Magnetic Resonance Imaging and Morphometric Quantitation of Cartilage Histology after Chronic Infusion of Interleukin 1 in Rabbit Knees (43569)

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Abstract. **Cartilage pathology in rabbit knees was monitored by noninvasive magnetic resonance imaging (MRI) and evaluated using morphometric histologic measurements. Infusion of rabbit knees with the cytokine interleukin 1 induces cartilage degradation and inflammation. A miniosmotic pump was implanted subcutaneously to deliver interleukin 1 through a polyethylene catheter inserted into the rabbit knee. Rabbit knees were imaged using MRI and prepared for histologic examination at 5 and 12 days after chronic infusion of interleukin 1. MRI obtained 0.7-mm sections for three-dimensional reconstruction of cartilage image. Cartilage deterioration near the site of infusion was visible on MRI. MRI measurements indicated a reduction in cartilage thickness. Histology revealed a loss of staining of cartilage matrix proteoglycan, synovial hypertrophy, and perichondral bone resorption. Morphometric analysis of cartilage histology indicated a reduction in both cellularity (chondrocytes/mμ^2 area) and cell to matrix area ratio. These observations suggest that a loss of proteoglycan, an early event in cartilage degeneration, can be detected by MRI.** [P.S.E.B.M. 1993, Vol 203]

Arthritis is characterized by inflammation and erosion of the articular cartilage. Both rheumatoid arthritis (RA) and osteoarthritis (OA) are multifactorial diseases leading to joint degeneration. The loss of the cartilage proteoglycan is an early event in the joint destruction seen in RA and OA. Chondrocytes synthesize proteoglycans in the extracellular matrix of cartilage. Binding of water by proteoglycans gives cartilage elastic recoil. Therefore, loss of proteoglycan leads to the inability of cartilage to distribute load. The organization of water in the cartilage matrix also determines the magnetic resonance properties of cartilage.

Synovial membranes secrete mediators that stimulate cartilage chondrocytes to degrade their surrounding proteoglycan matrix (1). The cartilage catabolic factor secreted by the synovium has been identified as the α form of interleukin 1 (IL-1) (2). IL-1 increases production of prostaglandins and metalloenzymes by chondrocytes and synoviocytes (3, 4). Induction of cartilage degradation and bone resorption by IL-1 may initiate the progressive degenerative changes in OA (5). Inflammation and cartilage proteoglycan loss can be induced in rabbits by intra-articular injection of IL-1 (6–12). The clearance rate of IL-1 injected into the synovial cavity was 23 to 29 min (8). Infusion of IL-1 (13) into rabbit knees produced an arthritis associated with a loss of proteoglycan staining of the cartilage and proliferation of synovial tissue. We used the IL-1 intra-articular infusion model to compare morphometric analysis of magnetic resonance (MR) images and cartilage histology (14).

To obtain an MR image, the subject must be placed in an electromagnetic field. Radio frequency pulses are

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focused by a surface coil located near area of interest (e.g., knee), producing resonance of proton nuclei. Magnetic resonance imaging (MRI) records excitation and relaxation of protons in tissues which depend on the physical properties and molecular environment. The protons in water in the cartilage extracellular matrix are associated with negatively charged sulfated glycosaminoglycan chains of proteoglycan. The organization of water in rabbit articular cartilage produces a rapid T2 relaxation time on MRI (15). Its ability to directly image articular cartilage (16) makes MRI a powerful research and clinical tool. MRI may be used to accurately diagnose knee pathology (17). In surgically induced OA and inflammatory models of arthritis, MRI detects lesions in noncalcified tissue and identifies developing bone changes at earlier stages than radiography (18). Magnetic resonance also allows the quantitation of joint degeneration resulting in altered T1 and T2 relaxation times measured from signal intensity changes (19). MR has been used to measure synovial inflammation in a rabbit model of arthritis (15). MRI detected reduction in cartilage thickness and signal intensity in rabbit knees injected with papain to induce proteoglycan degradation (20). MRI is a noninvasive diagnostic tool capable of quantitating joint changes during the longitudinal assessment of cartilage morphology in OA research.

