Different Levels of COX-1 and COX-2 Enzymes in Synoviocytes and Chondrocytes During Joint Contracture Formation

GUY TRUDEL, NANCY DESAULNIERS, HANS K. UHTHOFF, and ODETTE LANEUVILLE

ABSTRACT. ***Objective.*** To measure the levels of prostaglandin endoperoxide H synthase (PGHS) isozymes (or cyclooxygenase, COX) *in vivo* during the development of joint contractures secondary to immobilization in rats.

Methods. Rats had one knee joint immobilized for up to 32 weeks. Three groups were compared: 47 rats had knee joints immobilized, 38 animals had sham surgery, and 13 unoperated animals served as controls. Levels of PGHS-1 and PGHS-2 enzymes were characterized in the chondrocytes and synoviocytes of the knee joint by immunohistochemistry. Immunostaining intensity was quantified by microscopy using conventional analysis.

Results. PGHS-1 level was lower in synoviocytes of the anterior capsule compared with shams (1.3 vs 2.0; $p < 0.05$). PGHS-2 level was also lower in synoviocytes of the posterior capsule (1.8 vs 2.3; $p < 0.05$), but higher in chondrocytes at the anterior aspect of the tibia compared with shams (1.6 vs 0.8; $p < 0.05$). PGHS-2 staining was increased in chondrocytes at the posterior, opposed, and anterior aspects of the tibia compared with controls (1.1, 0.6, 0.8 vs 0.2, 0.1, 0.2, respectively; all $p < 0.05$).

Conclusion. Immobility induced joint contractures are characterized by a contrasting cellular pattern of PGHS enzyme levels: decreased in the synovium and increased in the chondrocytes. These findings suggest that chondrocytic PGHS isoenzymes are important in cartilage degradation of contractured joints. (J Rheumatol 2001;28:2066–74)

Key Indexing Terms:
PROSTAGLANDIN-ENDOPEROXIDASE SYNTHASE CONTRACTURE CARTILAGE
REST SYNOVIAL MEMBRANE

A contracture is defined as a loss in the passive range of motion of a joint. Contractures are characterized by capsule stiffness, articular cartilage degeneration, absence of pain, and loss of function of affected joints. Contractures develop in patients with acute illnesses associated with immobilization or with chronic diseases associated with decreased mobility[1-4]. There is no cure for contractures. This neglected form of joint disease causes important functional restrictions that very often become permanent.

Immobility produces joint contractures through a series of unelucidated events leading to 2 major pathologic alterations: capsular stiffness and articular cartilage degeneration. We and others have reported that capsule stiffness limits joint movement[5-9]. Moreover, in the capsule, immobilization results in degenerative changes such as synovial atrophy, retraction, fibrosis, and adhesion, explaining the ankylosis of immobile joints[7,10,11]. Cartilage degeneration has also been identified in joint contractures. A dramatic and widespread loss of metachromasia[12,13] has been correlated with a decrease in proteoglycan content[12,14-16]. The number of chondrocytes decreased[17], but localized increases have been reported[7,18]. Collagen contents were unchanged[16,19]. After 6 weeks, degenerative articular lesions and attempts at repair were widespread[7,20,21]. After immobility of 90 days' duration, remobilization, even of 200 days' duration, could not reverse the cartilage alterations[5]. The final stage of contractures involves cartilage ulceration with joint ankylosis. Cartilage degeneration in contractures can destroy the joint in 12 to 18 months, which is faster than many types of inflammatory arthritis[6,22,23].

Elevated biosynthesis of prostanoids has been associated with development and maintenance of the inflammatory response in the synovium and erosion of cartilage and bone in rheumatic diseases[24-30]. Inflammation, in turn, activates the hydrolysis of fatty acids from the cell membrane by

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phospholipases and thereby releases arachidonic acid. Prostaglandin endoperoxidase H synthase (PGHS) also referred to as cyclooxygenase (COX) catalyzes the rate-limiting step from arachidonate to prostaglandins and thromboxanes[31]. PGHS enzymes exist in at least 2 isoforms (PGHS-1 and PGHS-2) encoded by 2 separate genes located on human chromosomes 9 and 1, respectively[32,33]. The major difference between these isozymes is their dissimilar regulation of expression. PGHS-1 is ubiquitous while PGHS-2 is expressed only at low levels under homeostatic conditions, but is inducible by proinflammatory stimuli, like mitogens or cytokines[25,26].

Joint degeneration has been studied mostly in the context of inflammatory arthritis[24-26]. Whether inflammation mediators play a role in the pathogenesis of contractures is not known, since clinically affected joints present little or no external sign of inflammation. Contradictory experimental evidence exists. Michelsson, *et al* measured inflammation and obtained positive responses to treatment with antiinflammatory agents[34-36]. In contrast, patients immobilized for a mean of 106 days in a long leg cast for tibial fracture presented no increase in midpatellar circumference or in skin temperature[1].

Although PGHS enzymes are a potential target for treatment, their levels and roles have never been assessed in joint contractures. Thus we investigated the *in vivo* levels of PGHS-1 and PGHS-2 enzymes in rat knee joints subjected to immobility over a 32 week time course using immunohistochemistry.

