Major Role for Interleukin 1 but Not for Tumor Necrosis Factor in Early Cartilage Damage in Immune Complex Arthritis in Mice

PETER L.E.M. VAN LENT, FONS A.J. VAN DE LOO, ASTRID E.M. HOLTHUYSEN, LIDUINE A.M. VAN DEN BERSSELAAR, HARRY VERMEER, and WIM B. VAN DEN BERG

ABSTRACT. Objective. To determine the regulating role of interleukin 1α and β (IL-1α,β) and tumor necrosis factor α (TNF-α) on inhibition of proteoglycan synthesis and proteoglycan degradation in early immune complex arthritis (ICA) in the mouse.

Methods. In the early phases of arthritis, IL-1 and TNF were measured using cytokine specific bioassays, the NOB I EL-4 and L929 assay, respectively. The impact of IL-1 in proteoglycan synthesis was studied by neutralizing the formed IL-1 during early arthritis either by giving anti-IL-1 specific antibodies intravenously or IL-1 receptor antagonist (IL-1ra) intraperitoneally by osmotic pumps. TNF-α was neutralized by giving monoclonal antibodies directed against murine TNF-α. Synthesis of proteoglycans was measured ex vivo by uptake of 35S-sulfate by patellae derived from inflamed and control, noninflamed knee joints. In vivo formation of 35S-sulfate labeled proteoglycans was studied by autoradiography. Degradation of proteoglycans was measured by labeling patellae in vivo with 35S-sulfate before arthritis induction.

Results. High levels of IL-1 are formed during the first phase of immune complex arthritis (ICA). Neutralization of either IL-1α or β with specific polyclonal antibodies resulted only in partial blocking, whereas a combination fully blocked inhibition of proteoglycan synthesis. Full blocking was also found after systemic treatment with high amounts of IL-1 receptor antagonist (1.2 mg/day during 3 days). Influx of cells was also significantly reduced both in the anti-IL-1 as well as in the IL-1ra treated groups. Whether infiltrating cells are involved in inhibition of proteoglycan synthesis was further investigated in neutropenic mice. Significantly higher levels of IL-1 were found in arthritic joints of neutropenic compared with control mice. Suppression of proteoglycan synthesis was similar in arthritic knee joints of normal and neutropenic mice. However, only minor proteoglycan degradation was found in the latter. TNF-α was undetectable in the bioassay in early ICA and neutralization of TNF-α did not change either swelling, cell influx, proteoglycan synthesis or proteoglycan degradation.

Conclusion. Local production of IL-1 in ICA in knee joints seems directly responsible for inhibition of proteoglycan synthesis. A direct role of IL-1 in proteoglycan loss is unlikely, but indirectly IL-1 may be involved in proteoglycan breakdown by attracting inflammatory leukocytes and activating synovial cells. TNF-α seemed to have no effect on either cell influx, proteoglycan synthesis or proteoglycan degradation in this model. (J Rheumatol 1995;22:2250-8)