In the following study, MRI was used to reconstruct cartilage morphology to evaluate joint pathology. Subsequent to infusion of rabbit knees with IL-1, morphometric measurements of MR images of the rabbit knees were compared with morphometric measurements of histologic sections from the rabbit knees (14). Reduction of cartilage thickness on MRI was associated with loss of proteoglycan matrix in histology.

Materials and Methods

Materials. Eight male New Zealand White rabbits, HAR:PF/CF(NZW)BRSPF (2.5–3.0 kg), were purchased from H.A.R.E., Inc., Hewitt, NJ. Recombinant human interleukin-1 α (rhIL-1α) batch RNB81885/13 was supplied by Biogen (Geneva, Switzerland). Alzet miniosmotic pumps, model 2ML2 with mean (±SD) fill volume of 2139 ± 49 μl and mean pumping rate of 4.41 ± 0.16 μl/hr, were obtained from ALZA Corp., Palo Alto, CA. The infusate was delivered at a rate of 4.4 μl/hr in sterile saline containing 100 units/ml of penicillin and 100 μg/ml of streptomycin. Ketaset (100 mg ketamine HCL/ml injectable) was obtained from Aveco Co., Inc., Fort Dodge, IA. Rompun (20 mg xylazine/ml injectable) was purchased from Mobay Corp., Animal Health Division, Shawnee, KS.

Infusion. Each experimental knee was infused with 100 ng recombinant human IL-1α (Biogen) (2) per day for 5 or 12 days. The untreated right knee served as an internal control. A miniosmotic pump filled with 1 μg IL-1/ml was surgically implanted under the skin of the lower back. A polyethylene tube, PE-60 (o.d. = 1.22 mm, i.d. = 0.76 mm), was attached to the pump outlet and routed under the skin to the left knee. From the lateral side of the left knee, the tube was inserted directly under the fibular collateral ligament into the middle of the joint space. In preliminary experiments not reported here, infusion of the saline vehicle containing antibiotics did not produce inflammation, cellular infiltration, or cartilage degradation (21).

Magnetic Resonance Imaging. *Image acquisition.* Rabbits were anesthetized by intramuscular injection of 1 ml/kg body wt of solution of 10 mg xylazine + 50 mg ketamine/ml. Rabbits were scanned under anesthesia. Both knee joints were scanned in a 1.5 Tesla (General Electric, Milwaukee, WI) clinical magnet (19) using a 3-in surface coil. Rabbits were in the left decubitus position with the surface coil between the knees to visualize the articular cartilage in sagittal sections. For each rabbit, a T1 weighted scan, to localize the joints in the axial plane, followed by multiecho and gradient-refocused echo imaging sequences were obtained in the sagittal plane. For quantitation, the gradient refocused echo sequence was chosen because of thin slicing at 0.7 mm and resolution (300 microns). The gradient refocused echo sequence was obtained at 8 cm field of view, and using relaxation time/echo time (TR/TE) of 60/15 and flip angle of 15°, 256 × 256 matrix, and two averaging. For each knee joint, 28 two-dimensional slices were obtained in the lateromedial plane. For each rabbit, the total scan time was 45 min.

Image analysis. The two-dimensional sagittal images were transferred to a three-dimensional computer workstation (Dynamic Digital Displays, St. Davids, PA). The articular cartilage and the contour of the infusion tube were outlined in sequential 0.7-mm slices. Out of a total of 28 slices collected through each rabbit knee, the cartilage outline was visible in eight to 11 images of 0.7-mm sections. The software reconstructed structures in three dimensions. Cartilage thickness was measured manually by placing a cursor at the bone/cartilage interface and then generating a straight line to connect the cartilage surface. The cartilage thickness reported for the lateral and medial condyles is the mean cartilage thickness measured in three consecutive 0.7-mm sections in each condyle.

