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Molecular basis for differences between human joints

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Abstract. The molecular program of a cell determines responses including induction or inhibition of genes for function and activity, and this is true of the cells within articular cartilage, a major functional component of the joint. While our studies have previously focussed on differences in the molecular programs of the cells within the superficial and deep zones, we have recently begun to focus on relative differences between joints, such as the knee and ankle. In the human, these joints vary greatly in their susceptibility to joint diseases, such as osteoarthritis (OA). We have predicted that there would be a molecular basis for differences between joints that could lead to differences in susceptibility to OA, if inherent pathways locked into the resident cells induce differences in their response to their environment. We have been able to show that there are differences between the matrix components and water content; these properties correspond to a higher equilibrium modulus and dynamic stiffness but lower hydraulic permeability and serve to make the ankle cartilage stiffer, slowing movement of molecules through the cartilage. In addition to these biochemical differences in the cartilage matrix, we have also identified relative differences in the strength of the response to stimulation of chondrocytes from knee and ankle. The stronger response of the knee chondrocytes includes factors that increase damage to the cartilage matrix, such as a depression of matrix synthesis and increased enzyme activity. This response by the knee chondrocytes results in enzyme damage to the matrix that the cells may not be able to repair, while the weaker response of the ankle chondrocytes may allow the cells to repair their matrix damage.

Key words. Articular cartilage; knee; ankle; molecular differences.

Osteoarthritis (OA) is a slowly developing joint disease that affects at least 50 million adults in the United States alone and approximately 15% of the world's adult population with pain and disability. For as yet poorly understood reasons, the prevalence of OA is consistently higher in some joints than in others. The majority of patients are symptomatic in one joint only, with certain joints being affected more frequently than others [1]. In the hands, the distal and proximal interphalangeal joints and the carpometacarpal joint of the thumb are most often involved. Other joints commonly affected by this disease include the facet joints of cervical and lumbosacral spine, and in the lower extremity, the hips, the knees, and the first metatarsophalangeal joints. Some familial forms of precocious or early-onset OA appear to have a heritable component as dominant Mendelian traits; however, even in these individuals, some joints appear to be sites of increased susceptibility and are more predisposed to OA than others [2]. Other joints, such as the ankle, are spared for unknown reasons. In other joint diseases also, such as rheumatoid arthritis, the disease process selectively affects some joints while sparing others like the distal and proximal interphalangeal joints of the hand.

Our own studies have focussed on knee and ankle joints where differences in the prevalence of OA have been documented based on epidemiological, radiographic, and pathological data. These studies have shown that approximately 6% of the adult population is affected by symp-

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tomatic knee OA; in those over the age of 65, this percentage increases to almost 10% [3]. Risk factors vary for different joints; in the knee, the risk factors include not only age but also gender, abnormal biomechanics, trauma, obesity and perhaps exercise [1, 4]. OA in the knee is more frequent in women than in men and appears to be associated with trauma or occupations in which there is repetitive high stress in the knee [5–8]. OA also develops after mechanical insult following meniscectomy or with anterior cruciate ligament insufficiency. While symptomatic OA does develop in the ankle, it is quite rare (< 1%), even in advanced age [9]. In the ankle, the major risk factors are abnormal mechanics or trauma [10–14], and the only occupation that has been associated with ankle OA is ballet [15, 16].

The most widely used epidemiological criteria for OA are based on radiographic changes, including joint space narrowing that results from the self-destruction of articular cartilage. This is a tough yet elastic tissue whose cells, the chondrocytes, occupy only 5% of the total tissue and are responsible for maintaining their surrounding matrix composed predominantly of collagens and proteoglycans [17]. The combination of the collagens and proteoglycans allow the matrix to function as the cushion that absorbs the impact of load and force on the joint. This majority of the collagenous component of the extracellular matrix is exceptionally stable with a half-life exceeding the life span of the individual [18]. The half-life of proteoglycans is approximately 2 years [19], and these molecules are continuously turned over through a close coupling of synthesis (anabolism) and degradation (catabolism). If the molecular program of the chondrocytes is altered through an unbalanced equilibrium of synthesis and degradation, then enzymes, primarily matrix metalloproteinases (MMPs), released from these cells themselves cause damage to the matrix in a process called chondrocytic chondrolysis (fig. 1). The gradual self-destruction of the matrix is induced as the chondrocytes respond to catabolic mediators, such as interleukin-1 or to fragments of matrix components, including fibronectin [20], collagen [21], and hyaluronan [22]. With the loss of the matrix components, the cartilage is no longer able to function properly. It should be noted that while cartilage loss seen as joint space narrowing is useful as a radiographic feature that can be correlated with nonradiographic features to diagnose clinical OA, joint space narrowing is not the only feature that should be used. Felson et al. [23] suggested that radiographic OA should also be based on osteophytes and bony features as well.

