Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 8749–8753, November 1986
Medical Sciences

Interleukin 1 induces leukocyte infiltration and cartilage proteoglycan degradation in the synovial joint

(endotoxin/icosanoids/rheumatoid arthritis)

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Communicated by John Vane, July 14, 1986

ABSTRACT **Interleukin 1 (IL-1) is a polypeptide released by activated macrophages and is thought to be a key mediator of host responses to infection and inflammation. The availability of highly purified and recombinant material has now permitted the evaluation of IL-1 as a mediator of chronic inflammatory processes *in vivo*. We have demonstrated that intraarticular injection of IL-1 into rabbit knee joints induces the accumulation of polymorphonuclear and mononuclear leukocytes in the joint space and the loss of proteoglycan from the articular cartilage. The effects on cartilage could not be explained solely by the presence of leukocytes, since injections of endotoxin also stimulated leukocyte accumulation in the joint but had no effect on proteoglycan loss. Responses to IL-1 were not associated with increased production of the icosanoids prostaglandin E_2 or leukotriene B_4 and were not reduced by an inhibitor of their synthesis. The pattern of leukocyte infiltration and cartilage breakdown 24 hr after IL-1 injection was similar to that seen in animals with antigen-induced arthritis of 1 week's duration. These observations support the hypothesis that IL-1 acts directly to mediate the erosive processes of chronic arthritis.**

The interest in the role and biological activity of soluble products derived from mononuclear phagocytes has led to the detection of a number of factors, including endogenous pyrogen and lymphocyte-activating factor (LAF). The subsequent purification of these substances indicated a similar structural identity and the term interleukin 1 (IL-1) is now used to describe these closely related molecules (1). IL-1 plays a role in several pathophysiological events, often through the production of a secondary mediator. In the host response to infection, for example, the release of prostaglandin E_2 (PGE_2) by IL-1 mediates the development of fever (2). In addition, it has also been suggested that leukotrienes may be involved in IL-1-stimulated lymphocyte activation (3).

The proposal that IL-1 plays a major part in the erosive processes of chronic inflammation is based on a separate line of investigation. A substance isolated from synovial lining cultures and termed catabolin was found to promote cartilage breakdown (4). When purified to homogeneity, catabolin was shown to have properties identical to IL-1 (5). It is now known that IL-1 stimulates the release of metalloproteinases, such as stromelysin and collagenase, from synovial cells and chondrocytes in culture and that these enzymes degrade cartilage matrix (6). In these cultures, IL-1 also stimulates PGE_2 production (7).

We have now injected IL-1 into the knee joints or skin of New Zealand White rabbits and have measured inflammatory changes at intervals up to 3 days. Following intraarticular injection of IL-1, joint swelling, leukocyte accumulation, cartilage degradation, and icosanoid synthesis were measured. The responses induced by IL-1 were compared with changes seen in the joints of rabbits with antigen-induced arthritis (8) 1–14 days after antigen challenge. The effects of IL-1 on plasma exudation and leukocyte accumulation in rabbit skin were also compared with those induced by bradykinin (BK), a vascular permeability-increasing agent, and *N*-formyl-Met-Leu-Phe (fMet-Leu-Phe), a chemotactic agent.

MATERIALS AND METHODS

Intraarticular Injection of IL-1. Adult male New Zealand White rabbits (weight, 2.5–3.5 kg) were used. Highly purified or recombinant IL-1 (Genzyme, Suffolk, U.K.) was injected through the suprapatellar ligament into the joint space and the contralateral joint received an equal volume of the appropriate vehicle. The highly purified material was supplied in solutions of 100 units/ml in 5% fetal calf serum with a specific activity (quoted by Genzyme) of 8 units/pg of protein. Stock solutions were diluted with saline to give a constant injection volume of 0.5 ml and similar dilutions of fetal calf serum were made for control injections. Highly purified IL-1 was used in all of the experiments described, but in one experiment its activity after intraarticular injection was compared with recombinant β-IL-1. Recombinant β-IL-1 was supplied in solutions of 1000 units/ml in 0.1% ultrapure bovine serum albumin with a specific activity (quoted by Genzyme) of 0.1 unit/pg of protein. The contralateral joints received appropriate dilutions of bovine serum albumin vehicle.

