High collagen type I and low collagen type III levels in knee joint contracture

An immunohistochemical study with histological correlate

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ABSTRACT – We studied the levels of collagen type I and type III in the knee joints of rats immobilized for periods of 2, 4, 16 and 32 weeks and sham-operated controls. The intensities of immunostaining of the anterior and posterior synovial intima, anterior and posterior subintima, and patellar tendon were graded on a scale from 0 to 3. We found higher type I collagen levels in immobilized legs than in sham-operated legs in the anterior subintima after 4 and 16 weeks (mean score 2.1 vs 1.3, 2.2 vs 1.3, respectively) and posterior subintima after 2, 4 and 16 weeks of immobility (2.4 vs 1.7, 2.3 vs 1.5, 2.2 vs 1.3, respectively). Lower type III collagen levels were found in immobilized legs than in sham-operated legs in the anterior synovial intima after 32 weeks (1.3 vs 2.3), and posterior synovial intima and posterior subintima after 16 weeks of immobility (1.4 vs 2.8, 1.2 vs 1.7, respectively). The higher type I collagen levels in the subintima combined with lower type III suggests that the contracture process is marked by fibrosis, not new tissue proliferation. In this respect, contractures differ from granuloma, scar tissue and the pannus of inflammatory arthritis.

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The pathological changes in established joint contractures have been described, but the pathophysiology is incompletely understood. Microscopic changes in synovial joints subjected to immobilization have been described as global intraarticular tissue proliferation (Evans et al. 1960, Thaxter et al. 1965, Peacock 1966, Enneking et al. 1972, Finsterbush and Friedman 1973, Langenskiöld et al. 1979, Schollmeier et al. 1994), proliferation at the intercondylar region (Evans et al. 1960, Thaxter et al. 1965, Enneking et al. 1972, Akeson et al. 1973), and synovial adhesions to the articular cartilage followed by its degeneration (Evans et al. 1960, Salter and Field 1960, Thaxter et al. 1965, Enneking et al. 1972, Finsterbush and Friedman 1973). In contrast, other reports described neither pannus proliferation nor adhesion after joint immobility (Sood 1971, Akeson et al. 1973, Amiel et al. 1980). Using standardized and quantitative studies, we have been unable to measure any intraarticular pannus formation after immobility (Trudel et al. 1998). In fact, we found reduction in synovial intimal length and subintima after immobility which suggested that adhesions of the synovial intima rather than proliferative changes occurred in joint contracture (Trudel et al. 2000).

Determination of the levels of the various structural collagens is key to the understanding of joint capsule stiffness after immobilization. We found only two reports on the distribution of collagen types in periarticular connective tissue after joint immobilization. Amiel et al. (1980) suggested that type I, but no type III collagen was present in both 9-week immobilized rabbit knee capsules and control, using the standard differential salt precipitation technique. However, using immunohistochemistry, Schollmeier et al. (1996) reported high levels of type III collagen in the synovial intima of the canine shoulder after 12 weeks of immobilization. The determination of type III collagen levels is of value in deciding whether the changes in the capsule of a joint developing a contracture are of a proliferative/secretive or a degenerative/catabolic

nature. Using immunohistochemistry, we assessed the distribution of type I and type III collagen in the capsules of joints subjected to various periods of immobility.

Animals and methods

The protocol for this research project was approved by the University Animal Care Committee. We used 85 adult male Sprague-Dawley rats (average weight 350 g). 47 underwent unilateral internal, but extraarticular, knee joint immobilization at 135° of flexion according to a previously described method (Trudel et al. 1998). Briefly, rigid internal fixation was performed surgically with a plastic plate and two screws, inserted one each in the proximal femur and distal tibia away from all knee joint structures. The immobilized animals formed the experimental group. 38 rats underwent a sham operation after the same procedure in the experimental group, except that the plate was removed before inserting the screw. Surgery was performed under halothane anesthesia and completed in 20 minutes. Pre- and postoperative pain control were obtained with buprenorphine 0.05 mg/kg s.c. q12 hours for 48 hours. The animals were allowed unlimited activity and free access to water and food.

Rats were killed 2, 4, 16 and 32 weeks later. After harvesting the knee joint and surrounding soft tissue, the knees were fixed in Bouin's solution or in 10% formalin for 18–24 hrs at 41 °C. All knees were decalcified in 10% EDTA in Tris buffer 0.1 M (pH 7.2 adjusted with Tris base) at 41 °C for 2–3 months and embedded in low-melting point paraffin (Labware, St. Louis, MO, USA). 7 μm thick serial sections were made at the medial mid-condylar level in the sagittal plane.