Histology. At necropsy, knees were placed in 10% neutral buffered formalin. Joint tissues were demineralized using a 20% solution of formic acid overnight. Tissues were then collected from the weightbearing medial condyle of the femur and washed, and the acid was neutralized in 5% ammonium hydroxide. The tissues were again washed in running tap water for several hours and then processed for both paraffin and glycol methcrylate techniques (14). Tissues were then sectioned at 2–4 mμ and routinely stained with a safranin

O-fast green stain with a hematoxylin counterstain. The medial condyle processed in plastic was measured at three sites consisting of one middle and two side measurements avoiding the reactive margins of the cartilage. Full thickness, to the "tidemark," measurements were made. The measured areas represented approximately 15–20% of the total cross-sectional area. Measurements were performed using the American Innovision (San Diego, CA) morphometric system calibrated in millimeters using a stage micrometer and recorded in millimicrons. The following measurements and calculations were made: (i) total area measured ($m\mu^2$) (three observations/slide); (ii) total matrix ($m\mu^2$) of the area defined in (i) above; (iii) total chondrocyte area ($m\mu^2$) of the measured area; (iv) number of chondrocytes counted in the defined area; (v) chondrocytes/unit area; (vi) mean cell size ($m\mu^2$); and (vii) matrix to lacunae-chondrocyte area ratio. The normal range for the matrix to cell area ratio is from 10 to 20; a deviation from normal reflects hypocellular (>30) or hypercellular (<10) cartilage (Table I). In a previous study, morphometric analysis of articular cartilage from age- and sex-matched New Zealand White rabbits showed a variation of ±4% in the matrix to cell area ratio in knees from 36 control rabbits (14). The experimental design was such that one knee was untreated and one was infused with IL-1. Thus the contralateral knee served as an internal control and the comparison was made between the left and the right knees rather than from animal to animal. In this study, contralateral knees were not treated. Preliminary studies have shown that infusion of saline vehicle does not produce cartilage matrix degradation (21).

Results

MRI of IL-1α-treated knees showed inflammation marked by increased joint fluid compared with contralateral control knees (Fig. 1). The three-dimensional reconstructions of cartilage and the infusion tube were rotated in different planes to observe the entire length of the tube relative to the cartilage surfaces (Fig. 2). Different orientations were examined to precisely locate the tip of the tube where the IL-1 was delivered. Cartilage damage was observed at the site of IL-1 delivery. Morphometric measurements in histologic sections indicated a loss of proteoglycan matrix corresponding to MRI-measured reductions of cartilage thickness in IL-1-infused knees (Table I).

Treatment with IL-1α produced the following histologic changes in 5 days: reduced cellularity, increased chondrocyte cell size, a decrease in the matrix to cell area ratio (Table I), and a decrease in safranin O matrix staining (Fig. 3B). A loss of safranin O staining indicates proteoglycan depletion. Bone responses included a prominent increase of cellularity along the osseous subchondral plate. Multifocal areas of the osteoclastic-tidemark invasion were observed. In addition to the direct effect on chondrocytes, the chronic effect of IL-1α bone reabsorption is a consistent change noted in this study. At sites where bone resorption was occurring, the structural effects on cartilage were more pronounced (Fig. 4A).

After 12 days of IL-1α infusion, complete loss of safranin O staining was accompanied by a reduced matrix to cell area ratio (Table I). Reduction in the matrix to cell area ratio was a consistent finding observed in all eight of the knees infused with IL-1 for 5 or 12 days. In addition, a swelling of the chondrocytes ($m\mu^2$/cell) was noted after 12 days of IL-1α infusion (Fig. 3C). The subchondral bone response on Day 12 was more pronounced than that observed on Day 5 of IL-1α infusion (Fig. 4, B and C). Pannus formation was evident in one animal (Fig. 4C). Fibrosis of the subsynovial spaces was prominent, with the cellular infiltrate consisting of macrophages and lymphocytes. Lymphocyte perivascular accumulations were present in three of four animals.

Synovial membranes showed a mild synovial reaction after a 5-day infusion of IL-1α consisting of synovial cell activation, fibrosis, and a mixed cellular response of neutrophils and macrophages (Fig. 5A). After a 12-day infusion of IL-1α, the synovial membrane pathology progressed to include a pronounced perivascular lymphocyte infiltrate (Fig. 5B).

