Efficacy of daily compared to intermittent administration of IL-1Ra for protection against bone and cartilage destruction in collagen-challenged mice

K. Inoue, K. Masuko-Hongo, M. Okamoto, K. Nishioka

Rheumatology, Immunology and Genetics Program, Institute of Medical Science, St. Marianna University of Medicine, Kawasaki, Kanagawa, Japan

Abstract

Objective

To investigate the protective effect of interleukin-1 receptor antagonist (IL-1Ra) on bone and cartilage destruction in the induction phase of collagen-induced arthritis (CIA), an animal model of rheumatoid arthritis (RA).

Methods

DBA/1J mice were immunized with type II collagen for induction of collagen-induced arthritis (CIA) and simultaneously given different intraperitoneal doses of IL-1Ra daily, thrice weekly or once a week. Clinical symptoms of arthritis were noted daily and assessed using a scoring system during the course of disease. Bone and cartilage destruction in the mice was assessed by radiographic and histological methods respectively.

Results

Mice injected with IL-1Ra daily were completely protected from the occurrence of arthritis after immunization with type II collagen. Moreover, these mice were also protected against bone and cartilage destruction. However, weekly or thrice weekly treatment with IL-1Ra had no effect on arthritis and bone and cartilage destruction.

Conclusion

Daily administration of recombinant IL-1Ra, injected at the same time as arthritis induction, is effective in blocking the occurrence of inflammatory as well as destructive changes in CIA. Daily bolus injections of IL-1Ra may therefore be useful for protection against joint damage following minor joint injury, whereas the maintenance of appropriate blood levels of the antagonist may be critical for its therapeutic effect on chronic inflammatory arthritis.

Key words

IL-1Ra, collagen-induced arthritis, rheumatoid arthritis, bone destruction.

Kenji Inoue, PhD; Kayo Masuko-Hongo, MD, PhD; Masahiro Okamoto, PhD; Kusuki Nishioka, MD, PhD.

Introduction

Rheumatoid arthritis (RA) is characterized by a chronic inflammation and concomitant destruction of the bone and cartilage in synovial joints. The involvement of several proinflammatory cytokines, particularly interleukin-1 (IL-1) and tumor necrosis factor (TNF) , in the pathogenesis of RA is now well accepted based on numerous studies (1-10). For example, the level of IL-1 has been found to be significantly higher in synovial fluids, in synovium and also in sera from patients with RA (5-7). In addition, systemic administration of IL-1 was shown to accelerate and exacerbate arthritis in animal models of RA, such as type II collagen-induced arthritis (CIA) in mice (8, 9) and streptococcal cell wall-induced arthritis in rats (10).

Informatively, mice transgenic for the human IL-1 gene were found to develop arthritis in which cartilage degradation appeared to be more rapid and drastic than in TNF -transgenic mice (11, 12). These findings strongly suggest a dominant role for IL-1 in cartilage degradation in chronic arthropathy and imply that inhibition of the IL-1-signaling pathway may represent an effective therapy against cartilage degradation.

The human IL-1Ra was cloned as a specific receptor antagonist that competitively blocks binding of IL-1 and IL-1 to human IL-1 receptors type I and type II (13), as well as to the murine IL-1 receptor type I (14), and thereby inhibits IL-1 bioactivity. In addition to its therapeutic effect on IL-1-induced inflammation, several cartilage-protective effects of IL-1Ra have been reported. For example, IL-1Ra blocks the induction of several metalloproteinases (e.g. collagenase, gelatinase and stromelysin) and of PGE_2, and the release of glycosaminoglycan in bovine nasal cartilage explants *in vitro* (15). These findings suggest that one therapeutic application of IL-1Ra in arthritis may be to regulate both IL-1-induced inflammation and bone and cartilage degradation in the joint through inhibition of IL-1 binding (13, 16, 17).

The pharmacokinetics of IL-1Ra have been investigated using animal models of arthritis, such as adjuvant-induced arthritis and CIA (18). From these studies, a short half-life of IL-1Ra ($t_{1/2}$ = 2 hours) after injection *in vivo* was documented with rapid elimination via renal secretion (19). This finding suggested the necessity for maintaining effective blood levels of IL-1Ra for clinical application in arthritis. Thus, most of the previous studies on clinical effects of IL-1Ra in animal models employed continuous infusion (18, 20). However, continuous administration using an infusion pump would not seem practical for IL-1Ra therapy in RA patients. In addition, although most studies reported on the therapeutic effects of IL-1Ra in established CIA, its protective effects when applied to mice before disease onset have not been fully investigated.