Immunohistochemistry

Deparaffinized sections were treated with 0.2% trypsin in 0.1% $CaCl_2$, adjusted to pH 7.4 with 0.1 M NaOH for 1 hr at 37 °C. After washing in phosphate-buffered saline solution (PBS), sections were incubated in 3% H_20_2 in DH_20 for 30 min. To eliminate nonspecific antibody binding, the sections were incubated both with 5% skim milk for 30 min and Protein Block (normal goat serum diluted in PBS containing 1% bovine serum albumin, 0.09% sodium azide and 0.1% Tween-20; BioGenex, San Ramon, CA) for 20 min. The sections were washed in PBS between each treatment. Rabbit polyclonal antibodies against rat type I collagen (1: 200) (Chemicon Intern. Inc., Temecula, CA, USA) and type III collagen (1: 500) (Chemicon Intern. Inc.) were incubated on the slides overnight (about 18 hr) at 4 °C. After washing, all slides were exposed to biotinylated goat anti-rabbit Ig (Super Sensitive Rabbit Link, BioGenex) for 20 min. The sections were then incubated with peroxidase-conjugated streptavidin (Super Sensitive HRP Link, BioGenex) for 20 min, and incubated with 3,3'-diaminobenzidine for 4 min. The sections were counterstained with hematoxylin.

Control sections were exposed to nonspecific secondary antibodies. All slides were stained in one session (same day, same reagents, same protocol). The slides were assessed with optical microscopy, but the observer was blinded as to which specimen was being examined. The immunostaining intensity was reported at 5 sites: anterior and posterior synovial intima, anterior and posterior subintima, and patellar tendon. The immunohistochemical staining intensity of type I and type III collagen was graded on a scale of 0–3 where: no staining was 0, weak staining 1, moderate staining 2, and strong staining 3.

Intrarater reliability coefficient

After making all measurements, we randomly selected 9 sections each for collagen type I and type III and they were again blindly assessed again by the same observer.

Statistics

We used the software program SPSS 10.0 for Windows, (SPSS Inc., Chicago, IL, USA) for setting up the database and the statistical analysis. The inter-rater reliability coefficient was calculated, using the non-parametric Kendall's tau-b bivariate correlations. Using a within subjects analysis, multiple Mann-Whitney tests were employed to determine the significance of differences in staining intensity between the immobilized and the sham-operated legs at each time. The effect of time on staining intensity was studied, using the Kruskal-Wallis test with post-hoc Mann-Whitney tests. Post-hoc cor-

Table 1. Mean scores of staining intensity for type I collagen

Group	2 weeks		4 weeks		16 weeks		32 weeks	
	Mean score (SD)	n	Mean score (SD)	n	Mean score (SD)	n	Mean score (SD)	n
Anterior synovial intima								
Immobilization	2.7 (0.5)	15	2.4 (0.5)	8	2.7 (0.7)	10	2.9 (0.3)	9
Sham-operated	2.6 (0.5)	10	2.4 (0.5)	7	3.0 (0.0)	9	2.7 (0.5)	9
Anterior subintima								
Immobilization	2.0 (0.9)	15	2.1 (0.6) [a]	8	2.2 (0.6) [a]	10	1.9 (0.9)	10
Sham-operated	1.6 (0.7)	10	1.3 (0.8) [a]	7	1.3 (0.5) [a]	9	1.3 (0.7)	9
Posterior synovial intima								
Immobilization	3.0 (0.0)	15	2.6 (0.5)	11	2.6 (0.7)	9	2.8 (0.4)	9
Sham-operated	2.8 (0.4)	10	2.8 (0.5)	8	2.9 (0.3)	9	2.9 (0.3)	10
Posterior subintima								
Immobilization	2.4 (0.7) [a]	15	2.3 (0.7) [a]	11	2.2 (0.6) [a]	10	1.9 (0.7)	11
Sham-operated	1.7 (0.7) [a]	10	1.5 (0.8) [a]	8	1.3 (0.5) [a]	9	1.9 (0.7)	10
Patella tendon								
Immobilization	0.9 (0.5)	15	1.0 (0.6)	6	0.8 (0.8)	9	0.5 (0.5)	10
Sham-operated	1.1 (0.6)	10	0.8 (0.4)	6	0.8 (0.4)	9	0.6 (0.5)	8

[a] Significant difference between Immobilization and Sham-operated groups at each time ($p < 0.05$)

rection for multiple comparisons was done, using the control for False Discovery Rate (Benjamini and Hochberg 1995). Differences at $p < 0.05$ were regarded as statistically significant.

