ROLE OF INTERLEUKIN-1, TUMOR NECROSIS FACTOR α, AND INTERLEUKIN-6 IN CARTILAGE PROTEOGLYCAN METABOLISM AND DESTRUCTION

Effect of In Situ Blocking in Murine Antigen- and Zymosan-Induced Arthritis

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Objective. To determine the involvement of interleukin-1 (IL-1), tumor necrosis factor (TNF), and IL-6 in the cartilage pathology of murine antigen-induced arthritis (AIA) and zymosan-induced arthritis (ZIA).

Methods. Arthritis was induced by intraarticular injection of zymosan in naive mice or by subcutaneous injection of methylated bovine serum albumin in sensitized animals. Mini-osmotic pumps releasing human recombinant IL-1 receptor antagonist (IL-1ra) protein were implanted intraperitoneally 2 days before arthritis induction, and neutralizing antibodies directed against murine IL-1α, IL-1β, TNFα, or IL-6 were administered 1 day before. Proteoglycan (PG) synthesis and degradation were assessed in patellar cartilage.

Results. Murine IL-1α and IL-1β injected intraarticularly at doses of 0.1–100 ng suppressed chondrocyte PG synthesis. The highest dose of TNF tested (100 ng) decreased PG synthesis marginally. In contrast, the maximum dose of IL-6 (1 μg) stimulated PG synthesis 2 days after injection. Treatment of AIA with neutralizing monoclonal antibodies against either TNFα or IL-6 did not reduce either the PG degradation or the suppression of its synthesis. However, treatment with anti–IL-1 (α + β) polyclonal antibodies totally prevented PG suppression, although the initial breakdown of PG was unaffected. This effect was confirmed when IL-1ra was administered in high doses. Moreover, treatment of ZIA with anti–IL-1 (α + β), but not with anti-TNF, resulted in normal PG synthesis, confirming the key role played by IL-1 in the inhibition of PG synthesis. Treatment of AIA with anti–IL-1 did not affect inflammation during the acute phase, but a significant reduction of ongoing inflammation was noted at day 7, and there was a marked reduction in the loss of cartilage PG.

Conclusion. The suppression of PG synthesis in both ZIA and AIA in mice is due to the combined local action of IL-1 (α + β), and neither IL-6 nor TNF is involved. Moreover, the normalization of PG synthesis brought about by blocking of IL-1 ameliorates the cartilage damage associated with AIA.

Rheumatoid arthritis (RA) is a systemic illness characterized by chronic inflammation of the joints and severe cartilage abnormalities, such as joint space narrowing. Tumor necrosis factor (TNF), interleukin-1 (IL-1), and IL-6 are clearly involved in the arthritic process since all 3 cytokines are present in synovial fluid and can be detected immunohistochemically in the inflamed rheumatoid synovium (1,2). Furthermore, both local and systemic levels of each cytokine correspond to disease activity (3–5), and TNF and IL-1 have profound catabolic effects on articular cartilage explants from numerous species (6,7).

The spontaneous production of IL-1 by rheumatoid synoviocytes can be inhibited by anti-TNF antibodies (8), suggesting that the activity of TNF occurs earlier in the cascade than that of IL-1, whereas IL-6 occupies a position later in the cascade, being produced in response to either TNF or IL-1 (9,10).
Furthermore, IL-1 induces IL-6 synthesis by chondrocytes and is a cofactor in the IL-1-induced suppression of proteoglycan (PG) synthesis (11).

Intraarticular injections of TNF and IL-1 cause an influx of neutrophils into the joint and synovitis similar to that seen in experimental arthritis, but only IL-1 results in marked depletion of the cartilage matrix (12–15). In vitro, TNF is also less potent than IL-1 in suppressing PG synthesis in cartilage explants (16,17).