Key Indexing Terms:
EXPERIMENTAL ARTHRITIS
IL-1 RECEPTOR ANTAGONIST
CARTILAGE DAMAGE

One of the main features of joint inflammation is the alteration of homeostasis of chondrocyte function, which may lead to cartilage destruction. In normal cartilage a balance exists between anabolism and catabolism of proteoglycans, one of the main constituents of cartilage determining its compressive resilience. In experimental arthritides the disturbance of this balance is shown by severe inhibition of proteoglycan synthesis and enhanced degradation of proteoglycan1-3. Tumor necrosis factor α (TNF-α) and interleukin 1 (IL-1) are believed to play a crucial role in these effects. Enhanced cytokine levels are found during the early phases of experimental arthritides4-6. Moreover, it is known from in vitro experiments that both IL-1 and TNF-α added to living explants of cartilage induce breakdown and marked inhibition of proteoglycan synthesis, with IL-1 being most potent7-11. Moreover, relatively high doses of IL-1 are needed to cause breakdown, whereas low doses inhibit pro
teglycan synthesis. In vivo IL-1 was shown to cause joint inflammation and marked proteoglycan depletion after direct injection in the knee joints of rabbits, rats, and mice. Experiments blocking the influx of neutrophils revealed that cell influx had no direct effect on cartilage destruction. In experimental arthritis the role of IL-1 and TNF in cartilage destruction is less clear. Although IL-1 could be detected in the synovial tissue, the concentrations are probably too low to cause proteoglycan breakdown directly, whereas it may be sufficient to initiate inhibition of proteoglycan synthesis. In antigen-induced arthritis it was found that blocking of IL-1 with antibodies or IL-1 receptor antagonist (IL-1ra) prevented inhibition of proteoglycan synthesis, independent of joint inflammation. However, breakdown of proteoglycan was unaffected, suggesting the strong influence of other mediators. In contrast, IL-1 blocking in collagen arthritis was effective on both synthesis and breakdown but the latter result is probably related to the marked suppression of joint inflammation in this model. We attempted to further elucidate the role of IL-1 and TNF in the breakdown and synthesis of proteoglycan, and the influence of infiltrating leukocytes. We used the recently developed model of cationic immune complex arthritis (ICA) in mice, a model characterized by marked IL-1 production, pronounced IL-1 dependent cell influx, and early cartilage damage. Moreover, proteoglycan degradation is clearly dependent on infiltrating leukocytes, although no role could be attributed to PMN elastase. We studied the cartilage destruction and cytokine profiles in both normal and neutropenic mice and analyzed the effect of neutralization of IL-1ra, β and TNF-α using subtype specific antibodies and IL-1ra.

MATERIALS AND METHODS

Animals. Male C57 b/l mice were obtained from our breeding facilities (Overasselt, The Netherlands). They were fed a standard diet and tapwater ad libitum. Their age was between 8 and 12 weeks at the time of study. Chemicals. Poly-L-lysine (PLL), lysosome, and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) were obtained from Sigma Chemical Company, St. Louis, MO, USA. N,N-dimethyl-1,3 propandiamine was obtained from BDH Chemicals Ltd., Poole, England. Lysozyme coupling to PLL. Lysozyme was coupled to PLL according to the method of Danon using EDC as an activator and PLL as a nucleophile as described. Free carboxyl groups of the protein are then coupled to amine groups of PLL. The molecular weight was raised whereas the isoelectric point remained high as was determined in a 5% polyacrylamide slab gel with 0.8% ampholines (pH gradient from 3.5 to 9.5). The molecular weight appeared to be 74 kDa as determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis. Induction of ICA. PLL-lysosome (3 µg) was injected into the right knee joint of mice previously given specific antisera (0.2 ml) directed against lysosome intravenously (iv). The antisera, raised in rabbits, were decomplemented by heating at 56°C for 30 min. The left knee joint was injected with saline and served as a control. Polymorphonuclear (PMN) depletion. Mice were depleted of PMN by either total body irradiation or cyclophosphamide treatment. A single dose of 7.5 Gy was applied at a dose rate of 2 Gy/min, with a 13 MV electron beam, from a linear accelerator (CGS Saturne, Buc, France). Treatment was started 4 days before injection of the antisera. Mice were anesthesied by intraperitoneal (ip) injection of sodium pentobarbital, 0.03 mg/kg body weight (Narcoret, Aphares B.V., Arnhem, The Netherlands) and placed between 2 perspex plates, the upper one 1 cm thick. Cyclophosphamide (Endoxan-Asta, Asta Medica AG, Frankfurt am Main, Germany) was given ip at Day 4 (150 mg/kg) and Day 1 (100 mg/kg body weight) before induction of arthritis. To prevent infection in both irradiated and cyclophosphamide treated groups, mice were kept under aseptic conditions in aseptistirle room and received acidified drinking water and sterilized food. The irradiated mice were given daily supplements of 1 ml of glucose solution ip until injection of the antibodies. At this time, most mice had lower skin temperature and bodyweight loss up to 10%