Results

Microscopic examination

The microscopic appearance of the specimens from the immobilized and sham-operated knees differed. After 2 weeks of immobilization, moderate hypercellularity appeared in the posterior subintima. 4 samples of 15 showed loose reticular fibers at small synovial folds or at synoviocartilage recesses posteriorly. These new fibers contained mesenchymal cells that may have originated from the synovial intima. The posterior subintima showed high cellularity and new collagen fibers characteristic of a fibrotic reaction.

After 4 weeks of immobilization, the fibrosis increased in the posterior subintima. New loose collagen fibers obliterated the small synovial folds and synoviocartilage recesses in 4 samples of 11. 1 animal had a fibrotic bridge from the posterior synovial intima to the articular cartilage of the femur.

After long periods of immobilization (16 and 32 weeks), the synovial intima had atrophied markedly, and in some cases could not be identified. The posterior subintima was hypocellular with more organized connective tissue than specimens from animals that had been sham-operated or immobilized for a short period. The sites where loose collagen fibers were seen earlier (synovial folds and synoviocartilage recesses) were filled with more organized fibrous tissue.

The knee capsule of sham-operated legs showed a normal synovial intima with no adhesion to cartilage throughout the experiment. The subintima consisted of loose fibrous tissue and adipocytes of normal cellularity and vascularity.

Immunohistochemistry

We excluded some sites/slides from the final analysis for technical reasons, like failure to identify the synovial intima on some slides (mainly in the experimental group at 16 and 32 weeks), histological disruption or inadequate staining (e.g., had become dry during incubation overnight) (Tables 1 and 2).

The staining of type I collagen was strong in the anterior and posterior synovial intima, with no statistical difference between immobilized and sham-operated legs (Table 1). Type I collagen levels were higher in the anterior and posterior subintima of immobilized knees than in sham-operated ones (Figure 1). The differences were significant at 2, 4 and 16 weeks in the posterior subintima, and at 4 and 16 weeks in the anterior subintima (Figure 2).

Table 2. Mean scores of staining intensity for Type III collagen

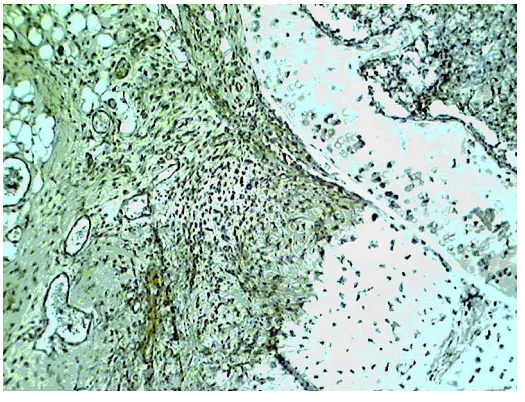
Group	2 weeks		4 weeks		16 weeks		32 weeks	
	Mean score (SD)	n	Mean score (SD)	n	Mean score (SD)	n	Mean score (SD)	n
Anterior synovial intima								
Immobilization	1.4 (0.6)	15	1.4 (0.8)	7	2.0 (1.0)	9	1.3 (0.5) [a]	9
Sham-operated	1.3 (0.5) [bd]	9	2.0 (0.9)	9	2.6 (0.5) [b]	8	2.3 (0.8) [ad]	10
Anterior subintima								
Immobilization	1.5 (0.6)	15	1.3 (0.8)	7	1.9 (0.9)	10	1.7 (0.8)	10
Sham-operated	1.3 (0.5)	9	1.3 (0.7)	9	1.8 (0.7)	8	1.7 (0.8)	10
Posterior synovial intima								
Immobilization	1.4 (0.7)	15	1.6 (0.7)	11	1.4 (0.5) [a]	8	1.5 (0.8)	8
Sham-operated	1.6 (0.7) [b]	10	1.7 (0.7) [c]	9	2.8 (0.4) [abc]	9	2.2 (0.8)	10
Posterior subintima								
Immobilization	1.5 (0.7) [a]	15	1.6 (0.7)	11	1.2 (0.4) [a]	10	1.6 (0.5)	11
Sham-operated	1.0 (0.0) [abd]	10	1.2 (0.4)	9	1.7 (0.5) [ab]	9	1.7 (0.7) [d]	10
Patella tendon								
Immobilization	0.5 (0.6)	15	0.6 (0.8)	7	0.5 (0.7)	10	0.4 (0.7)	10
Sham-operated	0.4 (0.5)	9	0.7 (0.7)	9	0.9 (1.0)	8	0.7 (0.7)	9

[a] Significant difference between Immobilization and Sham-operated groups at each time ($p < 0.05$)
[b] Significant difference between 2 weeks and 16 weeks in each group, using Mann-Whitney tests with False Discovery Rate correction ($p < 0.05$)
[c] Significant difference between 4 weeks and 16 weeks in each group, using Mann-Whitney tests with False Discovery Rate correction ($p < 0.05$)
[d] Significant difference between 2 weeks and 32 weeks in each group, using Mann-Whitney tests with False Discovery Rate correction ($p < 0.05$)

