeling [30]. The effects of bisphosphonates on osteoclasts is reflected by decreases in markers of bone turnover, including a decrease in the breakdown products of type 1 collagen, such as crosslinked N-telopeptide I (NTX-I). Even though the osteoclast is a principal target of bisphosphonates, additional antiproliferative and non-antiresorptive activities have also been seen in vitro. This suggests that bisphosphonates may also act on other cell types present in the joint, including macrophages and chondrocytes. In one study bisphosphonates prevented dexamethasone-induced bovine chondrocyte growth retardation and apoptosis [31]. Bisphosphonates also appear to exert a chondroprotective effect [32,33] and inhibit matrix metalloproteinases (MMPs), including MMPs 1, 2, 8, 9, 12, and 20 [34–37]. Other mechanisms under investigation include antiresorptive activity and cytokine inhibition [38–41]. In tumor models, nitrogen-containing bisphosphonates have been shown to increase production of osteoprotegerin (OPG), a locally produced antagonist of osteoclast formation [42]. In some systems they interfere with vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and angiogenesis [38,43–50].

11.2.2 Animal Models

In several animal models of spontaneous and induced OA, bisphosphonates have been shown to slow progression of the disease process. In animal models of human inflammatory RA, bisphosphonates have decreased bone and cartilage degradation and have reduced joint inflammation in adjuvant-induced arthritis of rats [51–53]. In a rabbit model of carageenan-induced inflammatory arthritis, zoledronate showed a partial chondroprotective effect [32]. In the tumor necrosis factor (TNF) transgenic mouse model of inflammatory arthritis, zoledronate inhibited bone and cartilage destruction without inducing a significant antiinflammatory response [54]. In several models of human OA, bisphosphonates have been found to have salutary effects.

Anterior cruciate ligament transection (ACLTX) models have been widely used to study the resultant degenerative OA, characterized by early periarticular bone loss and that in turn leads to eventual cartilage damage and osteophyte formation. In a rat ACLTX model, subcutaneous alendronate was shown [55] to have a chondroprotective effect, causing a reduction in collagen degradation and in subchondral bone remodeling and osteophyte formation. Cartilage damage was also diminished, as was the vascular invasion of calcified cartilage. Bisphosphonates have also been studied in rabbit ACLTX models, where periarticular bone loss is an early event. Doschak et al [56,57], using micro–computed tomography (CT) analysis, have reported that risedronate conserved the geometry of the bone mineral at sites where the medial cruciate ligament attaches to bone (enthesis), but was unaccompanied by osteophyte formation or angiogenesis. Bisphosphonates, therefore, may contribute to preserving mechanical strength of underlying bone by decreasing ligamentous laxity during loading.

Induction of experimental degenerative arthritis in rabbits by means of intraarticular instillation of chymopapain has been used to study bisphosphonates. Bone resorption was inhibited, accompanied by a reduction in the histologic severity of tibial cartilage lesions. In zoledronate-treated animals [58], proteoglycan loss appeared diminished.

The effect of the bisphosphonate risedronate has also been studied in the Duncan-Hartley

guinea pig model [59,60]. In one study the mean area of cartilage damage had doubled in the control group in a period of 12 months, whereas the area of the lesion had not changed in the risedronate group, suggesting an arrest of the OA disease process. In another experiment, different bisphosphonate compounds were tested over 24 weeks. Not all bisphosphonates affected the progression of OA lesions, and, interestingly, antiresorptive activity did not predict structure-modifying effects. Effective agents shared the common feature of nitrogen in a cyclic side chain, as in risedronate, zolendronate, and ibandronate, but not in alendronate. In an additional guinea pig study, the area of the cartilage lesion in the risedronate group was significantly smaller than in the control group [61]. This was not the case for the alendronate group.

11.2.3 Rationale for Bisphosphonates in Human Osteoarthritis

Several studies of human OA have pointed to subchondral bone as a site for intervention on the assumption that subchondral bone plays a role in the etiopathogenesis of the disease and may constitute a possible site to which to apply agents that modify the disease [cf. chapter 2].