MATERIALS AND METHODS

Immobilization. Eighty-five adult male Sprague-Dawley rats, average weight 355 g, were operated. Forty-seven animals had one knee joint immobilized unilaterally in flexion using an internal fixation system for 2, 4, 16, and 32 weeks. A rigid Delrin® plastic plate and 2 screws joined the proximal femur to the distal tibia. The knee joint capsule and the joint itself were untouched. Sham operated animals (n = 38) had holes drilled and screws inserted but none of these specimens were plated. The left and right hind legs were immobilized alternately to avoid potential systematic side differences. Sixteen knee joints from 13 non-operated rats were also studied.

Tissue processing. The rats were euthanized, and the immobilized and sham operated knees were harvested and fixed in Bouin or in 10% formalin for 18 h at 4ºC. After fixation, the specimens were decalcified in EDTA for 2 mo at 4ºC, with the EDTA solution changed every second day. The specimens were then embedded in low melting point paraffin (51–54ºC) (Oxford Labware, St. Louis, MO, USA). The embedded tissue was cut into 7 μm sagittal sections from the lateral to the medial side of the joint. Standardized serial sections were kept of the medial midcondylar regions of the knee.

Immunohistochemistry technique. The slides were processed to water then transferred to phosphate buffered saline (PBS). During the first staining session, slides from the 2 week, 4 week, and 16 week groups were stained. During the second staining session, slides from the 32 week group and controls as well as repeat slides from the 2, 4, and 16 week groups were stained using the standard protocol as follows: endogenous peroxidase blocking was done with 3% H_2O_2 for 30 min at room temperature, followed by PBS rinse for 20 min. The slides were incubated with 0.3% bovine serum albumin (BSA) in normal goat serum (Sigma, St. Louis, MO, USA) at room temperature for 20 min to block nonspecific protein and nonimmune immunoglobulin affinities. The slides were washed again in PBS for 20 min. The slides were first incubated with a polyclonal rabbit anti-murine PGHS-1 (1:40) or anti-murine PGHS-2 (1:80) antibody in PBS overnight at room temperature (Cayman Chemical, Ann Arbor, MI, USA), then rinsed in PBS. The slides were then incubated with a biotinylated goat anti-rabbit antibody for 30 min and then rinsed in PBS. A third incubation was done with streptavidin conjugated horseradish peroxidase solution for 45 min (Biogenex ESBE, Markham, ON, Canada). Chromogene marking was performed with 0.5 mg/ml 3,3'-diaminobenzidine tetrahydrochloride (DAB), 0.1 M imidazole, 0.03% H_2O_2 in PBS for 2–7 min. Counterstaining was with Mayer's hematoxylin for 10 min. For the negative controls, preincubation of either of the anti-PGHS-1 or anti-PGHS-2 antibodies with their cognate peptides was performed and, in other slides, the primary antibody was omitted.

Immunohistochemistry analysis. The intensity of the immunohistochemical staining was analyzed with a light microscope by conventional histological analysis, where grade 0 = absence of staining, 1 = weak staining, 2 = moderate staining, and 3 = intense staining. Staining intensity was assessed at 5 locations: (1) chondrocytes at the posterior femur; (2) chondrocytes at the anterior tibia; (3) chondrocytes at the region on the tibia where the femur contacted (opposed region); (4) synoviocytes of the posterior capsule; and (5) synoviocytes of the anterior capsule. Chondrocyte staining at the posterior femur was analyzed from the synovial cartilage junction. Chondrocytes from the transitional and radial layers but not the superficial and calcified layers were analyzed. The reason for excluding the chondrocytes from the superficial layer was the adhesions of synovium blurring the distinction between superficial layer and adherent synovium. Fifteen chondrocytes at each site were analyzed and the most consistent staining observed (mode) was the grade given. For the synoviocytes, the capsule was observed from the recess to the junction where the capsule and meniscus meet. The grade was determined by the most consistent level of staining displayed. The staining from the 2, 4, and 16 week repeats of the second session were compared with the first staining session to ensure consistency and reproducibility before analyzing the 32 week and normal groups.

The investigator was blinded to which knee was studied. The consistency of the results was verified by performing intraobserver and interobserver correlations of 8 randomly chosen histological sections.

Harvest of capsule and cartilage. Six additional rats were euthanized after 4 weeks of immobilization. A posterior longitudinal midline incision was performed to expose the capsule. The posterior capsule was released from its attachment at the tibial and femoral synovial cartilage junctions, then stored in PBS and frozen at –20°C until utilization. On the same animals, using a microscopic knife and a binocular microscope, the articular cartilage of the femur and the tibia was harvested by gentle radial peeling of cartilage from the subchondral bone (Carl Zeiss dissecting microscope, Germany). The cartilage was stored in PBS and frozen at –20°C until utilization.

Total protein concentration. The bicinchoninic acid (BCA) protein assay was used to determine the total protein concentration in the capsule and cartilage specimens (Pierce, Rockford, IL, USA). The absorbance was measured at 562 nm with a spectrophotometer (Dynatech Laboratories Inc., Chantilly, VA, USA) with BSA as the protein standard.

