INFECTION AND IMMUNITY, Dec. 1991, p. 4436–4442
0019-9567/91/124436-07$02.00/0

Vol. 59, No. 12

Pro- and Anti-Inflammatory Roles of Interleukin-1 in Recurrence of Bacterial Cell Wall-Induced Arthritis in Rats

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Received 10 June 1991/Accepted 10 September 1991

A specific interleukin-1 (IL-1) receptor antagonist (IL-1ra) was used to examine the roles of IL-1 in an experimental model designed to analyze the reactivation phase of erosive arthritis, induced in rats with peptidoglycan-polysaccharide polymers (PG-APS) isolated from cell walls of group A streptococci. Monoarticular arthritis was initiated by injection of a small dose of PG-APS into an ankle joint, and reactivation was induced by intravenous injection of PG-APS 20 days later. Human recombinant IL-1ra given at a dose of 2 to 3 mg/kg at the time of reactivation of arthritis and at 6-h intervals inhibits the increase in joint swelling by at least 60%. Joint swelling is suppressed 30 to 50% when the initial treatment with IL-1ra is delayed until 6 h after reactivation. IL-1ra is not effective when the initial injection is delayed 12 or 24 h. With an injection schedule of IL-1ra given at the time of reactivation and every 6 h, treatment can be stopped at 24 h and the suppression of swelling is no different from that in rats for which injections are continued for 4 days. The results indicate that IL-1 has a prominent, although not exclusive, role in initiating inflammation in this model and is involved in the amplifying processes in progressive inflammation and chronic erosive disease. An anti-inflammatory function of IL-1 is also indicated from data showing that IL-1ra treatment limited to 6 h or less after the induction of reactivation enhances joint swelling, whereas intravenous injection of human recombinant IL-1β 24 h before reactivation suppresses the reactivation of arthritis.

A single systemic injection of rats with an aqueous suspension of purified peptidoglycan-polysaccharide polymers (PG-APS) isolated from cell walls of group A streptococci can induce inflammation of peripheral joints characterized by repeated episodes of waxing and waning and progressing over a period of several months to joint destruction (9, 19). The most striking feature of this disease is the spontaneous recurrence of inflammation which, along with histological and radiological findings, resembles human rheumatoid arthritis (7, 10). Although most of the peripheral joints become involved following systemic injection of PG-APS, it is difficult to analyze the molecular events regulating recurrence, because recurrence is random and unpredictable for a given joint. Therefore, we have modified this model to synchronize reactivation. Monoarticular arthritis is induced by intra-articular (i.a.) injection of an ankle joint with a small dose of PG-APS, producing a synovitis which reaches a peak in 24 h and then recedes over the next several days (14). Reactivation is induced by intravenous (i.v.) injection of a normally subarthropathic dose of PG-APS (13) or lipopolysaccharide (23) 1 to 8 weeks later, producing a more prolonged, severe, chronic inflammation confined to the joint previously injured by PG-APS.

The involvement of interleukin-1 (IL-1) in inflammatory arthritis is suggested by reports of increased levels of IL-1 in joint effusions from rheumatoid arthritis patients (29) and a correlation of plasma levels with disease activity (11). i.a. injection of rabbits (18), mice (27), and rats (22) demonstrates the capacity of IL-1 to initiate inflammation which, however, is not sustained. We have reported that IL-1α or IL-1β can reactivate arthritis in a joint injured previously by PG-APS and that repeated injections can lead to marginal cartilage erosion (22).

Recent reports have described an IL-1 receptor antagonist (IL-1ra) produced by human monocytes grown on adherent immune complexes (1, 2). A cDNA encoding this protein has been isolated and expressed in *Escherichia coli* (12). The cDNA for an IL-1 inhibitor has subsequently been cloned from U937 cells (5), and this predicted protein is identical in sequence to that reported by Hannum et al. (15). The biological activity of the recombinant protein has been demonstrated in vitro by inhibition of IL-1 stimulation of prostaglandin E_2 production by human foreskin fibroblast cells (12, 15), synovial cells, and chondrocytes (3) and by inhibition of collagenase secretion by synovial cells stimulated by IL-1 (3). IL-1ra can block IL-1 action in vivo (17), prevent septic shock and death induced in rabbits by endotoxin (17) or *E. coli* (28), and reduce acute pulmonary inflammation induced by intratracheally administered endotoxin (26). We report here the use of this inhibitor of IL-1 activity in an examination of the role of IL-1 in the course of a progressive, chronic inflammatory response, namely, the bacterial cell wall model of reactivation of inflammatory arthritis. Two assessments, joint swelling and histological evidence of cartilage and bone erosion, were used.