Direct evidence that TNF and IL-1 play a role in the pathogenesis of experimental arthritis has been obtained in animal models in which blocking of the action of these cytokines has been shown to delay the onset of collagen-induced arthritis (CIA), suppress inflammation, and ameliorate cartilage destruction that corresponds to the antiinflammatory response (18–23). Those studies focused on macroscopic scoring of the affected joints and histologic evaluation, but did not analyze cartilage metabolism in detail, nor did they investigate the effect of anti-IL-6 treatment.

We therefore undertook the present investigation of the potency of IL-1, IL-6, and TNF in PG synthesis and degradation of murine cartilage, and the effect of blocking of endogenous IL-1, TNF, or IL-6 in murine arthritis induced either by antigen or by zymosan (24,25), a potent inducer of both IL-1 and TNF in vivo (26,27). The effect of treatment with neutralizing antibodies on acute joint swelling and inflammation was assessed, and PG synthesis and degradation were measured in cartilage.

MATERIALS AND METHODS

Animals. Male C57Bl/6 mice were obtained from our institution’s breeding facilities and were fed a standard diet and tap water ad libitum.

Cytokines. Purified and biologically active mature murine recombinant IL-1α and IL-1β were generously donated by I. G. Otterness (Pfizer Central Research, Groton, CT), purified murine recombinant IL-6 by G. Ciliberto (IRBM, Rome, Italy), and purified recombinant human IL-1 receptor antagonist (IL-1ra) by Synergen (Boulder, CO). Murine recombinant TNFα (carrier free) was purchased from R & D Systems (Minneapolis, MN).

Neutralizing anticytokine antibodies. Rat anti-mouse TNFα monoclonal antibody (V1q) was kindly donated by P. H. Krammer (German Cancer Research Center, Heidelberg), rabbit anti-mouse TNF polyclonal antisera by S. L. Kunkel (University of Michigan Medical School, Ann Arbor), and another rabbit anti-mouse TNF polyclonal antiserum by G. E. Grau (University of Geneva, Geneva, Switzerland). Rabbit anti-mouse IL-6 polyclonal antisera was donated by M. Fuller (University of Alabama at Birmingham). Rat anti-mouse IL-6 monoclonal antibody was purchased from Genzyme (Cambridge, MA). Neutralizing capacity was verified with specific bioassays for the cytokines.

Generation of rabbit anti-mouse IL-1 antiserum. Polyclonal antibodies directed against each type of murine recombinant IL-1 were prepared by immunization according to the method of Hogquist et al (28), with some modifications. Briefly, 250 μg IL-1 in 2.25 ml phosphate buffered saline (PBS) was suspended in 500 μl aluminum hydroxide (Imject Alum; Pierce, Rockford, IL), which was injected into female New Zealand white rabbits in 4 subcutaneous injections of 500 μl each. A further 4 subcutaneous injections of 500 μl Freund’s complete adjuvant (FCA)/PBS were given in sites adjacent to the IL-1/alum injection sites. Every 4–6 weeks, rabbits received 50 μg IL-1 suspended in alum in 3 divided doses, Freund’s incomplete adjuvant/PBS subcutaneously in adjacent sites, and 5 μg IL-1 intravenously. Ten days after every booster, 50 ml of blood was aspirated and consolated, and serum was stored at −70°C, and decomplemented at 56°C before use.

Purification and characterization of rabbit anti–IL-1 antibodies. Immunoglobulins were purified by affinity chromatography separation on a protein G-Sepharose CL-4B column. Immunoglobulins (IgG) were eluted from the column with 0.1M glycine HCl, pH 3.0, and immediately neutralized with 50 mM Tris HCl, pH 8.0. IgG fractions were then pooled, concentrated, and dialyzed against PBS at ambient temperature. Antibodies directed against the α or β form of IL-1 were tested in vitro for their neutralizing capacity, and it was found that dilutions in the range of 1:64,000 to 1:128,000 could fully block 5 pg/ml IL-1 (lowest plateau concentration) in the NOB1 proliferation assay. Antibodies showed no neutralizing cross-reactivity against each other or against IL-2 (which was tested in the CTLL proliferation assay), IL-4 (in the CT.4.S proliferation assay), IL-6 (in the B9 proliferation assay), or TNFα (in the L929 cytotoxicity assay). The anti–IL-1 antibodies had a half-life of >3 days in the circulation.