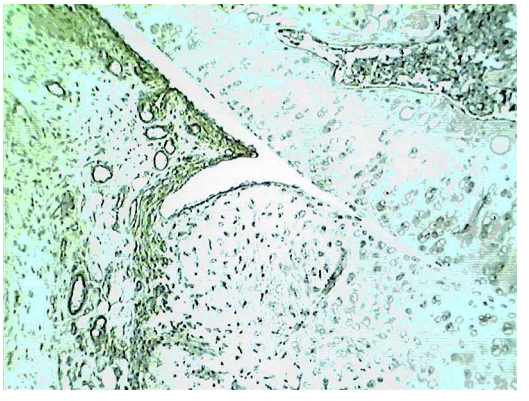
The staining intensity of type I collagen in the patellar tendon showed no difference between immobilized and sham-operated ones.

Time had no significant effect on type I collagen levels in the immobilized or sham-operated legs. Overall, the levels of type III collagen were lower than those of type I collagen at all capsular sites studied (Table 2). In the anterior and posterior synovial intima, type III collagen showed weaker staining in the immobilized than in the sham-

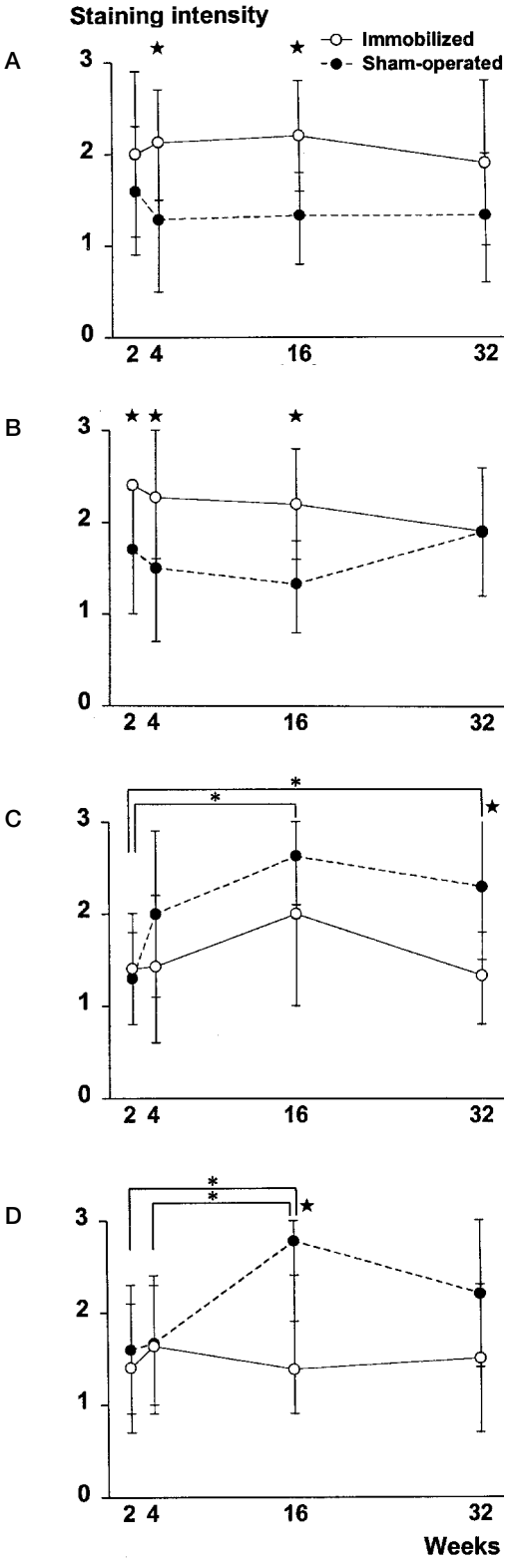
Figure 1. Type I collagen staining of immobilized and sham-operated legs. Microphotographs of the posterior capsule of a rat knee joint. Note the posterior aspect of the medial femoral condyle (top right), the posterior horn of the medial meniscus (bottom right), and the posterior capsule (left).



A. 2 weeks after immobilization. The subintima shows marked cellularity with organized connective tissue and strong type I collagen staining, ×25.



B. 2 weeks after sham operation. The knee capsule shows no proliferation or adhesion of synovial tissue to cartilage and weak type III collagen staining. Counterstained with hematoxylin, ×25.



operated legs (Figure 3). The differences were statistically significant at 32 weeks in the anterior synovial intima and at 16 weeks in the posterior synovial intima (Figure 2). In the posterior subintima, the intensity of type III collagen staining in the immobilized legs was higher than the sham-operated legs at 2 weeks, and lower at 16 weeks. In the anterior subintima and patellar tendon, the intensity of staining of type III collagen was about the same in the immobilized and sham-operated groups.