Similarities exist between osteoarthritic and osteoporotic bone. Bone mineral density (BMD) in subchondral regions of the knees in OA patients, whether or not they have osteoporosis (OP), is significantly reduced in OA patients [62]. Stiffness and bone mineral content are below normal in subchondral bone from femoral heads of patients with OA or OP [63–65]. The changes in subchondral bone stiffness and density may diminish structural support for the overlying cartilage. Subchondral bone from patients with OA has greater trabecular thickness and connectivity and fewer trabeculae with increased trabecular separation than is the case for normal individuals [66–69]. Fractal analysis of subchondral bone has further demonstrated loss of normal trabecular structure in OA [70–72].

Subchondral OA bone, like osteoporotic bone, is characterized by increased turnover. In one study, bone turnover in women with OA was compared with that in women with postmenopausal osteoporosis; bone resorption was evaluated with the aid of NTX-I and C-terminal type I collagen telopeptide (CTX-I) [73]. Median bone resorption levels were 31% to 87% higher in patients with either progressive OA or OP than in controls without OA or OP or in patients with nonprogressive OA. Bone turnover did not differ between women with progressive OA and those with OP or between controls and women with nonprogressive OA; therefore, altered bone turnover may be a marker for OA progression.

Imaging of the subchondral bone using MRI has shown that lesions described as bone marrow "edema" with an increased signal on fat-suppressed T2-weighted images are associated with OA pain [29]. These lesions have also been associated with progression in the ipsilateral compartment [28]. In another study, subjects with OA of the knee who took bisphosphonates had fewer bone marrow lesions, less bony attrition, and less OA pain than controls [74]. Similarly, patients on estrogen therapy also had fewer bone marrow lesions. Therefore, interventions that decrease bone turnover seem to lead to fewer bone lesions detected by MRI. Other reports have shown that agents directed toward subchondral bone have slowed progression of bone marrow lesions [75,76] and thus provide additional rationale for further study of these agents in OA.

11.2.4 Clinical Trials of Bisphosphonates in Osteoarthritis

The efficacy of risedronate in the treatment of OA of the knee has been investigated. An initial 1-year study, performed in the United Kingdom, British Study of Risedronate in Structure and Symptom of Knee OA (BRISK), evaluated 200 patients [27]. Radiographic outcomes were assessed using a highly standardized semiflexed fluoroscopically positioned radiograph; signs and symptoms were also followed. Patients were randomized to receive placebo or risedronate at 5 mg/d or 15 mg/d. On the basis of disease progression defined as greater than >0.75 mm joint space loss, fewer patients treated with the highest dose of risedronate demonstrated progression over a 1-year period compared to placebo groups, although the difference was not statistically significant. A reduction in bone turnover and cartilage degradation was reported. In two separate multinational 2-year

studies KOSTAR [75–78], more than 2400 patients were enrolled in the European Union (EU) and the United States. The EU patients were randomized into four treatment groups: placebo, and risedronate 5 mg/d, 35 mg/wk, and 15 mg/d. In the U.S. the four treatment groups were placebo, risedronate 5 mg/d, 50 mg/wk, and 15 mg/d. The study evaluated signs and symptomatic benefit, as well as structural changes. No symptomatic benefit was demonstrated for any dose of risedronate, compared to placebo. A clinically measurable improvement of ~20% in symptomatic end points was seen in all treatment and placebo groups. Even if radiographic progression is defined as >0.6 mm joint space loss, there was no beneficial effect of risedronate. However, only 13% of the total study population was defined as progressors [78]. A dose-dependent reduction in CTX-II was seen with risedronate. Elevated baseline levels of CTX-II were associated with the risk of progression in univariate and multivariate analysis. In a subset of patients in this study, MRI analysis showed that one patient group could be classified as rapid progressors, with loss of cartilage volume as an outcome measure. This loss did not, however, correlate with CTX-II levels [79]. On the other hand, a relationship between CTX-II levels and bone marrow changes detected with MRI was reported in another study [80]. An analysis of a subset of patient x-rays using fractal analysis showed that the highest dose of risedronate was associated with less trabecular change than was the case for the placebo group [81,82]. Even though the study results were largely negative, results from what to date is the largest study of OA of the knee may provide an opportunity for additional analyses that may lead to a better understanding of the disease process.