Immunoblotting. Fifty micrograms of total protein from a capsule homogenate and 12.5 μg from cartilage were electrophoresed in a 10% polyacrylamide gel in the presence of SDS prior to electroblot transfer onto a Trans-Blot® pure nitrocellulose membrane (Bio-Rad Laboratories, Hercules, CA, USA). Then nonspecific sites on the nitrocellulose membranes were blocked with 5% milk/PBS/0.1% Tween 20 at 4ºC overnight. Eight to 10 h later the blot was washed in PBS/0.1% Tween 20 for 2 × 5 min and 1 × 15 min. Immunodetection was conducted by incubation with the primary polyclonal rabbit anti-PGHS-1 or PGHS-2 antibodies

in 5% milk/PBS solution for 60 min at room temperature. After a second washing step (2 × 5 min and 2 × 15 min), the membranes were incubated with a polyclonal anti-rabbit IgG antibody labeled with horseradish peroxidase for 60 min at room temperature (Promega, Madison, WI, USA). After the final washes in PBS/0.1% Tween 20 (2 × 5 min and 3 × 15 min) and PBS (2 × 5 min), antibody binding was detected by developing the membranes in chemiluminescence blotting substrates for 15 min and exposing the membranes to uncoated black and white instant films (Boehringer Mannheim, Germany) in a camera. Purified ovine PGHS-1 and PGHS-2 enzyme were used as standards.

Data analysis and statistics. The software program SPSS for Windows™, 10.0 (SPSS Inc., Chicago, IL, USA) was used to create the database and perform statistical testing. Nonparametric statistics using ranks were used to account for unequal group sizes, ordinal variables, and many subgroups with small final sample sizes. Analysis by Kruskal-Wallis test was carried out to detect statistically significant comparisons related to intervention (immobilized, sham, or control) or effect of time postintervention (from 2 to 32 weeks). Post-hoc analyses of statistically significant comparisons were carried out with Mann-Whitney tests to detect the variable responsible for the initial difference in staining. The Benjamini-Hochberg false discovery rate controlling procedure for multiple comparisons was applied to the Mann-Whitney results[37]. A corrected p value < 0.05 was interpreted as statistically significant. Intra and interrater reliability were analyzed with nonparametric Kendall tau b correlations. To display the results graphically, chondrocyte data from all locations were combined in one group, and the same was done for synoviocyte data. Similar statistical analyses were conducted.

RESULTS

Characterization of PGHS antibodies. Although the PGHS-1 and PGHS-2 isoforms are 60% identical on the protein level within the same species, both antibodies were found to be highly isoform-specific. In Western blot experiments (Figure 1), the anti-PGHS-1 antibody detected a 72 kDa band in cellular extracts from the cartilage and capsule tissues, and a band of the same size was detected by the anti-PGHS-2 antibody in these tissues. The specificity of the 2 PGHS antibodies was visualized using commercial purified enzymes; the PGHS-1 antibody detected the PGHS-1 standard and not the PGHS-2 standard. Conversely, the PGHS-2 antibody detected the PGHS-2 standard and not the PGHS-1 standard.

Detection of PGHS-1 in synovial cells. Distribution of immunostaining for PGHS-1 was similar in each of the 3 animal groups. Most of the synovial lining cells showed immunostaining for PGHS-1 and intense staining was observed in synovial lining cells (Figure 2). Immobility of a joint decreased synoviocyte PGHS-1 levels compared to sham operated animals (Figure 3). Statistical analysis revealed that the type of intervention (immobilized vs sham vs normal) had a significant effect on PGHS-1 levels in the synoviocytes of the anterior knee capsule. The interactions responsible for this difference were a significantly lower PGHS-1 level in synoviocytes anteriorly in the immobilized knee capsule compared with sham operated (1.30 ± standard error of the mean 0.15 vs 1.95 ± 0.15; p = 0.005) and compared with non-operated legs (2.19 ± 0.21; p = 0.006) (Table 1). Alterations in PGHS-1 levels in synoviocytes of the posterior capsule did not reach statistical significance compared to sham operated or non-operated legs.

Detection of PGHS-1 in chondrocytes. Immunohistochemical staining for PGHS-1 was detected in chondrocytes. It was prominent in the superficial cartilage layer close to the synoviocartilage junctions (Figure 2). Increases in PGHS-1 levels in immobilized knees were observed at

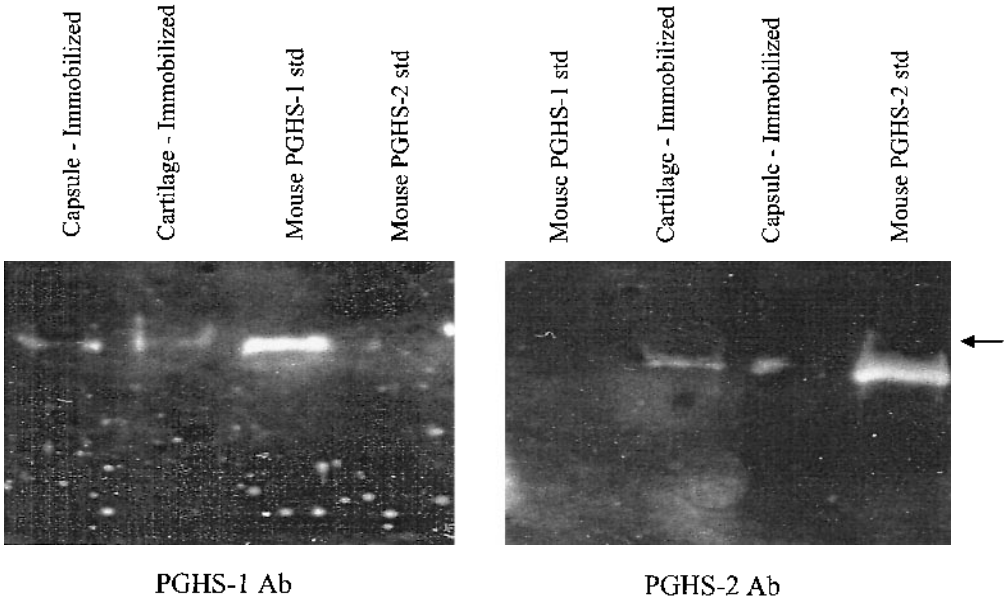
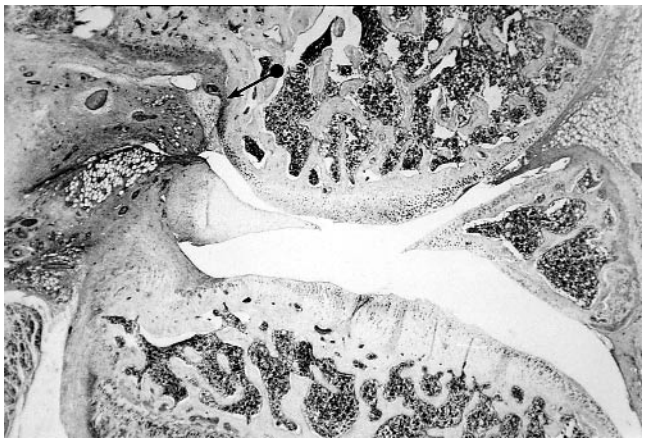