Poole et al. [24] have proposed that joints with different susceptibility to OA might be divided into those in which the repair process is dominant and those in which degradation is relatively more active. Our own studies have extended this concept to determine whether ankle cartilage is more resistant than knee cartilage to progressive degeneration due to: (i) increased synthetic ability, (ii) decreased matrix loss in response to catabolic factors, and (iii) differences in biochemical composition and/or biomechanical properties of the extracellular matrix.

Matched pairs of knee/ankle (donor population)

To demonstrate differences between the chondrocytes in joints with a high and low prevalence of OA, our studies have concentrated on human cartilages available within 24 h of death through collaboration with the Regional Organ Bank of Illinois. Studies of human articular cartilages are complicated by numerous factors including not only large intraindividual differences but also intraarticular differences. To standardize the criteria for knee and ankle cartilages used for the joint comparisons, the carti-

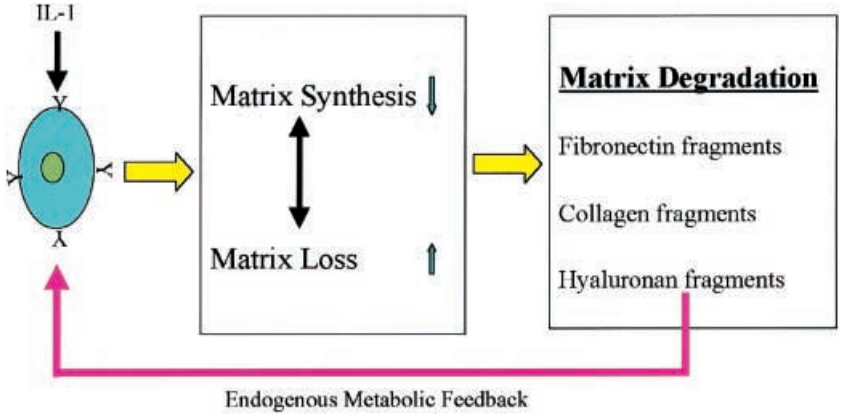

Figure 1. Chondrocytes respond to interleukin-1 through their receptors by shifting the maintenance of homeostasis through a decrease in matrix synthesis and an increase in matrix loss. Matrix loss occurs as the components become proteolytically degraded. The fragments generated through the degradation appear to generate an endogenous metabolic feedback that will generate a response from the chondrocytes. The effect of the fragments on the chondrocytes may result in greater matrix damage than interleukin-1 itself, apparently by acting through the cell surface receptors, activating degradative pathways, and further potentiating the process of matrix destruction.

