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Review
Cartilage degeneration in different human joints[1,2]

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Summary

Variations among joints in the initiation and progression of degeneration may be explained, in part, by metabolic, biochemical and biomechanical differences. Compared to the cartilage in the knee joint, ankle cartilage has a higher content of proteoglycans and water, as well as an increased rate of proteoglycan turnover and synthesis, all of which are responsible for its increased stiffness and reduced permeability. Chondrocytes within ankle cartilage have a decreased response to catabolic factors such as interleukin-1 and fibronectin fragments, compared to the chondrocytes of knee cartilage. Moreover, in response to damage, ankle chondrocytes synthesize proteoglycans at a higher rate than that found in knee cartilage chondrocytes, which suggests a greater capacity for repair. In addition to the cartilages of the two joints, the underlying bones also respond differently to degenerative changes. Taken together, these metabolic, biochemical and biomechanical differences may provide protection to the ankle.

Key words: Knee, Ankle, Cartilage, Degeneration, Repair.

Introduction

Osteoarthritis (OA) is a degenerative joint disease that involves not only articular cartilage but also synovium, joint capsule and bone. OA is a common joint disease of the elderly; however, it does not affect all joints equally[1,2], even in those individuals with generalized OA. Certain features have been associated with the onset of generalized OA, but the question of why some joints are affected while others are not remains unanswered[2,3]. The joints that are most often affected by OA include the hip, knee, spine and metatarsophalangeal joints, as well as both the distal and proximal interphalangeal joints of the hand. The ankle, wrist, elbow and shoulder are generally spared from symptomatic OA. In these nonsymptomatic joints, degeneration of the articular cartilage does occur, suggesting that this degeneration may be nonprogressive, while in susceptible joints degeneration progresses to the disease state.

Poole *et al.*[4] have suggested that an imbalance between degeneration and repair might help to explain, in part, the differences among joints in susceptibility to OA. There have been almost no studies that have compared the cartilages from different adult human joints in an attempt to identify features within joints that might either retard or stimulate cartilage degeneration. We had originally proposed that susceptibility to OA is genetically programmed into the chondrocytes of some but not all joints. Our laboratories have concentrated on human articular cartilages of the ankle (talocrural joint) and the knee (tibiofemoral joint) from the ipsilateral limb (matched pairs) (Fig. 1) that are available to us through collaboration with the Gift of Hope Organ and Tissue Donor Network.

The knee and ankle were chosen for these studies not only for their availability through the tissue bank but also because of the dramatic differences in the prevalence of OA and degeneration between the two joints. While symptomatic OA is extremely rare in the ankle joint ($<0.1\%$), almost 10% of the population will develop this disease in the knee[5,6]. Although cartilage degeneration cannot be equated with OA, there is general agreement that degeneration precedes OA. There have been several studies[7,8], including our own[9–11], that have shown that full thickness defects of the cartilage are higher in the knee compared to the ankle.

It is well accepted that there are numerous anatomical and biomechanical differences between the knee and ankle joints that could account for more frequent degenerative changes in the knee than in the ankle. The knee joint is a relatively unstable joint composed of the distal femur, proximal tibia and patella; it is non-congruent and partially stabilized by menisci and ligaments as well as muscles. Movement in the knee joint is a mixture of flexion/extension and rotation. The talocrural (ankle) joint connects the foot and leg and, like the knee, is also made up of three bones: the distal tibia, distal fibula and the dome of the talus. Ligaments and the interosseous membrane between the tibia and fibula help to make this an extremely stable joint where movement is limited to extension/flexion. Under high loads the articular surfaces become highly congruent, transmitting the weight of the body from the tibia to all the other weight-bearing bones of the foot. The fact that the ankle surface is exposed to higher loads per unit surface

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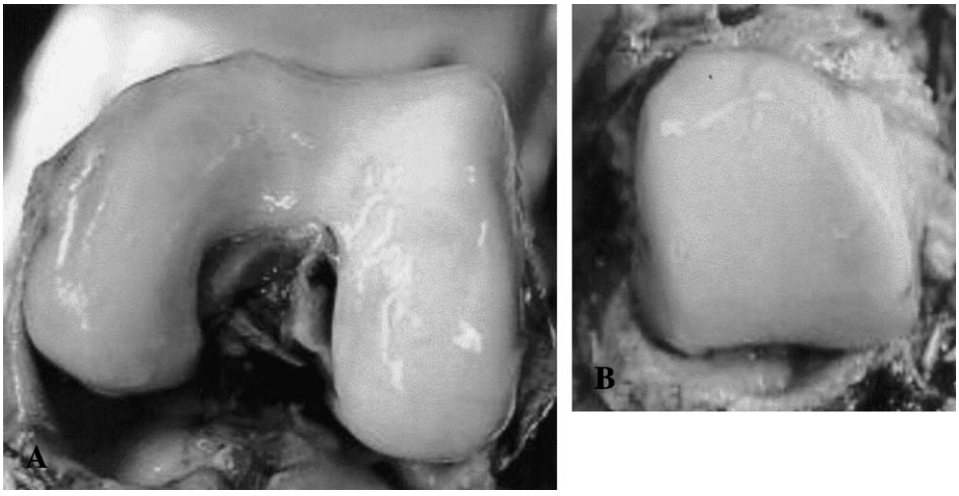

Fig. 1. Tibiofemoral and talocrural joints. (A) The distal femur and (B) the talus from a 36-year-old man. Both are Grade 0.

area than the knee in normal walking[12] would suggest that there are inherent properties of the ankle cartilage that protect it from the higher compressive loads it experiences.