11.2.5 Other Inhibitors of Osteoclasts

To date bisphosphonates have not been shown to affect OA as directly as SMAODs. Nevertheless, because interventions that affect bone turnover have caused CTX-II to decrease, interventions directed against subchondral bone may become OA therapy. Other agents, available or in development, that affect osteoclast function may become useful adjunct therapies.

The interaction of the receptor activator of nuclear factor-κB ligand (RANKL), expressed on osteoblastic stromal cells, with its receptor RANK, expressed on osteoclasts, is a primary requirement for osteoclast differentiation and activation [83,84]. Denosumab is a human monoclonal antibody that binds RANKL, thus blocking this interaction. In a recently reported study of 412 women with postmenopausal OP [85], denosumab was administered subcutaneously at 3- or 6-month intervals. Bone density was increased in trabecular and cortical bone in the denosumab groups, but not in the alendronate or placebo groups. Concomitant rapid and dose-dependent sustained reductions in bone turnover were also seen in the denosumab groups. The compound is being developed for treatment of OP, but no formal studies have been performed with OA patients. Because of its effects on osteoclasts and bone turnover, inhibition of the RANKL-RANK pathway may be of interest in targeting subchondral bone turnover in OA.

Intranasal calcitonin inhibits osteoclasts and is approved for treatment of Paget's disease, hypercalcemia, and postmenopausal osteoporosis. In the canine ACLTX model of OA, calcitonin decreased cartilage damage [86], cartilage lesions of ACLT knees were significantly reduced, and decreases in periarticular bone density were prevented [87]. Administration to postmenopausal women of a novel oral calcitonin led to a dose-dependent reduction in cartilage degradation, as evaluated by CTX-II, and the drug therefore may be of use in OA [88]. Inhibition of cathepsin K affects osteoclast function [89] and is addressed separately below.

11.3 Interleukin-1 as a Therapeutic Target

The cytokine IL-1 is an attractive drug for treatment of OA. It is a proinflammatory cytokine implicated in inflammatory responses [90–93] and in some additional aspects of OA pathobiology [94]. Inhibitors of IL-1 include the two naturally occurring inhibitors, IL-1 receptor antagonist (IL-1RA) and the "decoy receptor" IL-1 receptor type II (IL-1R type II). Compounds developed to inhibit the IL-1 pathway include recombinant IL-1RA, fusion proteins, consisting of IL-1 receptors attached to the F_c portion of a human immunoglobulin G (IgG) molecule, and monoclonal antibodies

with a high affinity for IL-1 or directed against the IL1 receptor. Small molecule inhibitors of the IL-1 converting enzyme (caspase-1) have also been developed and other therapeutic molecules that have been studied in OA act as IL-1 antagonists, including Diacerein.

Considerable evidence indicates that IL-1 produced by synovial cells and chondrocytes plays a prominent role in the pathogenesis of cartilage degradation in OA [90,95]. Interleukin-1 has many effects relevant to OA. These include the induction of other cytokines, proteases, nitric oxide, and prostaglandins. It acts on multiple cell types including chondrocytes, but also on macrophages, synovial fibroblasts, osteoclasts, and osteoblasts. Polymorphisms of the IL-1 gene complex have been associated with a risk for OA progression [96–98]. The effects of IL-1 on cartilage have been extensively studied in vitro and in explanted tissues from patients with OA. Basal production and expression of IL-1 is increased in OA chondrocytes and further upregulated in response to proinflammatory cytokines [99, 100]. Interleukin-1 is a potent inducer of inducible nitric oxide synthase (iNOS) that leads to expression of nitric oxide. The latter enhances chondrocyte apoptosis [101,102]. Interleukin-1 also induces cyclooxygenase-2 (COX-2) and certain forms of secretory phospholipase A_2 ($sPLA_2$) and may play a role in the generation of prostaglandins that mediate local inflammation. It is also involved in local and central pain. It induces chondrocyte and macrophage production of destructive MMPS, thus contributing to matrix degradation. It also inhibits cartilage repair by decreasing chondrocyte synthesis of proteoglycan [103] and type II collagen [104]. In addition, IL-1 activates osteoclasts and increases RANKL expression in stromal cells and osteoblasts, thereby hastening osteoclast maturation and activation [105]. It also induces endothelial adhesion molecule expression, possibly contributing to neovascularization seen in some cases of OA. By increasing production of other cytokines, including IL-6 and TNF, IL-1 contributes to the acute-phase response by inducing increases in C-reactive protein and erythrocyte sedimentation rate (ESR). These markers are associated with obesity, but have also been linked to OA progression. In animal models of inflammatory arthritis, IL-1 is an important cytokine that drives the inflammatory processes, along with bone destruction, erosions, and cartilage degradation [106–108].