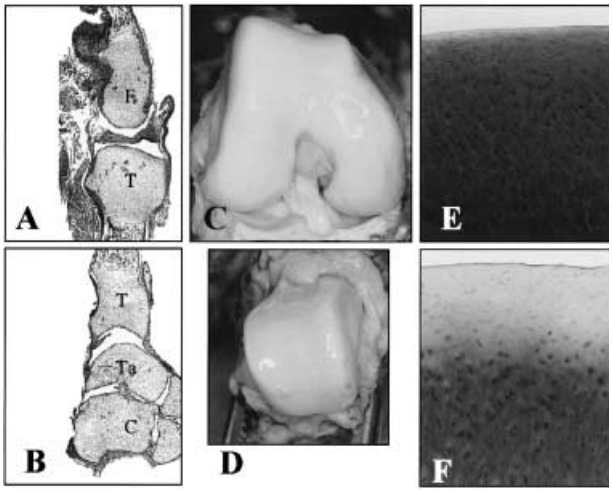

Figure 2. All comparisons are between the convex surface of the tibiofemoral or the talocrural joints. The cartilages were removed from the weight-bearing region of the femoral condyles from the tibiofemoral joint, and the weight-bearing region of the talus from the talocrural joint. Collins grade 0 and Mankin grades 0–3 are used to classify normal joints. (*A*) Tibiofemoral joint of 16-week fetus to show the femur (F) and the tibia (T) section through the medial compartment. (*B*) Talocrural and talocalcaneal joints from the 16-week fetus. The talus (Ta) is located between the tibia (T) and the calcaneus (*C*). (*C*, *D*) Femur and talus from a 19-year-old male showing Collins grade 0. (*E*, *F*) Knee articular cartilage reflecting Mankin grade 0 (*E*) and Mankin grade 3 (*F*).

lages were obtained from both joints of the same limb of each donor and are referred to as matched pairs. Cartilages are routinely removed from the convex surface of the weight-bearing region of the femur of the tibiofemoral compartment or the weight-bearing region of the talus in the talocrural joint (fig. 2). In addition, comparisons are made between joints that have normal cartilages defined by macroscopic and microscopic assessment.

Collins and mankin scales for grading joints and cartilages

The relationship between degenerative changes in the cartilages from undiagnosed donors and those from patients diagnosed with OA is not clear. OA is a clinical diagnosis based not only on radiographic evidence of cartilage loss seen as the narrowing of the joint space but also on other criteria such as pain and disability. Some studies from other laboratories have reported results with cartilages from donors with no indication of joint grade and so appear to ignore the effects that these degradative changes may have on the chondrocytes or their surrounding matrix, especially in the knee. Other studies [25–29] have considered these changes as an intermediate stage (pre-OA) in disease progression or OA itself; the study by Tshukahara [27] reported evidence of degeneration in 98% of the ankle joints examined in the study. Given the low prevalance of OA in the ankle, the degenerative changes obviously do not correlate exactly with clinical signs and symptoms of the disease. Until there is a better understanding of the effect of the degenerative changes on chondrocytes within the entire joint, studies of chondrocyte metabolism especially in the knees of older individuals should be limited to cartilages from joints with defined characteristics. Studies that employ chondrocytes from 'normal-looking' areas of joints obtained at the time of joint replacement from OA patients should be interpreted with great care.

To consistently define joint characteristics, all of the joints from the organ donors were graded macroscopically on the scale published by Collins [30] and modified by us [31]. The Mankin score [32] was adapted with slight modifications to the changes seen in cartilages from asymptomatic organ donors for microscopic assessment [33]. Using the two scales, normal cartilage was defined as Collins grade 0 or 1 and Mankin grade 0–3 (fig. 2). These Mankin grades were included since we had determined that they were present in the joints from individuals in their third decade of life with Collins grade 0 [34]. Although none of the donors from whom cartilages were obtained had been diagnosed with OA, not all of their cartilages were normal, even in the ankle (table 1). The presence of ankles with degenerative changes sup-

Table 1. Collins grades of knees and ankles available through the Regional Organ Bank of Illinois from March 1993 through November 2000: from 1711 donors, a total of 452 knees and 3,389 ankles were graded.

Collins grade	Criteria	Knee (%)	Ankle (%)
0	no cartilage degeneration or osteophytes	37	54
1	limited fibrillation of cartilage	18	23
2	deep fibrillation and fissuring	25	18
3	extensive fibrillation and fissuring of 30% or less of articular surface eroded to bone with osteophytes	14	4
4	greater than 30% of the articular surface eroded to bone with gross geometric changes including osteophytes	5	0.03

To ensure that the cartilages for the knee/ankle comparisons for these studies were taken from joints with known cartilage and osteophyte characteristics, all joints were graded on the scale of Collins [30] as previously modified [31]. Among the matched pairs, both the tibiofemoral and patellofemoral compartments of the knee, and both the tibial and talar surfaces of the ankle joints were used to determine the overall score in that joint.

ports the concept that these changes do not progress to OA and may represent a greater reparative response by the ankle cartilage than that of the knee.