Animals were killed 4 hr, 24 hr, or 3 days after a single injection and the joint diameters were measured with calipers. The joint space was then washed with 1 ml of saline and the resultant joint fluids from control and IL-1-injected joints were taken for total and differential leukocyte counts and for the assay of specific icosanoids. Samples of synovial lining were dissected and placed in buffered formalin for subsequent paraffin wax embedding and routine histological sectioning. The articular cartilage was dissected from the ends of the femurs for the assay of proteoglycan content. Blocks of articular cartilage, cut from the femoral condyles, were mounted in Tissue Tek (Bright, Cambridge, U.K.) and frozen in *n*-hexane at −70°C. Longitudinal sections cut through the cartilage were stained with safranin O to localize proteoglycan (9).

Assay of Icosanoids. PGE_2 and leukotriene B_4 (LTB_4) were assayed in the cell-free unextracted joint fluids as described (10, 11). Briefly, rabbit antiserum to each icosanoid was incubated with test samples and tritiated standards. Free radioactive material was removed with dextran-coated charcoal and the residual bound activity was measured in a scintillation spectrometer.

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Abbreviations: IL, interleukin; PMN, polymorphonuclear leukocytes; BK, bradykinin; fMet-Leu-Phe, *N*-formyl-Met-Leu-Phe; PGE_2, prostaglandin E_2; LTB_4, leukotriene B_4; LAF, lymphocyte-activating factor.

Assay of the Proteoglycan Content of Articular Cartilage. Articular cartilage was dissected from the ends of the femurs, blotted dry, weighed, and digested with papain at 65°C for 1 hr and the concentration of sulfated glycosaminoglycan (the polysaccharide side chains of proteoglycan) was measured by the 1,9-dimethylmethylene blue binding assay (12). The proteoglycan content was expressed as μg of glycosaminoglycan per mg of wet weight of cartilage and the IL-1-injected joint was compared with its contralateral control. The proteoglycan content was also determined relative to the collagen content of the cartilage in some experiments. The collagen content was assessed by measurement of the hydroxyproline content of the papain digest on an amino acid analyzer.

Control Experiments. The LAF activity of IL-1 is inhibited by the arginine binding agent phenylglyoxal (13). A solution containing 20 units of highly purified IL-1 per ml in 200 mM imidazole buffer (pH 8.0) containing 1% phenylglyoxal (Sigma) was incubated at room temperature for 4 hr. An identical solution, minus phenylglyoxal, was left at room temperature for 4 hr. Both solutions were then dialyzed against phosphate-buffered saline (0.137 M NaCl/0.003 M KCl/0.008 M Na_2HPO_4/0.016 M KH_2PO_4) at 4°C overnight. After filter sterilization these solutions either were tested in the LAF assay (14) or were injected intraarticularly. LAF activity was determined in cultures of thymocytes from C3H/HeJ mice aged 4–6 weeks. The cells were cultured in flat-bottomed 96-well microtiter plates (2×10^6 cells per well) in RPMI 1640 (GIBCO) containing phytohemagglutinin (1 μg/ml), 0.01 mM 2-mercaptoethanol, 10% (vol/vol) fetal calf serum, and IL-1 (0.5–5 units/ml). After 72 hr, cultures were pulsed for 16 hr with [^{3}H]thymidine (Amersham; 0.5 μCi per well; 1 Ci = 37 GBq). Subsequently, cells were harvested and washed and the uptake of [^{3}H]thymidine was measured by scintillation spectrometry.

The possibility that the IL-1 solutions used contained IL-2 (which may be arthritogenic) was controlled by injecting recombinant IL-2 (0.1 unit; Genzyme). The dose of IL-2 was chosen to be approximately equivalent on a weight basis to 10 units of IL-1. Endotoxin is another potential, biologically active, contaminant. The antibiotic polymyxin B (Sigma) binds and inactivates endotoxin (15) and was added to IL-1 solutions (2 μg/ml) prior to intraarticular injection. The content of endotoxin in the IL-1 solutions was assayed by using the limulus test (16) and levels were found to be <100 pg/ml. To determine if endotoxin had activity similar to that of IL-1, joints were injected with endotoxin in the range 0.1–100 ng (lipopolysaccharide B, *Escherichia coli* 0111: B4; Difco).