In the lower limb, the risk factors for OA include abnormal biomechanics and trauma, obesity, age, genetic predisposition and higher bone mineral density, as well as congenital and developmental disorders of bones and joints[13]. OA of the knee is more common in women than in men and is associated with occupations in which there is high repetitive stress on the joint. OA also develops with altered joint mechanics that result following menisectomy or damage to the anterior cruciate ligament. In the ankle, the major risk factors are abnormal mechanics or trauma, and the only occupations associated with OA are ballet and soccer[14–17].

The anatomical and biomechanical differences between the two joints alone do not explain why the knee joint is more susceptible to OA than the ankle joint. We have proposed that there must be biochemical and molecular biologic differences between these two joints that provide some protection to the ankle. Our studies were designed to determine whether ankle cartilage is more resistant than knee cartilage to progressive degeneration and OA due to either one or all of the following criteria: 1) differences in biochemical composition and/or biomechanical properties of the extracellular matrix; 2) decreased response to catabolic factors; and 3) increased synthetic ability to repair. At the present time we have data to support all three components of this hypothesis.

Donor population

In order to compare human adult cartilages from knee and ankle, we established collaboration with the Gift of Hope Organ and Tissue Donor Network that has continued for more than 15 years. During that period we have been able to acquire knees and ankles from the ipsilateral limb of donors ranging in age from fetal to 90+ years of age (Table I). In addition, we have acquired unmatched ankles for use in many of our cartilage studies including those on changes in cartilage with aging[18]. Our studies with the ankle cartilage have allowed us to establish the experimental parameters that we use for studying normal knee and ankle pairs that are available in more limited numbers.

The profiles of the donors from whom we receive joint tissue reflect the donor population available to the tissue bank[11]. That population consisted of 73% men and 27% women, of whom 91% were Caucasian, 6% African American, 2% Hispanic, and 1% Asian. The average age was 55.7 years. The most frequent causes of death were myocardial infarction or cardiopulmonary accident (72%), accidental death (16%) and stroke (12%). Of the adult donors that were assessed for weight, 33% of the women and 23% of the men were considered grossly overweight. Donor cartilages were excluded from our studies if the donor was being treated for joint disease (based on reports by the families of the donors) or had skeletal pathology, fractures, HIV, hepatitis or diabetes.

Joint grading scale

A five-point scale originally described by Collins[19] for OA of the knee was modified by Muehleman *et al.*[9] for use with the ankle (Fig. 2). In brief, the grades are as follows: Grade 0 joints display no signs of morphologic degenerative changes with a surface that is smoothly reflective and unfibrillated; Grade 1 joints have minimal fibrillations, shallow pits or grooves affecting the cartilage surface in the absence of degenerative changes in articular surface geometry; Grade 2 joints have deep fibrillations and fissuring, flaking, pitting and/or blistering, early marginal hyperplasia and, possibly, small osteophytes; Grade 3 joints have extensive fibrillations, fissuring, obvious osteophytes and 30% or less of the articular cartilage surface eroded down to the subchondral bone; and Grade 4 joints have prominent osteophytes and greater than 30% of the articular surface eroded down to the subchondral bone with gross geometric changes. Because osteophytes are extremely rare on the talus, the overall grading system for this

Table I
Total number of donors and joints from whom articular cartilage was available through the Gift of Hope Organ and Tissue Donor Network between 1993–2003

Donors (Total)	2953	Joints (Total)	5746
Knee/ankle pairs	271	Knee/ankle pairs	507
Ankles only	2682	Ankles only	5239

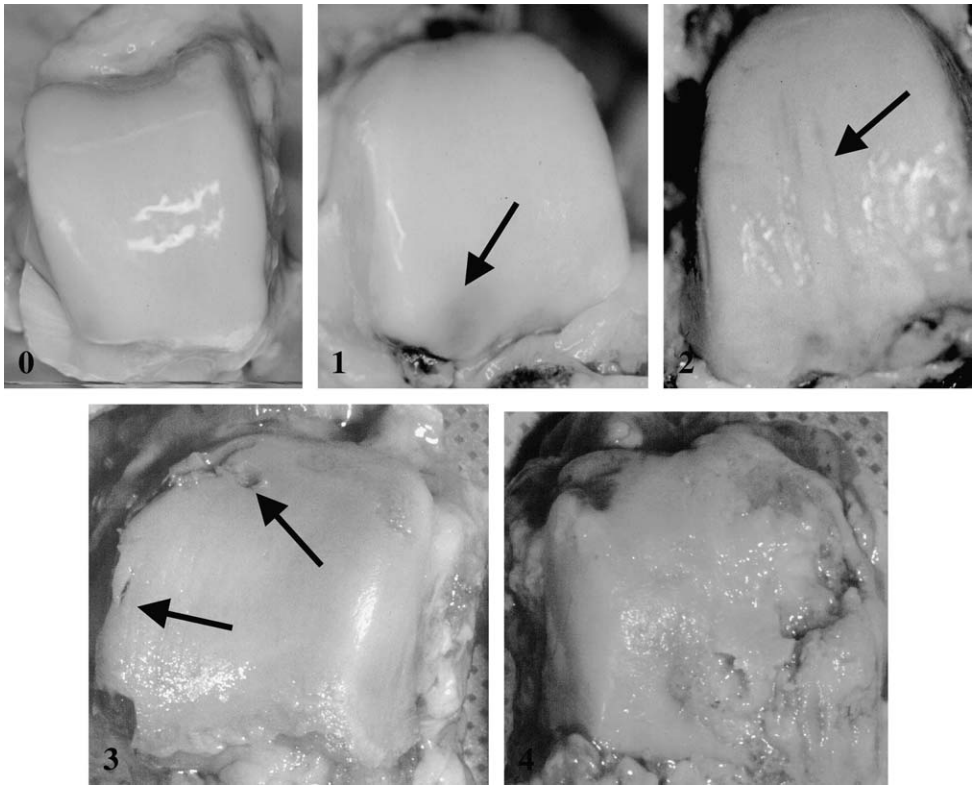

Fig. 2. The five-point grading scale applied to the talus. The grades are indicated as 0–4. Arrows point to areas of roughening (Grade 1), fissuring (Grade 2) or full thickness defect (Grade 3). On the Grade 4 talus, the dome is collapsed and more than 30% of the articular cartilage has been eroded to the subchondral bone. Reproduced from Cole *et al.*[11] with permission.

joint focused primarily on the disruption of the cartilage surface. The distribution of joint grades is listed in Table II. For our metabolic studies, we included both Grades 0 and 1 as normal because Grade 1 may simply represent normal senescent changes in the superficial cartilage that may not be progressive. Joints with Grades 2–4 were classified as degenerative.