Table I. Cartilage Measurements in Control and IL-1-Infused Knees on Day 5 and Day 12

Treatment	*n*	Histology matrix:cell area ratio[a]	*P*	MRI cartilage thickness[b] (mm)
Contralateral control	4	16.7 ± 2.3		0.37 ± 0.05
IL-1 infused Day 5	4	8.8 ± 0.9	0.0128	0.23 ± 0.05
Contralateral control	4	14.7 ± 1.1		0.37 ± 0.04
IL-1 infused Day 12	4	7.1 ± 0.2	0.0003	0.27 ± 0.02

[a] The mean matrix to cell ratio was calculated from total chondrocyte area ($m\mu^2$)/total area measured ($m\mu^2$) (three observations/slide) in histologic sections.

[b] Mean thickness was calculated using MRI measurements from three sequential 0.7-mm slices from the medial femoral condyle. Data are expressed as mean ± SE.

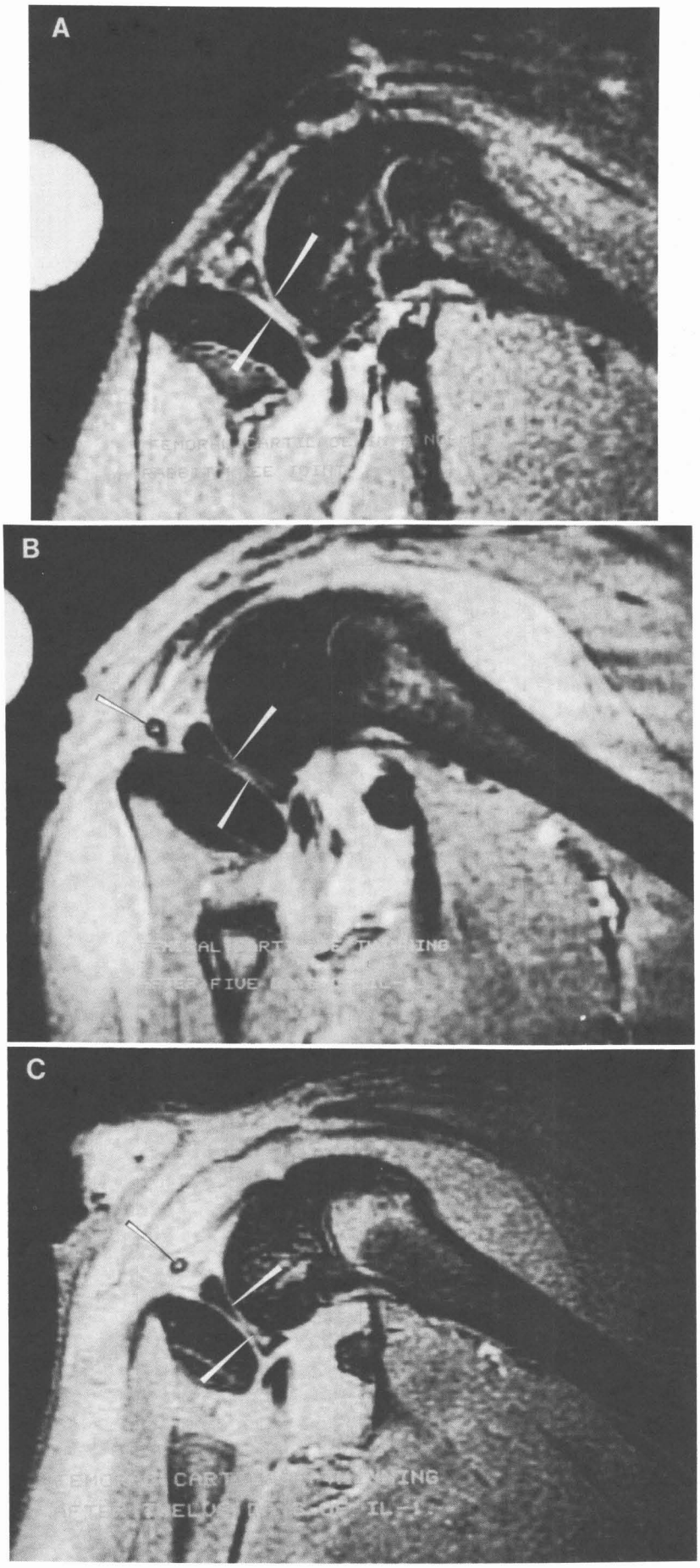

Figure 1. *A*, Two-dimensional MRI of control femoral and tibial cartilage (arrows) with normal thickness and intensity. *B*, Two-dimensional MRI of femoral and tibial cartilage (arrows) after 5 days of infusion with IL-1 shows thinning and hypointensity compared with normal control; note location of infusion tube (arrow) in this 0.7-mm section. *C*, Two-dimensional MRI of femoral and tibial cartilage (arrows) after 12 days of IL-1 infusion shows cartilage degeneration and inflammation; note location of infusion tube (outlined arrow) in this 0.7-mm section.

Figure 2. Three-dimensional MRI of rabbit knee infused with IL-1 red-reconstruction of infusion tube delivering IL-1 into the joint space, purple-femoral cartilage, blue-tibial cartilage, and aqua-femoral and tibial bones. Note the destruction of cartilage near the infusion site.

Little change in histologic cartilage matrix to cell area ratio and cartilage thickness on MRI occurred between Day 5 and Day 12 of IL-1α infusion (Table I). This apparent plateau in cartilage degeneration may reflect a defensive response to the IL-1 such as production of antibodies to rhIL-1α (13), enzyme inhibitors, or IL-1 receptor antagonists. However, there is an increased swelling of chondrocytes between Day 5 and Day 12 of IL-1α