Figure 1. Western blots in rat knee extracts from the cartilage and capsule. PGHS-1 and PGHS-2 antibodies (Ab) were highly isoform-specific. In these tissues 72 kDa bands were detected by anti-PGHS-1 and -2 antibodies. (Arrow indicates 70 kDa.) The PGHS-1 antibody identified the PGHS-1 standard and not the PGHS-2 standard. Conversely, PGHS-2 antibody identified PGHS-2 standard and not PGHS-1 standard.



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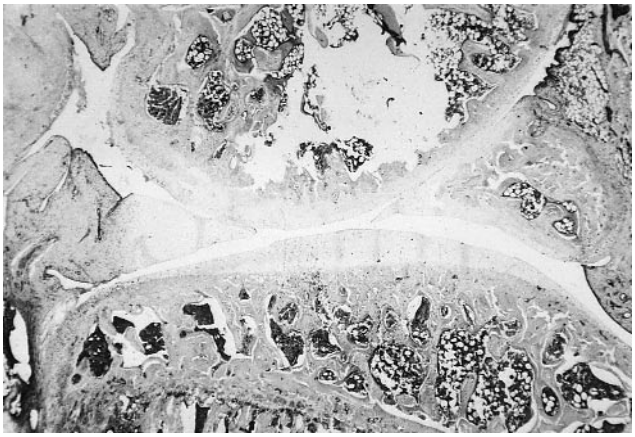

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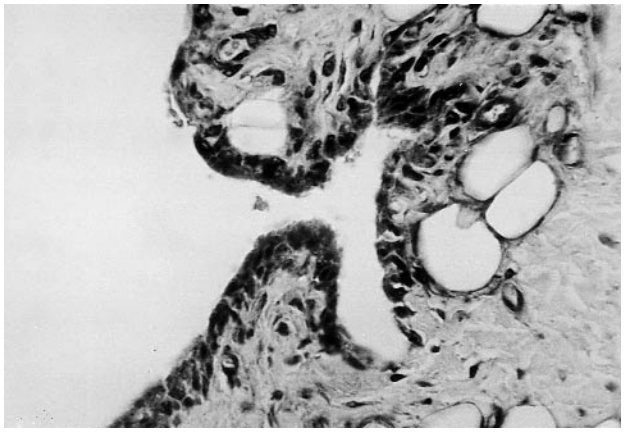

C

Figure 2. PGHS-1 immunostaining in rat knee synovium. Micrographs of (A) 4 week immobilization, (B) 32 week sham operated, and (C) 4 week sham operated specimens. Moderate staining was present in the synovial intima and subintima with either intervention. One adhesion of synovium to cartilage in the posterosuperior recess can be seen in the immobilized specimen (arrow, A). Note the greater chondrocyte staining in the immobilized specimen. At higher magnification (C), prominent synovial staining can be seen in a sham operated specimen 4 weeks after surgery. Original magnification A ×6.6, B ×6.6, C ×150.

early time points. Higher PGHS-1 levels in the 3 chondrocyte locations studied did not reach statistical significance compared to sham operated or non-operated legs (Table 1). When data from all chondrocyte sites were combined, immobilized chondrocytes showed significantly higher levels of PGHS-1 compared with sham operated animals 2 and 16 weeks after immobility (Figure 3).

Time after intervention had a significant effect on chondrocyte PGHS-1 levels in the posterior aspect of the femur (Table 1). Enzyme levels at 2 and 4 weeks were significantly higher than at 0 week (non-operated, time = 0) (1.38 ± 0.31 and 2.00 ± 0.33 vs 0.19 ± 0.14 arbitrary units; both $p < 0.05$). PGHS-1 levels decreased thereafter, and were significantly lower at 16 weeks compared to 4 weeks (0.60 ± 0.22 vs 2.00 ± 0.33; $p < 0.05$). PGHS-1 levels in the sham operated legs were not different from non-operated legs at any time point.