In this regard, Wooley *et al.* showed that intraperitoneal (i.p.) daily injection of IL-1Ra had a protective effect against arthritis in mice with CIA (21). To our knowledge, this is the only report showing that bolus injection of IL-1Ra can effectively suppress inflammation in the arthritis model. However, application by intermittent injection of IL-1Ra, using different and prolonged intervals between injections, has never been investigated in animal models of RA, in spite of its importance for clinical practice. In particular, it is not clear to what degree bolus injection of IL-1Ra may be able to prevent bone and cartilage destruction in the animal model. In human clinical studies, therapeutic effects of daily IL-1Ra treatment on joint damage were reported (22-24). However, the maximum interval between IL-1Ra injections retaining protection against joint damage in RA has not been determined.

In the present study, we investigated whether or not IL-1Ra treatment by bolus injection can protect bone and cartilage from destruction in mice in the early stages of CIA. Furthermore, to determine the maximum interval between IL-1Ra bolus injections which still shows a protective effect, we tested the effects of once or thrice weekly administration of IL-1Ra to the CIA mice.

Materials and methods

Animals

Female DBA/1J Ncrj mice (body weight 15-20 g) were purchased from Charles River Japan Inc., Kanagawa, Japan for use in this study. Animals were allowed to acclimate for 1 week prior to initiation of the experimentation. Mice were housed 4 animals per cage in clean cages (215 x 320 x 130 mm) and allowed access to food and water *ad libitum*. Animal care was in accordance with institutional guidelines.

Materials

Recombinant human IL-1Ra (ANA-KINRA) and its vehicle solution were provided by Amgen Inc., Thousand Oaks, CA. Freund's complete and incomplete adjuvants were obtained from Difco, Detroit. Bovine type II collagen was obtained from Collagen Research Center (Tokyo, Japan).

Induction of CIA

Bovine type II collagen was solubilized at 4 mg/ml in 0.05 N acetic acid and emulsified in an equal volume of chilled Freund's complete adjuvant (used for the first immunization) or chilled Freund's incomplete adjuvant (used for the second immunization). The mice were immunized intradermally at the base of the tail with 100 µl of the antigen emulsion on day 0. On day 21, the mice were given a second immunization intradermally in the same manner as the first immunization.

IL-1Ra treatment of CIA

Mice (n = 8) were injected intraperitoneally (i.p.) with IL-1Ra at either low (1.5 mg/kg; designated as the L group), medium (15 mg/kg; M group) or high doses (150 mg/kg; H group) daily, thrice weekly (on Tuesday, Thursday and Saturday) or once a week (Tuesday) from day 0 until day 77. Control mice (n = 8) were given i.p. injections of the vehicle solution daily or once a week. The protocol and denominations of each treatment group are summarized in Table I. The doses administered were determined according to a previous study (21).

Assessment of arthritis

The incidence of arthritis was assessed twice a week from the day of the second immunization until the end of the study. The clinical severity of arthritis was graded on a scale of 0-4 for each paw by the changes in redness and swelling, according to the following criteria: 0 = no change; 1 = swelling or redness at only one joint; 2 = swelling or redness at more than two finger joints or at an ankle joint; 3 = swelling or redness of the whole paw; 4 = maximal swelling and redness and later, ankylosis. The macroscopic score is given as the cumulative value for all paws, with a maximum of 16.

Assessment of bone destruction

On day 78, the mice were sacrificed by removal of blood from the abdominal vena cava, and were examined for bone destruction in the joints by radiography (35 keV X-rays for 1 min). The bone destruction score was graded using a scale (0-3) for each paw, according to the following criteria: 0 = no change; 1 = bone destruction at one finger joint; 2 = bone destruction at more than two joints; 3 = bone destruction at an ankle joint. The bone destruction score is given as the cumulative value for all paws, with a maximum of 12.

Histopathological analysis

The posterior limbs were fixed in 10% neutral buffered formalin solution. After decalcification of the tissues, the specimens were embedded in paraffin. Tissue sections in the finger joints were stained with Safranin O.

Statistical analysis

Values of clinical and bone destruction scores are shown as means ± S.E.M. The differences of the scores between each group were analyzed by Dunnett's test.