Although the frequency of degenerative changes did increase with age in both the knee and ankle, there were donors between 61–96 years of age with no visible degeneration in either the knee or ankle. We had previously reported [35] that among donors over 61 years of age, 38% of the ankles and 4% of the knees were graded as normal. Since the mean age of the donors was over 50 years of age, it is important to note that the cartilages from older individuals can be normal and are included in the studies.

Comparisons of knee and ankle cartilages

For the comparison studies of metabolic differences between the knee and ankle chondrocytes, two approaches were used: explant cultures and primary cultures in alginate beads. A third approach has been to compare gene expression, biochemical composition, and biomechanical properties of the two cartilages without culture to define in vivo differences between them.

Explant cultures

The cartilage is removed from the articular surface and divided into small pieces (3×3 mm^2) for culture so that the chondrocytes maintain their surrounding matrix. If our hypothesis that ankle has a higher potential for repair is correct, then ankle chondrocytes should have higher synthesis of matrix components than knee chondrocytes; in addition, ankle chondrocytes should respond less than knee chondrocytes to catabolic stimulation. Two different mediators of catabolic stimulation were compared: interleukin-1 and fibronectin fragments.

Interleukin-1 response

Interleukin-1 is a proinflammatory cytokine that is of physiological relevance, since there are intermittent inflammatory episodes in OA [36–40]. Chondrocytes are known to respond to interleukin-1 by decreasing synthesis of matrix components, increasing synthesis of proteolytic enzymes, and enhancing prostaglandin (PG) degradation [41, 42]. Thus, the metabolic balance between anabolism and catabolism is shifted in favor of matrix loss [43]. This cytokine has been used extensively in our own laboratories [44–48] to investigate its effects on cartilage.

In our studies [49], seven matched pairs of knee and ankle cartilages were incubated as explants with and without interleukin-1β (0.05–1000 pg/ml) for 72 h; and then exposed to ^{35}S-sulfate to label newly synthesized proteoglycans. When proteoglycan synthesis by knee and ankle chondrocytes was compared without interleukin-1 stimulation, synthesis by the ankle chondrocytes was higher than by the knee chondrocytes. At the lowest concentration tested (0.05 pg/ml), there was no decrease in proteoglycan synthesis by either; at this concentration, synthesis by the ankle chondrocytes remained higher than by the knee chondrocytes. When interleukin-1β (1–100 pg/ml) was added to the cultures, proteoglycan synthesis by both knee and ankle chondrocytes decreased; however, there were significant quantitative differences between the two. Interleukin-1β was approximately eight times more effective with knee than with ankle chondrocytes in reducing proteoglycan synthesis. With higher concentrations of interleukin-1β (250–1000 pg/ml), proteoglycan synthesis was reduced by the same amount in both knee and ankle, and there was no significant difference between the two. Although human chondrocytes are sensitive to concentrations of interleukin-1 as low as 1–5 pg/ml with respect to proteoglycan synthesis, we were unable to detect quantatively the proteoglycan loss even after 21 days of culture, regardless of the interleukin-1 concentration from 50 to 1000 pg/ml [unpublished data]. We were able to detect changes in MMP and aggrecanase activity, with an increase in neoepitopes generated by proteinase activity in the interglobular domain of aggrecan core protein, suggesting that their activity was upregulated but did not release the neoepitopes from the cartilage. We had previously reported that the cleavage products of proteoglycan were maintained in cartilages from normal as well as OA joints [50].