Induction of antigen-induced arthritis. Mice ages 8–10 weeks were immunized with 2 subcutaneous injections into the flank skin and 2 into the footpad of both forelegs, with a total of 100 μg methylated bovine serum albumin (mBSA; Sigma, St. Louis, MO) suspended in 100 μl FCA per animal. Heat-killed Bordetella pertussis at 2 × 109 organisms (National Institute of Public Health, Bilthoven, The Netherlands) was administered intraperitoneally as an additional adjuvant. Two subcutaneous booster injections with 100 μg mBSA/FCA were given in the neck region on day 7. Arthritis was induced 14 days after these injections, by intraarticular injection of 60 μg mBSA in 6 μl saline into the right knee joint.

Induction of zymosan-induced arthritis. A homogenous suspension of 30 mg zymosan A (Saccharomyces cerevisiae), dissolved in 1 ml endotoxin-free saline, was obtained by boiling twice followed by sonic emulsification. Arthritis was induced by intraarticular injection of 180 μg zymosan into the right knee.

Assessment of joint swelling. Animals were injected subcutaneously with 10 μCi 99mTc technetium pertechnetate (99mTc) in 0.2 ml saline in the neck region. After 15 minutes, the isotope accumulates in the knee due to the increased blood flow and edema. The amount of 99mTc was determined
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Histologic processing and analysis of knee joints. Knee joints were dissected, fixed, decalcified, dehydrated, and embedded in paraffin. Standard frontal sections of 7 μm were prepared, stained with Safranin O, and counterstained with fast green. cartilage deplentiation was visualized by diminished staining of the matrix and scored as 0 when normal and 1–3 according to the degree of depletion (loss of staining). For autoradiographic analysis of 35S-sulfate incorporation, radiolabeled sulfate was injected intraperitoneally 6 hours before dissection of the knee joints. Seven micrometer sections of paraffin-embedded joints were mounted on gelatin-coated slides which were immersed in K5 emulsion (Ilford, Basildon, Essex, England) and exposed for several weeks before being developed and stained with hematoxylin and eosin.

### Table 1. In vivo effects of interleukin-1 (IL-1), tumor necrosis factor (TNF), and IL-6 on proteoglycan (PG) synthesis

<table>
<thead>
<tr>
<th>Dose, ng/joint</th>
<th>IL-1α, day 1</th>
<th>IL-1β, day 1</th>
<th>TNFα, day 1</th>
<th>TNFα, day 2</th>
<th>IL-6, day 1</th>
<th>IL-6, day 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>70 ± 12 (6)†</td>
<td>90 ± 17 (6)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>0.3</td>
<td>65 ± 17 (6)†</td>
<td>72 ± 14 (6)†</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>1</td>
<td>50 ± 7 (6)†</td>
<td>54 ± 13 (6)‡</td>
<td>97 ± 27 (11)</td>
<td>148 ± 23 (6)</td>
<td>81 ± 10 (6)</td>
<td>94 ± 21 (6)</td>
</tr>
<tr>
<td>10</td>
<td>43 ± 9 (6)†</td>
<td>48 ± 15 (6)‡</td>
<td>91 ± 29 (24)</td>
<td>113 ± 19 (19)</td>
<td>105 ± 26 (6)</td>
<td>105 ± 33 (12)</td>
</tr>
<tr>
<td>100</td>
<td>47 ± 9 (6)‡</td>
<td>44 ± 7 (6)‡</td>
<td>80 ± 17 (12)†</td>
<td>79 ± 29 (25)</td>
<td>103 ± 23 (6)</td>
<td>120 ± 22 (12)</td>
</tr>
<tr>
<td>1,000</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>104 ± 24 (12)</td>
<td>132 ± 23 (18)‡</td>
</tr>
</tbody>
</table>

* Murine recombinant IL-1, TNF, or IL-6 was injected into the right knee joint cavity of mice. PG synthesis was determined by 35SO4 incorporation ex vivo and is expressed as a percentage of the normal synthesis in patellae of joints injected with saline. Values are the mean ± SD; values in parentheses are the number of animals per group. ND = not determined.
† P < 0.05 versus saline-injected joints, by Mann-Whitney U test of cpm values.
‡ P < 0.01 versus saline-injected joints, by Mann-Whitney U test of cpm values.