Results

Effect of IL-1Ra on the incidence of CIA

We produced CIA mice by immunizing DBA/1J animals with bovine type II collagen, and simultaneously injected recombinant IL-1Ra at different doses and time intervals to investigate the effect of intermittent bolus administration. The protocol for IL-1Ra adminis-

Table I. Development of CIA in the IL-1Ra-treated mice.

Treatment group	Days after the first immunization*									
	21	28	35	42	49	56	63	70	77	
V7 (Vehicle, daily), N=8	0	2	4	5	7	8	8	8	8	(100%)
V1 (Vehicle, once a week), N=7	1	4	5	5	6	6	6	6	6	(86%)
L7 (1.5 mg/kg, daily), N=8	0	2	4	5	5	8	8	8	8	(100%)
L3 (1.5 mg/kg, thrice a week), N=7	0	1	3	5	6	6	6	6	6	(86%)
L1 (1.5 mg/kg, once a week), N=8	0	4	5	8	8	8	8	8	8	(100%)
M7 (15 mg/kg, daily), N=8	0	3	6	6	7	7	7	8	8	(100%)
M3 (15 mg/kg, thrice a week), N=8	0	1	5	5	6	6	6	7	7	(88%)
M1 (15 mg/kg, once a week), N=7	0	4	6	7	7	7	7	7	7	(100%)
H7 (150 mg/kg, daily), N=8	0	0	0	0	0	0	0	0	0	(0%)
H3 (150 mg/kg, thrice a week), N=8	1	3	5	5	5	7	7	7	7	(88%)
H1 (150 mg/kg, once a week), N=8	1	1	6	8	8	8	8	8	8	(100%)

*Each number indicates the number of mice in each group that developed arthritis on the indicated day.

tration is summarized in Table I.

The overall occurrence of arthritis, without taking severity into account, is shown in Table I. Most notably, mice that were administered 150 mg/kg IL-1Ra daily were completely protected from the occurrence of arthritis during the entire observation period (0% incidence; Table I). There were also some mice that did not become arthritic in groups V1, L3, M3 and H3, although this could not be definitively attributed to the IL-1Ra administration. In the remaining groups, however, IL-1Ra did not show any protective effect. There was no significant retardation in the onset of inflammation in the animals in the different groups. Therefore, it appears that the daily injection of IL-1Ra protects against arthritis.

Next we investigated whether IL-1Ra decreases the severity of arthritis in each group treated with different doses and at different intervals. The cumulative arthritis scores of the groups treated daily are shown in Figure 1. It was confirmed that mice in the H7 group were completely protected from arthritis (Fig. 1). In addition, mice in groups M7 and L7, which received a daily IL-1Ra injection at a lower dose, also showed a decreased severity of arthritis, although the differences did not reach statistical significance.

In contrast, when the mice were treated with IL-1Ra only once a week, no effect on the arthritic score was observed in either group (Fig. 2). Additionally, thrice weekly treatment even with high-dose IL-1Ra (150 mg/kg, group H3) had no effect on the scores (data not shown). These results indicate that daily, but not thrice weekly or once weekly injection of IL-1Ra effectively suppressed inflammation in CIA.