MATERIALS AND METHODS

Preparation of PG-APS. *Streptococcus pyogenes* type 3 strain D-58 was grown in Todd-Hewitt broth (BBL, Cockeysville, Md.) to the early stationary phase. Cells were harvested, washed by centrifugation, and broken in a Dyno-Mill (Glen Mills, Inc., Maywood, N.J.). The cell wall fragments were collected by differential centrifugation, extracted three times with 2% sodium dodecyl sulfate, washed

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extensively with pyrogen-free water, and lyophilized. The cell wall particles, consisting of large PG-APS polymers, were further fragmented by sonication and separated by centrifugation into a fraction referred to as 100p PG-APS, which was soluble at 10,000 × *g* but sedimented at 100,000 × *g*. PG-APS was quantitated on the basis of its rhamnose content, which was approximately 30%. Aseptic techniques were used throughout. The details of the methods used to monitor purification have been recently described (24).

Induction of recurrence of arthritis. Female Lewis rats (Charles River, Triangle Park, N.C.) weighing about 175 g were injected i.a. under ether anesthesia above the calcaneus through the Achilles tendon into the tibiotalar (ankle) joint on day 0 with 2.0 μg of rhamnose equivalents (approximately 6.0 μg [dry weight]) of 100p PG-APS suspended in 10 μl of pyrogen-free saline as described previously (5, 6). Right or left joints were injected with PG-APS in alternate animals, and contralateral joints were injected with 10 μl of pyrogen-free saline. Thus, in each group, one half of the animals were given PG-APS in the right joint and saline in the left joint, and the other half received the opposite treatments. On day 20 all rats (average weight at this time, 195 g) were injected i.v. with 100p PG-APS in a dose of 100 μg of rhamnose to induce a recurrence of arthritis in the joint injured earlier by PG-APS. The increase in the diameter of saline-injected joints was minimal and transient, rarely exceeding 0.2 mm at 24 h. Rats were randomly divided into groups of 8 to 12 animals on day 0 and designated to be treated with vehicle control (pyrogen-free phosphate-buffered saline [PBS] or human serum albumin [HSA]) or with IL-1ra on day 20. We have seen no difference between control groups given repeated subcutaneous (s.c.) injections of PBS or 2 mg of HSA per kg every 6 h for 7 days post-i.v. PG-APS. This schedule of HSA neither affected reactivation of arthritis by PG-APS nor itself induced reactivation of arthritis.

The lateral diameter of the ankle joints was measured with a Fowler Ultra-Cal II digital caliper (Lux Scientific Instrument Corp., New York, N.Y.). The precision of this method has been described previously (22, 24). To reduce bias, we coded all groups, and the person holding the rat selected the animal from cages at random and presented it so that no identification was visible to the person making the measurement. Joints were measured before i.a. and i.v. injections and daily thereafter. The data are expressed in Fig. 1 as joint diameter to illustrate the course of the disease and in subsequent figures as the change in the joint diameter from the pre-i.v. measurement, providing a control for individual animal variations.

For the histological study of joint sections, rats were killed with CO_2 and ankle joints were removed, skinned, fixed in Formalin, decalcified, embedded in paraffin, sectioned sagitally, and stained with hematoxylin and eosin. To limit sampling variability, we marked the middle of each tissue from the center of the heel with black ink before embedding was done, and rough cuts were made to this mark before sections were removed for staining. Sagital sections through or near the center of each ankle joint were graded 0 to 4+ for the severity of synovitis in the involved joint spaces, the degree of bursitis surrounding the Achilles tendon, and the amount of cartilage and bone erosion in the ankle joint. Observer bias was reduced by blindly reevaluating each coded section after the initial histological assessment.

Statistical analysis. The level of significance of differences between groups was calculated by Student's two-tailed *t* test.

IL-1ra. Human recombinant IL-1ra was prepared from lysates of *E. coli* transformed with a plasmid containing a modified IL-1ra cDNA under the control of the *tac* promoter. The expression of IL-1ra was induced by the addition of isopropylthio-β-galactoside for several hours, and the cells were collected by centrifugation and lysed in a pressure cell. The recombinant protein was purified by successive cation- and anion-exchange chromatography and was found more than 95% pure by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and by reverse-phase high-pressure liquid chromatography. It contained less than 0.2 endotoxin units per mg of protein, as measured by the *Limulus* clotting assay. The biological and physical properties of this protein have been described in a series of papers (1–3, 5, 12, 15). IL-1ra was stored frozen as a sterile solution in PBS (pH 7.0).