11.4 Diacerein

Diacerein (4,5-bis(acetyloxy)-9,10-dioxo-2-anthracene carboxylic acid) is an oral OA drug that inhibits IL-1 in vitro. Rhein, the active breakdown product of Diacerhein, is an anthraquinone found in Cassia plants. It has moderate antiinflammatory and analgesic activity and is a weak laxative [109]. Diacerein inhibits IL-1 produced by human chondrocytes and acts on downstream inflammatory mediators, including MMPs and other proteases. It also stimulates chondrocyte metabolism to increase proteoglycan and collagen production [110,111]. Diacerein inhibits IL-1 production by lipopolysaccharide (LPS)-stimulated macrophages and synovial cells [112], and reduces cartilage damage in animal models [113].

A recent review indicates that Diacerein decreases pain in knee and hip OA to about the same extent as NSAID [114]. Gastrointestinal side effects are commonly reported with Diacerein. These include diarrhea, heartburn, and abdominal pain, but no peptic ulceration or infection. Diacerein induces structural changes in studies of hip OA [115]. As a result, it has been approved as an SMOAD for the treatment of hip OA in some countries. In the hip study, Evaluation of the Chondro modulating effect of Diacerein in OA of the Hip (ECHODIAH), 507 patients with primary OA of the hip received either a placebo or Diacerein, 50 mg b.i.d. Progression was defined as loss of joint space greater than 0.5 mm. A significant slowing of radiographic progression was demonstrated in the hip study over 3 years. However, the rate of patient attrition was very high, only 51% of Diacerein-treated patients completing 3 years, and only 55% of the placebo-treated patients. Fewer patients in the Diacerein group (14.5%) underwent hip arthroplasty (an indication of joint failure) than in the placebo treated group (19.8%). The effects of Diacerein on CTX-II and serum hyaluronic acid (HA) levels were not reported [116], nor are data available to evaluate Diacerein by more sensitive methods of imaging. One group has reported negative findings when knee OA was treated with Diacerein [117], whereas other studies have demonstrated efficacy in improving OA signs and symptoms [118].

11.4.1 Anakinra (IL-1 Receptor Antagonist)

Anakinra is a recombinant form of a naturally occurring inhibitor of IL-1, the IL-1 receptor antagonist. Anakinra has been approved for the treatment of RA. It inhibits RA disease progression as evaluated radiographically. Decreased acute-phase reactants were seen along with a modest reduction in clinical disease activity. Radiographic joint damage was reduced within 6 months [119], with less joint space narrowing and fewer erosions or bony destructive lesions. Biopsy studies carried out on human RA synovium have shown that anakinra decreased MMP expression, macrophage infiltration, and inflammatory cytokine expression [120,121]. In the treatment of RA, daily doses of anakinra are administered subcutaneously, with the major side effects reactions to the injections and systemic bacterial infection. More recently dramatic efficacy has been reported for anakinra in the treatment of children with Muckle-Wells syndrome, a disease characterized by high spiking fever, rash, and arthritis with a marked elevation of acute-phase reactants [121]. This finding again demonstrates the critical role IL-1 plays in the disease process.