Anticytokine treatment. Antibodies were injected intravenously into the orbital plexus of the mice, 18–24 hours before induction of arthritis. Each experimental group consisted of at least 7 animals.

**Anti-IL-1 (α + β) antibody treatment.** Mice were injected intravenously with a 200-μl standard dose of 2 mg purified rabbit anti-IL-1 antibodies (with a total neutralizing capacity of 32 ng of both subtypes of IL-1 when tested in the NOB1 assay; this was sufficient to completely block the effect of 1 ng of IL-1α and β on PG synthesis in vivo). Normal rabbit IgG or polyclonal anti-ovalbumin antibodies were used as controls for the nonspecific effects of treatment.

**Anti-TNF and anti-IL-6 treatment.** Mice were given a dose of 90,000 units of neutralizing monoclonal antibody V1q directed against TNFα (which was sufficient to block 7.8 µg of TNFα in the L929 bioassay). Some mice were given rat anti-mouse IL-6 IgGl (with a total neutralizing capacity of 175 ng IL-6 in the B9 bioassay); others received normal rat IgG as a control.

**IL-1ra treatment.** Mini-osmotic pumps (Alzet 1007D; Alza, Palo Alto, CA) were implanted into the peritoneal cavity 2 days before arthritis induction, and set to release 37.5 µg of IL-1ra per hour for the next 7 days. The mean steady-state level of IL-1ra in the blood was 4.7 µg/ml from the first day after implantation, as measured in the NOB1 assay.

### RESULTS

**Effects of IL-1, TNF, and IL-6 on PG metabolism.** In vivo. Intraarticular injection of IL-1α or IL-1β suppressed PG synthesis in patellar cartilage within a day of administration, in a dose-related manner (Table 1). Marked suppression of 50–60% lasted at least 2 days, and recovery occurred thereafter (13). Chondrocyte PG synthesis was suppressed by TNFα only at 100 ng, with lower doses having no effect after 1 day and actually stimulating PG synthesis after 2 days. Low doses of IL-6 also had no effect, and doses of 1 µg significantly enhanced PG synthesis by 32% after 2 days.
Effect of anticytokine pretreatment on PG synthesis in murine antigen- and zymosan-induced arthritis. Chondrocyte PG synthesis was markedly suppressed in both murine arthritis models. Pretreatment of the mice with anti-IL-6 or anti-TNF antibody did not reverse the inhibition of PG synthesis on day 2 of AIA and ZIA, whereas pretreatment with anti-IL-1 (α + β) did (Figure 1A). Selective elimination of either IL-1α or IL-1β did not prevent the suppression of PG synthesis (Figure 1B), indicating that both subtypes reached optimal effective concentrations in the joints. On the second day of arthritis, the acute joint swelling in AIA was as severe as that in ZIA. In AIA, joint swelling was not significantly affected by treatment with anti-IL-1, anti-TNF, or anti-IL-6, and in ZIA it was reduced only moderately (18%) by anti-TNF, but not by anti-IL-1.

Effect of IL-1ra on PG synthesis in AIA. In initial experiments, mice received bolus injections of either 2 mg/kg or 10 mg/kg IL-1ra into the peritoneal cavity, before and during arthritis, and PG synthesis remained significantly suppressed on day 2 of arthritis (Table 2). In further experiments, the high amount of IL-1ra delivered to mice via mini-osmotic pumps prevented the inhibition of PG synthesis on day 2 of AIA, confirming the results obtained after pretreatment with anti-IL-1 antibody in AIA.