Antiserum treatment of mice. Anti-IL-1 antibodies. Mice were given 400 µl of a mixture: 200 µl of anti-IL-1α and β antisera (1 to 1 ratio) and 200 µl of antilysozyme, 16 h before arthritis induction as described. Culturing arthritic patellae of anti-IL-1 treated mice revealed that IL-1 could not be detected in the NOB assay (data not shown). IL-1ra. IL-1ra (100 mg/ml) kindly provided by Synergen, Boulder, CO, USA (Dr. Thompson), was brought into micro-osmotic minipumps (Alzet model 1007D; Alza Corporation, Palo Alto, CA, USA). These pumps release 0.5 µl/h during 7 days in a standard manner. The pumps were implanted 2 days before starting the experiment to minimize the effect of stress. As controls empty pumps were implanted. Anti-TNF antibodies. Neutralizing monoclonal antibodies (Mab) directed against TNF-α were injected iv. The 50,000 units of Mab VIIQ19 given were sufficient to fully block biologic activity of 4500 ng of TNF in the L929 bioassay. Cytokine production by synovial tissue. Synovial tissue was isolated in a standard manner by dissection of patellar tendon and patellar plate. The tissue specimen contained the patella, tendons and synovium. Six synovial specimens from knee joints were washed in 2 ml culture medium (RPMI 1640 medium; Dutch modification) for 1 h at room temperature. Washouts (undiluted or diluted 10,20,40 and 80-fold) were tested for IL-1 and TNF activity by sensitive bioassays. Also higher dilutions were tested (160-320-fold) but in most washouts, no IL-1/TNF signal was detected.

Bioassay for IL-1. IL-1 activity was measured in the one stage bioassay for IL-1 as described by Gearing, et al. The assay is performed as a coculture of the IL-1 specific subclone of the murine thymoma cell EL-4, designated NOB-1 cell, producing IL-2 and IL-4, with the lymphokine responder CTLL line. In brief EL-4 cells were washed twice and resuspended at 5 × 10⁶ cells per ml RPMI containing 5% fetal calf serum (FCS). The cells were distributed into 96 well microtiter plates at 2 × 10⁴ cells/well in 100 µl volumes. CTLL cells (4 × 10⁴) were added in 50 µl RPMI followed by appropriate dilutions of test sample to a final volume of 200 µl. After 20 h, 0.5 µCi [³H]-thymidine (Dupont, NEN producers, Boston, MA, USA; specific activity 20 Ci/mmol) was added to each well, and the contents harvested 3 h later, and the incorporated activity determined. The EL-4.6.1 line from which NOB-1 was derived does not incorporate thymidine since it is deficient in thymidine kinase and therefore only CTLL proliferation is measured by [³H]-thymidine incorporation. Maximal [³H]-thymidine incorporation in the bioassay in the presence of IL-1 was between 10-12000 cpm. CTLL alone served as control and incorporated only 100-1000 cpm, indicating that washouts contain only low IL-2 or IL-4 concentrations. Bioassay for TNF-α. TNF-α activity was measured as described. Briefly, 1 × 10⁵ L929 cells were brought into a flat bottom 96 well microtiter plate. A standard curve was made by adding serial 2-fold dilutions of recombinant mouse TNF-α (range 0.6 to 0.002 ng/ml). Arthritic patella washouts (dilution 0–3) were tested. After incubation of 20 h at 37°C and 5% CO₂, TNF-α mediated cytotoxic effects on L929 cells were evaluated. The above dilution was gently discarded and the remaining cells fixed by adding methanol (96%) during 1 min. After drying of the cells, crystal violet (Sigma Chemical Co., St. Louis, MO) was added and after 5 min, the excess of crystal violet washed away. The plate was then dried, 100 µl of acidic acid

van Lent, et al: Mechanisms in cartilage damage
added, and, after shaking of the plate, the extinction determined on an ELISA reader at 540 nm.