Fibronectin-fragment response

To test whether proteoglycan loss from cartilage could be induced, we tested a second mediator, fibronectin fragments. The amino-terminal thrombin-generated 29-kDa fibronectin fragment (fn-fs) had previously been shown to be very potent in both inducing chondrocytic chondrolysis in bovine cartilage explant cultures [51] and causing severe depletion of cartilage proteoglycan when injected into the knee joints of rabbits [52]. In addition, fn-fs elevates MMPs [53] and suppresses proteoglycan synthesis [54]. These combined activities cause 30–50% degradation and loss of proteoglycan from bovine metatarsalphalangeal explant cultures. This mediator was chosen further because the damage appears to be driven by catabolic cytokines that are relevant to arthritic diseases [20] and because fn-fs is found at elevated levels in the synovial fluids of patients with OA as well as rheumatoid arthritis [55].

We have reported [56] the results of studies using seven matched pairs of knee and ankle cartilages cultured with the 29-kDa fn-fs for up to 28 days. From the knee cartilage, there was damage, defined as a 30–50% decrease in proteoglycan content, between 7 and 14 days; however, in the ankle cartilages from the same donor, there was

little detectable decrease in proteoglycan content even after 28 days. The study was then extended to include additional cultured knee cartilages from 13 donors for whom ankles were not available; in most cases, proteoglycan content was decreased by day 7. However, with the ankle cartilages from additional donors for whom knees were not available, damage was still not detectable by day 21 or 28. As with the interleukin-1 stimulation, there was an accompanying increase in aggrecanase activity in the knee compared to the ankle. These data clearly show differences between ankle and knee cartilage in susceptibility to fn-fs and suggest the feasibility of using fn-fs for discerning differences in ankle and knee cartilage homeostasis.

Primary cultures in alginate beads

The chondrocytes are enzymatically released from their matrix and then resuspended in alginate without allowing the cells to dedifferentiate, in order to increase cell number. In the alginate beads with 40,000 chondrocytes/bead, the cells maintain their chondrocytic phenotype, and will rebuild their matrix with many characteristics of the in vivo cartilage [57]. If the ankle chondrocytes have a greater ability than the knee chondrocytes to synthesize the matrix components, then that greater synthetic ability should be maintained when the cells are removed from their intact matrix and allowed to build a new one. The differential response to catabolic stimulation should also be maintained.

This study [58] confirmed that when chondrocytes are compared in primary cultures within alginate beads, the molecular differences between knee and ankle cells are maintained. The study additionally showed that in the presence of the catabolic cytokine, interleukin-1, both the knee and ankle chondrocytes decrease proteoglycan synthesis, but the intensity of the response varies, with the knee chondrocytes, showing a much stronger response than the ankle chondrocytes, just as in the explant cultures. Additionally, the receptor antagonist protein was able to overcome the effects of interleukin-1 in the ankle cartilage but not in that of the knee; the study reported that ankle chondrocytes contain fewer interleukin-1 receptors than do knee chondrocytes. The significance of this component of the knee/ankle comparison shows that the molecular program resulting in a differential response of the knee and ankle chondrocytes is not merely a result of differences in biomechanical factors that have developed over the lifetime of the individual. But, significantly, this program is maintained even after the cells are released, reseeded in a different matrix, and allowed to build a newly formed matrix.

Different molecular programs have also been described for the superficial and deep zones within each articular cartilage. The synthesis and secretion of a novel proteoglycan, the superficial-zone protein (SZP), from chondrocytes of the superficial zone but not from the middle or deep zone chondrocytes has previously been described [59, 60]. These zonal differences in metabolism have not only been demonstrated in explant cultures of articular cartilage but also from chondrocytes which were enzymatically released from their matrix and maintained in culture suspended in agarose [61] or alginate beads [48]. The superficial and deep-zone chondrocytes differ in their response to interleukin-1, in their high-affinity binding sites for interleukin-1, and in their response to interleukin-1 receptor antagonist protein [48]. Another component of the differences between knee and ankle may reside in the relative content of superficial and deep cells.

In vivo

To investigate the molecular program that existed in the living individual as closely as possible, cartilage is removed from the joint within 12–24 h of death and processed immediately without culture, for assays comparing gene expression and for biochemical or biomechanical testing. Higher synthesis of matrix components by ankle chondrocytes should be reflected in differences in the biochemistry and biomechanics of the cartilage.

