Age-related Changes in the Response of Human Articular Cartilage to IL-1α and Transforming Growth Factor-β (TGF-β)

CHONDROCYTES EXHIBIT A DIMINISHED SENSITIVITY TO TGF-β*

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Cartilage glycosaminoglycan (GAG) synthesis and composition, upon which its structural integrity depends, varies with age, is modified by anabolic and catabolic stimuli, and is regulated by UDP-glucuronate availability. However, how such stimuli, prototypically represented by transforming growth factor-β (TGF-β1) and IL-1α, modify GAG synthesis during aging of normal human articular cartilage is not known. Using explants, we show that chondroitin sulfate (CS)-total GAG ratios decrease, whereas C6S:C4S ratios increase with cartilage maturation, and that chondrocytes in the cartilage mid-zone, but not the superficial or deep zones, exhibit uridine 5′-diphosphoglucose dehydrogenase (UDPGD) activity, which is also increased in mature cartilage. We also show that IL-1α treatment reduces both total GAG and CS synthesis, decreases C6S:C4S ratios (less C6S), but fails to modify chondrocyte UDPG activity at all ages. On the other hand, TGF-β1 increases total GAG synthesis in immature, but not mature, cartilage (stimulates CS but not non-CS), age-independently decreases C6S:C4S (more C4S), and increases chondrocyte UDPG activity in a manner inversely correlated with age. Our findings show that TGF-β1, but not IL-1α, modifies matrix synthesis such that its composition more closely resembles “less mature” articular cartilage. These effects of TGF-β1, which appear to be restricted to periods of skeletal immaturity, are closely associated although not necessarily mechanistically linked with increases in chondrocyte UDPG activity. The antianabolic effects of IL-1α are, on the other hand, likely to be independent of any direct modification in UDPG activity and manifest equally in human cartilage of all ages.

Aggrecan, the large aggregating proteoglycan (PG),† is a vital component of the articular cartilage extracellular matrix (ECM), providing it with many of its characteristic physicochemical properties (1). The carbohydrate component of aggrecan, which constitutes at least 90% of its molecular mass, consists of many long, predominantly chondroitin sulfate (CS) and keratan sulfate (KS), unbranched glycosaminoglycan (GAG) chains covalently linked to a core protein (2). The contribution of these GAGs to PG function has been divided into two categories: firstly, maintenance of hydration, a property dependent on their polyanionicity, which engenders in cartilage the ability to withstand compression (3), and secondly, interactions with other macromolecules, which depend on the specific distribution of GAG negative charges and their carbohydrate backbone conformation (4). Thus, the importance of these GAG moieties is clear; however, neither their specific role nor the mechanisms regulating their synthesis are fully understood.