Based on the data collected since 1993, we have been able to gain new insights into the degenerative changes in a population that has not been diagnosed with OA. While an increase in degenerative changes in the knee with age has long been accepted[20], it was not previously known whether the same was true for the ankle. Approximately 50% of the tali had articular surfaces with no macroscopically visible surface disruptions (Grade 0) compared with 30% of the femurs. When the ankle grades are analyzed by decade, it is clear that the percentage of those with Grade 0 decreased with increasing age; however, compared with the knee, progression is slower (Fig. 3)[11]. Interestingly, the percentage of Grade 0 ankles in the donors 90 years or older increased to over 50% while only 29% in the 80 year olds received Grade 0. In fact, none of the tali from donors in the ninth or tenth decades were Grade 3 or 4. This increase in Grade 0 tali may be due to the fact that cartilage degeneration in the ankles of those donors who live longer lives (>90 years of age) may be progressing even more slowly.

Table II
Distribution of joint grades. Percentage is based on 4005 ankles and 405 knees that have been graded

Joint/Grade	0	1	2	3	4
Knee (%)	34	20	25	14	7
Ankle (%)	52	25	19	4.7	0.3

In the ankle, more men (53%) received Grades 2–4 than women (34%); in the knee, the percentage of higher grades was also greater in men but the difference was smaller (60% for women, 67% for men). When the gender differences were analyzed based on weight, overweight men had higher grades than normal weight men for both the knee and ankle. Nearly 50% of the Grade 4 tali were obtained from overweight men.

In addition to the gender differences, there were other unexpected findings[10]. Our data show that the degree of degeneration in ankle cartilage is similar in both limbs in the majority of cases (79% had symmetrical scores). Additionally, in those donors with ankle cartilage degeneration, the ipsilateral knee cartilage also showed degenerative changes of an equal or higher grade. These two unexpected findings suggest that factors, such as altered biomechanics, responsible for degeneration in one limb also cause changes in the contralateral limb and influence both the ankle and knee ipsilateral joints.

Adult articular cartilage from the knee and ankle

Both knee and ankle cartilages have general features that are typical of articular cartilage[21] in that there are no blood vessels or nerve supply, and nutrition is derived from the synovial fluid. Articular cartilage protects the underlying more rigid bone by providing elasticity and resistance to compressive forces. These properties are conferred by the two major matrix constituents of the cartilage extracellular

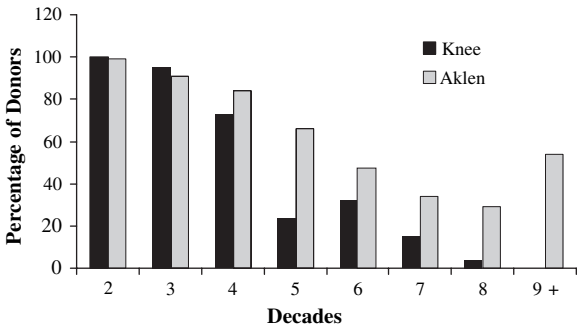

Fig. 3. Percentage of Grade 0 by decade in knee and ankle. The distribution of grades is based on data from 4005 tali and 409 femurs. Donors included in the ninth+ decade were 81 to 96 years of age. Adapted from Cole *et al.*[11] with permission from the Orthopaedic Research Society.

matrix: collagens and proteoglycans. The abundant extracellular organic matrix (98% by volume) of cartilage is synthesized and maintained by a few sparsely distributed cells, the chondrocytes.

In both joints, the articular cartilage can be divided into three main layers – the superficial, middle and deep (Fig. 4). The matrix of the superficial layer, which contains flat, disc-shaped chondrocytes, has a relatively low proteoglycan content and collagen fibers parallel to the surface. In both joints, these specialized superficial layer cells, but not the chondrocytes deeper in the cartilage, secrete the superficial zone protein, or SZP, into the synovial fluid[22,23]. In the middle and deep zones, the chondrocytes are more spherical; the matrix has a higher content of proteoglycan and the collagen orientation changes from isotropic in the middle to perpendicular to the surface in the deep layers. The articular cartilage in the ankle is fairly uniform in thickness (1–1.5 mm) across the entire surface, while the cartilage of the knee varies from 2–6 mm in thickness. The major difference between the two cartilages appears to be the thickness of the middle and deep layers[24,25]. In the superficial layer, the chondrocytes are organized into chondrons[26]; the chondrons in the knee are composed primarily of single cells, while the chondrons in the ankle are organized into clusters of two to four cells each. While there is a dramatic decline in cellular density between young infants and adult cartilage in both the superficial and deep layers[27], in the adult the cell density does not change with age in normal cartilage. The cell densities of the superficial layers of the knee and ankle are similar, as are the cell densities of the deep layers[28].