Effects of anti-IL-1 pretreatment on cartilage pathology during the chronic phase of AIA. In AIA, PG synthesis was suppressed for at least the first 7 days of arthritis, whereas the suppression in ZIA was more transient. A single intravenous injection of anti-IL-1 antibodies prior to arthritis induction fully prevented the suppression of PG synthesis for this whole period (results not shown). Autoradiography of 35S pulse-labeled knee joints on day 4 of AIA demonstrated marked inhibition of label incorporation, indicating reduced PG synthesis in the cartilage matrices. Normalized incorporation was noted after anti-IL-1 pretreatment of the arthritic mice (Figure 2).

In the next experiment, cartilage degradation was accelerated; the 35S-sulfate content in prelabeled patellar cartilage on day 2 was reduced by a mean ± SD of 54 ± 10% in AIA and 43 ± 11% in ZIA. Anti-IL-1 (α + β) pretreatment did not prevent this in either form of arthritis, with losses of 50 ± 12% and 35 ± 9% being measured; this suggests that IL-1 was not involved in the initiation of this process.

The combined action of reduced synthesis and enhanced degradation led to a profound loss of cartilage PG in AIA, which was substantially reduced by pretreatment with anti-IL-1, as reflected by PG measurements of cartilage on days 4 and 7 of AIA (Table 3). Histologic analysis of whole knee joints showed significant amelioration of the damage to the cartilage. Safranin O staining of the articular cartilage matrices was greater in the arthritic joints of anti-IL-1-treated as compared with control treated mice, indicating a higher PG content (Figure 2).

Although blocking of IL-1 had no antiinflamma-
Table 2. Effects of interleukin-1 receptor antagonist (IL-1ra) protein on proteoglycan (PG) synthesis in antigen-induced arthritis (AIA)*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Experiment 1, 2 mg/kg IL-1ra†</th>
<th>Experiment 2, 10 mg/kg IL-1ra‡</th>
<th>Experiment 3, 37.5 µg/hour IL-1ra§</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal AIA</td>
<td>Normal AIA</td>
<td>Normal AIA</td>
</tr>
<tr>
<td>Saline</td>
<td>1,172 ± 101</td>
<td>ND</td>
<td>1,270 ± 139</td>
</tr>
<tr>
<td>IL-1ra</td>
<td>1,107 ± 141</td>
<td>431 ± 69‡</td>
<td>500 ± 153§</td>
</tr>
</tbody>
</table>

* PG synthesis was determined by 35S04 incorporation in patellae ex vivo 48 hours after induction of AIA. Values are the mean ± SD cpm in at least 6 patellae.
† IL-1ra (2 mg/kg) or saline was injected into the peritoneal cavity 2 hours before arthritis induction and at 4, 10, 16, 22, 28, and 46 hours after arthritis induction. Total cumulative dose was ~0.35 mg/mouse.
‡ IL-1ra (10 mg/kg) or saline was injected into the peritoneal cavity 2 hours before arthritis induction, every 3 hours the first day after arthritis induction, and every 6 hours the second day after arthritis induction. Total cumulative dose was ~3 mg/mouse.
§ Mini-osmotic pumps were implanted intraperitoneally 2 days before arthritis induction and set to deliver 37.5 µg of IL-1ra per hour or saline (control group) for the next 7 days. Total cumulative dose was ~3.6 mg/mouse. Values are representative of 3 experiments.
‖ P < 0.001 versus normal contralateral joints, by Mann-Whitney U test.

The study effect on the acute inflammation, ongoing inflammation of the joint was much lower in later phases of AIA after pretreatment with anti-IL-1. Joint swelling and synovitis were significantly reduced on day 7 of AIA (Table 3).

DISCUSSION

Direct evidence for the involvement of cytokines in the process of joint inflammation and cartilage destruction in RA is still lacking. We therefore undertook to investigate, in a comparative study, the potency of TNFα, IL-1, and IL-6 in influencing chondrocyte function in vivo, and the effect of selective blocking, in murine arthritis.