Time had no effect on the levels of type III collagen in the immobilized knees, but they increased with time after sham surgery. The staining intensity was stronger at 16 weeks than at 2 weeks in the anterior synovial intima, posterior synovial intima and posterior subintima (Figure 2). Type III collagen staining was also stronger at 16 weeks than at 4 weeks in the posterior synovial intima and stronger at 32 weeks than at 2 weeks in the anterior synovial intima and posterior subintima (Figure 2).

Intrarater reliability

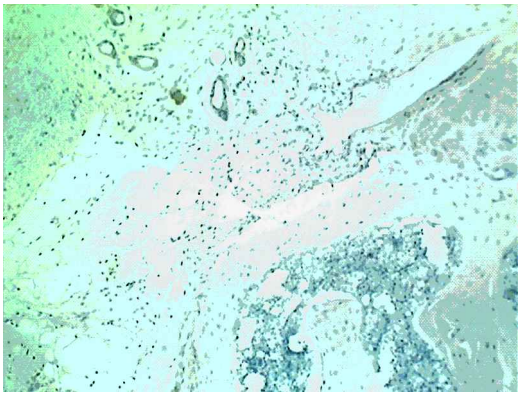
The intrarater reliability coefficients for immunohistochemical scoring of type I and type III collagen were 0.92 and 0.91, respectively (both $p = 0.001$).

Discussion

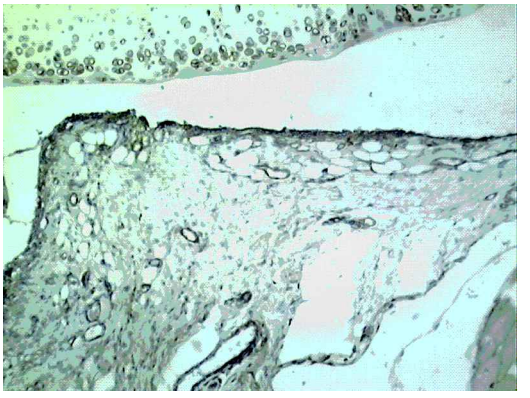
The distribution of collagen type I and type III in the joint capsule provides information about the pathophysiology of joint contracture secondary to immobility. We report a complete study where rat knees were immobilized for up to 8 months and

Figure 2. Type I and Type III collagen levels in rat knees. Mean scores of staining intensity of the immobilized and the sham-operated legs.
A. Type I collagen in the anterior subintima.
B. Type I collagen in the posterior subintima. Type I collagen levels are higher in the anterior and posterior subintima of immobilized than in sham-operated legs.
C. Type III collagen in the anterior synovial intima.
D. Type III collagen in the posterior synovial intima. Type III collagen levels are lower in the immobilized than in the sham-operated legs.
✱: Significant difference ($p < 0.05$) between the immobilized and the sham-operated legs at various times.
★: Significant effect of time ($p < 0.05$).
Error bar = 1SD.

Figure 3. Type III collagen staining of immobilized and sham-operated legs. Microphotographs of the posterior capsule of the rat knee joint. Note the posterior femoral condyle (top left) and posterior synovium (right).



A. 16 weeks after immobilization.



B. 16 weeks after sham operation. The synovial intima in the immobilized joint shows atrophy and weaker staining for type III collagen than in the sham-operated leg. Counterstained with hematoxylin, ×33.

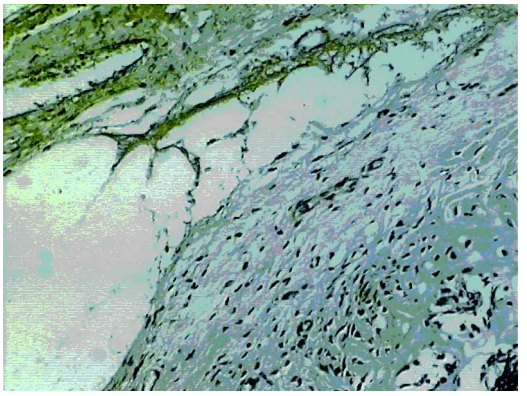
developed a flexion contracture. Throughout this period, the levels of type I and type III collagens were assessed with immunohistochemistry. Standardized methods and the use of a sham-operated group allowed us to distinguish between the effects of immobility and experimental artifacts.