infusion. The area/cell was 9.2 ± 0.5 mμ^2/chondrocyte in the contralateral control compared with 14.7 ± 1.1 mμ^2/chondrocyte after chronic exposure to IL-1 for 12 days. The animal to animal variability in cellularity (chondrocytes/mμ^2) and area/cell (mμ^2/chondrocyte) with only four rabbits per group necessitates further study to verify and statistically analyze these changes.

Discussion

A rabbit model of joint pathology provided a tool for comparison of changes in MRI and histology of articular cartilage. In a recent study, degenerative changes in articular cartilage detected by MRI of cadaveric lumbar facet joints were correlated with histopathology (21). In this study, knees of anesthetized rabbits were imaged by MRI using parameters routinely applied in clinical practice, followed by microscopic histologic examination.

IL-1 infusion and papain injection (20) caused a reduction in cartilage thickness on MRI and loss of proteoglycan staining in histologic sections. Cartilage thickness is not measured in fixed histologic sections. The change in cartilage thickness *in vivo* on MRI of articulating knees with ligaments and muscles intact may be due to an increase in compressibility caused by loss of elastic recoil after proteoglycan depletion.

Changes in proteoglycan fixed charge density are correlated with changes in the relaxation time and/or signal intensity of water in MR images taken *in vitro* (22). Use of a volume coil to surround the knee with a uniform magnetic field is required to measure signal intensity accurately. In these preliminary studies, a surface coil used clinically for the temporomandibular joint was adapted to image rabbit knees. The surface coil did not create uniform conditions to compare readings at various locations within the imaged knees. Future application of volume coils to surround the rabbit knee or custom surface coils for imaging rabbit knees will allow measurement of MR changes due to changes in the physical properties and biochemical composition of cartilage (23).