In the Lowell et al study, a single dose of 150 mg of anakrina was administered directly into the knee of 13 patients with OA [121]. Pain was decreased from baseline by 42% after 3 months on average. These preliminary results are intriguing, but more work is needed to determine longer term efficacy and safety.

11.5 IL-1 Antagonists in Development

11.5.1 IL-1-Trap (rilonacept)

A novel IL-1 inhibitor, IL-1-Trap, is a recombinant molecule whose high affinity for IL-1 consists of a dimer of the IL-1 receptor, fused to the IL-1 receptor accessory protein, all fused to the Fc portion of IgG1 [122]. In a 12-week study of patients with RA, varying doses of IL-1-TRAP were administered subcutaneously [123,124], with modest efficacy at the highest dose. The reduction in acute-phase reactants and other systemic biomarkers, including MMPs, was dose-dependent. No radiographic evaluation was performed. The efficacy of IL-1-TRAP has also been evaluated in patients with periodic fever syndromes.

11.5.2 Interleukin-1 Converting Enzyme (ICE) Inhibitor

Interleukin-1β is fully activated posttranslationally as a result of the cleavage of a leader sequence. The enzyme responsible for this cleavage is a cysteine protease caspase-1, known as interleukin-1β converting enzyme (ICE). Pralnacasan, an oral inhibitor of ICE, has been tested in animal models of OA and in clinical trials of RA and OA. In the mouse model of collagen-induced arthritis, the inhibitor significantly delayed the onset of inflammation and reduced disease severity by 50 to 70%. Cartilage damage was reduced by 60% and bone erosion by 80%. Pralnacasan has also shown beneficial effects in two mouse models of knee OA [125].

A 12-week phase II study of pralnacasan was carried out with more than 500 OA subjects, with results reported only in press releases. The primary pain end point, Western Ontavie McMasters University Osteoarthritis index (WOMAC), was improved by 29 to 30% in all treatment groups, as well as in the controls. Some urine and serum markers of bone and cartilage turnover differed statistically in the two groups. A phase IIa study in 285 RA patients showed the compound to be effective in reducing symptoms and acute-phase reactions. Additional phase III studies in RA were discontinued because of concerns regarding hepatotoxicity observed in animal studies. Other inhibitors of ICE have been developed, but whether they will be studied in OA or RA is unclear [126].

11.5.3 Antibody to IL-1 Receptor

A monoclonal antibody, AMG 108, which binds to the IL-1 receptor and blocks IL-1 from being bound, has been investigated, but findings have not been reported except in press releases. An 8-week phase IIa study of 146 patients with knee OA has been performed to investigate the efficacy and safety of monthly subcutaneously administered AMG 108 [127]. AMG 108 was found not to be effective at the end of 6 weeks in terms of the WOMAC pain score. Whether radiographic or biomarker analyses were performed is unknown.

11.5.4 Gene Therapeutic Approaches

The effect of gene therapy utilizing the anti-inflammatory IL-1 RA in human joints has been tested in patients with RA, but not in OA patients. Exciting preliminary results have been obtained in rabbit models of inflammatory arthritis with the aid of an adenoviral vector that encodes the soluble TNF receptor, as well as IL-1RA [128]. More recently a small study of nine patients undergoing metacarpophalangeal (MCP) arthroplasty for RA has shown effective transduction of synovial cells, with a construct encoding IL-1RA in patients [129]. Such studies are encouraging, but whether local delivery leads to systemic consequences is not known [130–134]. The use of other constructs using adeno-associated vectors in clinical trials of other diseases have been reported [135] and are under investigation for an RA phase I clinical trial. Preliminary results are available from a 15-patient phase I clinical trial of an adeno-associated virus type 5, encoding a soluble p75 TNFR:Fc fusion construct, tgAAC94. The patients had inflammatory arthritis, including RA, psoriatic arthritis, and ankylosing spondylitis, and received escalating doses of tgAAC94 that were injected into the arthritic joint. The construct encodes a protein that inhibits the action of TNF. It was well tolerated at doses up to 1×10^{11} DRP per mL of joint volume. In 9 of 11 patients followed for 4 weeks, signs and symptoms showed sustained improvement [136].