Measurement of Plasma Exudation and Leukocyte Accumulation in Rabbit Skin. Plasma exudation was measured by the detection of extravascular leakage of labeled albumin (17). The backs of rabbits were shaved prior to an intravenous injection of 50 μCi of ^{125}I-labeled human serum albumin (Radiochemical Centre, Amersham, England) in a 2.5% solution of Evans blue dye, dissolved in sterile saline. Solutions of IL-1, BK, fMet-Leu-Phe, or PGE_2 were diluted with sterile saline and 100-μl volumes were injected intradermally. Each test solution was injected at five sites on the back and each animal received up to 50 intradermal injections. After 30 min, three blood samples were taken from an ear vein and the animals were killed by an overdose of anesthetic. The skin was removed and injection sites were separated with a 1.68-cm steel punch. Radioactivity in plasma and skin samples was measured and the plasma volume at each injection site was calculated.

The effects of IL-1, BK, or fMet-Leu-Phe on leukocyte accumulation in the dermis 30 min, 4 hr, and 24 hr after injection were also estimated. Skin samples were fixed, processed, cut, and stained for microscopic examination. Total leukocyte numbers were counted in five high-power fields (×1000) arranged vertically through the dermis.

Induction of Antigen-Induced Arthritis. Rabbits were immunized with subcutaneous injections of 4 mg of ovalbumin (Sigma) in 1 ml of Freund's complete adjuvant (GIBCO). Animals were reimmunized 14 days later in the same way. Five days after the second immunization, arthritis was induced in the right knee joint by injecting 1 ml of a sterile solution of ovalbumin (5 mg/ml) into the joint cavity. The contralateral joint was injected with sterile saline to act as a within-animal control. Animals were killed 1, 7, or 14 days after joint challenge, the cellular infiltrate in the joint fluid was enumerated, and the proteoglycan content of the articular cartilage was measured.

RESULTS

Inflammatory Changes Induced by Intraarticular IL-1. Intraarticular injection of IL-1 (1–20 units) caused a dose-dependent infiltration of leukocytes into the joint space (Fig. 1). The number of leukocytes in the synovial cavity was highest 4 hr after injection and at this time most of the cells were polymorphonuclear leukocytes (PMN: >90%, Fig. 2). After 24 hr, the total leukocyte numbers had declined although there were still significantly ($P < 0.01$) more cells in the IL-1-injected than in the contralateral vehicle-injected joints. At this time the infiltrating cells were predominantly mononuclear (60–80%). After 3 days, the cell counts in the synovial fluid were not significantly different from control values. Examination of hematoxylin/eosin-stained sections of synovial lining revealed extensive infiltration of PMN at 4 hr. Many vessels showed perivascular collections of cells. Large numbers of PMN were also found at the surface layer

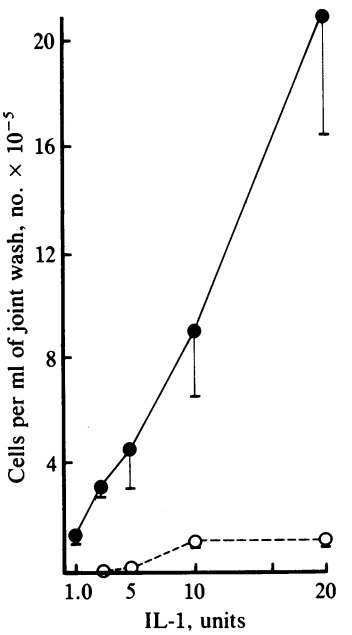

FIG. 1. Total leukocyte numbers in joint washes 24 hr after injection of highly purified human IL-1 into rabbit knee joints (●). Stock solutions of IL-1 [100 units/ml (1 unit of activity is defined as that amount of IL-1 required to double the proliferative response of mouse thymocytes stimulated with phytohemagglutinin alone at 1 μg/ml)] in 5% fetal calf serum were diluted with saline to give a constant injection volume of 0.5 ml. The contralateral joints received similar injections of diluted fetal calf serum alone (○). Each point is the mean ± 1 SEM of 4–10 estimations from separate animals. For all points, the total number of cells present in the IL-1-injected joints was significantly higher ($P < 0.01$) than in the vehicle-injected controls. The leukocytes present were predominantly (60–80%) mononuclear.