Synovial cell proliferation, loss of cartilage matrix staining, with cartilage and bone erosion after 14 days of infusion of 200 ng of rhIL-1α/day into rabbit knees has been described by Feige *et al.* (13). In our studies reported here, noninvasive MRI detected a reduction in cartilage thickness after 5 and 12 days of infusion with 100 ng of rhIL-1α/day. These MRI changes corresponded to synovial proliferation, loss of proteoglycan staining, and a reduced cell to matrix area ratio in histologic sections. We noted a swelling of chondrocytes after 12 days of IL-1 infusion. The area/cell was 9.2 ± 0.5 mμ^2/chondrocyte in the control compared with 14.7 ± 1.1 mμ^2/chondrocyte after 12 days of chronic exposure to IL-1. The slower rate of progression of cartilage destruction observed by MRI and/or histologic examination between Days 5 and 12 may represent a plateau or be due to the instability of rhIL-1α ($t_{1/2}$ = 7 days) (13), production of antibodies to rhIL-1α (13), or induction of an endogenous rabbit IL-1 receptor antagonist. The rabbits in this study were not tested for antibodies to rhIL-1α or IL-1 receptor antagonists. The experiment was designed to compare IL-1-infused knees with an internal contralateral control. There may be systemic effects of IL-1 inducing changes in the contralateral knees that are masked by calculations based on the comparison of treated/control knee of each rabbit. No pathologic changes in the contralateral knees were noted. Histologic data on control knees from previous studies, this study, and future studies will be compiled and analyzed for any changes due to IL-1 infusion of the contralateral knee.

Infusion of the rabbit knee using nonmagnetic surgical materials permits noninvasive verification of the site of infusion and evaluation of joint pathology using MRI. MRI and histology can be employed to evaluate joint degeneration induced with infusions of graduated doses of cytokines and enzymes implicated

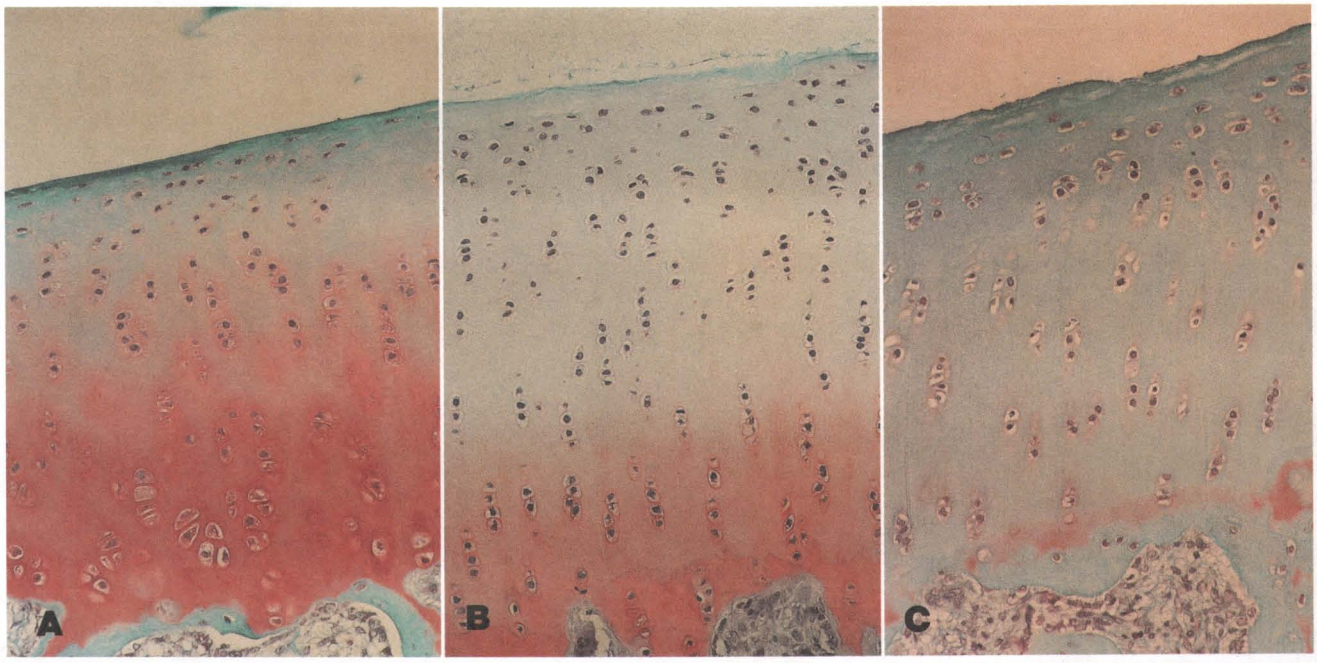

Figure 3. *A*, Right medial-femoral condyle from noninfused control rabbit, safranin O, ×250. *B*, Left medial-femoral condyle after 5 days of infusion with IL-1; a loss of safranin O matrix staining and cell swelling is evident, ×250. *C*, Left medial-femoral condyle after 12 days of IL-1 infusion; note loss of safranin O staining and cell swelling, ×250.