Measurement of chondrocyte proteoglycan synthesis. Quantitative. Mice were sacrificed by ether anesthesia. The patellae from arthritic joints and their contralateral nonarthritic knee joints either treated with anticytokine antibodies, or controls were dissected leaving the cartilage intact. After incubation for 3 h at 37°C in RPMI with 20 μCi Na35SO4/ml, tissue specimens were washed, fixed in 4% buffered formalin for 20 h and decalcified in 5% formic acid for 4 h. The patella could thus be punched out and the surrounding connective tissue removed. Punched patellae, consisting of cartilage and bone were digested in solute (Lumac LSC bv, Olen, Belgium) for 3 h at 60°C and subsequently dissolved in Lipofluor (Lumac LSC bv, Groningen, The Netherlands) for liquid scintillation counting. The washing, fixation, and decalcification procedures removed all free 35S-sulfate from the tissue. The amount of 35S-sulfate retained is a measure of 35S proteoglycans. More than 90% of the incorporated radioactivity was confined to the patellar cartilage layer. Comparison of radioactivity in the patella and that in quantitatively isolated glycosaminoglycans (GAG) [more than 95% of the incorporated label was liberated by overnight pepsin digestion (1 mg/ml)] revealed that for 35S-sulfate incorporation studies the whole patella can be used as a reliable measure for sulfated GAG synthesis.

Qualitative. Autoradiography. Whole knee joint sections of joint specimens (6 μM) were prepared 2 days after arthritis induction in immune mice. Three hours prior to sacrifice, 75 μCi 35S-sulfate was injected ip. Knee joint sections were coated on gelatine coated slides. These were dipped in K5 emulsion (Ilford Basildon, Essex, England) and exposed for 1–4 weeks. After this period the slides were developed and stained with hematoxylin and eosin.

Measurement of proteoglycan degradation. Patellae were prelabelled by ip injection of radiolaucate (50 μCi/mouse) 4 h before induction. More than 90% of the incorporated radioactivity was confined to the patellar cartilage layer if compared to whole patellae. At various days after arthritis induction, patellae were isolated, fixed in 4% buffered formalin for 20 h and decalcified in 4% formic acid. The patella was punched out and the labelled proteoglycan content was determined. Cartilage degradation was defined as enhanced loss of 35S from the inflamed patella compared to the normal loss in the contralateral, noninflamed patella.

RESULTS

Production of IL-1 and TNF in ICA. Biologically active IL-1 and TNF production during early arthritis was measured with bioassays using washouts of standardized patella specimens. Detectable IL-1 levels were found as early as 3 h after arthritis induction (data not shown). Markedly enhanced levels were found at 6 h, whereas at 24 h no IL-1 was detected (Figure 1). Production at 6 h varied between 10–30 pg/specimen. Biologically active TNF-α was measured using the L929 bioassay, which has a sensitivity of 1–2 pg/ml. TNF-α could not be detected in inflamed joints, 1,3,6, or 24 h after onset of arthritis (data not shown), indicating that the TNF-α production is below 0.3 pg/patella. No IL-1 nor TNF-α was detected in washouts of contralateral noninflamed joints.

Suppression of proteoglycan synthesis in ICA. ICA was characterized by early joint swelling and suppression of proteoglycan synthesis. Joint swelling, which was correlated to cell influx, reached maximal values already at Day 2 (Figure 2). Swelling varied from 1.5–1.8 at this time. At Day 7, no significant swelling remained. Marked suppression of proteoglycan synthesis was already seen at Day 1 (Figure 2).

Maximal suppression (55%) was seen at 48 h and declined thereafter. At Day 4, there was no more evidence of suppression, whereas at Day 7 and Day 10, surplus production of proteoglycan of respectively 68 and 44% was found.
In vivo effect of blocking IL-1 on proteoglycan synthesis. IL-1 production was neutralized in 2 ways. Firstly by iv injection of polyclonal antibodies directed against the α and β form iv shortly before arthritis induction; secondly, by giving IL-1 receptor antagonist (IL-1ra) systemically by implanting osmotic pumps ip.