Detection of PGHS-2 in synovial cells. As observed for PGHS-1, immobility led to decreased PGHS-2 levels in synoviocytes of immobilized knees at all locations studied (Table 2). Statistical analysis revealed that the type of intervention (immobilized vs sham vs no operation) had a significant effect on PGHS-2 levels in synoviocytes of the posterior capsule. Post-hoc analyses revealed that PGHS-2 staining was significantly lower in synoviocytes of the posterior aspect of the knee capsule compared with sham operated legs (1.82 ± 0.13 vs 2.29 ± 0.15; $p = 0.016$). The sham surgery increased PGHS-2 levels in synoviocytes of the posterior capsule compared with non-operated legs (2.29 ± 0.15 vs 1.69 ± 0.20; $p < 0.05$).

Detection of PGHS-2 in chondrocytes. As for PGHS-1, immobility increased PGHS-2 levels in chondrocytes at all locations compared to sham and non-operated animals (Table 2). The immunostaining was localized to the perinuclear region and endoplasmic reticulum (Figure 4). Statistical analysis revealed that the type of intervention (immobilized vs sham vs no operation) had a significant effect on PGHS-2 levels at the 3 chondrocyte locations studied. Post-hoc analyses revealed that PGHS-2 staining was significantly higher in the chondrocytes of the anterior aspect of the tibia in immobilized knees compared with sham operated legs (1.61 ± 0.15 vs 0.77 ± 0.18; $p < 0.001$). Immobilization increased chondrocyte levels of PGHS-2 compared with non-operated legs in the posterior aspect of the femur, opposed surfaces, and anterior aspect of the tibia (1.45 ± 0.15, 1.06 ± 0.18, and 1.61 ± 0.15 vs 0.19 ± 0.14,

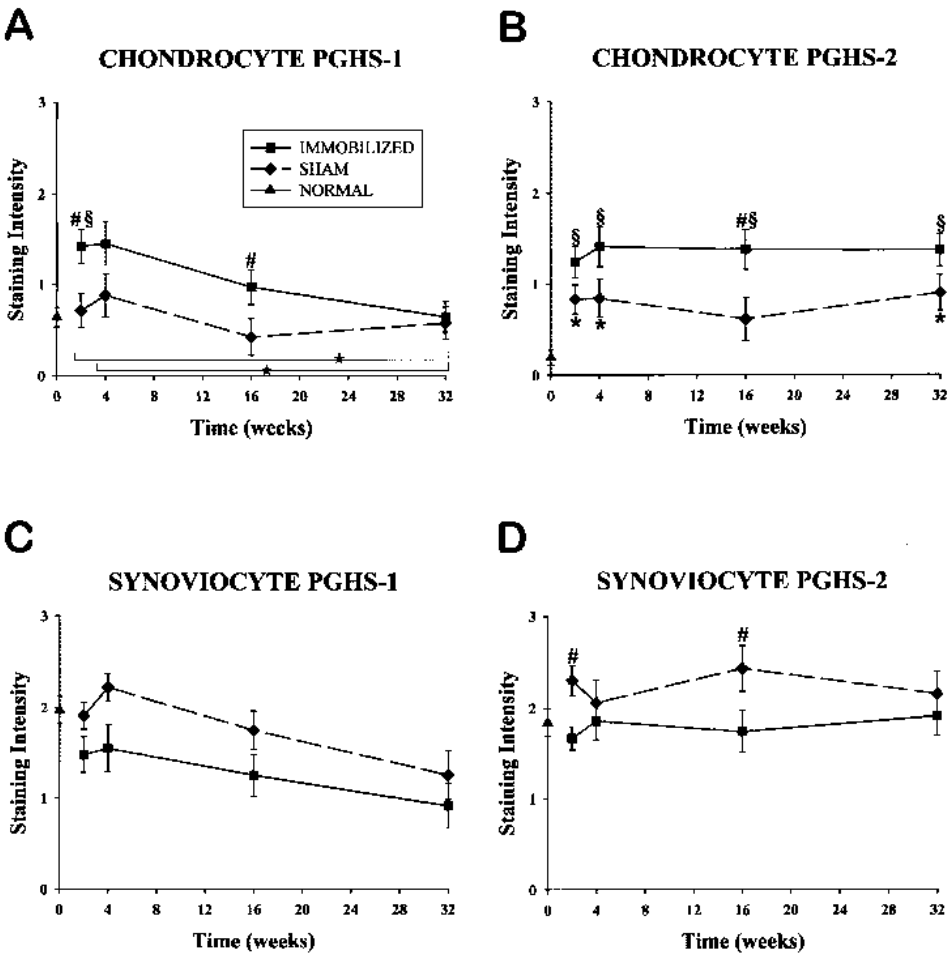

Figure 3. PGHS immunostaining intensity in rat knee joint after immobilization. A and B. Data from all articular chondrocyte sites (posterior femur, anterior tibia, opposed tibia) were combined to produce these graphs. Immobilization increased PGHS levels compared with sham operated legs and non-operated legs. C and D. Data from all synovial sites (posterior and anterior) were combined to produce these graphs. Immobilization decreased PGHS levels compared to sham operated animals. There was significant difference ($p < 0.05$) between immobilized and sham (#), immobilized and normal (§), and sham and normal (*), and between different time points (★) on Mann-Whitney test after Benjamini-Hochberg false discovery rate controlling procedure for multiple comparisons.

0.13 ± 0.12, and 0.25 ± 0.14, respectively; all $p < 0.005$). The sham surgery also led to increased PGHS-2 levels in chondrocytes of the posterior aspect of the femur compared with non-operated legs (1.14 ± 0.16 vs 0.19 ± 0.14; $p < 0.05$).