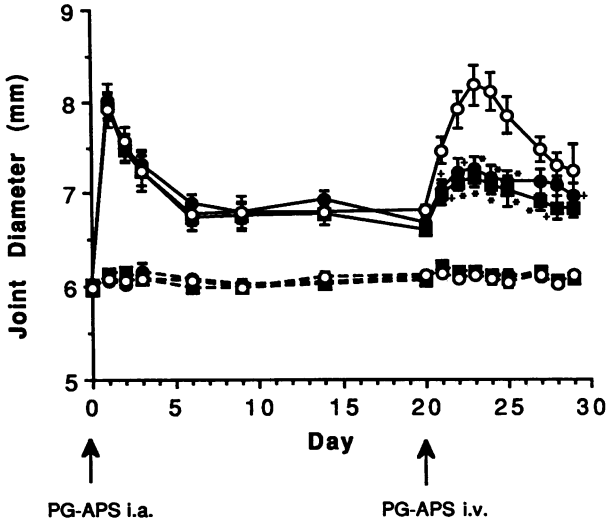

FIG. 1. Effect of IL-1ra on recurrence of joint inflammation. One ankle joint of each rat was injected i.a. with 100p PG-APS on day 0 to induce initial joint inflammation, and the contralateral ankle joint was injected with 10 μl of pyrogen-free saline. On day 20, all rats were injected i.v. with 100p PG-APS to induce a recurrence. Rats were divided into groups of eight and, beginning on day 20, were injected with IL-1ra in accordance with the following schedules: group I, vehicle control, 0.2 ml of PBS (pH 7.0) i.p. 5 min before reactivation and then s.c. every 6 h for 84 h (○); group II, IL-1ra, 2.0 mg/kg i.p. 5 min before reactivation and then s.c. every 6 h for a total of 15 injections over 84 h (●); group III, IL-1ra, 8.0 mg/kg i.p. 5 min before reactivation and then s.c. every 12 h for a total of 8 injections over 84 h (■). *, $P < 0.01$; +, $P < 0.05$. Bars represent 1 standard error of the mean. The increase in the diameter of saline-injected joints is shown by the broken lines. Rats were sacrificed 9 days after reactivation (day 29) to obtain joint specimens for histopathology.

RESULTS

Proinflammatory role of IL-1 in the recurrence of joint swelling. To determine whether IL-1 is involved in the reactivation of articular inflammation by PG-APS, we treated rats with IL-1ra immediately before the i.v. injection of PG-APS and at different times thereafter. When IL-1ra was given in a dose of 2 mg/kg at the time of PG-APS injection and at intervals of 6 h over a period of 84 h, the increase in joint swelling was reduced by as much as 64%, with an average of 55% inhibition over the first 5 days after reactivation (Fig. 1). Joint swelling was also effectively

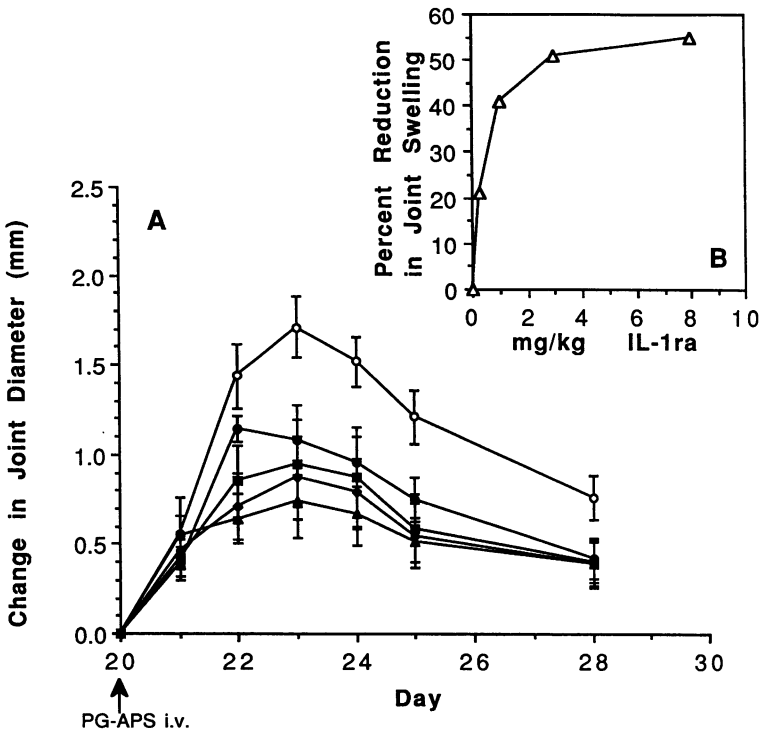

FIG. 2. (A) Effect of dose of IL-1ra on recurrence of joint inflammation. Results for the joints injected i.a. with PG-APS on day 0 are shown as increased joint diameter (difference between pre- and postreactivation) after reactivation with PG-APS i.v. on day 20; there were eight rats per group. IL-1ra was injected i.p. 5 min before reactivation and then s.c. at 4 h, 10 h, and every 12 h for 94 h at 0 (○), 0.3 (●), 1.0 (■), 3.0 (◆), and 8.0 (▲) mg/kg. All mean values for each group from days 22 to 28 were significantly lower than control group values at $P < 0.05$ or less, except for 0.3 mg of IL-1ra at 22 days. Rats were sacrificed for histopathology 9 days after reactivation (day 29). The changes in the diameter of saline-injected joints were minimal and are not shown. (B) Percent inhibition of recurrence as a function of dose of IL-1ra 2 days after reactivation (day 22). Data from 2 to 5 days after reactivation are comparable.