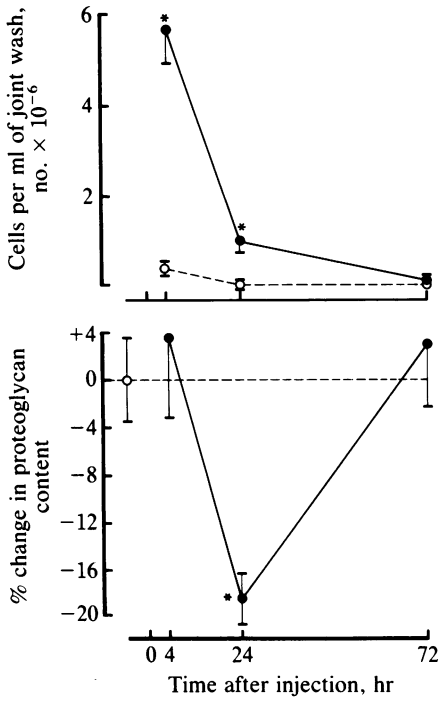

FIG. 2. Effects of intraarticular injection of 10 units of highly purified human IL-1 on leukocyte accumulation and proteoglycan loss from articular cartilage 4–72 hr after a single injection. (*Upper*) IL-1-injected joints (●) contained significantly more leukocytes ($P < 0.01$) than control joints receiving vehicle alone (○) at 4 hr and 24 hr but not at 72 hr. (*Lower*) Articular cartilage (20–40 mg) was digested by incubation with papain for 1 hr. The digests were then allowed to react with 1,9-dimethylmethylene blue (DMB), which binds to sulfated glycosaminoglycan (GAG) derived from cartilage proteoglycan (12). The absorbance at 540 nm of the DMB–GAG complex was determined immediately after mixing. The proteoglycan content of cartilage from IL-1-treated joints (●) was calculated as μg of GAG per mg of wet weight and expressed as a percentage of control values (○) in the same animals. Each point is the mean of 5–10 estimations; bars represent ±1 SEM and the * indicates $P < 0.01$ compared to controls.

of cells—the synovial lining cell layer. PMN were still observed at 24 hr but their numbers were considerably reduced. Although large numbers of mononuclear cells were found in the synovial cavity at this time, there were only relatively small numbers of mononuclear cells visible in the synovial lining. This partly reflects the difficulty in recognizing mononuclear leukocytes in connective tissue. By 72 hr, the tissues had returned to normal.

Although large numbers of cells infiltrated the IL-1-injected joints, at no time was there a significant increase in joint diameter. Joint washes from vehicle- or saline-injected joints did not contain detectable concentrations of PGE_2 or LTB_4 (<0.05 ng/ml). The injection of IL-1 did not result in the appearance of PGE_2 or LTB_4 in the joint fluid, and pretreatment of animals with BW755C (100 mg/kg *per os*; ×3 in 24 hr), the dual inhibitor of prostaglandin and leukotriene synthesis (18), did not significantly alter the responses to IL-1 at 24 hr.

Influence of IL-1 on Articular Cartilage Proteoglycan Content. Articular cartilage from untreated joints or joints receiving control injections (vehicle or saline) contained a mean concentration of proteoglycan of 21.3 ± 1.0 μg/mg of wet weight (mean ± SEM; $n = 9$). There was no change in proteoglycan content 4 hr after injection of 10 units of highly purified IL-1 but at 24 hr the cartilage proteoglycan content had decreased to 17.3 ± 0.7 μg/mg. Three days after injection of IL-1, proteoglycan concentrations had returned to control values (Fig. 2). Although there was a large variation in the effects induced by individual doses of IL-1, the proteoglycan loss was dose-dependent. Injection of 0.2–1 unit had no effect, whereas 1–5 units had a small but variable effect. Consistent cartilage degradation could always be observed with injections of 5–20 units of IL-1. This loss of proteoglycan was normally related to the wet weight of cartilage. A similar degree of loss was also observed when the proteoglycan content was related to the collagen content measured by hydroxyproline assay of cartilage.

Histological assessment of safranin O-stained sections of articular cartilage from animals receiving IL-1 24 hr previously revealed a major loss of proteoglycan from the midzone of the cartilage with little loss from the superficial layer or from cells lying deeper to the midzone.

Specificity of IL-1 Response. Contaminants in physicochemically purified IL-1 preparations that could be biologically active include other interleukins (e.g., IL-2) and endotoxin. Injection of IL-2 (0.1 unit) had no effect on cartilage proteoglycan content and did not induce any significant inflammatory infiltrate. Injection of 10 units of cloned β-IL-1 (Genzyme) resulted in a similar response to that seen with the highly purified material. Treatment of IL-1 with phenylglyoxal inhibited the LAF activity and the *in vivo* chemotactic activity of this molecule by 80–90% and almost totally abolished its ability to promote cartilage breakdown (Fig. 3).