In the immobilized group, time after immobility had a statistically significant effect on chondrocyte PGHS-2 levels. Articular chondrocytes of the posterior aspect of the femur and anterior aspect of the tibia displayed significantly higher levels of PGHS-2 at Weeks 2, 4, 16, and 32 compared with Week 0 (posterior femur 1.15 ± 0.25, 2.00 ± 0.30, 1.20 ± 0.39, and 1.33 ± 0.26 vs 0.19 ± 0.14; anterior tibia 1.33 ± 0.26, 1.00 ± 0.33, 1.90 ± 0.35, and 1.92 ± 0.23 vs 0.25 ± 0.14, respectively, all $p < 0.05$) (Table 2). In the sham operated legs, PGHS-2 levels were also significantly increased in the chondrocytes of the posterior aspect of the femur at Weeks 2, 4, and 32 compared to non-operated legs (1.27 ± 0.27, 1.00 ± 0.29, and 1.10 ± 0.35 vs 0.19 ± 0.14; all $p < 0.05$) (Table 2).

Intra and interobserver reliability. Intra and interobserver correlation coefficients for the 5 locations on 8 randomly chosen slides were, respectively, 0.863 and 0.858.

DISCUSSION

In an animal model of joint contracture induced by immobilization, the levels of PGHS-1 and -2 enzymes were measured in synoviocytes and chondrocytes using selective antibodies and immunohistochemistry. Levels of both enzymes were increased in chondrocytes and reduced in synoviocytes from contractured joints compared to sham or non-operated animals. Changes in the levels of PGHS-2 enzyme were more important and widespread than changes in PGHS-1.

Table 1. Staining for COX-1 in rat knee joint contractures.

Intervention			Chondrocyte Posterior Femur		Chondrobyte Anterior Tibia		Chondrocyte Opposed Tibia		Synoviocyte Posterior		Synovicocyte Anterior	
	Week	n	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Immobilized	2	13-14	1.38†	0.31	1.31	0.33	1.36	0.34	1.50	0.27	1.50	0.27
	4	10-11	2.00†	0.33	1.30	0.42	1.00	0.45	1.45	0.41	1.64	0.34
	16	10	0.60†	0.22	1.60	0.40	0.60	0.27	1.20	0.33	1.30	0.33
	32	11-12	0.58†	0.29	0.75	0.35	0.58	0.26	0.91	0.41	0.92	0.31
Total		45-47									*,**	
Sham operated	2	10-11	0.80	0.33	1.00	0.40	0.45	0.31	1,.64	0.24	2.18	0.18
	4	8-9	1.00	0.42	0.67	0.44	1.00	0.44	2.11	0.26	2.33	0.17
	16	8	0.63	0.32	0.75	0.49	0.00	0.00	1.25	0.31	2.25	0.16
	32	10	0.70	0.30	0.80	0.42	0.20	0.13	1.30	0.40	1.20	0.39
Total		36-38										
Non-operated	0	16	0.41	0.12	1.13	0.20	0.41	0.18	1.75	0.19	2.19	0.21

*,**, † $p < 0.05$ after correction for multiple comparisons, * different from sham operated, ** different from non-operated, † different for various time points (see text). n varies slightly if the staining in one location could not be assessed precisely due to anomalies (e.g., fold in cartilage).

Table 2. Staining for COX-2 in rat knee joint contractures.

Intervention			Chondrocyte Posterior Femur		Chondrobyte Anterior Tibia		Chondrocyte Opposed Tibia		Synoviocyte Posterior		Synovicocyte Anterior	
	Week	n	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Immobilized	2	12-14	1.15†	0.25	1.33†	0.26	1.14	0.35	1.79	0.19	1.64	0.20
	4	9-11	2.00†	0.30	1.00	0.33	1.20	0.44	1.82	0.30	1.90	0.31
	16	9-10	1.20	0.39	1.90†	0.35	1.00	0.41	1.40	0.31	2.11	0.31
	32	11-12	1.33†	0.26	1.92†	0.23	1.00	0.39	2.27	0.30	1.58	0.29
Total		43-46	**		*,**		**		*			
Sham operated	2	11	1.27†	0.27	0.64	0.31	0.64	0.28	2.09	0.25	2.45	0.21
	4	7-9	1.00†	0.29	0.57	0.43	0.89	0.39	2.44	0.24	2.45	0.21
	16	6-7	1.00	0.52	0.67	0.49	0.17	0.17	2.57	0.30	2.29	0.42
	32	9-10	1.10†	0.35	0.89	0.35	0.70	0.37	2.10	0.41	2.20	0.29
Total		33-37	**						**			
Non-operated	0	16	0.19	0.14	0.25	0.14	0.13	0.12	1.69	0.20	2.00	0.22

*,**, † $p < 0.05$ after correction for multiple comparisons, * different from sham operated, ** different from non-operated, † different for various time points (see text). n may vary slightly if the staining in one location could not be assessed precisely due to anomalies (e.g., fold in cartilage).

The use of sham operated legs as control allows us to distinguish the effect of immobility versus that of surgical trauma. Comparing PGHS-1 staining in sham operated versus non-operated knees, no significant changes were observed for chondrocytes and synoviocytes. The significant reduction in PGHS-1 levels observed in synoviocytes of immobilized joints can therefore be attributed to immobility and not to a nonspecific surgical artifact. However, the surgical trauma, although performed distant to the knee, induced a significant increase of the PGHS-2 protein. PGHS-2 staining was higher in synoviocytes and chondrocytes of sham operated legs compared to non-operated animals. The mechanisms by which an injury distant to a joint alters its homeostasis are not known. In humans, knee joint effusions have been reported secondary to a diaphyseal femur or tibia fracture or superficial leg injuries[38]. Our observations suggest that increased PGHS-2 enzyme levels in the synoviocytes could be involved. This postsurgical increase in synoviocyte PGHS-2 levels after distant surgery was prevented by immobility.

