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A monoarthritis model in rabbits induced by repeated intra-articular injections of lipopolysaccharide

HIROKAZU IDOGAWA*, ATSUSHI IMAMURA*,
KONOMI MATSUO†, KUNINORI YOSHITAKE†,
TAKASHI UMEMURA‡ AND MASAMI OHASHI*

**Department of Pharmacology, New Drug Discovery Research Laboratory and*
†New Drug R & D Laboratory, Kanebo, Ltd., Osaka, Japan, and
‡Department of Veterinary Pathology, Faculty of Agriculture, University of Tottori, Tottori, Japan

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Summary. We attempted to develop a monoarthritis model using repeated intra-articular injections of high-dose Lipopolysaccharide (LPS). Furthermore, the effect of dexamethasone on this arthritis model by intra-articular administration was studied to examine whether the model can be used to screen anti-rheumatic drugs in a short time. Arthritis was induced by one, two or three intra-articular injections of LPS (5–50 μg/joint) at 4-day intervals into the knee joint. The rabbits were sacrificed at 7 days following the last injection of LPS. Three intra-articular injections of LPS at 50 μg/joint resulted in persistent joint swelling. Hyperplasia of synovium with some discolouration was macroscopically observed. Infiltration of mononuclear cells and lymphoid follicles were histologically observed as the synovial lesions. Concerning the articular bone/cartilage, trabecular destruction of gastrocnemius sesamoid bone and severe loss of safranin-O staining of articular cartilage were observed. Immunohistochemical analysis revealed that inflammatory cells and lymphoid follicles in the synovial lesions consisted predominantly of $CD4^+$ T cells, with few $CD8^+$ T cells. Treatment with dexamethasone markedly reduced the joint swelling and the articular destruction. The results suggest that this arthritis model in rabbits can be utilized to screen anti-rheumatic drugs as a model of rheumatoid arthritis.

Keywords: arthritis, rabbits, experimental model, dexamethasone, lipopolysaccharide

Rheumatoid arthritis (RA) is a systemic inflammatory disease characterized by chronic inflammation in the synovial tissue. Adjuvant arthritis in rats (Pearson & Wood 1963) and collagen arthritis in mice (Courtenay *et al.* 1980) have been developed as representative models of chronic inflammatory arthritis (which closely resemble RA). These models are frequently used for the evaluation of anti-rheumatic drugs. Using rabbits, antigen-induced arthritis (Dumonde & Glynn 1962) and *E. coli* O:14 sensitizing arthritis (Aoki *et al.* 1972, 1985) also have been reported as experimental animal models for RA, but it takes a few months to induce the lesion in these models and they are thus not easy to use for the screening of anti-rheumatic drugs.

Correspondence: Hirokazu Idogawa, Department of Pharmacology, New Drug Discovery Research Laboratory, Kanebo, Ltd. 5-90, Tomobuchi-cho 1-chome, Miyakojima-Ku, Osaka 534, Japan.

An experimental model of arthritis in rabbits which can be produced in a short time is lipopolysaccharide (LPS)-induced arthritis by direct intra-articular injection of LPS (Akahoshi *et al*. 1994; Goldenberg *et al*. 1984; Matsukawa *et al*. 1993a,b). In studies using a single intra-articular injection of low-dose LPS (*E. coli*, 10 ng/joint) (Matsukawa *et al*. 1993a,b), it was reported that a large amount of tumour necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) were produced in synovial fluid and such cytokines acted as key mediators of the induction of this arthritis. Both cytokines are also seen to be important mediators in the articular destruction of RA (Arend & Dayer 1995; Cush & Lipsky 1991; Zvaifler 1995). However, this arthritis was an acute synovitis which consisted mainly of polymorphonuclear cells, with no destruction of the articular bone/cartilage observed histologically (Matsukawa *et al*. 1993a,b). In contrast to low-dose LPS, arthritis in rabbits induced by a single intra-articular injection of high-dose LPS (*Neisseria gonorrhaeae*, 5–20 μg/joint) caused an acute, polymorphonuclear synovitis, eventually evolving to chronic mononuclear cell infiltration (Goldenberg *et al*. 1984). In these studies, however, the histological destruction of articular bone/cartilage, which was observed in RA, was not reported. Antigen-induced arthritis in rabbits was observed as chronic synovitis following a single intra-articular injection of antigen, and the destruction of the articular bone/cartilage became serious by repeated intra-articular injections of antigen (Goldlust *et al*. 1978).