To analyze changes in cartilage with age in our initial studies[18], we used ankle cartilage because 50% of the donor talar cartilages were normal (Grade 0). These analyses show that neither the content of type II collagen (128 ± 14 μg/ml wet weight) nor sulfated glycosaminoglycans (40.6 ± 14 μg/mg wet weight) changed with increasing age between 13 and 75 years. However, there is continuing synthesis and degradation of the matrix components throughout life that is primarily associated with the cell and its pericellular matrix. Type II collagen synthesis was identified by mRNA and the C-propeptide (CPII) by immunoassay. The initial proteolytic processing of the collagen by collagenases and its subsequent denaturation were also measured by immunoassay for the collagenase cleavage and the denaturation neoepitope. While the cleaved type II collagen showed a trend toward increasing with age, the ratio of cleaved to denatured type II collagen did show a significant increase. These data suggest that in normal articular cartilage a homeostasis exists with regard to the turnover of matrix components and that this predominantly pericellular turnover continues throughout life. These studies would further suggest that the talar cartilage may provide an excellent model for studying changes in cartilage with aging that are distinct from changes with degeneration as are often found in the knee.

Comparison of knee and ankle cartilage

BIOCHEMICAL COMPOSITION AND BIOMECHANICAL PROPERTIES

Our data have shown that there are significant differences in the biochemical composition and biomechanical properties of the ankle cartilage matrix compared to that of the knee (Fig. 5)[29]. For example, in the ankle, the sulfated glycosaminoglycan content is significantly higher and the water content is significantly lower; however, there is no difference in collagen content. The dynamic stiffness is higher in ankle cartilage than in knee cartilage, while the

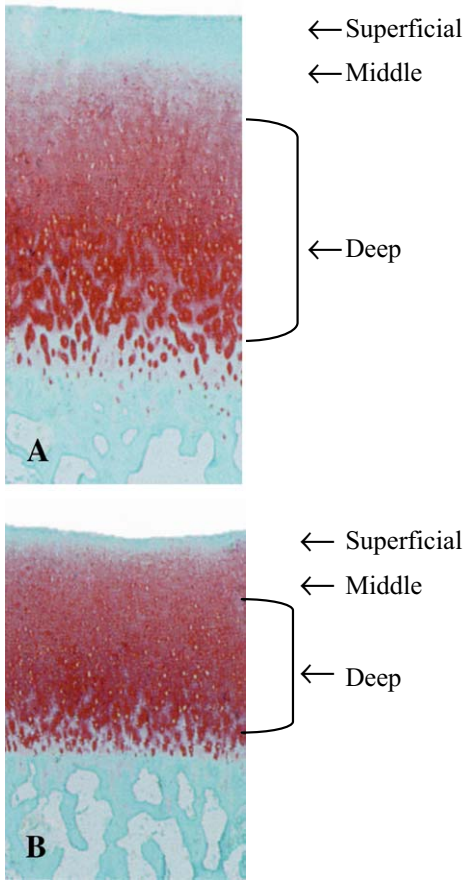

Fig. 4. Histological sections of full thickness articular cartilage and subchondral bone from the femur (A) and talus (B) of a 52-year-old man that were stained with Safranin O and fast green. The relative positions of the superficial, middle and deep layers of the cartilage are shown. (Original magnification = 4×.)

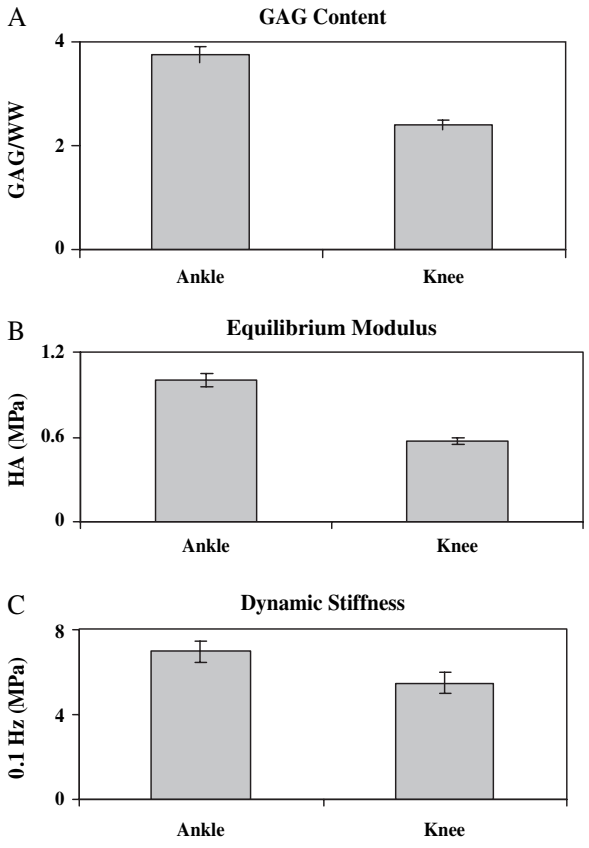

Fig. 5. A comparison of glycosaminoglycan (GAG) content (A), equilibrium modulus (B), and dynamic stiffness (C) of the femur of the knee and the talus from the ankle. Bars correspond to the mean + S.E.; differences were assessed by analysis of variance and Fisher's least significant difference test, *indicated $P < 0.01$. Adapted from Treppo *et al.*[29] with permission from the Orthopaedic Research Society.

hydraulic permeability is lower, reflecting the decreased water content and higher sulfated glycosaminoglycan content of the ankle cartilage. These properties could benefit the ankle cartilage by increasing cartilage stiffness, thereby protecting the cartilage from the deleterious effects of higher compressive forces.