It is however, well established that all these carbohydrate components are synthesized from UDP-sugar nucleotide precursors and assembled onto the core protein by a system of specific glycosyl transferases (5). The efficiency of this system depends on UDP sugar availability, and it has been proposed that one of the key regulatory points in this synthetic pathway is the utilization of UDP-glucos (UDPGal) (6). Therefore, it is pertinent that UDPGlc can be converted to either UDP-glucuronic acid (UDP-GlcUA) by UDP-glucuronic acid dehydrogenase (UDPGD; EC 1.1.1.22) activity or UDP-galactose by UDPGlc 4′-epimerase. UDPGlcUA is a precursor of GlcUA in the synthesis of CS disaccharides (with GalNAc) that culminates in C-4 or C-6 sulfation of the hexosamine by specific sulfotransferases (5). It is known that GAG composition can be altered through the modulation of such pathways and that this can have a major influence on the physicochemical properties of the tissue (13, 14). Thus, the control of UDP-sugar precursor availability may permit cells to direct their synthetic activity toward particular types of GAG chain. In addition, cellular regulation of GAG chain sulfation represents a further means by which PG content can be specifically modified to meet the requirements of the ECM (15). Changes in cartilage GAG chain composition can result from chondrocyte activation and induction of anabolic or...
catabolic responses, and much evidence indicates that both cytokines and growth factors influence these responses (16). Indeed, members of the transforming growth factor-β superfamily, such as transforming growth factor-β1 (TGF-β1), exhibit chondrogenic properties and stimulate PG synthesis by articular chondrocytes in vitro and in vivo. In addition, TGF-β1 can counteract the suppression of cartilage PG synthesis that is induced by the cytokine, interleukin-1α (IL-1α) (17). The influence of these cytokines and growth factors on sulfation patterns has been examined in several tissues (18). However, few studies have evaluated such responses in normal human cartilage, and fewer still have addressed the possibility that they may be modulated by aging. A study of the modulation of GAG chain synthesis and sulfation patterns by TGF-β1 and IL-1α in normal human tissue is therefore highly relevant to place in perspective the changes in GAG structure that occur in diseases such as osteoarthritis (OA). In the present study, we have examined age-related changes in GAG synthesis and composition, as well as changes induced in response to exogenous treatment with recombinant human (rh) TGF-β1 or rhIL-1α. To evaluate the contribution of individual chondrocytes to such changes in GAG synthesis, we have used quantitative cytochemistry to evaluate age-related and TGF-β1 or IL-1α-induced alterations in resident chondrocyte UDPGD activity in distinct regions of articular cartilage.

EXPERIMENTAL PROCEDURES

Materials—Dulbecco’s modified Eagle’s medium (DMEM) and supplements were from Imperial Laboratories. Sephadex G25 and G50 was from Amersham Biosciences. Recombinant human IL-1α and rhTGF-β1 were a gift from Genentech. [35S]Na2SO4 was from Amersham Biosciences. Sodium bicarbonate, 4.5 mg/ml D-glucose, 2 mM L-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, and modified Eagle’s medium non-essential amino acids (100×) solution (Invitrogen). Medium was not supplemented with serum. For assessment of PG synthesis, cartilage was then diced into 1–2-mm3 pieces and randomized to account for variations in proteoglycan content in different regions across the surface of the condyle (19).

Assessment of PG Synthesis—Rates of proteoglycan synthesis were determined using 20–30 mg of cartilage placed in each well of a Linbro 24-well culture plate (2 cm2/well) containing 900 μl of DMEM and 100 μl of phosphate-buffered saline containing 0.1% (w/v) bovine serum albumin. Explants were preincubated at 37 °C in a humidified atmosphere of 5% CO2 for 48 h to attain a stable rate of proteoglycan metabolism; in all cases, segments were cultured for a 2-day “wash-out” period prior to experimental treatment to minimize the effects of medication and surgical isolation. Explants were then labeled with 10 μCi/ml [35S]sulfate during the final 6 h of culture after incubation in control medium or medium supplemented with either 1.0 ng/ml rhIL-1α or 10.0 ng/ml rhTGF-β1 for 48 h (20). Subsequently, explants were digested in papain and 500-μl aliquots of [35S]-labeled digest eluted from a 9.1-ml Sephadex G25 column equilibrated with 2 M guanidinium chloride, 50 mM sodium acetate, pH 5.8. Radiolabel incorporated into the V0 peak was determined by scintillation counting. In all experiments, media were refreshed daily, and those required for analysis were frozen and stored at −70 °C. Preliminary studies found that ~85% of human articular cartilage samples exhibited no induction of GAG release from either endogenous or newly synthesized pools in response to IL-1α treatment and that this was not dependent on the age of the samples. It is therefore likely that the effects of IL-1α treatment are not the product of PG degradation. GAG chain structure was evaluated using 150–200 mg of cartilage cultured in 1800 μl of DMEM and 200 μl of phosphate-buffered saline with 0.1% (w/v) bovine serum albumin and 50 μCi/ml [35S]sulfate for 6 h.