Neutralizing the α form of IL-1 resulted in a significant decrease of swelling (46%). The potency of anti-IL-1α was comparable and neutralization resulted in a 44% decrease in swelling. A combination of anti-IL-1 α and β was most effective and reduced swelling by 90% (Table 1). Total knee joint sections showed that influx of cells was significantly lowered. The α and β form were equipotent in this respect. Both antibodies given together resulted in significantly reduced cell influx.

To confirm findings with neutralizing antibodies, IL-1 bioactivity was blocked with IL-1ra. Osmotic pumps were implanted in the peritoneal cavity and set to release constant amounts of IL-1ra for 7 days (1.2 mg IL-1ra/day). In the control group empty pumps were implanted. Two days after arthritis induction, swelling in the IL-1ra treated group was significantly decreased (83% inhibition). Influx of cells was also significantly decreased (Figure 3). Only in the synovial layer small numbers of inflammatory cells were present. Suppression of proteoglycan synthesis was fully blocked by the IL-1ra as measured in the patella assay (Table 2). Both anti-IL-1 and IL-1ra treated groups synthesis of proteoglycan in the contralateral nonarthritic knee joint was higher — although values did not reach statistical significance — compared to the contralateral joints of control mice. IL-1ra given to normal mice had no effect on proteoglycan synthesis (data not shown).

In additional studies, mice treated with anti-IL-1 antibodies, IL-1ra, or control phosphate buffered saline (PBS) were given 35S-sulfate ip at Day 2 after ICA induction. Three hours thereafter total knee joints were processed for autoradiography and proteoglycan synthesis studied. Figure 3C shows that patellar and femoral cartilage surfaces in control, arthritic knee joints show fewer black spots around the chondrocytes, indicating low proteoglycan synthesis (Figure 3C). Both anti-IL-1 antibodies and IL-1ra treated groups (Figure 3D) showed normal synthesis, comparable to non-artritic joints injected with PBS.

In vivo effect of blocking TNF on proteoglycan synthesis. Although no free biologically active TNF-α could be detected in early immune complex arthritis, this cytokine might function locally. To investigate the in vivo role of local TNF-α production in suppression of proteoglycan synthesis, an excess of neutralizing anti-TNF-α Mab (50,000 U) was given iv before arthritis induction. Swelling measured by 99mTc uptake was not changed (Table 3). Total knee joint sections stained by hematoxylin/eosin also showed that influx of cells in the synovium and joint cavity was not changed. Measuring proteoglycan synthesis revealed that neutralization of TNF-α could not block suppression (Table 3). Similar suppression was found in control (rat IgG treated) and anti-TNF treated groups.

Table 1. Inflammation and inhibition of proteoglycan synthesis in ICA. Effect of neutralization of α and β IL-1 with anti-IL-1 antibodies

<table>
<thead>
<tr>
<th></th>
<th>ICA</th>
<th>Anti-IL-1 α</th>
<th>Anti-IL-18</th>
<th>Anti-IL-1α+β</th>
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<tr>
<td>90mTc uptake</td>
<td>L/R</td>
<td>1.72 ± 0.04</td>
<td>1.39 ± 0.16</td>
<td>1.40 ± 0.02</td>
</tr>
<tr>
<td>Proteoglycan</td>
<td>L</td>
<td>915 ± 259</td>
<td>1431 ± 238</td>
<td>1432 ± 264</td>
</tr>
<tr>
<td>synthesis (cpm)</td>
<td>R</td>
<td>1735 ± 236</td>
<td>2012 ± 64</td>
<td>1979 ± 358</td>
</tr>
<tr>
<td>Inhibition (%)</td>
<td></td>
<td>29*</td>
<td>28*</td>
<td>-29*</td>
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Swelling was measured using the 99mTc uptake method. L/R — ratios indicating amount of swelling; > 1.1 indicates significant swelling. Proteoglycan synthesis was measured by uptake of 35S by patellae from the arthritic knee joint compared to the contralateral nonarthritic (R) knee joint. Values represent the mean ± SD of 3 experiments. In each experiment at least 6 mice were tested. Data were statistically evaluated by Mann-Whitney U test. * p < 0.05.