Effect of IL-1Ra on bone destruction
As the effects of IL-1Ra on inflammation and on bone and cartilage may differ from its effects in the rat adjuvant arthritis model (19), we next investigated the degree of joint destruction in these mice. The effect of IL-1Ra on bone was assessed using an X-ray grading scale. The results are summarized in Figure 3. It was demonstrated that the daily administration of IL-1Ra at
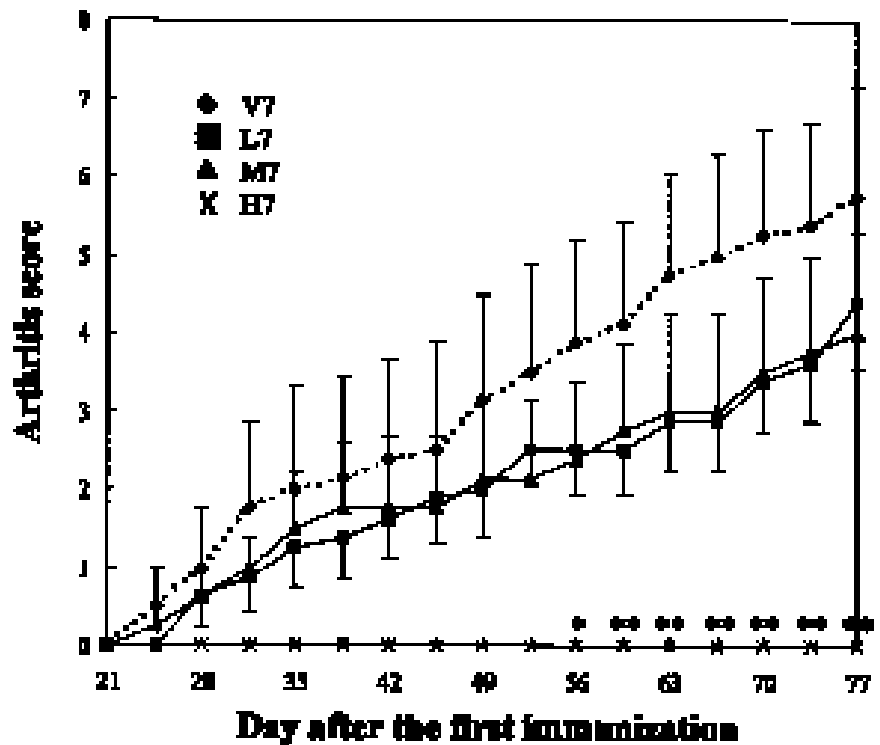

Fig. 1. Effect of daily administration of IL-1Ra on CIA. Closed diamonds, closed squares, closed triangles, and crosses indicate group V7 (Vehicle), L7 (1.5 mg/kg IL-1Ra), M7 (15 mg/kg IL-1Ra) and H7 (150 mg/kg IL-1Ra), respectively. Arthritis scores were visually determined twice a week in the peripheral joints, according to the criteria in the protocol. Values are the means ± S.E.M. *P < 0.05 versus vehicle, daily group by Dunnett's test. **P < 0.01 versus vehicle, daily group by Dunnett's test.
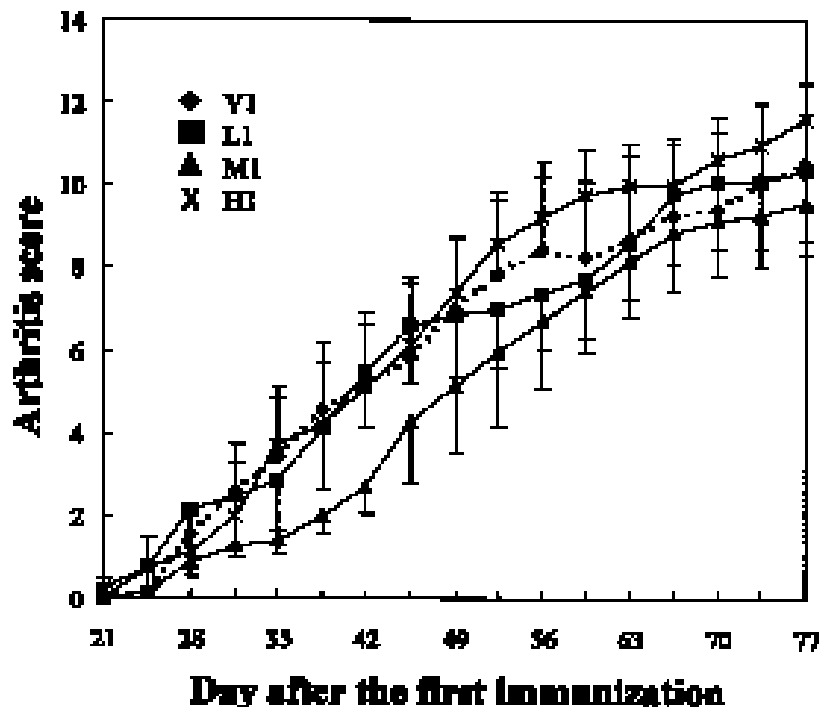