Biomechanical as well as evolutionary and anatomical differences between joints, including the knee and ankle, [62] are well attested; however, there has been little information on biochemical differences. Also well accepted is that biomechanical and biochemical properties of cartilage are interdependent (see further discussion in the contribution by Kerin et al.). Studies of the etiopathogenesis of OA have pointed to multiple factors involved in the interaction of biomechanical and biological aspects of the destruction of articular cartilage. A biomechanically driven etiopathogenesis of OA must also be biochemically mediated; therefore, OA diseases must result from both a combination of mechanical and biologic events.

Our studies [63] have supported the concept that a combination of biochemical and biomechanical factors affects the chondrocytes and their matrix thus making the ankle tissue more resistant to enzyme damage and disease progression than that of the knee. Using matched pairs from seven donors, cylindrical disks were removed from ten sites within the tibiofemoral and femoropatellar joint surfaces and four sites within the talocrural joint. For both cartilages, only the top 1 mm was compared. For the ankle, the cartilage is approximately 1 mm in total thickness while for the knee, this represents only the top portion of the entire cartilage; it does represent the relative regions that are subjected to compressive deformation during cyclic loading. Uniaxial confined compression measurements were performed to determine equilibrium modulus, hydraulic permeability, dynamic stiffness, streaming potential, electrokinetic coupling coefficient, and electrical conductivity. The content of proteoglycan

[measured as glycosaminoglycans (GAG)], collagen (measured as hydroxyproline), DNA, and water, as well as hypotonic swelling behavior were determined for each cartilage sample on which the biomechanical and electromechanical tests were conducted. The ankle cartilage appeared denser, with a higher GAG content, lower water content, higher equilibrium modulus and dynamic stiffness and lower hydraulic permeability. The equilibrium modulus increased with increasing GAG/wet weight and decreased with increasing water content for all joint surfaces. More than 80% of the variation in the equilibrium modulus could be accounted for by variations in the biochemical parameters (water content, GAG/wet weight, and hydroxyproline content/wet weight) for each joint surface. Another striking finding was the consistency of the differences between the properties of ankle and knee cartilages from each of the seven individual donors. The implication of these findings to the cartilage would appear to be that the denser, more highly charged ankle matrix may retard the progression and therefore the exposure of chondrocytes to catabolic mediators. The biomechanical properties and biochemical composition of talar cartilage may additionally endow it with an increased resistance to loading, making it less sensitive to damage and ultimately to the progression of OA.

The effects of catabolic mediators on chondrocytes include not only decreased matrix synthesis and increased loss of matrix components but also changes in the activities of proteolytic enzymes, both MMPs and aggrecanases. Little is known about the activity of these proteolytic enzymes in normal cartilages, especially ankle cartilages. Expression of MMPs has been extensively studied in relation to the development of OA. One MMP differed between the knee and ankle cartilages as well: collagenase 2 or MMP-8 mRNA was detectable in normal knee chondrocytes but not in ankle chondrocytes unless the ankle chondrocytes were stimulated in culture with interleukin-1 or the ankle cartilage originally showed signs of degeneration in vivo [33, 34, 50, 64]. The differences in detectable expression of MMP-8 were also reported for the first and fifth metatarsalphalangeal joints [65].

Summary

The results of our research have provided evidence that the lower frequency of osteoarthritis in the human ankle than in the knee may be due to a combination of factors including metabolic, biochemical, and biomechanical differences between the cartilages from the two joints. The chondrocytes within each joint cartilage build a matrix around themselves that confer different properties to their tissues, with the ankle tissue being tougher, more elastic, and more resistant to damage. Even when isolated from that matrix, the cells maintain differences in their response to stimulation. By expanding our understanding of how the two cartilages differ from one another, we come closer to identifying early stages of damage that may precede OA. Our goal in OA research is to identify the early stages of disease, develop means of blocking the progression of the disease process, and reversing its effects. One method of reversing the effects of early disease processes is to decrease the response of chondrocytes to catabolic stimulation and to stimulate the chondrocytes to rebuild their matrix. A better understanding of these differences between chondrocytes in different joints should facilitate development of therapeutic strategies for early detection and prevention of OA.

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