van Lent, et al: Mechanisms in cartilage damage
Fig. 3. (A) and (B). Total anterograde tract injection at days 2 and 4 (C). Immunocytochemistry for PGP (control) or IL-1β. (D) and (E) Immunocytochemistry for IL-1β. (F) and (G) Immunocytochemistry for IL-1β and IL-1Ra. Immunocytochemistry for IL-1β and IL-1Ra.
Cell influx and inhibition of proteoglycan synthesis. We investigated whether influx of cells in arthritic knee joints impairs proteoglycan synthesis. To investigate this, mice were made neutropenic either by total body irradiation or cyclophosphamide treatment. Both methods induced more than 98% depletion of peripheral PMN. IL-1 levels in arthritic neutropenic joints, measured at 6 h, were significantly higher compared to control arthritic knee joints. Maximal levels were found at 6 h and remained high at 24 h (Figure 4). Production of IL-1 at 6 h varied between 70–120 pg and at 24 h between 10–30 pg/specimen. At 48 h only minimal amounts of IL-1 were detected in both groups. Again no biologically active TNF-α was detected in neutropenic arthritic joints. No IL-1 nor TNF-α was detected in washouts of contralateral nonarthritic neutropenic joints.

Total knee joint sections showed that only minimal amounts of PMN were observed in arthritic knee joints. Influx of monocytes was also minimal. Total body irradiation had no significant (18% inhibition) side effects on proteoglycan synthesis as measured in the contralateral nonarthritic knee joint (Table 4). Cyclophosphamide treatment raised proteoglycan synthesis by 33%. Arthritis induction in control and neutropenic arthritic knee joints showed comparable suppression of proteoglycan synthesis (Table 4). This suggests that local production of mediators by resident cells is sufficient for proteoglycan suppression.

**Table 4. Inhibition of proteoglycan synthesis in neutropenic arthritis mice**

<table>
<thead>
<tr>
<th>Animals</th>
<th>35S-PG Synthesis</th>
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<tbody>
<tr>
<td></td>
<td>Left</td>
</tr>
<tr>
<td>Control</td>
<td>1236 ± 290</td>
</tr>
<tr>
<td>neutropenica</td>
<td>847 ± 175</td>
</tr>
<tr>
<td>Control</td>
<td>814 ± 262</td>
</tr>
<tr>
<td>neutropenicb</td>
<td>1188 ± 182</td>
</tr>
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</table>

Mice were made neutropenic by either total body irradiation (750 rad) or cyclophosphamide treatment. Patellae were dissected 2 days after induction of ICA. Proteoglycan synthesis was measured by uptake of 35S by patellae derived from the arthritic (L) knee joint and compared to contralateral nonarthritic (R) knee joint. Data represent the mean ± SD of 2 experiments. In each experiment at least 6 mice were tested. Data were statistically evaluated by Mann-Whitney U test.

Direct role of IL-1 in proteoglycan degradation. We studied the degradation of cartilage proteoglycan by prelabeling of the cartilage proteoglycans with 35S-sulfate and analyzing the loss of 35S-proteoglycan after arthritis induction. In irradiated and cyclophosphamide treated mice less 35S uptake by the patellae was observed before induction of ICA, compared to the control group. Proteoglycan loss among groups was compared. Loss of proteoglycan from patella in arthritic joints was compared to that from patella in contralateral nonarthritic joints. At Day 2, a 60% loss of proteoglycan was found in normal mice with ICA. Despite high IL-1 levels.
in neutropenic mice (Figure 1), we observed only minor proteoglycan degradation in neutropenic mice with ICA (Table 5). This suggests that the direct effect of IL-1 on proteoglycan breakdown is marginal and the main effect of IL-1 is through indirect regulation of cell influx and amplification of activation of synovial cells.