In parallel experiments, segments (at least triplicate) of each cartilage sample (0.5 mm3), with as much of the subchondral bone removed as possible, were cultured after appropriate preincubation (see above) in the presence and absence of 1 ng/ml rhIL-1α or 10 ng/ml rhTGF-β1 for 48 h. They were then immediately snap-chilled in N-hexane at −70 °C (grade low in aromatic hydrocarbons) and stored at −70 °C until cryostat sections were cut for assessment of UDPGD activity (see below).

Sulfated Glycosaminoglycan Analysis—Distribution of [35S]sulfate-labeled CS/DS and residual chondroitinase ABC lyase-insensitive GAG pools was determined by eluting chondroitinase-digested material on a 2 M HCl–hexane at 50 °C.
Sephadex G50 (145/11003 0.6 cm) column, equilibrated with 0.05 M NaAc, pH 5.0, and calibrated by the methods of Wasteson (21). [35S]Sulfate incorporation into GAGs was determined by scintillation counting.

Disaccharide Analysis—Chondroitin 4- and 6-sulfate patterns were determined by HPLC separation. Briefly, cartilage samples were digested with 250 g/ml papain in 0.1 M sodium acetate, 2.4 mM EDTA, and 10 mM L-cysteine, pH 5.8, at 65 °C for 24 h and then boiled for 15 min. Samples were dialyzed into 0.1 M Tris acetate, pH 8.0, and concentrated in a Centricon ultrafiltration device containing a M_r 10,000 exclusion membrane (spun at 4600 × g for 45 min). After digestion with 0.2 units of chondroitinase ABC lyase (EC 4.2.2.4) at 37 °C overnight, samples were removed, ethanol-precipitated (4× volume), and stored overnight at 4 °C. After removal of a small pellet containing undigested material and residual enzyme, the supernatant was eluted from a Partisil 5-PAC column equilibrated in Tris borate buffer (22, 23). Mon-

![FIG. 3. Effect of rhIL-1α and rhTGF-β1 on [35S]sulfate incorporation (percentage of control untreated incorporation, ± S.E.) into CS and non-CS within GAG chains, in cartilage from immature (<20 years) and mature (>20 years) individuals. Tissue was either untreated (open) or cultured in the presence of 1 ng/ml rhIL-1α (hatched) or 10 ng/ml rhTGF-β1 (cross-hatched). Elution of chondroitinase ABC-digested tissue on a Sephadex G50 column separated the GAG chains into peak I (residual, chase-insensitive, non-CS, including KS/heparan sulfate (HS)) and peak II (CS/DS). The proportion of counts within each n was expressed as percentage of the control.](image)

![FIG. 4. Changes in ΔDi-6SO₄:ΔDi-4SO₄ CS disaccharide ratio. A, age-related change in endogenous (open circles) and newly synthesized (filled circles) ΔDi-6SO₄:ΔDi-4SO₄ ratios. B, differences in endogenous and newly synthesized ΔDi-6SO₄:ΔDi-4SO₄ ratios (± S.E.) in immature (open) and mature (hatched) cartilage. Statistical significance denotes comparison between distinct ages, where ** denotes p < 0.001. No differences were evident between endogenous or newly synthesized ratios in either age group.](image)

![FIG. 5. Changes in ΔDi-6SO₄:ΔDi-4SO₄ CS disaccharide ratio induced by rhIL-1α and TGF-β1 treatment. A, effect of rhIL-1α and rhTGF-β1 on [35S]sulfate incorporation (percentage of untreated, ± S.E.) into ΔDi-6SO₄:ΔDi-4SO₄ CS disaccharides in endogenous (hatched) and newly synthesized (open) pools. ** denotes p < 0.0001, and * denotes p < 0.005 from controls. B, age-related changes in ΔDi-6SO₄:ΔDi-4SO₄ ratios in human cartilage induced by treatment with rhIL-1α and rhTGF-β1. Open circles, control untreated samples; open squares, samples treated with rhIL-1α; closed triangles, rhTGF-β1.](image)
Statistics—All results are reported as mean ± S.E. Comparisons were evaluated by Wilcoxon's signed-rank test, by paired or unpaired Student's t test on original numerical data (as appropriate) and not the percentage figures illustrated. In all cases, p < 0.05 was considered statistically significant.