PGHS enzymes have been detected in all normal joint tissues in cartilage, capsule, and in the synovial fluid[28,39,40]. Primary cultures of normal human articular chondrocytes stained positive for PGHS-1 protein and negative for PGHS-2[39]. In animal models, the expression and modulation of PGHS-1 and PGHS-2 enzymes vary according to the species used. Cartilage samples from rabbits have a different profile of PGHS enzyme expression than human cells, and PGHS-2 protein is detected in unstimulated rabbit chondrocytes[41]. Results from our study are in agreement with reports from experiments in rat species showing both PGHS-1 and PGHS-2 enzymes in both the chondrocytes and synoviocytes[24,42].

Specimens from patients with inflammatory joint diseases (rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis) and from animal models of arthritis have

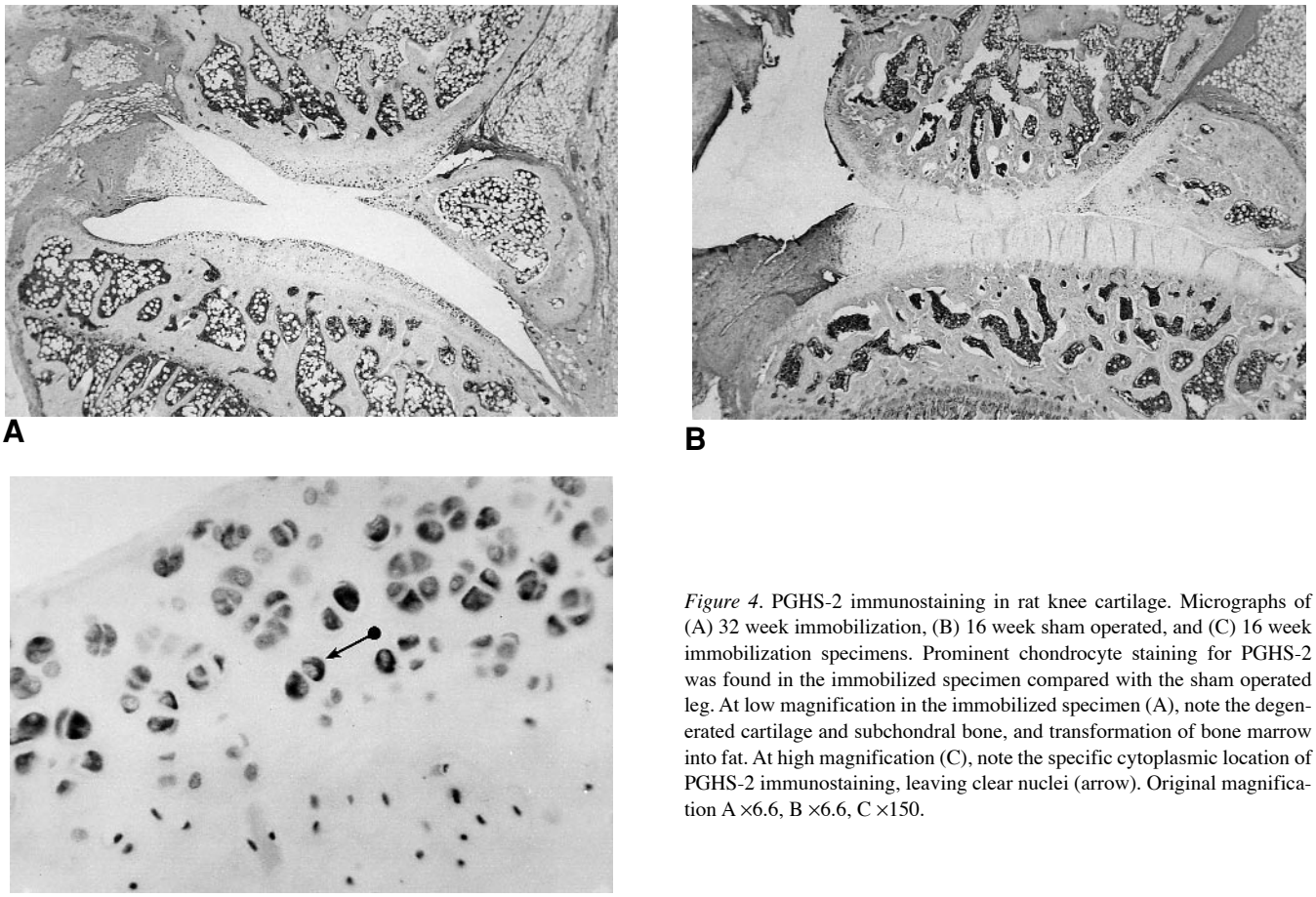

Figure 4. PGHS-2 immunostaining in rat knee cartilage. Micrographs of (A) 32 week immobilization, (B) 16 week sham operated, and (C) 16 week immobilization specimens. Prominent chondrocyte staining for PGHS-2 was found in the immobilized specimen compared with the sham operated leg. At low magnification in the immobilized specimen (A), note the degenerated cartilage and subchondral bone, and transformation of bone marrow into fat. At high magnification (C), note the specific cytoplasmic location of PGHS-2 immunostaining, leaving clear nuclei (arrow). Original magnification A ×6.6, B ×6.6, C ×150.

been studied for their PGHS enzyme content. Immunostaining for PGHS-2 but not PGHS-1 increased in a disease dependent manner in synoviocytes, chondrocytes, and mononuclear cells of synovial fluid[27,30,40]. PGHS-2 levels were also higher compared to patients with osteoarthritis (OA)[27,30,40]. OA is viewed as a degenerative disease of the cartilage, where inflammatory features of morning stiffness and acute inflammatory synovitis are mild compared to RA. The general conclusion was that PGHS-2 enzyme levels correlated with inflammatory joint diseases.