**DISCUSSION**

Our results suggest that IL-1 but not TNF-α is the main cytokine involved in cartilage damage during experimental CIA. IL-1 directly mediates suppression of proteoglycan synthesis. Its direct effect on loss of proteoglycan seems only limited but as the most important regulator of cell influx IL-1 mediates cartilage degradation indirectly.

Neutralization of IL-1 in ICA leads to full blockade of inhibition of proteoglycan synthesis but also to significantly lowered influx of inflammatory cells. The same observations were made in the collagen type II induced arthritis and cyclophosphamide (Day −4, 100 mg/kg; Day −1, 80 mg/kg) treatment. Patellae, prelabeled with 35S (50 μCi/mouse) in vivo before arthritis induction, were dissected 48 h after ICA induction. The 35S content of the arthritic patella is expressed as % of 35S content of the contralateral noninflamed patella. Values represent the mean ± SD of 2 experiments, each consisting of at least 7 animals. Data were statistically evaluated by Mann-Whitney U test.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control (%)</th>
<th>Neutropenic (%)</th>
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<tr>
<td>Total body irradiation (750 rad)</td>
<td>56 ± 7</td>
<td>10 ± 6*</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>60 ± 7</td>
<td>11 ± 14*</td>
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Effect of PMN depletion on proteoglycan degradation. Mice were made neutropenic either by total body irradiation (750 rad) or cyclophosphamide (Day −4, 100 mg/kg; Day −1, 80 mg/kg) treatment. Patellae, prelabeled with 35S (50 μCi/mouse) in vivo before arthritis induction, were dissected 48 h after ICA induction. The 35S content of the arthritic patella is expressed as % of 35S content of the contralateral noninflamed patella. Values represent the mean ± SD of 2 experiments, each consisting of at least 7 animals. Data were statistically evaluated by Mann-Whitney U test.

*p < 0.05.

In contrast to anti-IL-1 antibodies, high amounts of IL-1ra are needed for optimal neutralization of the IL-1 effects. To block IL-1 effects on murine cartilage a 1000-fold excess of IL-1ra was necessary. Like IL-1, 17 kDa IL-1ra has a high clearance rate of 30 min. We found that the effects of an intraarticular injection of 1 ng could be blocked by the IL-1ra levels released by the osmotic pumps. Since the amount of IL-1 formed during ICA is much less than 1 ng, these IL-1ra blood levels were sufficient for neutralization. A similar level is not easily achieved with repeated injections. Because of the high removal rate of IL-1ra, multiple injections are necessary to reach sustained, high concentrations. Multiple injections may, however, alter the course of arthritis by stress induction. Of interest, proteoglycan synthesis of contralateral nonarthritic joints was higher in IL-1ra treated mice compared to control mice. This suggests that IL-1ra may elevate normal proteoglycan synthesis. However, IL-1ra treatment of normal animals, in which no arthritis was induced, had no effect on proteoglycan synthesis. This indicates that systemic effects are induced by a surplus of IL-1, generated in the arthritic knee joint and that this is effectively blocked by IL-1ra.

In contrast to IL-1, TNF-α could not be detected in early arthritis. No detectable biologically active TNF-α levels could be found in control or neutropenic arthritic knee joints. This indicates that TNF-α production is below 0.3 pg/patella. High amounts of neutralizing anti-TNF-α antibodies given before arthritis had no effect on either cell influx or suppression of proteoglycan synthesis. Other species of anti-TNF-α Mab showed similar results (data not shown). In vitro studies have shown that TNF-α may cause significant proteoglycan suppression. TNF-α was however less potent than IL-1, and large amounts of TNF-α (100 ng/ml) were necessary to induce suppression. In our model, TNF-α production is too low to induce suppression. In other arthritis models like collagen type II both TNF-α and IL-1 seem to be important in cell influx and blockade of IL-1 increased influx of cells and normalized suppression of proteoglycan synthesis, whereas blockade of TNF-α had only moderate effects. In bacterial induced joint inflammation, TNF-α seems to be the more dominant cytokine involved in cell influx. Furthermore cytokine production by immune complexes and its effect on tissue damage might be tissue dependent. Im-
mune complex (IC) mediated inflammation in the skin seemed predominantly regulated by IL-1 whereas in the lung both TNF and IL-1 were important\textsuperscript{11}.