Therefore, in the present study, we induced arthritis in rabbits by repeated intra-articular injections of high-dose LPS (5–50 μg/joint) in a short time and observed the destruction of articular bone/cartilage. The articular destruction was evaluated by measuring the joint swelling, by macroscopic and histopathological examination, and by immunohistochemical analysis of inflammatory cells of the synovial lesions. We compared the articular lesions of this arthritis with those of RA. We also examined whether this model can be utilized to screen anti-rheumatic drugs in a short time, by assessing the effects of dexamethasone treatment.

Materials and methods

Experimental schedule for chronic arthritis model (Experiment A)

Eighteen male New Zealand white rabbits (Charles River Co., Atsugi, Japan) weighing 2.5–3.0 kg were used. Rabbits were housed individually in stainless steel cages with wire floors. The lighting was 12 h cycles of illumination/darkness. Approximately 150 g of commercial feed was provided daily; water was available *ad libitum*. Room temperature was maintained at 20 to 22°C, relative humidity was 40 to 50%, and the room was ventilated at a rate of 12 air changes/hr. Arthritis was induced by intra-articular injection of one of three dosages of LPS ($n = 2$) (5, 20 or 50 μg, *E. coli* O111, B4 (Sigma Chemicals, St. Louis, MO, USA) suspended in 0.1 ml of saline) once (Exp. 1: LPS was injected on day 0), twice (Exp. 2: LPS was injected on day 0 and 4) or three times (Exp. 3: LPS was injected at day 0, 4 and 8) at 4-day intervals into the left knee joint. An equal volume of saline was injected at the same schedule in the right knee of each rabbit as a normal control. The rabbits were sacrificed by rapid intravenous injection (i.v.) of sodium pentobarbital at 7 days following the last injection of LPS (Exp. 1: Rabbits were sacrificed at day 7, Exp. 2: Rabbits were sacrificed at day 11, Exp. 3: Rabbits were sacrificed at day 15) (Figure 1a).

Experimental schedule for the prophylactic effect of dexamethasone (Experiment B)

Ten male New Zealand white rabbits weighing 2.8–3.5 kg were used. Arthritis was induced by intra-articular injection of LPS (50 μg, *E. coli* O111, B4; (Sigma) suspended in 0.1 ml of saline) three times at 4-day intervals into the left knee joint (LPS was injected at day 0, 4, and 8). An equal volume of saline was injected at the same schedule in the right knee as a normal control. In control animals ($n = 5$), 0.5 ml of distilled water was injected into the left knee joint immediately after each of the three LPS injections and at 4 days after the last injection of LPS (Distilled water was injected at day 0, 4, 8, and 12). In the dexamethasone-treated group ($n = 5$), dexamethasone (0.3 mg, (Decadron-A, Banyu, Tokyo, Japan) suspended in 0.5 ml of distilled water) was injected into the left knee joint immediately after each of the three LPS injections and at 4 days after the last injection of LPS (Dexamethasone was injected at day 0, 4 8, and 12). As a normal control, an equal volume of distilled water was injected into the right knee joint immediately after each saline injection and at 4 days after the last saline injection. The rabbits were sacrificed by rapid intravenous injection of sodium pentobarbital at 3 days after the last injection of dexamethasone or distilled water (Rabbits were sacrificed at day 15) (Figure 1b).

Measurement of joint swelling

The degree of swelling on the knee joints (joint swelling) was determined by measuring the diameter of joint periodically with the use of a caliper. The diameter of the pre-injected joint (day 0) was subtracted from that of