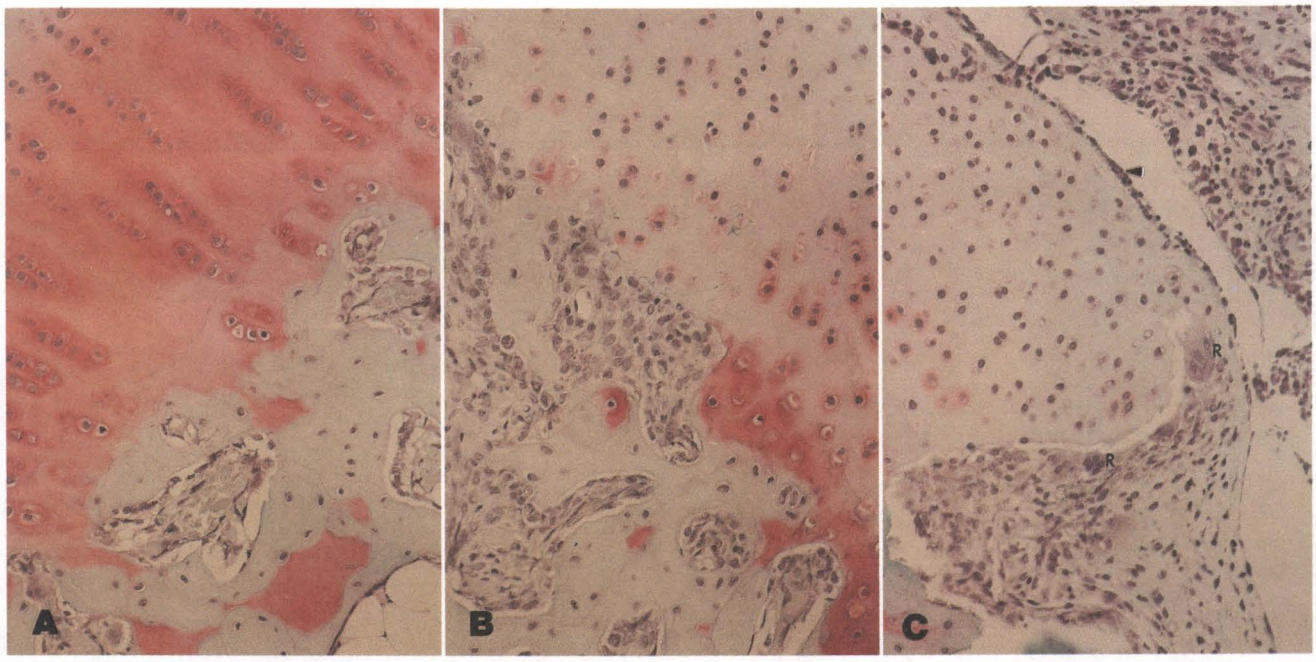

Figure 4. *A*, Subchondral region from right medial-femorotibial articulation from control rabbit; note the intact subchondral osseus plate, safranin O, ×250. *B*, Subchondral invasion by osteoclast-macrophage type cells in rabbit infused with IL-1 for 12 days; note the complete loss of safranin O stain in the overlying cartilage, ×250. *C*, Left medial-femoral condyle from rabbit infused with IL-1 for 12 days; note marginal osteoclastic resorption (R), pannus formation (arrow), and loss of safranin O staining, ×250.