PG synthesis was markedly suppressed after intraarticular injections of IL-1α or IL-1β, whereas higher doses of TNFα were needed for this. In previous studies, we and others demonstrated that intraarticular injection of IL-1 into synovial joints causes marked cartilage PG degradation and cartilage depletion in various species (12–14). Of interest, this effect seems to be independent of the inflammatory reaction (13,30). A single intraarticular injection of TNFα did not cause edema or enhanced PG degradation in the tested dose range of 1–100 ng in mice (results not shown). Although TNF may cause cartilage destruction in vitro (16), evidence for such a role in vivo is lacking (14,15).

TNF and IL-1 are potent inducers of IL-6 production, but we clearly showed that IL-6 is not a destructive mediator in the murine joint and may even play a protective role in the joint since it stimulates PG synthesis. Moreover, IL-6 enhances the expression of inhibitors of cartilage destructive enzymes (e.g., tissue inhibitor of metalloproteinases) in synovial fibroblasts (31).

It has been shown in studies of rheumatoid synovium that TNF may be an important driving force in the production of IL-1 (8). Although TNF does not seem to be a major cartilage destruction mediator, its regulating role makes it an interesting target for therapy. Recent studies with chimeric antibodies to TNFα demonstrated their efficacy in suppressing signs of inflammation in short-term trials (32), but protection against ongoing cartilage destruction has yet to be demonstrated. Given the existence of separate pathways of IL-1 production in addition to TNF-driven pathways, it is tempting to suggest that blocking of IL-1 would be a more valid approach with respect to amelioration of cartilage destruction.

Pretreatment of mice with rabbit polyclonal anti-IL-1 antibodies protected chondrocyte function in both ZIA and AIA. The IL-1 subtypes had to be blocked together to prevent suppression of PG synthesis in arthritis, whereas pretreatment with anti-TNF or anti-IL-6 antibodies had no effect. This result is consistent with the relative potencies of these cytokines after intraarticular injection into the murine knee joint. Although it is reported that IL-6 is a cofactor in IL-1–induced suppression of PG synthesis in human cartilage explants (11), this has yet to be confirmed in vivo.

A major role of TNF and/or IL-1 in cartilage PG degradation during the acute phase of murine AIA or ZIA cannot be deduced from the present study, and this result is in accord with observations on AIA in the
rabbit (33,34). This may merely indicate that other mediators are playing a more important role, or it may imply that IL-1 levels in AIA are too low. In that respect it is intriguing to note that inhibition of chondrocyte PG synthesis can be achieved with relatively small doses of IL-1, whereas considerably high IL-1 levels are needed to cause substantial PG degradation (13,16,17).
Table 3. Effects of anti-interleukin-1 (anti-IL-1) treatment on inflammation and cartilage loss in antigen-induced arthritis (AIA)*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Joint swelling (Tc ratio), day 7 of AIA†</th>
<th>Synovitis (0–3 score), day 7 of AIA‡</th>
<th>Glycosaminoglycan content (µg/patella)§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rabbit IgG</td>
<td>1.44 ± 0.25</td>
<td>2.3 ± 0.8</td>
<td>1.56 ± 0.50</td>
</tr>
<tr>
<td>Anti-IL-1</td>
<td>1.23 ± 0.16</td>
<td>1.3 ± 0.9†</td>
<td>2.30 ± 0.46†</td>
</tr>
</tbody>
</table>

* Normal rabbit IgG or antibodies against IL-1 (α + β) were injected intravenously into mice 1 day before arthritis induction. Values are the mean ± SD.
† Joint swelling is expressed as the ratio of the enhanced uptake of 99mtechnetium pertechnetate in the arthritic joint to that in the normal contralateral joint (see Materials and Methods for details).
‡ Whole knee joint sections were analyzed for leukocyte infiltration into the synovium and arbitrarily scored from 0 (no cells) to 3 (large number of cells).
§ Glycosaminoglycan content was measured as described in Materials and Methods. Content in patellar cartilage of the contralateral knee joint was 2.58 ± 0.39 µg on day 4, and 2.45 ± 0.31 µg on day 7.