reduced when the interval between injections was 12 h, when an initial injection was given at the time of induction of the recurrence, and when a dose of IL-1ra of 8 mg/kg was used (Fig. 1). When the intervals were increased to 24 h, for a total of four injections over 96 h, reactivation was not suppressed, even with a dose of 32 mg/kg per injection (data not shown). These results indicate that IL-1 plays a critical role in the early stages of recurrence of joint swelling and that, when IL-1 activities are unchecked for an interval of more than 12 h, it is no longer possible to intervene by blocking only IL-1 binding to its receptor.

Consistent with the assumption that IL-1ra works through blocking of the IL-1 receptor is the observation that the suppression of the increase in ankle joint swelling was dependent on the dose of IL-1ra, which was effective over a range of 0.3 to 8.0 mg/kg, employing the schedule described in the legend to Fig. 2. The maximum suppression of ankle joint swelling was 55% and the 50% effective dose was 0.5 mg/kg per injection with this schedule.

To further establish at what stages of the evolving process IL-1 plays a prominent role, i.e., initiating and/or maintaining inflammation, we next determined how long the initial IL-1ra treatment can be delayed or how long it must continue to be given. Definite but reduced suppression (average inhibition from days 1 to 11 after reactivation, 38%) was achieved when the initial injection of IL-1ra was delayed until 6 h after the reactivation stimulus, at which time the increase in joint swelling was already evident (Fig. 3A, group II). When the initial injection of IL-1ra was delayed until 12 or 24 h after the reactivation stimulus, further recurrence of inflammation was not suppressed (data not shown). Thus, although IL-1 plays an important role in early joint swelling, by 12 h other pathways have become involved and blocking only IL-1 is not sufficient.

To try to answer the question of the requirement for IL-1 in sustaining more prolonged inflammation, we determined the effect of stopping IL-1ra treatment at 24 h. When IL-1ra was begun at the time of PG-APS reactivation and continued every 6 h at a dose of 2 mg/kg, treatment could be stopped at 24 h and the subsequent course of joint swelling was no different from that in rats for which injections were continued every 6 h for 4 days (Fig. 4). Thus, continued blocking of IL-1 beyond 24 to 36 h is not required for continued suppression of soft tissue swelling.

Anti-inflammatory role of IL-1. We were surprised to find that when IL-1ra administration was limited to a more brief period, there was a trend toward enhanced rather than depressed inflammation. When IL-1ra treatment was stopped at 6 h after reactivation, joint swelling became more severe than in the vehicle control group (Fig. 3A). This difference from the control group was consistent and statistically significant at day 4. The severity of joint swelling in the group in which IL-1ra was stopped at 6 h after reactivation was significantly greater on all days from 1 to 11 than it was in the group in which IL-1ra injections began at 6 h (Fig. 3A).

In a separate experiment, in which injections of IL-1ra were given at the time of reactivation and only at 2 h later