The higher compressive stiffness of the ankle cartilage compared to that of the knee was apparent when the two cartilages were subjected to injurious compression[30]. In order to produce levels of peak stress and visible damage to human cartilage, a 65% final strain was required producing peak stresses of 11 ± 1 MPa in knee cartilage and 16 ± 1 MPa in ankle cartilage. This compression resulted in macroscopic tissue changes to 81% of the knees but only 17% of the ankles.

DIFFERENCES IN SYNTHESIS OF PROTEOGLYCANS

The higher sulfated glycosaminoglycan content of ankle cartilage correlates with a higher rate of synthesis by ankle chondrocytes[31]. When cartilages from the two joints were assayed in explant culture for differences in synthesis, ankle cartilage had a higher incorporation rate (mean = 28,799 cpm/μg DNA) than those from the knee (mean = 15,510 cpm/μg DNA) (Fig. 6). These differences were maintained over 25 days in culture[27] and begin early in development because they were found in donors as young as 1-year old[31]. In addition, ankle cartilage has also been shown to have higher levels of protein synthesis than knee cartilage[32].

When the turnover of proteoglycans in knee and ankle cartilages was compared[33], the half-life in knee cartilage was 22.68 days while that in ankle cartilage was 16.58 days. This reduction in the half-life implies that the ankle is metabolically more active not only in synthesizing proteoglycans but also in their degradation. While we do not know at the present time which proteoglycans are responsible, our data have shown that there are 2.1 times higher levels of aggrecan mRNA in ankle chondrocytes compared to those in the knee. Because aggrecan contains the majority of the sulfated glycosaminoglycans in the cartilage, it is probable that this proteoglycan is principally responsible for the differences.

MODULATION OF SYNTHESIS BY MECHANICAL COMPRESSION

The synthesis of proteoglycans, collagens and other proteins can be modulated by static as well as dynamic mechanical compression; static compression (>10%) has been previously shown to inhibit proteoglycan and protein synthesis in a dose-dependent manner in bovine calf cartilage[34,35]. When knee and ankle cartilages were subjected to increasing static compression (0–50%), proteoglycan, collagen and protein synthesis in both the knee and ankle were altered[32,36]. In the ankle, collagen synthesis was suppressed by 15% compression while the synthesis of proteoglycans and protein were not significantly suppressed until the higher strain of 25–50% compression was applied. Without compression, ankle chondrocytes synthesized significantly more protein than those of the knee; with compression, protein synthesis was increased in knee chondrocytes to levels similar to those of the ankle chondrocytes. In the ankle, dynamic compression caused

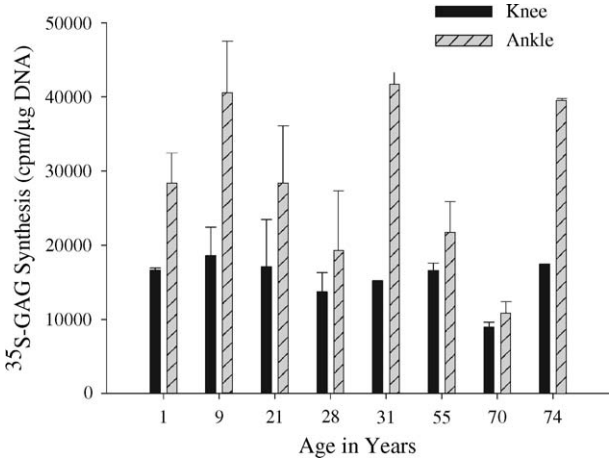

Fig. 6. Synthesis of glycosaminoglycans (GAG) by matched pairs of knee and ankle chondrocytes. ^{35}S-incorporation into GAG as cpm per μg DNA was measured in explants for 4 h after 3 days in culture. Each measurement is the mean ± S.D. in the knee (closed columns) and ankle (hatched columns) of triplicate samples from matched pairs ($P = 0.047$). Reprinted from Eger *et al.*[31] with permission from the Orthopaedic Research Society.

a significant increase in the synthesis of collagen and total protein, but not of proteoglycans. Dynamic loading caused an increase in protein synthesis across all ages tested, emphasizing the importance of regular loading to maintain cartilage integrity. If knee and ankle chondrocytes were first stripped of their matrices, then seeded into agarose and allowed to resynthesize a matrix before compression, the response to loading by both knee and ankle chondrocytes was no longer significantly different. These data suggested that the differences between knee and ankle chondrocytes are not genetically programmed into the cells and in addition, this supports a role of the extracellular matrix in regulating cellular activity. Because adult articular cartilage provides a stable microenvironment for the chondrocytes with extremely low rates of turnover[37,38], the presence of the native matrix is thought to be important in regulating chondrocyte metabolism.

MAINTENANCE OF CHONDROCYTE PHENOTYPE

Further data from knee and ankle chondrocytes provide additional supporting evidence that the extracellular matrix plays a role in influencing synthesis. If that matrix is first removed and the denuded chondrocytes are allowed to synthesize a new matrix in alginate beads, there were no longer significant differences in glycosaminoglycan synthesis[39], nor were there significant differences in their half-lives. However, differences in the response of knee and ankle chondrocytes to the catabolic cytokine, interleukin-1β (IL-1β), were maintained.

DIFFERENCES IN RESPONSE TO CATABOLIC FACTORS

Interleukin-1 (IL-1) at low concentrations is a proinflammatory catabolic cytokine that has two distinct effects on human chondrocytes (Fig. 7): at lower concentrations it suppresses the production of matrix components and in higher concentrations stimulates the synthesis of proteolytic enzymes and the degradation of extracellular matrix components, thereby shifting the metabolic balance from anabolism to catabolism[40]. OA may be regarded as a disorder of cartilage matrix metabolism with intermittent inflammatory episodes superimposed. During these inflammatory events, IL-1 is released from the inflamed synovial tissue where it is capable of entering the cartilage. The concentration of IL-1 measured in the synovial fluid from the joints of patients with arthritis ranges from 13–402 pg/ml[41]. The response of chondrocytes to IL-1 in this dose range has been used to study the effects of a catabolic stimulus on the cartilage matrix and its ability to repair.