RESULTS

Sulfated GAG Synthesis

Changes Associated with Cartilage Aging—To establish age-related changes in GAG synthesis, newly synthesized [35S]sulfate-labeled GAG chains were isolated from chondroitinase ABC (Chase)-digested articular cartilage from patients ranging from 9 to 53 years of age (see Table I). These were found to produce two peaks when fractionated; peak I represents newly synthesized residual Chase-insensitive, non-CS chains (peak I, K\text{av} = 0.11), and peak II represents the CS (and DS, peak II, K\text{av} = 0.83) chains. Pooling of peak I and additional Chase digestion failed to yield extra CS chains, indicating that digestion was complete (not shown). The content of the Chase-insensitive, non-CS, peak I was not investigated. This analysis indicated that synthesis rates for both CS and non-CS GAGs declined with increasing age (Table I), and although total CS GAG ratios decreased with age, this did not reach levels of statistical significance (R² = 0.8551; p = 0.09).

Changes Induced by Treatment with rhIL-1α or rhTGF-β1—We then examined whether treatment with rhIL-1α or rhTGF-β1 modified such GAG synthetic rates in immature (<20 years; 28,29) and mature (>20 years) samples of articular cartilage and found that rates of GAG synthesis in both immature and mature samples of cartilage were significantly reduced by treatment of the explants with rhIL-1α (Fig. 2). In contrast, rhTGF-β1 treatment produced statistically significant increases in the rate of GAG synthesis in immature, but not mature, samples (Fig. 2). Detailed analysis of the specific changes in newly synthesized non-CS and CS pools showed that rhIL-1α treatment significantly decreases the synthesis of both in cartilage from young and old specimens (Fig. 3). In contrast, treatment with rhTGF-β1 failed to significantly modify non-CS synthesis but produced statistically significant increases in the level of sulfate incorporated into CS in immature cartilage, but not older, mature cartilage (Fig. 3).

Ratio of Chondroitin-4-sulfate to Chondroitin-6-sulfate

Changes Associated with Cartilage Aging—Before examining whether treatment with rhIL-1α or rhTGF-β1 also modified CS sulfation, we determined age-related differences in CS sulfation patterns in cartilage and found that both newly synthesized and endogenous GAG chains show increases in C6S:C4S ratios associated with cartilage aging (Fig. 4A). A significant correlation with age in both endogenous (p < 0.01, R² = 0.639) and newly synthesized (p < 0.02, R² = 0.589) pools (Fig. 4A) was observed. In addition, a curve fit indicates the presence of a plateau in C6S:C4S ratios after the age of 20 years, as well as a rapid rise in these ratios during puberty (Fig. 4A). Evaluation of C6S:C4S ratios in distinct age groups indicates that the endogenous ratio of these isomers is significantly higher in older individuals (p < 0.001, from 0.90 ± 0.25 in immature to 4.00 ± 0.25 in mature samples (Fig. 4B). Although ratios of newly synthesized C6S:C4S showed a similar trend (1.30 ± 0.31 increasing to 2.83 ± 0.46), these did not reach statistical significance (Fig. 4B).

Changes Induced by Treatment with IL-1α or TGF-β1—Examination of the C6S:C4S ratio of cartilage GAGs (without reference to age) showed that treatment with rhTGF-β1 (67 ± 4% of controls), and more markedly, rhIL-1α (37 ± 4% of controls) significantly reduces C6S:C4S ratios in the newly synthesized pool (Fig. 5A). Neither of these agents altered the
ratio in the endogenous pool (Fig. 5A), but both rhTGF-β1 and more markedly rhIL-1α reduce C6S:4S ratios in newly synthesized GAGs (Fig. 5B).