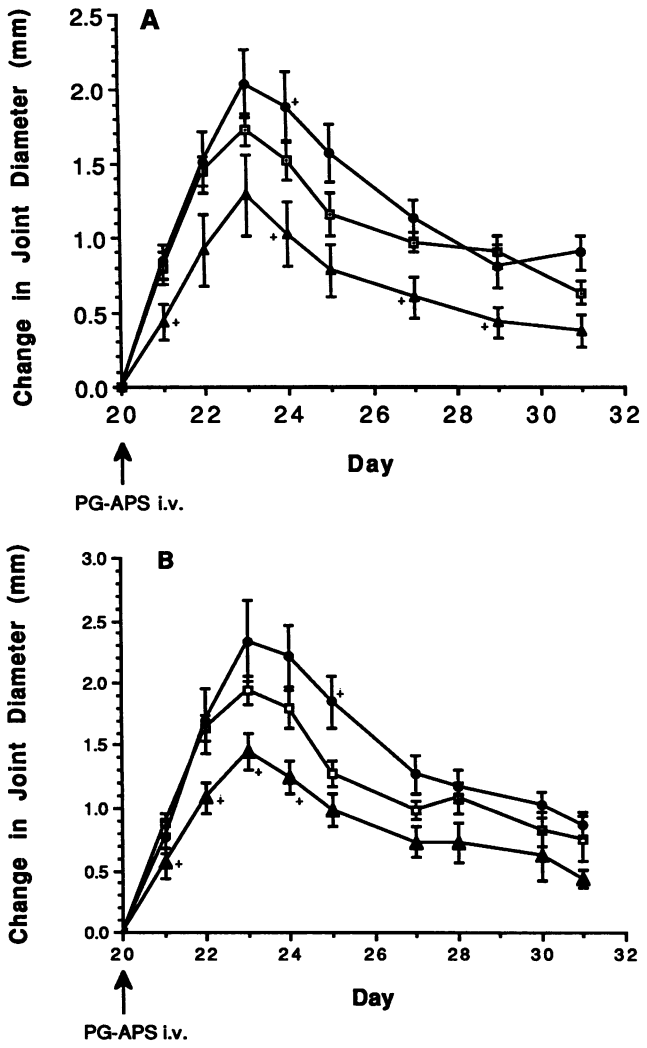

FIG. 3. (A) Comparison of IL-1ra treatment stopped at 6 h or begun at 6 h after reactivation. Rats were injected i.a. on day 0 with PG-APS or saline in the contralateral joints. The changes in the diameter of the PG-APS-injected joints following i.v. PG-APS administration on day 20 were recorded; there were eight rats per group. Symbols: ●, group I, IL-1ra, 3.0 mg/kg s.c. immediately before PG-APS i.v. and then 2 and 6 h later; ▲, group II, IL-1ra, 3.0 mg/kg s.c. begun 6 h after PG-APS i.v. and continued every 6 h for 24 h and then every 12 h for 96 h; □, group III, PBS s.c. on the same schedule as that of group II. Bars are standard errors of the mean. The two treatment groups (I and II) were different from each other at $P < 0.05$ or less at all times. +, $P < 0.05$ compared with control group III. (B) Effect of IL-1ra treatment stopped 2 h after reactivation compared with more prolonged treatment. PG-APS was injected as in panel A (eight rats per group). Symbols: ●, group I, IL-1ra, 3.0 mg/kg s.c. immediately before PG-APS i.v. and 2 h later; ▲, group II, IL-1ra 3.0 mg/kg s.c. begun 4 h after PG-APS i.v. and then given at 10 h and continued every 12 h for 10 days; □, group III, PBS s.c. on the same schedule as that of group II. Bars are standard errors of the mean. The two treatment groups (I and II) were different from each other at $P < 0.05$ or less at all times, except for days 21 and 30. $P < 0.05$ compared with control group III.

(Fig. 3B), joint swelling was again consistently more severe than it was in the vehicle control group (significant at day 5) and the severity was significantly greater from days 2 to 11 than it was in the group given more prolonged IL-1ra

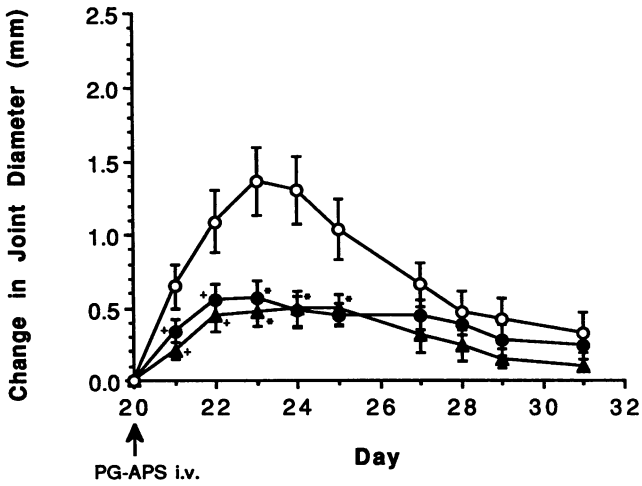

FIG. 4. Effect of stopping treatment with IL-1ra 24 h after reactivation. Rats were injected i.a. on day 0 with PG-APS or saline in the contralateral joints. The changes in the diameter of the PG-APS-injected joints following i.v. PG-APS administration on day 20 were recorded; there were eight rats per group. Symbols: ▲, group I, IL-1ra, 2.0 mg/kg i.p. immediately before PG-APS i.v. and then s.c. every 6 h for 24 h, and then PBS every 6 h for 84 h; ●, group II, IL-1ra, on the same schedule as that of group I, except that IL-1ra was continued for 84 h; ○, group III, PBS on the same schedule as that of group I. *, $P < 0.01$; +, $P < 0.05$ (both compared with PBS control).

treatment (Fig. 3B). In another experiment, in which IL-1ra was injected only once, at the time of reactivation, joint swelling was not different from that in the control group at any time in the succeeding 16 days (data not shown).

These results suggested that since brief, early suppression of IL-1 activity by IL-1ra actually increased joint inflammation, endogenous IL-1 might play a role in down-regulating some facets of the very early inflammatory response. Both pro- and anti-inflammatory consequences were detected when IL-1β was injected i.v. 24 h before reactivation by i.v. injection of PG-APS (Fig. 5). Injection of 1.0 μg of human recombinant IL-1β i.v. induced renewed swelling, which was detectable by 1 h and reached a peak at 6 h, in the joint previously injected i.a. with PG-APS. However, the recurrence of joint inflammation subsequently induced by i.v. injection of PG-APS was significantly lower than that in the control group at 3 and 6 days after the induction of a recurrence (Fig. 5).