11.5.5 Unanswered Questions Regarding IL-1 in Osteoarthritis

Several questions remain concerning IL-1 as a target to inhibit arthritis. All agents studied so far have shown only modest reductions in clinical disease activity in RA, but gave rise to marked reductions in acute-phase reactants and the systemic inflammatory response. Anakinra has shown efficacy in inhibiting disease progression, evaluated in terms of cartilage loss and reduction in bony erosions, even though the effect on signs and symptoms was modest. This may indicate a possible dissociation between these two outcomes. It is as yet unclear whether intraarticular or systemic administration is most appropriate to deliver a cytokine antagonist, and many questions remain regarding drug distribution in the body, the pharmacokinetics and pharmacodynamics of intraarticular anticytokine therapies. Neither the safety aspects of the long-term administration of IL-1 inhibitors, nor the infection risks for the OA population are known. Also needed is better understanding of the best methods to assess outcome, whether by proof-of-concept studies, evaluation of signs and symptoms, or assessment of structure modification. Developing appropriate outcome measurements for single joint therapies is an area of discussion for Outcome Measures in [136b] Rheumatology Clinical Trials OMERACT8 (http://www.omeract.org/).

11.6 Matrix Metalloproteinase Inhibitors

Matrix metalloproteinases (MMPs) are a group of more than 20 zinc-containing endopeptidases that are important in connective tissue remodeling, wound healing, angiogenesis, and metastasis, with activities against a number of structural proteins. The MMPs include gelatinases A (MMP-2) and B (MMP-9), fibroblast collagenase (MMP-1), neutrophil collagenase (MMP-8), collagenase-3 (MMP-13), stromelysin 1 (MMP-3), stromelysin 2 (MMP-10), stromyeslin 3 (MMP-11), and matrilysin (MMP-7) [137,138]. In synovium and cartilage from OA and RA patients, as well as in response to proinflammatory cytokines, including IL-1 and TNF, the activities of several MMPs are upregulated and appear to contribute to the destruction of cartilage. Several endogenous inhibitors of MMPs, including the tissue inhibitor of metalloproteinases, are also present and increased in arthritis; however, their activities do not seem to compensate for the concomitant upregulation of the MMPs themselves, with net destruction that results from overabundance of MMPs. The MMPs are also important in metastatic tumor growth, and much of the drug development work has been directed toward the use of these compounds in cancer. There is significant overlap in terms of substrate for the different MMPs such that development of specific inhibitors has faced challenges.

A number of MMP inhibitors have been developed that have been tested in various animal models of arthritis. An inhibitor of the collagenases (MMP-1, -8, and -13) decreased

cartilage degradation with IL-1 in bovine cartilage explants and also in animal models of inflammatory arthritis [139]. Interestingly, but perhaps not surprisingly, while cartilage damage was decreased, inflammation was not significantly affected. Tetracyclines and their derivatives have been demonstrated to inhibit collagenases including MMP-2 and -9 [140]. Tetracycline derivatives have activity that is independent of their antimicrobial effects in periodontal disease [141]. Doxycycline decreases collagenase and gelatinase production from OA cartilage explants [142]. Several tetracycline antibiotics have also been tested in human arthritis, including minocycline in RA and doxyxycline in OA. Other MMP inhibitors have been used in arthritis clinical trials, but side effects have largely precluded their further development. Finally, one mechanism postulated for the effectiveness of bisphosphonates has been that they act as MMP inhibitors, because they chelate bivalent cations, including the zinc required for MMP activity [37].