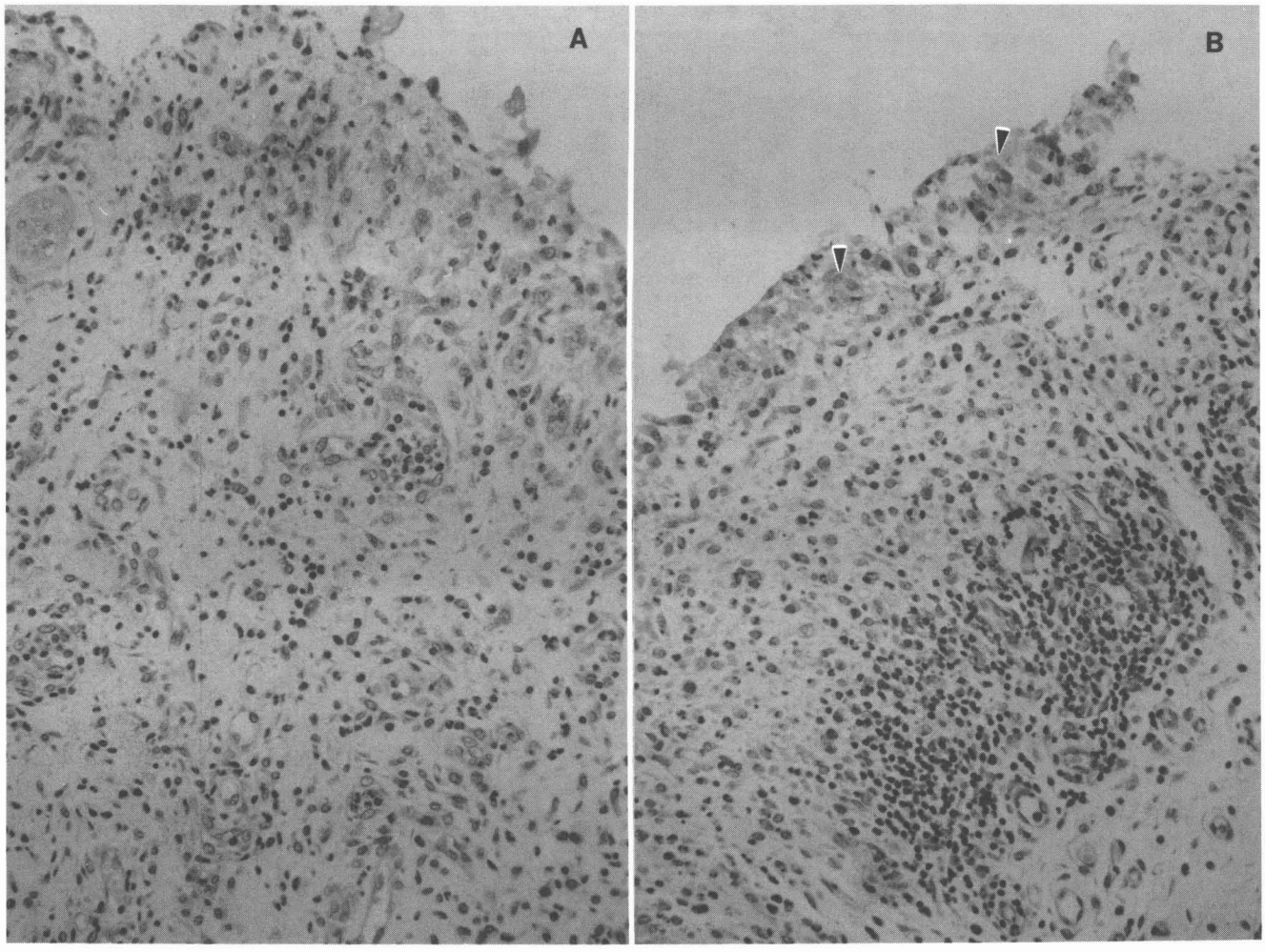

Figure 5. *A*, Synovial membrane from rabbit infused with IL-1 for 5 days; fibrosis with mixed type cellular infiltrate, ×250. *B*, Synovial membrane from rabbit infused with IL-1 for 12 days; note the increase in perivascular lymphocyte accumulation and macrophage type synovial cells (arrows), ×250.

in the pathogenesis of OA and RA. Development of imaging techniques for articular cartilage and detection of cartilage metabolites (24, 25) will enable evaluation of therapeutic agents for effects inhibition of degeneration and regeneration of cartilage.

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