To test the possibility that the effects of IL-1 may be due to endotoxin contamination, IL-1 was injected after treatment with polymyxin B, which inactivates endotoxin (15). Although polymyxin B was able to reduce the activity of intraarticularly injected endotoxin, it had no effect on IL-1. Furthermore, injections of endotoxin (1–100 ng) caused a dose-dependent infiltration of the joint with leukocytes but this was not accompanied by a loss of proteoglycan from the cartilage (Fig. 3).

Intradermal Injection of IL-1. Intradermal injections of IL-1 (10 units) or fMet-Leu-Phe (20 ng) stimulated an infiltration of the dermis with PMN, which was evident after 30 min, increased at 4 hr, and had subsided by 24 hr. At 30 min, IL-1 and fMet-Leu-Phe induced a similar response (≈40 cells

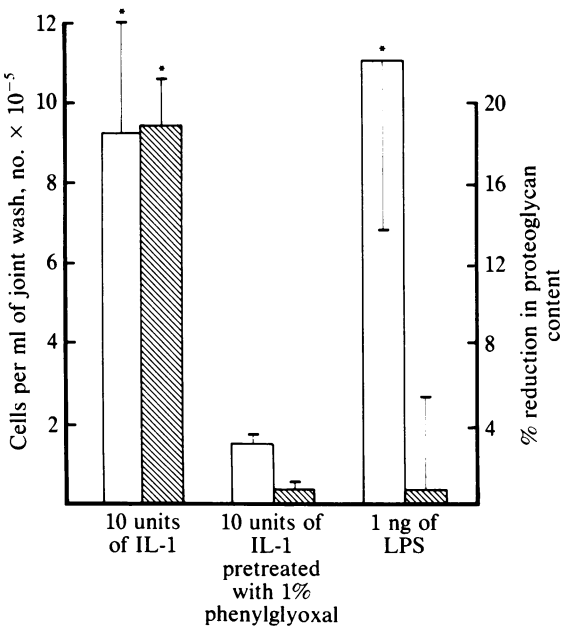

FIG. 3. Effects of intraarticular injections of highly purified human IL-1 or endotoxin lipopolysaccharide (LPS) on leukocyte infiltration (open columns) and proteoglycan loss from cartilage (hatched columns). The effects of pretreatment of IL-1 with phenylglyoxal are also shown. Each column is the mean of 4–10 estimations; bars represent ±1 SEM and the * indicates $P < 0.05$.

in five high-power fields) but at 4 hr mean leukocyte numbers at IL-1-injected sites were 128 ± 22 and at fMet-Leu-Phe injected sites were 76 ± 12. At 30 min and 4 hr, IL-1 and fMet-Leu-Phe responses were significantly greater than vehicle controls. No significant infiltration of the dermis was seen after injections of BK (0.5 μg) or PGE_2 (10 ng).

Intradermal injections of BK (0.5 μg) caused a mean extravasation of 91.3 ± 9.8 μl of plasma and this was increased to 168 ± 11.9 μl when BK was injected in combination with PGE_2 (10 ng). fMet-Leu-Phe (20 ng) induced plasma extravasation of 30.2 ± 6.5 μl, which was increased by PGE_2 to 81.7 ± 12.0. However, IL-1 (10 units) or PGE_2 (10 ng) alone had no effect on plasma extravasation and combinations of IL-1 and PGE_2 produced significantly less plasma extravasation (39.3 ± 2.9 μl) than combinations of PGE_2 with either fMet-Leu-Phe or BK.

Antigen-Induced Arthritis. In a separate series of experiments, leukocyte accumulation and cartilage degradation was assessed in a model of antigen-induced arthritis in the rabbit that closely resembles rheumatoid arthritis in man (8). Intraarticular injection of antigen (ovalbumin) into the knee joints of sensitized animals resulted in a marked joint swelling that was sustained for at least 14 days. Joint washes collected 24 hr after injection contained immunoreactive PGE_2 (9.1 ± 1.7 ng/ml), immunoreactive LTB_4 (0.5 ± 0.2 ng/ml), and large numbers of PMN (Fig. 4). After 14 days, the total leukocyte count had not diminished but the proportion of mononuclear leukocytes had risen to ≈20%. LTB_4 was undetectable after 24 hr and PGE_2 concentrations declined to 0.5–1.0 ng/ml between 7 and 14 days. Twenty-four hours after antigen injection the proteoglycan content of articular cartilage was not significantly different from control joints. Between 7 and 14 days, however, there was significant loss of proteoglycan from the cartilage of the arthritic joint (Fig. 4) and the effect at 7 days was similar to that seen 24 hr after a single injection of 5–20 units of IL-1 into normal animals.