Contractures, clinically, are not accompanied by the cardinal signs of inflammation: pain, redness, heat, and edema. Our animal model mimics the clinical condition of contractures developing after immobilization and does not present detectable inflammatory signs. The decreases in levels of both PGHS-2 (significant) and PGHS-1 (not significant) enzymes we measured in synoviocytes from joints with contractures support the absence of an inflammatory reaction in immobilization induced contractures. It is anticipated that the net production of prostanoids by synovial cells would be less important in contractured joints than in normal controls. Using an animal model for joint contractures, we observed that the joint capsule was significantly atrophied, in agreement with previous studies[10,43], and that synovial adhesion rather than proliferation is the hallmark of synovial intima alteration in contractures[11]. The role of PGHS enzymes in synovial adhesion to cartilage observed in contractured joints remains to be determined.

The elevated levels of PGHS-2 enzymes in chondrocytes of joints with contractures are more likely to be associated with articular cartilage destruction than inflammation. Similar contrasting increases of interleukin 1ß, tumor necrosis factor-α, and inducible NO synthase in chondrocytes but not in synoviocytes have been reported in noninflammatory human arthropathies (OA and trauma)[44]. Elevated biosynthesis of prostanoids is considered to mediate the erosion of cartilage and juxtaarticular bone[29]. High levels of prostaglandin E_2 (PGE_2) in the articular cartilage also inhibit cell proliferation as well as the synthesis of cellular matrix components[45]. In joint contractures, cartilage degeneration appears rapidly, is severe, and causes irreversible joint damage[5-7,10,12-23]. Although the production of PGE_2 by articular chondrocytes of contractured joints has not been measured, our observation that levels of both PGHS-1 and -2 increase suggests that more PGE_2 is produced, causing cartilage degeneration.

Elevations in PGHS protein levels in chondrocytes from contractured joints are not likely to be mediated by inflam-

matory cytokines, as suggested in inflammatory conditions[27,28,44]. The absence of mechanical stimulation is likely to be the stimulus for increasing PGHS enzyme levels in chondrocytes. Cyclical and static mechanical stimulation alters chondrocyte gene expression and synthesis of proteins involved in cartilage structure and function, such as proteoglycans[46]. The levels of PGHS-1 mRNA are increased in primary cultures of stretched rat osteocytes[47]. Also, the expression of PGHS-2 enzyme and production of prostaglandin F_{2alpha} were reported to increase after repetitive mechanical stimulation of differentiated skeletal muscle[48,49]. Our observations support the concept that expression of PGHS genes is influenced by mechanical stimulation. The exact mechanisms by which mechanical stimulation, or lack of it, leads to altered gene expression, particularly of PGHS, remain to be established.

Treatment with rest versus motion has always been a dilemma for physicians treating musculoskeletal conditions[50,51]. Immobilization was shown to be of benefit in the treatment of inflammatory arthritis[52,53]. On the other hand, early mobilization and passive motion devices have successfully prevented deleterious effects of immobility[54,55]. Our results elucidate the effects of rest and motion on joints and may suggest appropriate clinical indications. We found that the 2 major tissues involved with articular homeostasis, cartilage and synovium, respond very differently to immobility: PGHS enzymes increase in the chondrocytes while decreasing in the synoviocytes. Therefore in a case of acute inflammatory synovitis, rest will decrease PGHS. Consequently, the net decrease in prostanoid production can explain the clinical improvement[52,53]. In this respect, immobilization can be viewed as a specific synoviocyte-PGHS inhibitor. Our results suggest that for chondrocytes of the articular cartilage, degenerative PGHS-2 modulated effects will be aggravated by rest and prevented by motion. This correlates with a study showing continuous passive motion preserved cartilage after antigen induced synovitis[54]. Motion can then be seen as a specific chondrocyte-PGHS inhibitor. Given the opposite behaviors of synoviocytes and chondrocytes, clinical indications for rest or motion can be refined. Persons with inflammatory arthritis decrease the use of affected joints as a strategy to decrease pain. In these patients, inhibition of PGHS action in the chondrocytes should be pursued by maintaining motion. Meanwhile, increased production of PGHS in the synovium can be controlled pharmacologically (e.g., with nonsteroidal antiinflammatory drugs).

Joint contractures secondary to immobility are characterized by cell-specific alterations: PGHS enzyme levels are decreased in the synoviocytes and increased in chondrocytes. Our results support the direct role of chondrocytes in the cartilage damage of a noninflammatory joint disease. Our results also provide guidelines for immobility as a treatment. In joint contractures the roles of PGHS-1 and -2 enzymes and their prostanoid products and the benefit of PGHS inhibitors remain to be determined.

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