Fig. 2. Effect of once a week administration of IL-1Ra on CIA. Closed diamonds, closed squares, closed triangles, and crosses indicate group V1 (Vehicle), L1 (1.5 mg/kg IL-1Ra), M1 (15 mg/kg IL-1Ra) and H1 (150 mg/kg IL-1Ra), respectively. Arthritis scores were visually determined twice a week in the peripheral joints, according to the criteria in the protocol. Values are the means ± S.E.M.
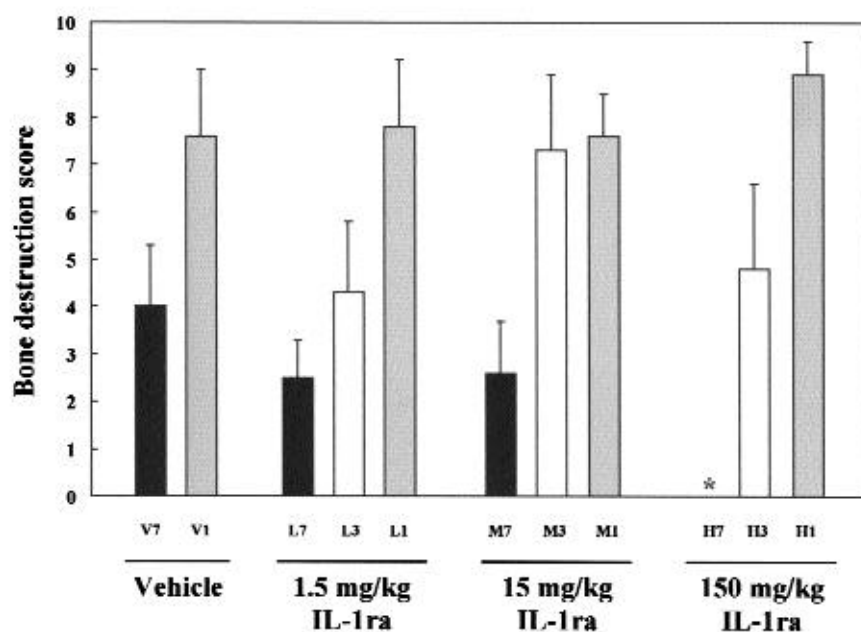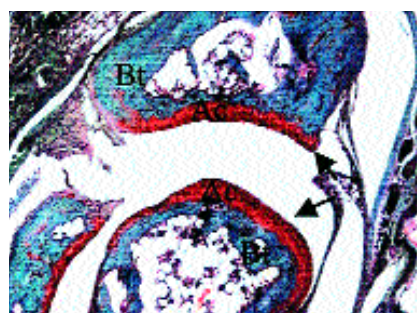
Fig. 3. Effect of IL-1Ra on bone destruction in CIA mice. Bone destruction in the joints was visually determined by radiography, according to the criteria described in the Materials and Methods. Values are the means ± S.E.M. *P < 0.05 versus vehicle, daily group by Dunnett's test.
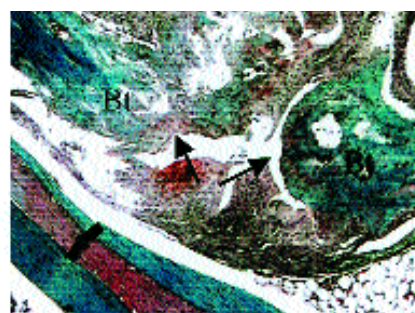
A.

B.

Fig. 4. Histological analysis in joints of CIA mice treated with IL-1Ra. Articular cartilage was stained with Safranin O as described in the *Materials and Methods*. (**A**) Section of a joint in mice from group H7; arrows indicate the articular cartilage. (**B**) Section of a joint in mice in group V7; arrows indicate the morphological changes in the cartilage. Ac and Bt indicate articular cartilage and bone tissue, respectively. (Original magnification x 200.)

150 mg/kg (H7) completely prevented bone damage. Mice receiving 1.5 or 15 mg/kg of IL-1Ra daily (L7 and M7) also showed slightly decreased scores for bone destruction (not significant) compared to the control. However, treatment with IL-1Ra three times a week or once a week did not have any significant impact on bone destruction scores.

These results indicate that daily, but not once weekly or thrice weekly, injection of IL-1Ra effectively prevented not only the inflammation but also bone destruction in CIA.

Effect of IL-1Ra on cartilage destruction

The effect of IL-1Ra on cartilage damage in CIA mice was examined by histological analysis using Safranin O staining. As shown in Figure 4B, the articular cartilage in the mice treated with vehicle (group V7) was severely damaged. Daily administration of IL-1Ra at 150 mg/kg (group H7) markedly inhibited the destruction of articular cartilage (Fig. 4A). However, the mice in the other IL-1Ra-treated groups still showed complete destruction of the articular cartilage (data not shown).