† P < 0.05 versus normal rabbit IgG-treated mice, by Mann-Whitney U test.
# P < 0.01 versus normal rabbit IgG-treated mice, by Mann-Whitney U test.

The overall effect of cytokine neutralization seems to depend on both the type and the phase of the arthritis. Anti-TNF treatment in CIA resulted in marked amelioration of joint inflammation and cartilage destruction in a prevention protocol, but was markedly less efficient when treatment was started during established disease (35). In contrast, neutralization of IL-1 was still efficacious when treatment was started late, and marked protection of cartilage damage was observed (19,36). In the present study, anti-TNF pretreatment using the same antibody (V1q) as was used in CIA did not diminish acute joint swelling, nor did it protect against cartilage damage in murine AIA. Furthermore, neutralization of IL-1, also using the same anti-IL-1 antibody as in the CIA model, did not reduce acute joint inflammation either, yet markedly ameliorated cartilage destruction after day 2 of AIA.

These findings in the various models suggest that protection against cartilage destruction with neutralizing anticytokine antibodies can be obtained in two ways. First, there is an indirect effect, when IL-1 and/or TNF are key elements in the inflammatory process. Second, there is a direct effect, when inflammation is caused by an excess of other mediators yet elimination of a destructive mediator like IL-1 may still cause substantial protection. In the first situation a direct, destructive role of IL-1 can never be proven. However, in the present study of AIA and ZIA, in which the acute inflammation seems highly IL-1 and TNF independent, a key role of IL-1 in the suppression of PG synthesis has now been established. Nevertheless, we cannot exclude the possibility that the amelioration of cartilage degradation in the anti-IL-1-treated mice was due to decreased PG degradation caused either directly or as a consequence of relief of the ongoing inflammation, as was shown on days 4 and 7 of AIA.

The role of IL-1 in PG synthesis suppression was confirmed in experiments with IL-1ra-treated mice. Conflicting results have been reported in the past. Lewthwaite et al (34) demonstrated blocking of IL-1-induced inflammation in the rabbit, but were unable to show protection of PG synthesis in rabbit AIA, in studies in which the animals were given repeated subcutaneous injections of human IL-1ra. Wooley et al (18) also reported a failure to modulate murine AIA. We had similar, negative observations with repeated injections of IL-1ra, but efficacy was clearly proven when continuous high levels were generated using Alzet mini-osmotic pumps. Using a similar approach, we recently proved efficacy also in murine CIA and immune complex arthritis (van Lent PLEM et al and Joosten LAB et al: manuscripts in preparation). The relevance of this approach is obvious, given the poor pharmacokinetic profile of IL-1ra. Moreover, continuous blocking of almost all IL-1 receptors is needed to prevent cell activation, necessitating a continuous excess of at least 1,000–10,000-fold amounts of IL-1ra (35).

In RA, much emphasis is now focused on blocking of TNFα using either chimeric antibodies or fusion proteins with TNF soluble receptor. In addition, initial trials are under way with IL-1ra. Our experimental studies suggest that IL-1 is an important target in protection against cartilage destruction. Moreover, continuous administration of high levels of
IL-1ra is needed to control IL-1 in arthritic processes, and it is debatable whether the IL-1ra dosages used in clinical trials are high enough. It is hoped that antagonists proteins with a better profile, or selective inhibitors of IL-1 production, will become available in the near future.

Our present study does not demonstrate efficacy of anti-IL-6 antibody treatment. Given the high levels of IL-6 in the circulation and in inflamed joints, the possibility cannot be excluded that some IL-6 escapes neutralization. This would be particularly relevant with regard to local production in cartilage, since antibodies will not penetrate to a great extent. The role of IL-6 remains to be elucidated, but we expect that it plays a protective role in arthritis.

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