Closer examination, however, disclosed that the C6S:4S ratio in newly synthesized and endogenous pools exhibits different age-related trends. Immature cartilage (<20 years) shows higher, and mature cartilage shows lower, C6S:4S ratios in the synthesized pool of GAGs than in the endogenous pool (Fig. 4B). Absolute rates of 4S and 6S synthesis show that the rhIL-1-induced reduction in C6S:4S ratios is achieved by diminution in both 4S and 6S at all ages, with consistently greater reduction in C6S (Fig. 6A). However, the rhTGF-β1-induced reduction in C6S:4S ratios is nearly always associated with increased synthesis of both 4S and 6S, with consistently greater increases in 4S synthesis (Fig. 6B).

**UDPGD Activity/Cell in Different Cartilage Zones**

Changes Associated with Chondrocyte Position and Cartilage Age—To determine the potential contribution of individual chondrocytes to such changes in GAG synthesis, we measured resident chondrocyte UDPGD activity using a quantitative cytochemical method and made an evaluation of age-related and TGF-β1 or IL-1α-induced alterations in distinct regions of articular cartilage. We found that chondrocytes resident in distinct cartilage zones showed statistically significant differences in UDPGD activity (Fig. 7, A and B), with lowest activities (MIE × 100/cell) evident in the superficial zone (Zone 1) and highest activities in deep cartilage (Zone 4). In all zones, but most markedly in the mid-zones (118 and 98% increase in zones 2 and 3, p < 0.001 and 0.0001, respectively), chondrocyte UDPGD activity was higher in mature than in immature cartilage (Fig. 7C). Indeed, an analysis of mid-zone chondrocyte UDPGD activity (Fig. 8A) discloses a significant correlation with age (R² = 0.880), which is more accurately described by a binomial distribution (R² = 0.988). Chondrocytes in both deep and superficial (not shown) regions fail to show this change (Fig. 8B).

We therefore examined only zones 2 and 3 in subsequent analyses. To examine whether chondrocyte UDPGD activity is modified by maintenance of cartilage explants in vitro, we measured UDPGD activity (zones 2/3) in fresh tissue (chilled immediately after isolation) and paired samples chilled after 2 days in culture. This showed (Fig. 9) that mid-zone chondrocyte UDPGD activity was unaffected by such treatment (Fig. 10).

Changes Induced by Treatment with rhIL-1α or rhTGF-β1 (Immature versus Mature)—Chondrocyte UDPGD activity/cell in zones 2 and 3 of immature, as well as mature cartilage was...
unaffected by rhIL-1α treatment. In contrast, rhTGF-β1 treatment of immature cartilage produced significant increases in chondrocyte UDPGD activity (Fig. 10, Zone 2 > Zone 3). Furthermore, although rhTGF-β1 treatment of mature cartilage also increased chondrocyte UDPGD activity in the mid-zone, these changes were always significantly smaller than in the equivalent zone of immature cartilage (Fig. 10). Evaluation of this age-related decline in sensitivity of chondrocytes to rhTGF-β1 indicates a significant (p < 0.0001 and p < 0.01) negative correlation between the magnitude of rhTGF-β1-induced increases in chondrocyte UDPGD activity and cartilage age (Fig. 11, A and B).

**DISCUSSION**

This study shows that treatment of cultured segments of human articular cartilage with rhIL-1α results in: (i) a reduced total GAG synthesis, with depression in both CS and non-CS pools; (ii) a decrease in the ratio of C6S:C4S that consistently involves a greater diminution of C6S; and (iii) no modification in the maximum capacity of resident chondrocyte UDPGD activity. In contrast to such IL-1α-induced changes that were almost entirely independent of age, rhTGF-β1 treatment results in several age-dependent responses with: (i) increases in total GAG synthesis in immature but not mature cartilage, involving a significant and predominant stimulation in CS synthesis; (ii) marked (age-independent) decreases in C6S:C4S ratios involving greater enhancement in C4S synthesis; and (iii) increases in chondrocyte UDPGD activity that are most marked in immature cartilage.