In our study, the microscopic appearance of the immobilized specimens did not show marked pannus proliferation. This is contrary to some previous reports (Evans et al. 1960, Thaxter et al. 1965, Finsterbush and Friedman 1973, Langenskiöld et al. 1979, Michelsson and Hunneyball 1984, Schollmeier et al. 1994, 1996) but consistent with others (Sood 1971, Akeson et al. 1973, Amiel et al. 1980). The difference between our study and others can be related to the methods used. Some interventions may have damaged the knee joint or capsule whereas our fixation was completely extraarticular. In other studies, immobilization was achieved by pinning, casting, splinting, bandaging, denervation, a combination of these, or were case reports. These methods may have permitted joint movement thereby promoting the formation of repair tissue. Villous synovial lining hyperplasia was found on remobilization after immobilization (Evans et al. 1960, Michelsson and Hunneyball 1984). Our internal fixation by plate and screws was more rigid.

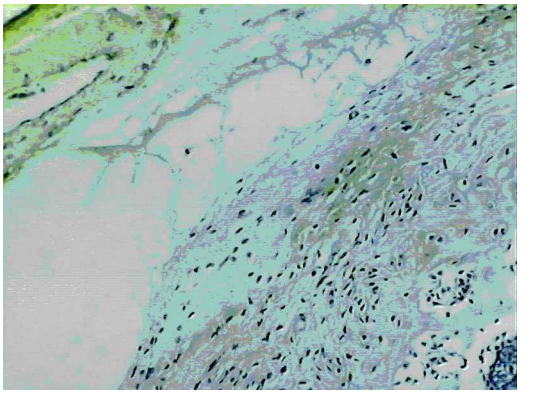
The biochemical changes leading to pathological alterations are poorly understood. After immobilization, periarticular connective tissues including tendon, ligament, capsule and synovial tissue lose significant amounts of glycosaminoglycans and water (Akeson et al. 1973, Woo et al. 1975). Lower concentrations of glycosaminoglycans and water are thought to reduce plasticity and resilience of connective tissue matrices and lubrication efficiency (Akeson et al. 1973). An increase in the total collagen in the posterior capsule has been found after immobilization (Peacock 1966). In contrast, others have detected no change in total collagen as reflected by the hydroxyproline concentration (Akeson et al. 1973, Woo et al. 1975). Moreover, the levels of salt-soluble collagens (mainly procollagen) fell (Woo et al. 1975). These data indicate that the contracture process can not be due to accelerated collagen synthesis alone.

We observed higher levels of type I collagen in our model after immobilization which was mainly located in the anterior and posterior subintima. This correlated with histological findings of subintimal fibrosis. The fibrotic reaction started early (2 weeks), increased during the first 16 weeks and plateaued thereafter. In the patellar tendon, the type I collagen levels were not affected. This accords with other reports, showing no statistical change in the total collagen mass of the patellar tendon after 9 weeks of immobilization (Amiel et al. 1982, Harwood and Amiel 1992, Bland and Ashhurst 1997). After 12 weeks of immobilization, significant losses in total collagen mass have been noted (Harwood and Amiel 1992).

Figure 4. Type I and type III collagen staining of the postero-inferior recess of an immobilized joint. Serial sections of the same specimen 2 weeks after immobilization.



A. Type I collagen.



B. Type III collagen. New loose fibers are seen. Type I collagen shows strong staining of the new fibrous tissues, but weak staining for type III collagen. Counterstained with hematoxylin, ×50.

Type III collagen localizes in tissues and organs that require a structural scaffolding able to respond to varying tensions, which has been postulated to maintain the structure in expansible organs (Montes et al. 1984), and stabilize new matrices (Cheung et al. 1983). We confirmed that it is a major constituent of normal synovium (Eyre and Muir 1975, Linck et al 1983, Ashhurst et al. 1991, Bland and Ashhurst 1997) and present in inflamed synovium (Weiss et al. 1975) and proliferative rheumatoid synovium (Eyre and Muir 1975, Weiss et al. 1975, Adam et al 1976). Large amounts of type III collagen are found in late embryonic and early postnatal life in the human dermis (Epstein 1974). In adults, it is synthesized in large amounts in granulomatous tissue (Bailey et al. 1975), during wound healing and fracture repair (Lane et al. 1986). In wound healing, the initial proliferative phase is marked by a peak expression of type III collagen (Barnes et al. 1976, Gay et al. 1978, Clore et al. 1979, Lane et al. 1986, Haukipuro et al. 1991, Rasmussen et al. 1992, Betz et al. 1993, Cornelissen et al. 2000). Later, in the organization phase, type I collagen fibers replace type III collagen fibers (Gay et al. 1978, Rasmussen et al. 1992).