These results indicate that daily, but not once weekly or thrice weekly, injection of IL-1Ra could also completely protect the cartilage from destruction in CIA mice.

Discussion

We demonstrated that daily injection of high-dose IL-1Ra into DBA/1J mice at the induction phase of CIA dramatically reduced the incidence of inflammatory arthritis, and furthermore completely protected the bone and cartilage from destruction. These findings strongly support the clinical application of IL-1Ra at intermittent intervals *in vivo* for protection against the joint destruction which occurs following the disease-inducing event.

We investigated the preventive effect of recombinant IL-1Ra administration, intraperitoneally in bolus form, at prolonged intervals. The doses administered were determined in a previous study (21). The high-dose (150 mg/kg) level was determined as the dose that had a therapeutic effect on inflammation in CIA mice. This dose is higher than that used in human RA therapy (~200 mg/day), but the pharmacokinetics of IL-1Ra in mice and humans may differ. The present study demonstrated that daily injection of the highest dose (150 mg/kg) of IL-1Ra had a significant protective effect on bone and cartilage destruction. We also confirmed the significant effect in a separate experiment using mice which received an intermediate dose (75 mg/kg) of IL-1Ra (unpublished data), indicating that the protection was dose-dependent.

In this regard, although the half-life of intraperitoneally injected IL-1Ra in bolus form has not been reported thus far, from a study of s.c. injection of IL-1Ra into rats it can be estimated to be about 2 hours ($t_{1/2}$ = 2 hours) (19). Specifically, Bendele *et al.* showed that the anti-arthritic effect of s.c. injected IL-1Ra required a blood level of 0.2 µg/ml in the CIA rat (19). In their study, the effective blood level of IL-1Ra was reported to be maintained for about 12 hours after 100 mg/kg s.c. injection. Considering these data, it is assumed that the circulating level of IL-1Ra in the group receiving 150

mg/kg daily in our present study would be maintained for about 24 hours after the injection. On the other hand, the levels of IL-1Ra in other groups (L, M, H1 and H3) seemed to be below the effective level a day after injection, suggesting that the continuous maintenance of IL-1Ra blood levels above the effective threshold is required for the anti-arthritic effect and protection against bone and cartilage degradation. Plasma clearance of IL-1Ra in human is as rapid as in mice (18, 25). The frequency of dosage is important in the therapy of human RA, with daily treatment providing the most benefit, and our results support this therapeutic protocol for RA.

It has been suggested that the signaling cascade following IL-1 binding to the IL-1R could play an important role in cartilage destruction *in vivo* by modulating proteoglycan synthesis and the production of several metalloproteinases by chondrocytes (15, 26). Joosten *et al.* reported that IL-1 blockade using anti-murine IL-1 antibodies can result in the reduced release of cartilage oligomeric matrix protein, a circulating marker of cartilage turnover (27), and ameliorate inflammatory arthritis in CIA mice. In contrast,TNF- blockade can ameliorate only inflammatory arthritis (28). Thus IL-1Ra is expected to protect against such mechanisms leading to cartilage deterioration by inhibiting IL-1/IL-1R binding. Interestingly, IL-1Ra-deficient mice were found to suffer severe arthropathy with cartilage destruction, supporting a crucial role of IL-1Ra in protection (29).

As for human RA, a wide array of modulators of inflammation, in addition to IL-1, are known to be involved in the pathogenesis of RA. For example, proinflammatory cytokines TNF- and IL-6 have been focused on as a therapeutic target (30, 31). In addition, hormones and neurotransmitters are suggested to play an important role in RA, since in RA patients the hypothalamic-pituitary-adrenal system and hypothalamic-autonomic nervous system are altered (32). These facts make the pathological mechanism of RA much more complex than that of animal models of arthritis such as CIA.

In human clinical studies, daily treatment with IL-1Ra significantly reduced the erosive joint counts and Larsen scores in RA (23, 24). In contrast, the same protocol of IL-1Ra therapy had only a limited effect on inflammation in RA (30). IL-1 may therefore play a more important role in joint damage than inflammation in RA. On the other hand, IL-1 may also be a major mediator of both inflammation and joint damage in the CIA model.