11.6.1 MMP Inhibitors in Arthritis

11.6.1.1 Minocycline

Minocycline is commonly used in the treatment of acne and inhibits MMPs in vitro, but mostly at supraphysiologic doses [143,144]. In a rat model of experimental inflammatory arthritis, minocycline decreased damage scores [145]. Because RA may represent an immunologic response to an ongoing infectious nidus and because minocycline is also a putative MMP inhibitor, several studies have investigated the effects of minocycline in the treatment of rheumatoid arthritis. In the Minocycline in Rheumatoid Arthritis (MIRA) study, 219 patients with active RA received minocycline 100 mg b.i.d. or placebo for 48 weeks [146]. Small but significant differences compared to placebo were reported for patients with at least a 50% improvement in swollen joints (54% vs. 39%) and tender joints (56% vs. 41%). Significant differences were reported for the acute-phase response using ESR and for rheumatoid factor. The standard composite measure of clinical response in RA clinical trials (ACR20/50/70) and in European League Against Rheumatisive (EULAR) responses were not reported. That 39% and 41% of placebo-treated patients experienced a 50% improvement in these swollen and tender joint assessments, respectively, indicates a very robust placebo response in the study. In other words, the effect of the minocycline intervention was small. Radiographic analysis failed to show any evidence of minocycline modifying RA in terms of erosions or joint space narrowing over 1 year [147]. Another 2-year study compared minocycline to hydroxychloroquine in patients with early RA, using the ACR50 response criteria as a measure of primary outcome [148]. In this trial, an impressive 60% of patients treated with minocycline, compared to 33% of patients receiving hydroxychloroquine, achieved at least a 50% improvement in a composite measurement of swollen and tender joints. This applied also to other variables, including acute-phase reactants, function, and global assessments by patient and physician.

Common side effects of minocycline include photosensitivity and nonspecific gastrointestinal (GI) complaints. Many patients treated with minocycline develop a skin hyperpigmentation that resembles ochronosis [149]. Drug-induced lupus and antinuclear antibody (ANA) positivity are also seen with minocycline.

11.6.1.2 Doxycycline

Doxycycline is a common tetracycline antibiotic that is used in small oral doses and by local instillation to treat periodontal disease. In several animal models of OA, including the dog meniscectomy model, the Duncan Harley guinea pig spontaneous OA model, and a rabbit model, doxycycline treatment led to a decrease in OA progression [150]. Doxycycline 100 mg b.i.d. was compared to placebo in 431 obese females, ages 45 to 64, with unilateral OA of the knee [151]. In this well-designed NIH-sponsored, clinical trial that involved several centers, patients were selected who were at higher risk of developing progressive disease over the 30 months of the study. It showed that obese women with established OA were more likely to show x-ray progression. Both the index (OA) knee and the contralateral knee with minimal OA were imaged at baseline, at 16 and 30 months. Patients were allowed to continue with background analgesics except for a proscribed washout before each study visit. The study did not demonstrate any effect of doxycycline on OA symptoms, but in patients treated with doxycycline, there was

less overall radiographic progression than in the controls. However, this effect was seen only in the index knee with preexisting OA, with no effect in the contralateral knee. The authors note that treatment may affect only the knee with more advanced disease. However, OA was also detected in the lateral and patellofemoral views of the contralateral knee. The rate of radiographic progression demonstrated in the overall cohort was 0.45 mm over 30 months, with no difference between the placebo-treated OA knees and the contralateral knees.

Patients with higher levels of baseline pain were more likely to progress. Interestingly, baseline levels of the cartilage turnover marker CTX-II were not predictive of progression [152], nor were other markers except for baseline levels of MMP-3 [153,154].

The results of the doxycycline studies are important and demonstrate the potential feasibility of conducting a study of a DMOAD using plain radiography. However, the limited progression and the small ultimate sample size raise questions about the outcome. It is uncertain, for example, whether the results apply to men, to older or to nonobese women. Furthermore, long-term use of doxycycline at the doses studied has been associated with a significant increase in photosensitivity, GI distress, and monilial vaginosis.