We found lower type III collagen levels in immobilized rat knee capsules than in sham-operated legs in the anterior and posterior synovial intima after 16 and 32 weeks of immobility. In our model, synovial folds and joint recesses were of special interest. These were the only areas where microscopic adhesions were found. In those areas during immobilization, the intensity of staining of type III collagen was very weak while immunostaining of type I collagen was strong (Figure 4). These findings suggest that the contracture process does not involve an initial phase of type III collagen deposition in the synovial or subintimal tissues. Type III collagen consists of three α1(III) chains, while type I collagen is made of one α2(I) and two α1(I) chains. The genes coding for these three chains are located on different chromosomes: the human genes for α1(I), α2(I) and α1(III) are on chromosomes 17, 7 and 2, respectively (Uitto and Chu 1989). The systematic sequential expression first of type III collagen, then of type I collagen in repair processes suggests the temporally ordered expression of these genes. Our findings indicate that joint immobilization stimulates type I collagen synthesis directly. In this respect, the capsular reaction during contractures seems to differ from the wound healing and fracture repair processes.

In the sham-operated legs, type III collagen levels were higher in the synovial intima and posterior subintima after long periods of immobilization. One reason may be the increase in activity that was temporarily reduced after the surgical procedure. This was not seen in immobile joints since no remobilization took place.

Our findings support the view that periarticular changes in joint contractures do not resemble those in wound healing or inflammatory proliferative

processes, but are more characteristic of a fibrotic process. Future research should aim to quantify biochemically the type I and type III collagen levels in the capsules of joints with contractures.

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Adam M, Vitasek R, Deyl Z, Felsch G, Musilova J, Olsovska Z. Collagen in rheumatoid arthritis. Clin Chim Acta 1976; 70 (1): 61-9.

Akeson W H, Woo S L, Amiel D, Coutts R D, Daniel D. The connective tissue response to immobility: biochemical changes in periarticular connective tissue of the immobilized rabbit knee. Clin Orthop 1973; 93: 356-62.

Amiel D, Akeson W H, Harwood F L, Mechanic G L. The effect of immobilization on the types of collagen synthesized in periarticular connective tissue. Connect Tissue Res 1980; 8 (1): 27-32.

Amiel D, Woo S L, Harwood F L, Akeson W H. The effect of immobilization on collagen turnover in connective tissue: a biochemical-biomechanical correlation. Acta Orthop Scand 1982; 53 (3): 325-32.

Ashhurst D E, Bland Y S, Levick J R. An immunohistochemical study of the collagens of rabbit synovial interstitium. J Rheumatol 1991; 18 (11): 1669-72.

Bailey A J, Sims T J, Le Lous, Bazin S. Collagen polymorphism in experimental granulation tissue. Biochem Biophys Res Commun 1975; 66 (4): 1160-5.

Barnes M J, Morton L F, Bennett R C, Bailey A J, Sims T J. Presence of type III collagen in guinea-pig dermal scar. Biochem J 1976; 157 (1): 263-6.

Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Statist Soc B 1995; 57 (1): 289-300.

Betz P, Nerlich A, Wilske J, Tubel J, Penning R, Eisenmenger W. Analysis of the immunohistochemical localization of collagen types III and V for the time-estimation of human skin wounds. Int J Legal Med 1993; 105 (6): 329-32.

Bland Y S, Ashhurst D E. Fetal and postnatal development of the patella, patellar tendon and suprapatella in the rabbit; changes in the distribution of the fibrillar collagens. J Anat 1997; 190 (3): 327-42.

Cheung D T, DiCesare P, Benya P D, Libaw E, Nimni M E. The presence of intermolecular disulfide cross-links in type III collagen. J Biol Chem 1983; 258 (12): 7774-8.

Clore J N, Cohen I K, Diegelmann R F. Quantitation of collagen types I and III during wound healing in rat skin. Proc Soc Exp Biol Med 1979; 161 (3): 337-40.

Cornelissen A M, Stoop R, Von den Hoff H W, Maltha J C, Kuijpers-Jagtman A M. Myofibroblasts and matrix components in healing palatal wounds in the rat. J Oral Pathol Med 2000; 29 (1): 1-7.

Enneking W F, Horowitz M, Florida G. The intra-articular effects of immobilization on the human knee. J Bone Joint Surg (Am) 1972; 54 (5): 973-85.

Epstein E H Jr. $[\alpha 1(III)]_3$ human skin collagen. Release by pepsin digestion and preponderance in fetal life. J Biol Chem 1974; 249 (10): 3225-31.

Evans B E, Eggers G W N, Butler J K, Blumel J, Texas G. Experimental immobilization and remobilization of rat knee joints. J Bone Joint Surg (Am) 1960; 42 (5): 737-58.