Effect of IL-1ra on histological changes in joint tissue. Destruction of cartilage and bone is a feature of rheumatoid arthritis and of joint disease induced by PG-APS in rats. In vitro, IL-1ra can prevent the IL-1-induced resorption of cultured bone (20). Therefore, we assessed the influence of IL-1ra on the cartilage and bone erosion accompanying arthritis induced by PG-APS. Histological sections of decalcified ankle joints were prepared from rats sacrificed 4 to 11 days after reactivation. The severity of changes in the synovium, tendons, cartilage, and bone was scored blindly from coded slides. Vehicle-treated positive control rats had extensive synovitis, particularly around the ankle joints, with infiltration of the stroma by mononuclear cells and infiltration of the joint space by neutrophils. Chronic inflammation involved the entire synovial membrane up to and usually including the edge of the articular cartilage. In seven experiments in which ankle joints from positive control rats

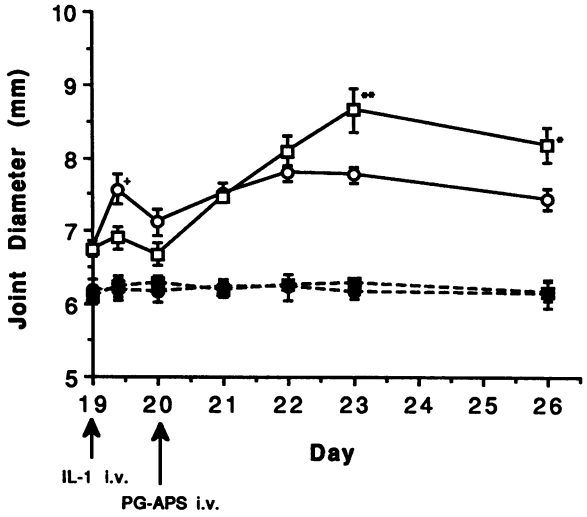

FIG. 5. Recurrence of joint inflammation induced by IL-1 and suppression of subsequent reactivation induced by PG-APS. The mean diameter of the joints ± the standard error of the mean for eight rats per group was recorded. Rats were injected i.a. on day 0 with PG-APS. Saline was injected into the contralateral ankle joints. On day 19, rats in group I (○) were injected i.v. with 1.0 μg of human recombinant IL-1β and rats in control group II (□) received PBS. A transient increase in joint swelling, peaking at 6 h ($P < 0.03$), was induced in the PG-APS-treated joints by IL-1. At 24 h later (day 20) rats in both groups were injected i.v. with PG-APS. **, $P < 0.001$; *, $P < 0.02$; +, $P < 0.03$ (group I versus group II). Solid symbols indicate the mean diameter of joints injected with saline.

were examined microscopically, 51 of 70 joints (73%) also had early damage of the articular surfaces, with marginal erosion of cartilage and sometimes adjacent bone (Fig. 6).

The joints of rats treated with IL-1ra had the same morphological changes as did control joints, but the severity of synovitis and the incidence of erosion were lower (Fig. 6A and B). The reduction in the severity of cartilage and bone erosion depended on the intensity of the suppression of IL-1 activity. As noted above, although less intense schedules of IL-1ra treatment could reduce joint swelling, they did not convincingly affect the less sensitive and precise histological assessment. Thus, in comparison with an untreated positive control group, the average score for the severity of synovitis was reduced 63% in a group injected with IL-1ra (2 mg/kg) 5 min before and 2 and 6 h after reactivation and then every 6 h for 4 days. The incidences of cartilage erosion were three of nine in the treated group and seven of nine in the control group. In another experiment with a comparable schedule of IL-1ra, the incidence of erosion was reduced from 8 of 11 to 4 of 11 and the average score for the severity of synovitis was reduced 59%. In these experiments, joint swelling was also reduced 50 to 60%. However, with a smaller dosage (2 mg/kg at 4 and 10 h after reactivation and then every 12 h for 80 h), the reduction in the severity of synovitis was only 30% and the incidences of erosion were the same in both the control and treated groups. With even less frequent treatment (8.0 mg of IL-1ra per kg 5 min before reactivation and then every 12 h for 84 h), the reduction in histological severity was only 11% and the incidences of ankle joints with marginal erosion were five of eight in the treated group and six of eight in the control group, despite the fact that joint swelling was significantly suppressed.