11.6.1.3 Other MMP Inhibitors

The results of a phase I study of an MMP inhibitor BAY 12-9566 in OA have been reported [155]; 35 patients received three doses of the drug or placebo given 3 weeks before planned total joint arthroplasty. Biomarkers and levels of the drug were evaluated. The highest doses led to a higher proteoglycan turnover and an increase in new matrix synthesis. Studies of other MMP inhibitors were terminated early due to drug toxicities, but the results of these studies have not been published. One MMP inhibitor, Marimastat (BB-2516), has been evaluated in phaseII/III cancer studies. Dose-dependent musculoskeletal pain was seen in >60% of patients. Discontinuation of the medication stopped the pain [156,157]. Use of MMP inhibitors has led to fibrosing conditions, including adhesive capsulitis of the shoulder, Peyronie disease, and concerns of retroperitoneal and pulmonary fibrosis. These adverse effects indicate the importance of MMPs, not only in pathologic states, but also in normal tissue remodeling. Whether these side effects can be overcome with more selective inhibitors requires further study. However, because a number of MMPs are involved in the pathogenic arthritis states, with overlapping substrate specificity, inhibition of a single MMP may have no clinical effect.

11.7 Cathepsin K Inhibitors

Another group of proteases of importance in bone health and contributing to the pathobiology of arthritis are the cathepsins, a family of cysteine proteases of the papains [89]. Cathepsin K is the major cysteine protease of osteoclasts. It is also produced by synovial cells. It functions as a collagenase, degrading type I collagen in bone and type II in cartilage, and acts on other extracellular matrix proteins, including aggrecan. Cathepsin K cleaves triple helical collagens in their helical domains [156] and is upregulated in articular chondrocytes in a mouse transgenic OA model. Overexpression of cathepsin K results in spontaneous synovitis with joint destruction [158,159]. Upregulation of cathepsin K in response to TNF and IL-1 has been demonstrated in RA, OA synovium, and in synovial cells [160]. Because osteoclasts produce cathepsin K, its synthesis has become an important target for bone remodeling and for pharmacologic inhibition, with possible effects in osteoporosis, as well as in OA. Overexpression of cathepsin K in mice results in a phenotype with increased bone turnover at the metaphysis of trabecular bone. Cathepsin K deficiency in humans leads to a bony sclerotic disease known as pycnodysostosis. This and the role enzyme plays in bone turnover make cathepsin K an attractive pharmacologic target [161,162].

Several cathepsin K inhibitors have been developed for use in OA and osteoporosis. The cathepsin K inhibitor SB 553484 is effective in a dog medial meniscectomy model, inducing less cartilage damage [163]. Another compound, relacatib (462795), is currently under study in OA and osteoporosis [164]. The results of studies of these and related cathepsin K inhibitors will help in defining the role of interventions intended to alter bone turnover and OA progression.

11.8 Conclusion

The recognition of OA as a potentially modifiable disease is extremely exciting. Unfortunately, it is difficult to detect disease worsening over a short periods with existing methodology and plain radiography, unless a large number of patients and highly standardized methods of assessment are used. Because in many studies only a small number of patients exhibit substantial worsening, evaluation of a putative treatment as a DMOAD becomes difficult. Therefore, it becomes imperative to define subsets of patients who are at enhanced risk of progression, even if those criteria do not necessarily apply to the general population.

The role of other imaging modalities needs further definition, specifically MRI, which assesses the components of the joint, including bone, synovium, and cartilage (thickness, volume). The use of functionally enhanced MRI can assess cartilage proteoglycan content [165], but the time-dependent changes of this and comparable measures need to be related to plain radiography, before regulatory agencies will embrace these methodologies. The ongoing work of Outcome Measures in Rheumatology Clinical Trials OMERACT and Osteoarthritis Research Society Intervention OARSI will help to define the roles for these various techniques. Furthermore, the NIH and industry-sponsored Osteoarthritis Initiative will provide a rich clinical, imaging, and biologic specimen repository in the public domain that should greatly enhance the efforts of the international community in moving these efforts forward. The studies that have been conducted using other putative DMOADs should also be explored, in the hope they will yield additional clinical and biologic predictors for disease progression [166]. Similarly, the pharmaceutical industry should be encouraged to make available the data from these studies, thereby furthering international efforts in developing drugs to combat osteoarthritis.

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