Apart from local IL-1 production by resident cells, infiltrating inflammatory cells may also significantly contribute to the cytokine load since these cells contain small amounts of IL-1 and TNF. Local activation of these infiltrated cells, e.g., with cationic IC\textsuperscript{12} may release these cytokines. In our study we found however that in the absence of PMN even higher IL-1 levels were expressed for longer periods. This indicates that the PMN, which is the predominant cell during early arthritis, affects biologically active IL-1 levels by clearing the triggering IC responsible for IL-1 production\textsuperscript{16}. Furthermore, apart from releasing proinflammatory cytokines, the PMN also produce cytokine inhibitors such as IL-1ra\textsuperscript{33} or enhance expression and shedding of IL-1 receptor type II. IL-1 receptor type II may act as a decoy to catch IL-1, thus preventing biological activity\textsuperscript{34}. IL-18 has a higher affinity for type II than the α form\textsuperscript{27} and binds in larger amounts in the presence of PMN. This may also explain why 80% of the IL-1 signal we detected in the bioassay contained the α form.

So although PMN downregulate IL-1 the remaining IL-1 is still sufficient for maximum suppression of proteoglycan synthesis. Apart from reducing synthesis, IL-1 can resorb cartilage in 2 ways: first, within the cartilage, by activating chondrocytes that resorb cartilage from the inside and second from outside by directing the influx of infiltrating cells. The latter may release enzymes into the synovial fluid, which may degrade cartilage starting at the cartilage surface. Our study indicates that in arthritic joints in the absence of inflammatory cells, IL-1 levels were even higher. Nevertheless IL-1 levels seem insufficient to induce the loss of proteoglycan observed in control arthritic knee joints. Earlier studies revealed that higher doses of IL-1 are needed for cartilage resorption than for suppression of proteoglycan synthesis\textsuperscript{11}. This indicates that IL-1 levels secreted during arthritis may be too low to cause cartilage resorption directly, and influx of cells is necessary to cause degradation. The influx of cells during ICA is for the greater part dependent on IL-18. Blockade of IL-1 by anti-IL-1 antibodies or IL-1ra stops the influx of PMN. Although there is little effect on the influx of monocytes, cartilage degradation remained at 50%\textsuperscript{8} or even 75% in IL-1ra treated arthritic animals (manuscript in preparation). This suggests that the interaction between infiltrating monocytes and synovial cells may be the most important mechanism in cartilage degradation during ICA. On one hand, synovial macrophages and fibroblasts activated by IC release cartilage degrading enzymes like metalloproteinases\textsuperscript{39}. On the other hand infiltrating monocytes have the capacity to release proteoglycan degrading enzymes. Cationic IC, which strongly attach to synovial tissues due to their charge\textsuperscript{8}, may activate infiltrating monocytes to release these enzymes as well as other factors that activate synovial fibroblasts. Leakage of synovial vessels elevates the presence of fibrinolytic enzymes like plasmin, which may transform latent cartilage degrading enzymes in its active form. Whether monocytes and/or synovial cells in this model are responsible for external cartilage degradation is currently under investigation.

Our results indicate that in ICA, IL-1 is the major cytokine involved in suppression of proteoglycan synthesis. Inflammatory cells seem not to be involved in suppression of proteoglycan synthesis. These cells are however involved in proteoglycan loss\textsuperscript{16}. Since IL-1 is the main cytokine involved in cell influx, this cytokine also indirectly regulates proteoglycan loss. Neutralizing the surplus of IL-1 generated in arthritic joints may be an important approach to stimulate reconstruction of damaged cartilage.

ACKNOWLEDGMENT
The authors thank Dr. Ottenness, Pfizer Central Research, Groton, CT, for the gift of murine recombinant IL-1α and IL-1β and Dr. Thompson Syenergen, Boulder, CO, USA) for providing IL-1ra.

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