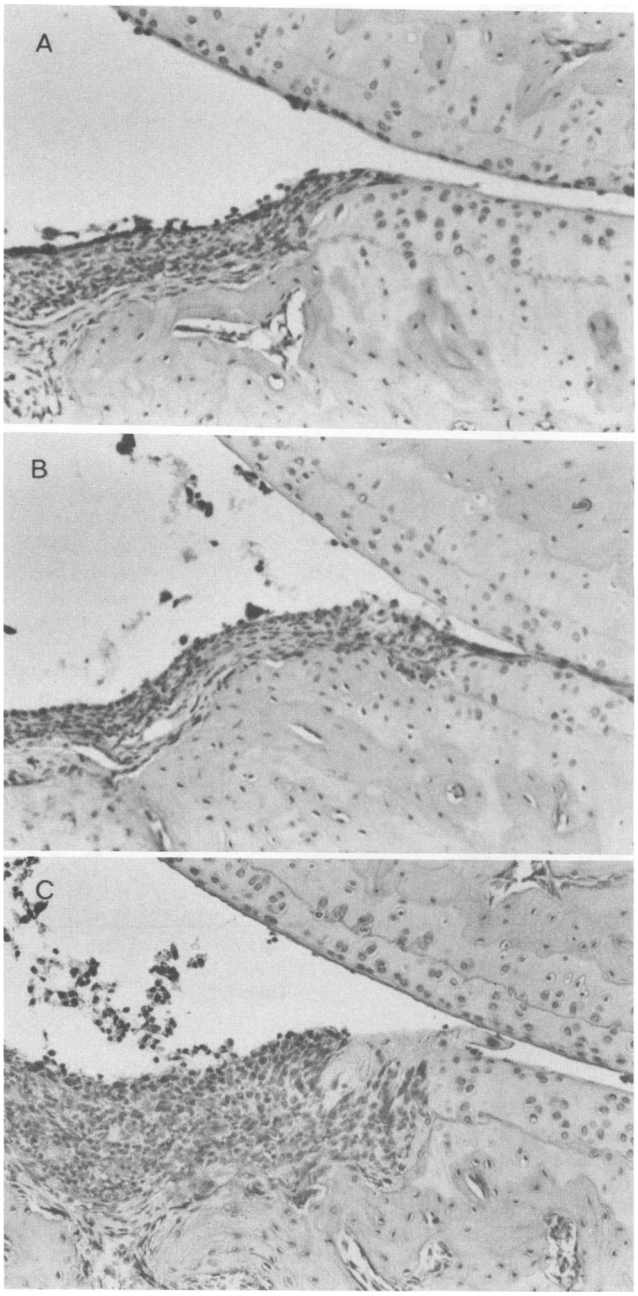

FIG. 6. Inflammation of the posterior edge of ankle joints from three rats (from the same experiment) injected i.a. with PG-APS on day 0 and i.v. with PG-APS on day 20 and sacrificed 8 days later. (A) IL-1ra (1.0 mg/kg) injected s.c. 1 h before PG-APS i.v. and at 10 min, 2 h, and 6 h and then every 6 h for 4 days post-PG-APS. The synovitis extends from the left up to the edge of the articular cartilage but does not erode it (1+ synovitis, 0 erosion). The opposing cartilage surface is uninvolved. (B) Ankle joint from another PG-APS-injected arthritic rat treated with the same regimen of IL-1ra. The inflammatory reaction in the synovium extends into the edge of the cartilage, with fragmentation and erosion of the articular surface. The underlying bone is intact (1+ synovitis, 1+ erosion). (C) Ankle joint from a PG-APS-injected arthritis-positive control rat injected with buffered saline on the same schedule as that described for panel A. The inflammatory process has led to destruction of the edge of the cartilage and erosion of the underlying bone (2+ synovitis, 2+ erosion). All sections were stained with hematoxylin and eosin. Magnification, ×130.

DISCUSSION

IL-1 has been implicated in rheumatoid arthritis by results indicating that circulating IL-1 levels increase in arthritis patients (11), by the measurement of IL-1 in joint effusions (29), and by the observation that inflammatory cell accumulation and cartilage degradation can be induced by i.a. injection of IL-1 in rabbits (18), mice (27), and rats (22). However, it is likely that a large number of cytokines and other mediators, acting antagonistically or in concert and in an uncertain sequence, determine the course of a process as complex as human rheumatoid arthritis or the experimental arthritis that we examined. Direct evidence of the importance of IL-1 in inflammatory arthritis required a specific inhibitor which could function in vivo. IL-1ra binds to the class of IL-1 receptor found on rodent T cells, fibroblasts, and endothelial cells and less avidly to the class of receptor found on rodent pre-B cells, B cells, and neutrophils (4, 6, 15). This may be an additional reason why IL-1ra is unable to provide more complete suppression of joint inflammation. Nevertheless, our results indicate that IL-1 is one of the primary regulators in an animal model of arthritis, as indicated by the capacity of the specific inhibitor to reduce the reactivation of inflammation by 60% when given at the time of reactivation and continued at 6-h intervals. This reduction is significant for an agent which blocks only one component in the milieu of inflammatory mediators. Furthermore, because IL-1ra has limited biological persistence in tissues of rodents, in contrast to neutralizing antibodies, for example, it can be used as a "pulse" inhibitor. Therefore, it is feasible to use this agent to help determine at which points in evolving inflammation IL-1 has important roles.