DISCUSSION

The single injection of highly purified or recombinant IL-1 into knee joints of rabbits causes a marked cellular infiltrate in synovial lining and fluid and the loss of proteoglycan from the articular cartilage. Surprisingly, the IL-1-induced accumulation of inflammatory cells was not associated with plasma extravasation or with swelling in either the joint or the skin. Nor was there stimulation of icosanoid synthesis. The response within the joint was almost totally abolished by preincubation of IL-1 with the arginine-binding reagent phenyglyoxal. IL-2 was without effect and the response to IL-1 was not abolished by preincubation with polymyxin B. These experiments demonstrate that the *in vivo* response is specific to IL-1.

The loss of proteoglycan from cartilage does not appear to be dependent solely upon the presence of migrating leukocytes since endotoxin stimulated a similar cell response without cartilage breakdown. It is likely, therefore, that IL-1 directly stimulates the joint tissues to release degradative enzymes. In autoimmune diseases such as rheumatoid arthritis, it is possible that the resident synovial cells produce IL-1 and other cytokines, which then stimulate cartilage breakdown (4, 19).

Unlike BK, IL-1 does not increase vascular permeability in the skin, although it does induce accumulation of leukocytes in the dermis. Chemotactic factors such as fMet-Leu-Phe or LTB_4, in combination with a vasodilator prostaglandin, cause plasma extravasation that is dependent upon circulating neutrophils (20). However, in experiments in which IL-1 caused a greater leukocyte infiltrate than fMet-Leu-Phe, combination of IL-1 and PGE_2 produced significantly less plasma extravasation than combinations of fMet-Leu-Phe and PGE_2. This is further evidence that leukocyte accumulation is not directly associated with other inflammatory processes (21).

The results reported in this paper demonstrate that IL-1 induces responses after 24 hr that are characteristic of chronic erosive arthritis of 7–14 days' duration and that these actions are distinct from the properties of mediators of acute inflammation. In preliminary experiments we have found inhibitors of IL-1 action in synovial washes from rabbits with antigen-induced arthritis. This has frustrated attempts to measure endogenous concentrations of IL-1 in this model. However, the detection of IL-1-like activity in synovial fluids from patients with rheumatoid arthritis (22) and the ability of IL-1 to mimic the symptoms of chronic inflammation strongly support its role as a key mediator of chronic destructive arthritis.

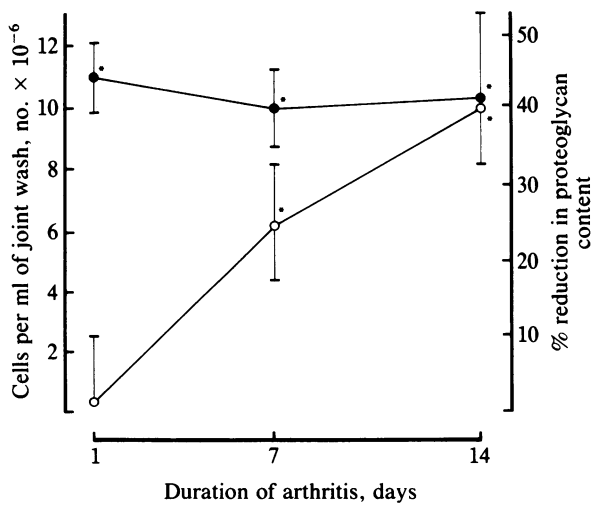

FIG. 4. Total leukocyte numbers in joint washes (●) and percent proteoglycan loss from articular cartilage (○) in rabbits with developing antigen-induced arthritis. Groups of five animals were killed 1, 7, or 14 days after antigen challenge and joint washes were taken for total leukocyte estimations. The proteoglycan content of cartilage from both joints was measured and the loss of proteoglycan was expressed as percentage loss compared with control values in the same animals. Bars represent ±1 SEM and the * indicates $P < 0.05$.

We thank Anita Caffery and Karen Jackson for excellent technical assistance.

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