Eyre D R, Muir H. Type III collagen: a major constituent of rheumatoid and normal human synovial membrane. Connect Tissue Res 1975; 4 (1): 11-6.

Finsterbush A, Friedman B. Early changes in immobilized rabbit knee joints: a light and electron microscopic study. Clin Orthop 1973; 92: 305-19.

Gay S, Vijanto J, Raekallio J, Penttinen R. Collagen types in early phases of wound healing in children. Acta Chir Scand 1978; 144 (4): 205-11.

Harwood F L, Amiel D. Differential metabolic responses of periarticular ligaments and tendon to joint immobilization. J Appl Physiol 1992; 72 (5): 1687-91.

Haukipuro K, Melkko J, Risteli L, Kairaluoma M, Risteli J. Synthesis of type I collagen in healing wounds in humans. Ann Surg 1991; 213 (1): 75-80.

Lane J M, Suda M, von der Mark K, Timpl R. Immunofluorescent localization of structural collagen types in endochondral fracture repair. J Orthop Res 1986; 4 (3): 318-29.

Langenskiöld A, Michelsson J E, Videman T. Osteoarthritis of the knee in the rabbit produced by immobilization. Attempts to achieve a reproducible model for studies on pathogenesis and therapy. Acta Orthop Scand 1979; 50 (1): 1-14.

Linck G, Stocker S, Grimaud J A. Porte A. Distribution of immunoreactive fibronectin and collagen (types I, III, IV) in mouse joints. Fibronectin, an essential component of the synovial cavity border. Histochemistry 1983; 77 (3): 323-8.

Michelsson J E, Hunneyball I M. Inflammatory involvement in rabbit knee following immobilization and resulting in osteoarthritis. Scand J Rheumatol 1984; 13 (3): 273-81.

Montes G S, Bezerra M S F, Junqueira L C U. Collagen distribution in tissues. In: Ultrastructure of the connective tissue matrix (Eds. Ruggeri A, Motta P M). Martinus Nijhoff Publishers, Boston 1984; 3: 66-88.

Peacock E E Jr. Some biochemical and biophysical aspects of joint stiffness: role of collagen synthesis as opposed to altered molecular bonding. Ann Surg 1966; 164 (1): 1-12.

Rasmussen L H, Jensen L T, Avnstorp C, Karlsmark T, Peters K, Horslev-Petersen K. Collagen types I and III propeptides as markers of healing in chronic leg ulcers. A noninvasive method for the determination of procollagen propeptides in wound fluid--influence of growth hormone. Ann Surg 1992; 216 (6): 684-91.

Salter R B, Field P. The effects of continuous compression on living articular cartilage. J Bone Joint Surg (Am) 1960; 42 (1): 31-49.

Schollmeier G, Uhthoff H K, Sarkar K, Fukuhara K. Effects of immobilization on the capsule of the canine glenohumeral joint. A structural functional study. Clin Orthop 1994; 304: 37-42.

Schollmeier G, Sarkar K, Fukuhara K, Uhthoff H K. Structural and functional changes in the canine shoulder after cessation of immobilization. Clin Orthop 1996; 323: 310-5.

Sood S C. A study of the effects of experimental immobilisation on rabbit articular cartilage. J Anat 1971; 108 (3): 497-507.

Thaxter T H, Mann R A, Anderson C E. Degeneration of immobilized knee joints in rats; histological and autoradiographic study. J Bone Joint Surg (Am) 1965; 47 (3): 567-85.

Trudel G, Jabi M, Uhthoff H K. Intraarticular tissue proliferation after immobility: methods of assessment and preliminary results in rat knee joints. J Rheumatol 1998; 25 (5): 945-50.

Trudel G, Seki M, Uhthoff H K. Synovial adhesions are more important than pannus proliferation in the pathogenesis of knee joint contracture after immobilization: an experimental investigation in the rat. J Rheumatol 2000; 27 (2): 351-7.

Uitto J, Chu M L. Regulation of collagen gene expression in human skin fibroblasts and its alterations in diseases. In: Collagen Volume IV: Molecular Biology (Eds. Olsen B R, Nimni M E). CRC Press, Inc. Boca Raton, Florida 1989; 7: 109-24.

Weiss J B, Shuttleworth C A, Brown R, Sedowfia K, Baildam A, Hunter JA. Occurrence of type III collagen in inflamed synovial membranes: a comparison between non-rheumatoid, rheumatoid, and normal synovial collagens. Biochem Biophys Res Commun 1975; 65 (3): 907-12.

Woo S L, Matthews J V, Akeson W H, Amiel D, Convery F R. Connective tissue response to immobility. Correlative study of biomechanical and biochemical measurements of normal and immobilized rabbit knees. Arthritis Rheum 1975; 18 (3): 257-64.