IL-1ra treatment limited to only the first 6 h or less after reactivation does not suppress the increase in joint swelling; in fact, inflammation is enhanced (discussed below). On the other hand, when the initiation of IL-1ra treatment is delayed for 6 h after the induction of a recurrence of arthritis, some protection is still provided, but effectiveness is reduced. No protection is provided when treatment is delayed for more than 6 h or when the interval between injections of IL-1ra exceeds 12 h. This result means that although IL-1 has a role in the early stages of inflammation in this model, blocking IL-1 only over the first 6 h is not sufficient; rather, IL-1 is also involved in the amplifying and recruiting processes (6 to 24 h) important in evolving inflammation, as suggested by Pettipher et al. (18). With sufficiently intense treatment, IL-1ra injections could be stopped at 24 h, with no emergence of joint swelling over the next 10 days.

We conclude that when early IL-1 activity is adequately suppressed, alternate or dependent pathways are minimally activated. However, when the suppression of early IL-1 activity is inadequate, delayed for 12 h, or unchecked for an interval exceeding 12 h, alternate pathways become involved in maintaining inflammation, so that blocking only IL-1 binding to its receptor is no longer sufficient to influence the process. An alternative interpretation could be that early unchecked IL-1 production may induce more IL-1 receptor expression, thus decreasing the effectiveness of IL-1ra. While these interpretations apply to the acute phase reflected in joint swelling (edema, infiltration, and synovitis), an early and continued intensive schedule of IL-1ra is required to reduce the incidence and severity of cartilage and bone erosion. Thus, IL-1 is involved not only in directing the initial phases of the inflammatory response but also in participating in the maintenance of the chronic reactions leading to bone and cartilage destruction.

The findings that IL-1ra administration limited to the first 6 h actually increases reactivation of joint swelling and that a single injection of IL-1 24 h before reactivation decreases joint swelling indicate that in certain circumstances IL-1 can function as a down-regulator of inflammation. Although subject to alternative interpretations, such as altered expression of IL-1 receptors or endogenous inhibitors, our results are consistent with other reports that IL-1 can have an anti-inflammatory function. Jacobs et al. (16) reported that three weekly i.a. injections of 100 ng of IL-1 reduced the joint swelling induced by subsequent injection of methylated bovine serum albumin in a model of antigen-induced arthritis in rats. Using a model of colitis in rabbits, Cominelli et al. (8) depressed inflammation by injecting IL-1 24 h (but not 30 min) before the induction of colitis. The timing and route of injection of IL-1 are important since, in our studies, i.v. injection 1 h before PG-APS or i.a. injection 24 h before PG-APS did not influence the recurrence of joint swelling (18a).

It is not clear which, if any, of the many activities of IL-1 in vivo are responsible for the proposed anti-inflammatory effect. The fact that IL-1 is effective 24 h prior to the induction of recurrence suggests that suppression is a consequence of a cascade triggered by IL-1. Ulich et al. (25) have reported that IL-6 can inhibit tumor necrosis factor and IL-1 expression and have proposed that IL-6 may be part of a negative feedback mechanism. Cominelli et al. (8) have ascribed suppression induced by IL-1 in the colitis model to prostaglandin production, since suppression correlated with colonic prostaglandin E_2 production and was reversed by ibuprofen.

The model of reactivation of arthritis in a previously injured joint by i.v. injection of a relatively small dose of PG-APS is a self-limiting disease, possibly because there is no reservoir of PG-APS in the liver or spleen (13, 19). This self-limiting arthritis is in contrast to the progressive, destructive arthritis characteristic of our earlier model of streptococcal cell wall-induced arthritis produced by a single intraperitoneal (i.p.) injection of PG-APS (9, 19). However, the PG-APS reactivation model has the features of a chronic inflammatory process, since the histopathology reveals extensive infiltration of the synovium with mononuclear cells and since reactivation requires T lymphocytes and can be suppressed by cyclosporin A (24a). The distinct advantage of the reactivation model is that it provides predictable synchronized recurrences, allowing more precise analysis of the regulation of recurrences. In addition, because it is of a shorter duration (1 to 2 weeks versus several months), the relatively brief administration of a specific inhibitor can yield significant results.

IL-1ra provides a unique tool for dissecting the functions of IL-1 in vivo. IL-1ra is probably part of a physiological system for regulating normal and pathological activities of IL-1 and other cytokines, since it is an endogenous inhibitor of IL-1, is made by the same cells which secrete IL-1 (15), and appears to be the same molecule as that found in the urine of human febrile patients and patients with myelomonocytic leukemia (21).

ACKNOWLEDGMENTS

This work was supported by grant AR 39480 from the National Institutes of Health and funds from Synergen, Inc., Boulder, Colo.

We thank T. A. Ayers for excellent technical assistance.

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