Normal human articular cartilage explants are known to exhibit aging-related changes in GAG composition and synthesis, including decreased rates of sulfate incorporation into PGs and decreased KS:CS and increased C6S:C4S ratios (30–34), providing a confirmed reference for interpreting our findings. Further, it is recognized that chondrocytes can express catalytic (degradatory) or anabolic (synthetic) phenotypes and that IL-1α and TGF-β, respectively, are prototypic inducers of such characteristics. However, the evaluation of our findings will be restricted to describing their influence on GAG synthesis and whether this is subject to modification by cartilage age.

Our studies confirm that the relative content of newly synthesized CS in human cartilage decreases with sample age (30, 31). Furthermore, our studies confirm that TGF-β1 treatment of cartilage from immature, but not mature, individuals promotes an increasingly immature pattern of GAG synthesis. This relative refractivity of mature, older cartilage to TGF-β1-induced changes may reflect differences in chondrocyte sensitivity or variations in growth factor presentation that are a consequence of age-related changes in ECM composition. These possibilities are supported by studies showing reduced IGF-1-induced sulfate incorporation in chondrocytes isolated from aged femoral condyles (35). Moreover, responses of porcine chondrocytes to TGF-β and IGF-1, measured in terms of extracellular inorganic pyrophosphate elaboration and cartilage intermediate layer protein/nucleotide pyrophosphohydrolase expression, also exhibit marked age-related differences in sensitivity (36). These studies support the notion that isolated aged chondrocytes have inherently different sensitivities. Our studies indicate this also appears to be the case for chondrocytes resident in cultured segments; however, it is possible that ECM composition confers specific sensitivities to these exogenous factors.

In contrast, we show that IL-1α inhibits both CS and non-CS synthesis to a similar extent in cartilage of all ages, suggesting that neither age nor ECM composition modifies chondrocyte sensitivity to IL-1α. IL-1α also diminishes newly synthesized C6S:C4S ratios by greater reduction in C6S than C4S (Fig. 5B). Although have previously found that the effects of IL-1α on sulfation and C6:C4 ratios are not entirely dependent upon core protein synthesis (19) and that IL-1α has little effect on GAG chain hydrodynamic size, either in the presence or in the ab-
sence of xyloside. Previous studies have shown that the effects of IL-1\(\alpha\) does indeed induce core protein inhibition (38). It therefore remains possible that IL-1\(\alpha\) may diminish PG synthesis at the level of sulfation; however, our current findings make it likely that decreases in PG synthesis induced by IL-1\(\alpha\) do not involve changes at the UDP-glucose branch point.

Consistent with early studies, we show increases in C6S:C4S ratios with age, in both endogenous and synthesized GAG chains (32–34). Such changes may be related to the aging process or, alternatively, indicate profound differences between the behavior of cartilage taken from joints before or after the age at which skeletal maturity is reached (34). Changes in GAG composition in OA also include increased 4-sulfation of CS (39, 40), which resembles the patterns of CS composition evident at puberty, with high C6S:C4S ratios and low 4,6-disulfated-galNAc non-reducing termini characteristic of the adolescent transition (41). In our studies, TGF-\(\beta\) also diminishes C6S:C4S ratios, demonstrating an active restoration toward those ratios found in immature cartilage and involving greater stimulation of C4S than C6S. Our analysis of GAG chain hydrodynamic size from TGF-\(\beta\)-treated tissue suggest that longer GAG chains arise as a result of stimulation of UDP-sugar precursor levels, possibly via enhancement of UDPGD activity.

It was recently demonstrated that CS-disaccharide sulfation patterns in human articular cartilage vary with joint topography and zone (depth) (33). It is therefore important to stress that our biochemical analyses are restricted to full-depth segments of femoral condyles but that in all cases, these samples are removed, diced, and randomized to account for such variations in composition. On the other hand, our quantitative cytochemical studies address such zonal variations directly and have shown that UDPGD activity per cell is lowest in the superficial zone (zone 1) and highest in deeper layers of cartilage and that such zonal variation is most apparent in mature cartilage.