In cartilage explant cultures, the response of ankle chondrocytes to IL-1β at concentrations of 1–250 pg/ml was significantly different from that of knee chondrocytes (Fig. 8), demonstrating a reduced response by ankle chondrocytes to catabolic stimulation compared to knee chondrocytes[31]. While both knee and ankle chondrocytes responded to IL-1 by decreasing ^{35}S-sulfate incorporation into glycosaminoglycans in a dose-dependent manner, the dose at which the knee chondrocytes responded was lower than that for the ankle chondrocytes. At the lowest IL-1 doses tested, 0.1 and 0.5 pg/ml, glycosaminoglycan synthesis was unaffected in both knee and ankle chondrocytes. However, at an IL-1 dose of 1 pg/ml, glycosaminoglycan synthesis was reduced by 67% in the knee, but the ankle chondrocytes were not affected by a similar reduction until the IL-1 dose was 5 pg/ml. Between IL-1 doses of 5 and 250 pg/ml, there were significant differences in the

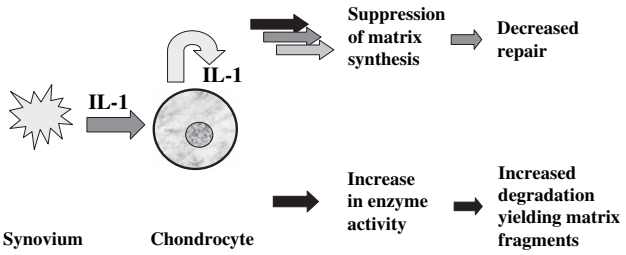

Fig. 7. Diagrammatic representation of the influence of IL-1 on the chondrocyte. The chondrocyte responds to IL-1 either from the synovium or from chondrocytes themselves. In human cartilage the primary response of the chondrocytes is to suppress synthesis of matrix components at low levels of IL-1; at higher levels of IL-1 enzyme activity is elevated, shifting the metabolic balance between anabolism and catabolism in favor of matrix degradation. Both results can be deleterious to the matrix, whether by decreasing repair or increasing degradation. OA may be considered a disorder of cartilage matrix metabolism with the superimposition of intermittent inflammatory episodes.

suppression of glycosaminoglycan synthesis by both knee and ankle chondrocytes. At the highest IL-1 concentrations tested (500–1000 pg/ml) there was no longer a significant difference in the suppression of glycosaminoglycan synthesis between knee and ankle; the synthesis in both was reduced to that of 10% of the control.

The differences in response to catabolic stimulation between knee and ankle chondrocytes were maintained when the chondrocytes were released from their native matrix and cultured in alginate beads. The IC_{50} for decreased glycosaminoglycan synthesis by knee chondrocytes in alginate was 11.8 pg/ml and was significantly different from that of the ankle, which was 56.1 pg/ml[39]. Both of these values are similar to the IC_{50} found, respectively, for knee and ankle cartilage explants. The data showing that both knee and ankle chondrocytes maintain differences in response to increasing concentrations of IL-1β support our hypothesis that ankle chondrocytes

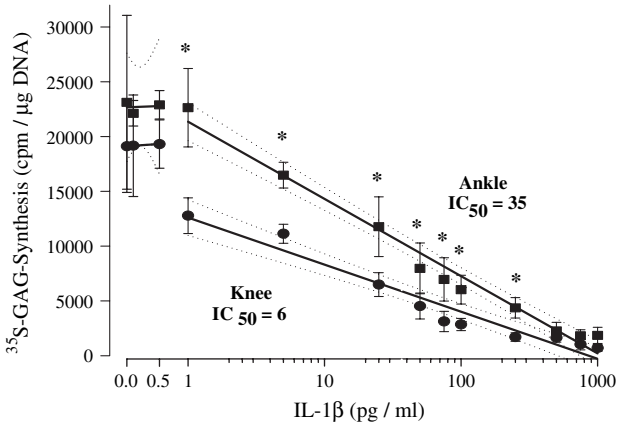

Fig. 8. The effect of interleukin-1β on proteoglycan (PG) synthesis. The effect of IL-1β on proteoglycan synthesis was evaluated by ^{35}S-incorporation into GAG as cpm per µg DNA. The results (mean ± S.D.) are from matched pairs of knee (●) and ankle (■) cartilages of six different donors. Differences were considered significant at $P < 0.05$ and are marked with an asterisk (*). The IC_{50} for inhibition in the explant cultures of knee and ankle cartilages are indicated. Reprinted from Eger *et al.*[31] with permission the Orthopaedic Research Society.

are less responsive to catabolic stimulation, at least with regard to decreasing proteoglycan synthesis. These differences in response to IL-1 may have been caused by either the number or types of IL-1 receptors[42]. The chondrocytes of the superficial zone are more responsive to IL-1 than are cells from the deeper cartilage. This difference may result from the higher number of receptors for IL-1 on chondrocytes from the articular surface than on those from the deep tissue. It is noteworthy that the IL-1 receptor antagonist protein could completely block the IL-1 induced inhibition of proteoglycan synthesis in cells from the ankle joint, but was only partially effective in knee cartilage cells.

Following injurious compression, knee cartilage, but not ankle cartilage, responded to treatment with IL-1[30]. When the cartilage was injured and then exposed to IL-1, there was a synergistic increased loss of glycosaminoglycan in the knee. Unlike the results from knee cartilage, in ankle cartilage from the same donor there was no statistically significant interaction between injury and IL-1 treatment. These data demonstrate a synergistic effect of cytokines and cartilage injury found in the adult human knee but not the ankle, leading to the hypothesis that the interaction between joint injury and cytokines may be an underlying difference in the progression to OA of knee and ankle cartilage after injury.