Our present study demonstrated that the daily administration of 150 mg/kg IL-1Ra dramatically inhibited bone and cartilage destruction in CIA mice. These results suggest that the daily injection of IL-1Ra (despite rapid clearance) may be applicable not only for rheumatoid arthritis but also for the treatment of acute arthropathy, at least in those patients who can tolerate daily injections and need the definite cartilage protective effect. Moreover, it is expected that IL-1Ra could be used to prevent further cartilage damage, for example after acute traumatic injury or intra-articular surgical manipulation, because we have demonstrated complete protection from arthropathy in mice treated daily with IL-1Ra after arthritis induction. On the other hand, a modified form of IL-1Ra with a longer half-life, if applicable, would be clinically more relevant for the long-term therapy of chronic arthritis.

In conclusion, we have demonstrated that the daily administration of recombinant IL-1Ra effectively blocks not only inflammation but also the destructive joint changes in mice during induction of CIA. Maintenance of appropriate blood levels may be critical for the therapeutic effect of IL-1Ra on chronic inflammatory arthritis.

References
1. AREND WP, DAYER JM: Inhibition of the production and the effects of interleukin-1 and tumor necrosis factor in rheumatoid arthritis. *Arthritis Rheum* 1995; 38: 151-60.
2. VAN DE LOO FAJ, JOOSTEN LA, VAN LENT PL, ARNTZ OJ, VAN DEN BERG WB: Role of interleukin-1, tumor necrosis factor and interleukin-6 in cartilage proteoglycan meta-
bolism and destruction: Effect of *in situ* blocking in murine antigen- and zymosan-induced arthritis. *Arthritis Rheum* 1995; 38: 164-72.
3. EASTGATE JA, WOOD NC, DIGOIOVINE FS, SYMONS JA, GRINLINTON JAA, DUFF GW: Correlation of plasma interleukin-1 levels with disease activity in rheumatoid arthritis. *Lancet* 1988; 24: 706-9.
4. KHALE P, SAAL JG, SCHAUDT K: Determination of cytokines in synovial fluids: Correlation with diagnosis and histomorphological characteristics of synovial tissue. *Ann Rheum Dis* 1992; 51: 731-4.
5. NOURI AME, PANAYI GS, GOODMAN SM: Cytokines and the chronic inflammation of rheumatic disease. I. The presence of interleukin-1 in synovial fluids. *Clin Exp Immunol* 1984; 55: 295-302.
6. WOOD DD, IHRIE EJ, DINARELLO CA, COHENOK: Isolation of an interleukin-1-like factor from human joint effusions. *Arthritis Rheum* 1983; 26: 975-83.
7. HOPKINS SJ, HUMPHREYS M, JAYSON MIV: Cytokines in synovial fluid. I. The presence of biologically active and immunoreactive IL-1. *Clin Exp Immunol* 1988; 72: 422-7.
8. HOM JT, BENDELE AM, CARLSON DG: *In vivo* administration with IL-1 accelerates the development of collagen-induced arthritis in mice. *J Immunol* 1988; 141: 834-41.
9. KILLAR LM, DUNN CJ: Interleukin-1 potentiates the development of collagen-induced arthritis in mice. *Clin Sci* 1989; 76: 535-8.
10. STIMPSON SA, DALLDORF FG, OTTERNESS IG, SCHWAB JH: Exacerbation of arthritis by IL-1 in joints previously injured by peptido-glycan-polysaccharide. *J Immunol* 1988; 140: 2964-9.
11. NIKI Y, YAMADA H, SEKI S *et al.*: Macrophage- and neutrophil- dominant arthritis in human IL-1 transgenic mice. *J Clin Invest* 2001; 107: 1127-35.
12. KEFFER J, PROBERT L, CAZLARIS H *et al.*: Transgenic mice expressing human tumor necrosis factor:a predictive genetic model of arthritis. *EMBO J* 1991; 10: 4025-31.
13. EISENBERG SP, EVANS RJ, AREND WP *et al.*: Primary structure and functional expression from complementary DNA of a human interleukin-1 receptor antagonist. *Nature* 1990; 343: 341-5.
14. HANNUM CH, WILCOX CJ, AREND WP *et al.*: Interleukin-1 receptor antagonist activity of a human interleukin-1 inhibitor. *Nature* 1990; 343: 336-40.
15. SMITH RJ, CHIN JE, SAM LM, JUSTEN JM: Biologic effects of an interleukin-1 receptor antagonist protein on interleukin-1-stimulated cartilage erosion and chondrocyte responsiveness. *Arthritis Rheum* 1991; 34: 78-83.
16. AREND WP, JOSLIN FG, MASSONI RJ: Effects of immune complexes on production by human monocytes of interleukin 1 or an interleukin 1 inhibitor. *J Immunol* 1985; 134: 3868-75.
17. CARTER DB, DEIBEL MR, DUNN CJ *et al.*: Purification, cloning, expression and biological characterization of an interleukin-1 receptor antagonist protein. *Nature* 1990; 344: 633-8.
18. BENDELE A, MCABEE T, SENNELLO G,