These differences in UDPGD activity may reflect zonal modification in GAG synthesis. Indeed, UDPGD activity has been linked to the control of CS and KS synthesis (11, 42), with high and low activity promoting CS (heparan sulfate and hyaluronan) and KS synthesis, respectively. Low UDPGD activity near the cartilage surface is consistent with reports of predominant KS immunolabeling in these regions (43–45). However, it should be noted that the relationship between KS immunolabeling and KS tissue content are not always in agreement (46). Antisense oligonucleotides directed to the ATG region of human UDPGD can, however, inhibit \(^{35}\)S-sulfate incorporation into PG, suggesting that UDPGD regulates GAG synthesis (47). Studies of sugarless, a Drosophila mutation that encodes a protein with substantial UDPGD homology, also support its role in controlling CS synthesis (48). Moreover, rapid increases in UDPGD mRNA levels following cycloheximide treatment of orbital fibroblasts indicate that UDPGD has immediate early gene characteristics (12). Our results differ...
from some others in which KS synthesis increases relative to CS in TGF-β-treated bovine explants (49), and despite the fact that TGF-β1 increases both UDPGD activity and CS synthesis, the extent to which these are mechanistically linked is not established, and these areas warrant further study.

It is worth emphasizing that daily measurements of GAG synthesis rates (35S incorporation) in our serum-free culture system indicate a stable steady state in all specimens (50). It has been shown that an as yet unidentified factor in serum increases the UDP-glucuronate pool, without dramatically affecting flux (13). Increases in glucosamine precursor pool size have been described in response to long term IL-1α treatment (52), indicating that such factors may modulate UDP-sugar availability or UDPGD expression, but this has not been addressed directly. Nonetheless, the cytochemical method for UDPGD activity is a dynamic assay in which added substrate and cofactor concentrations are sufficient to not limit enzymatic rate significantly. Furthermore, growth factors effects, which are monitored in presence of serum, with effects of its own, are likely to be difficult to interpret.

Using this culture system, we found that UDPGD in mid-zone chondrocytes exhibits increased activity but decreased sensitivity to TGF-β1 in cartilage from mature joints. It is somewhat paradoxical that high UDPGD activity in untreated mature cartilage is associated with the synthesis of decreased CS:total GAG and increased C6S:C4S ratios, whereas in contrast, TGF-β1-induced increases in UDPGD activity in immature cartilage correspondingly involves increasing CS:total GAG ratios and decreasing C6S:C4S ratios. It is possible, however, that this paradox may be explained by an understanding of such quantitative cytochemical techniques. These are optimized in order that maximum activity is demonstrable (25). Thus, although maximal UDPGD activity is raised, chondrocytes in mature cartilage may fail to utilize its full potential. This provides the basis for alternative interpretation of our findings regarding cartilage aging.

Our findings may indicate that UDPGD activity is tonically suppressed in immature chondrocytes and that this suppression is removed by treatment with TGF-β1. Chondrocytes in mature cartilage, on the other hand, may lose the capacity to regulate UDPGD activity in this way, suggesting that aging-related deregulation is the basis for the altered GAG synthesis profile that mature cartilage exhibits. This is supported by the loss of several other NAD- and NADP-linked dehydrogenase enzyme activities in OA-prone C57B1 mouse strains (54), indicating that such factors may modulate UDP-sugar sensitivity to TGF-β1 in cartilage from mature joints. It is possible, however, that these are mechanistically linked with the effects of TGF-β1 to restore GAG synthesis in circumstances where they are diminished by IL-1α. It is also tempting to speculate that the anabolic effects of agents with TGF-β-like activity in cartilage will be restricted to an age before skeletal maturity is reached. On the other hand, the antianabolic effects of IL-1α are likely to be manifest equally in human cartilage of all ages.

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