RESPONSE TO MATRIX FRAGMENTS

Fragments of matrix components, such as fibronectin and collagen, can have biological activity by activating pathways that are not part of the native molecule's normal function[43]. A 29 kDa fragment from the amino terminal of fibronectin has been shown to modulate proteoglycan synthesis at low doses and degradation at high doses[44,45]. This fragment, which can be generated by MMP-3 or other catabolic proteinases, is elevated in OA synovial fluid and cartilage[46,47]. An injection of the 29 kDa fibronectin fragment (Fn-f) into the rabbit knee joint causes severe cartilage damage with characteristics of OA[48].

When the Fn-f were incubated with matched pairs of knee and ankle cartilages, both anti-anabolic as well as catabolic pathways were activated[33,49]. Proteoglycan synthesis was reduced in a dose-dependent manner by both knee and ankle chondrocytes; however, just as with the response to IL-1, there was a difference in the concentration at which proteoglycan synthesis was significantly reduced by Fn-f. In the knee, 1 nM Fn-f significantly suppressed proteoglycan synthesis, while in the ankle, 100 nM was required. There was also a significant difference in the catabolic response of knee and ankle chondrocytes to stimulation by the 29 kDa Fn-f. There was greater proteoglycan loss and an increased activity of aggrecanase in the knee compared with the ankle; the ankle cartilage was not damaged even after 28 days in culture. The knee cartilage showed a significant loss of 30–50% of its proteoglycan as early as day 7. When the rate of degradation, measured as proteoglycan loss to the media during 7 days of explant culture, was determined, the rates for the knee and ankle were nearly identical. In the presence of the 29 kDa Fn-f, the degradation rates significantly increased to 0.68 ± 0.28 μg proteoglycan/mg cartilage/day for knee cartilage and 0.58 ± 0.14 μg proteoglycan/mg cartilage/day for ankle cartilage. In addition, the 29 kDa Fn-f significantly decreased the half-life of ^{35}S-labeled proteoglycan by 1.87-fold in the knee and 2.36-fold in the ankle, both compared to the control.

While the influence of Fn-f has primarily focused on proteoglycan regulation, studies of type II collagen fragments have focused on collagen synthesis. It was previously thought that adult human cartilage no longer synthesized type II collagen[50,51]; however, it has recently been shown that collagen synthesis evaluated by mRNA and the CPII continues in articular cartilage, albeit at low levels[18]. Collagen is also continuously turned over throughout life as well[18], such that collagen fragments accumulate in the normal matrix at a concentration of 1–4 mg/g wet weight of the cartilage; OA cartilages contain between 7 and 17 mg/g wet weight[52]. Incubation of knee and ankle explants with collagen fragments for 21 days results in a significant loss of glycosaminoglycans as well as collagen from the explants; however, there were no significant differences in the loss between knee and ankle[52].

ATTEMPTED REPAIR

Another reason for the lack of damage progression in ankle cartilage may be that ankle chondrocytes, which are more responsive to anabolic agents than knee chondrocytes, are thus able to repair early cartilage lesions. The anabolic factor osteogenic protein-1 (OP-1, also known as bone morphogenetic protein-7) caused a marked stimulation of proteoglycan and collagen synthesis (mostly aggrecan and type II collagen) in human articular chondrocytes. OP-1 had a much more potent anabolic effect compared to other factors including TGF-1β, activin A, and IGF-1[53]. When OP-1 was tested in combination with low concentrations of IL-1, OP-1 was able to overcome the inhibitory effect of IL-1 on aggrecan synthesis. When knee and ankle explants were first incubated with IL-1β and then allowed to rebound without IL-1β, the synthesis of proteoglycans was significantly elevated by ankle chondrocytes within 5 days; knee chondrocytes were unable to significantly increase synthesis even after 8 days. In alginate beads no differences were found in the response of either knee or ankle chondrocytes to a monoclonal anti-OP-1 neutralizing antibody that suppressed proteoglycan synthesis and endogenous OP-1 content[54].

Differences in knee and ankle cartilage response to degenerative changes

In an attempt to compare the knee and ankle *in vivo* response to damage, we analyzed markers of synthesis and degradation in cartilage from joints that were Grade 0 (normal) and Grade 2 (early lesions). The results indicate a net anabolic response in the ankle cartilage lesion and a net catabolic response in knee cartilage lesions[55]. In ankle cartilage lesions, there was an upregulation of the markers of collagen synthesis and of proteoglycan synthesis which were downregulated in the knee cartilage lesions. In lesions of knee cartilage, but not ankle cartilage, there was a five-fold upregulation of collagen degradation markers. Compared to the ankle, there was a 24-fold increase in cleavage epitopes compared to denaturation epitopes in knee cartilage lesions. The upregulation of matrix turnover that was seen in early cartilage lesions of the ankle would appear to represent an attempt to repair the damaged matrix. The increase in collagen synthesis and aggrecan turnover seen in ankle lesions was absent from the knee lesions. Instead, there was an increase in type II collagen cleavage. Taken together with the differences in collagen denaturation, these changes point to the stimulation of matrix assembly during early lesion development in ankle cartilage and of degradation of the matrix in knee

cartilage. The result is a fundamental difference in matrix turnover within lesions in ankle and knee cartilage. The increase in type II collagen cleavage in early lesions of knee cartilage, but not in ankle cartilage, suggests an early predisposition to degenerative pathology in the knee, while in the ankle the early emphasis is on repair.