FRAZIER J, CHLIPALA E, McCABE D: Efficacy of sustained blood levels of interleukin-1 receptor antagonist in animal models of arthritis. *Arthritis Rheum* 1999; 42: 498-506.

19. BENDELE A, McABEE T, WOODWARD M *et al.*: Effects of interleukin-1 receptor antagonist in a slow-release hylan vehicle on rat type II collagen arthritis. *Pharm Res* 1998; 15: 1557-61.

20. JOOSTEN LAB, MONIQUE MAH, VAN DE LOO FAJ, VAN DER BERG WB: Anticytokine treatment of established type II collagen-induced arthritis in DBA/1 mice. *Arthritis Rheum* 1996; 39: 797-809.

21. WOOLEY PH, WHALEN JD, CHAPMAN DL *et al.*: The effect of an interleukin-1 receptor antagonist protein of type II collagen-induced arthritis and antigen-induced arthritis in mice. *Arthritis Rheum* 1993; 36: 1305-14.

22. CAMPION GV, LEBSACK ME,LOOKABAUGH J, GORDON G, CATALANO M: Dose-range and dose-frequency study of recombinant human interleukin-1 receptor antagonist in patients with rheumatoid arthritis. *Arthritis Rheum* 1996; 39: 1092-101.

23. BRESNIHAN B, ALVARO-GRACIA JM, COBBY M *et al.*: Treatment of rheumatoid arthritis with recombinant human interleukin-1 receptor antagonist. *Arthritis Rheum* 1998; 41: 2196-204.

24. JIANG Y, GENANT HK, WATT I *et al.*: A multicenter, double-blind, dose-ranging, randomized, placebo-controlled study of recombinant human interleukin-1 receptor antagonist in patients with rheumatoid arthritis. *Arthritis Rheum* 2000; 43: 1001-9.

25. GRANOWITZ EV, PORAT R, MIER JW *et al.*: Pharmacokinetics, safety and immunomodulatory effects of human recombinant interleukin-1 receptor antagonist in healthy humans. *Cytokine* 1992; 4: 353-60.

26. AREND WP, DAYER JM: Cytokines and cytokine inhibitors or antagonists in rheumatoid arthritis. *Arthritis Rheum* 1990; 33: 305-15.

27. SAXNE T, HEINEGARD D: Cartilage oligomeric matrix protein:A novel marker of cartilage turnover detectable in synovial fluid and blood. *Br J Rheumatol* 1992; 31:583-91.

28. JOOSTEN LAB, HELSEN MMA, SAXNE T, VAN DE LOO FAJ, HEINEGARD D, VAN DEN BERG WB: IL-1 blockade prevents cartilage and bone destruction in murine type II collagen-induced arthritis, whereas TNF-blockade only ameliorates joint inflammation. *J Immunol* 1999; 163: 5049-55.

29. HORAI R, SAIJO S, TANIOKA H *et al.*: Development of chronic inflammatory arthropathy resembling rheumatoid arthritis in interleukin 1 receptor antagonist-deficient mice. *J Exp Med* 2000; 191: 313-20.

30. VAN DEN BERG WB: Anti-cytokine therapy in chronic destructive arthritis (review). *Arthritis Res* 2001; 3: 18-26.

31. NISHIMOTO N, KISHIMOTO T, YOSHIZAKI K: Anti-interleukin 6 receptor antibody treatment in rheumatic disease (review). *Ann Rheum Dis* 2000; 59: i21-i27.

32. STRAUB RH, CUTOLO M: Involvement of the hypothatalmic-pituitary-adrenal/gonadal axis and the peripheral nervous system in rheumatoid arthritis (review). *Arthritis Rheum* 2001; 44: 493-507.