Response of subchondral bone to degenerative changes in cartilage

OA is characterized by both articular cartilage degeneration and subchondral bone changes; early degenerative changes in the cartilages of the knee, hip, and finger joints with progression to OA show increased subchondral bone density[56]. We therefore investigated whether the degree of degeneration of ankle cartilage (mild or moderate) correlates with an elevated bone density in the ankle[57]. Using peripheral quantitative computed tomography, we found that even moderate degeneration of the articular cartilage of the ankle is not accompanied by an increase in local bone density, but rather shows an associated decrease. In the ankle, cartilage degeneration appears to precede thickening of the subchondral bone. Because the ankle joint is generally not affected by progressive OA, the lack of association between increased bone density and cartilage degeneration would appear to support the idea that progressive degeneration of the cartilage requires bone involvement.

Conclusions

Our studies comparing knees and ankles have shown that there are differences not only in the cartilage and subchondral bone but also in the chondrocytes themselves, which may contribute to degeneration in the knee and repair in the ankle (Table III). The accumulation of this data has been possible through collaboration with the Gift of Hope Organ and Tissue Donor Network and continuous support through a grant from NIH for a SCOR in OA. The studies have been conducted not only by intramural investigators but also by a number of extramural investigators who have contributed their expertise and unique techniques and reagents to compare knee and ankle cartilages as well as bone. The success of these studies may be attributed to the fact that we have been able to study normal knee and ankles from the ipsilateral limb of a large number of adult donors. Each of these studies has taken 2 to 3 years to complete and, thus, has proceeded at a slower pace than the average successful research project.

Our most convincing data were obtained from experiments with cartilage explants where the chondrocytes remained in their accumulated native matrix. The proteoglycan content of the extracellular matrix is significantly higher in ankle cartilage than in knee cartilage. The higher proteoglycan content, along with lower water content, provide an increased stiffness and lower hydraulic permeability to ankle cartilage. The result is a higher compressive stiffness that could protect ankle cartilage from continuous microtrauma. The lack of a synergistic response of ankle chondrocytes to injurious compression and IL-1 suggests that, even with traumatic injury, ankle cartilage is more resistant to the progression of degeneration. Together, these data show that the extracellular matrix plays a significant role in protecting the cartilage in the ankle.

The extracellular matrix also plays a role in maintaining the differences between knee and ankle cartilage. In a number of our studies, the chondrocytes were first

Table III
Summary of knee and ankle differences

Feature	Knee	Ankle
Joint stability	Relatively unstable Non-congruent	Highly stable Highly congruent
Joint motion	Flexion/extension Rotation	Flexion/extension
Cartilage		
Cellularity	Same	Same
Cartilage thickness	2–6 mm	1–1.5 mm
Superficial chondrons	Single cells	Clusters of 2–4 cells
Sulfated glycosaminoglycan content	Lower	Higher
Water content	Higher	Lower
Collagen content	Same	Same
Dynamic stiffness	Lower	Higher
Hydraulic permeability	Higher	Lower
Peak stress with 65% final strain	11 MPa	16 MPa
Glycosaminoglycan synthesis		
In explants	Lower	Higher
In alginate	Same	Same
Proteoglycan half-life	22.68 days	16.58 days
Protein synthesis	Lower	Higher
IC_{50} for IL-1 reduction of proteoglycan synthesis		
In alginate	11 pg/ml	56 pg/ml
In explants	6 pg/ml	35 pg/ml
Influence of Fn-fs on		
Proteoglycan synthesis (anti-anabolic)	Low dose (1 nM)	High dose (100 nM)
Proteoglycan loss (catabolic)	Significant at 7 days	Not significant after 28 days
Attempted repair (Response to anabolic factors following catabolic stimulation)	No significant rebound	Significant rebound
Response to degeneration	Upregulation of collagen degradation	Upregulation of matrix synthesis

released from their native matrix and allowed to reform a new matrix in either alginate or agarose. In the newly formed matrix, there were no longer significant differences in response to compression, nor were the differences in proteoglycan content or synthesis maintained. However, there were differences that were not lost, such as the dose response to IL-1, suggesting that some of these differences were programmed into the chondrocytes themselves regardless of their extracellular matrix, just as was previously shown for superficial and deep chondrocytes. Differences in response to IL-1 may be due to differences in either the number or types of IL-1 receptors that are maintained in both superficial and deep chondrocytes taken from knee and ankle.

Ankle chondrocytes display a decreased response to not only IL-1 but also to other catabolic factors, such as Fn-f in explant cultures. In addition, ankle chondrocytes have an increased response to anabolic agents, such as OP-1. These data are supported by our analyses of markers of degradation and synthesis *in vivo*. Damaged (Grade 2) ankle cartilage contains higher levels of CPII, indicative of collagen synthesis, and 846-epitope, indicative of proteoglycan synthesis, accompanied by lower levels of degradation neoepitopes. Although degeneration occurs in ankle cartilage, its progression may be slower compared to the knee due to the continuing attempts to repair.

The ankle appears to provide an *in vivo* model for studying reparative response mechanisms that are not present in the knee. Our current data suggest that there is not a single property, but numerous subtle differences between the cartilages, as well as the bone, from the two joints, which could help protect the ankle cartilage from progressive degenerative changes. By understanding how knee and ankle cartilages differ from one another, we can begin to identify factors active in the early stages of cartilage damage that may precede OA. Our ultimate goal in OA research is to develop the means of blocking the progression of the disease process and to reverse its effects. One method of reversing the effects of early disease processes is to decrease the response of chondrocytes to catabolic factors and to stimulate the chondrocytes to rebuild their matrix. Effectively, simulating the characteristics of ankle chondrocytes in different joints should facilitate the development of therapeutic strategies for the early detection and prevention of OA. The ankle may provide a model for studying reparative response mechanisms that are absent or diminished in the knee.

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