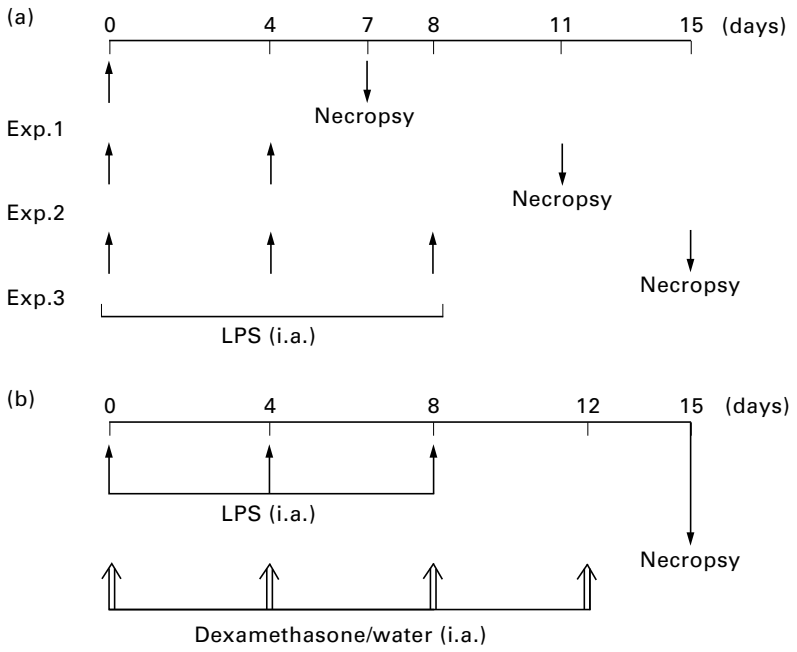

Figure 1. The schedules for the induction of arthritis and the treatment of dexamethasone. a, Experimental schedule of the chronic arthritis model by repeated intra-articular injection (i.a.) of LPS in rabbits (↑). Two rabbits were used for each dosage (5, 20, and 50 μg/joint) of Exp. 1–3. LPS (*E. Coli* O111.B4) was injected into the left knee joint. The right knee joint was injected with saline as a normal control; Animals were sacrificed 7 days after final LPS injection (↓). b, Experimental schedule of the prophylactic effect of dexamethasone in the LPS-induced arthritis in rabbits. Five rabbits each were used in control and dexamethasone treatment groups. Induction of arthritis: 50 μg of LPS (*E. Coli* O111.B4) was injected into the left knee joint three times at 4-day intervals (↑). The right knee joint was injected with saline as a normal control. In control animals, distilled water was injected into the left knee joint immediately after each LPS injection and at 4 days after the last LPS injection and in the dexamethasone-treated group, dexamethasone (0.3 mg/joint) was injected into the left knee according to the same schedule (⇑). The right knee joint was injected with distilled water as a normal control. Animals were sacrificed 7 days after final LPS injection (↓).

the post-injected joint (day 1–15) to determine the increase in the diameter of joint. The joint swelling was calculated as the difference between the increase in the diameters of saline-injected joints and those of LPS-injected joints. Measurement of the diameter of joint was done by a blind observer.

Macroscopic examination

Macroscopic examination of the joint tissue was conducted by the method of Blackham (1978). Each of the following 12 parameters was assessed on a 0–3 rating by a blind observer: synovial fluid volume, viscosity, discoloration and tissue debris; synovium petechiae, hyperplasia, vascularization and discoloration; cartilage and bone pitting, erosion and new bone formation and capsule fibrosis.

Histopathological examination

Each knee joint was excised by the method of Edward *et al.* (1988). The dissected joints were fixed in 10% neutral-buffered formalin, decalcified with EDTA, and embedded in paraffin. Sections were stained with haematoxylin-eosin (HE), and safranin-O and fast green. The tissues were evaluated histopathologically using a 0–3 rating for the following 9 parameters by a blind observer: Synovium proliferation of the lining layer, oedema, fibrin, fibrosis, inflammatory cell infiltration, vascularization and lymphoid follicles; cartilage loss of safranin-O staining and bone (gastrocnemius sesamoid bone) trabecular destruction.

Immunohistochemical examination

The right and left synovial tissues from the infrapatellar region were taken from rabbits which received LPS injections (50 μg/joint, three times; experiment A3) at the time of necropsy and kept at -70°C until cryosectioned. Details of monoclonal and polyclonal antibodies used in this study are shown in Table 1. The antibodies KEN-4 (Kotani *et al.* 1993) and 12.C7 (De Smet *et al.* 1983; Wilkinson *et al.* 1993) were purchased from Spring

Table 1. Antibodies used in the immunohistochemical staining

Antibody	Raised in	Specificity	Cellular distribution
Monoclonal antibodies			
KEN-4	Mouse	CD4	Helper/inducer T cells
12.C7	Mouse	CD8	Suppressor/cytotoxic T cells
198	Mouse	CD11b	Macrophages and pseudoeosinophils
2C4	Mouse	MHC class II (RLA-DQ)	
Polyclonal antibody			
RTLA	Goat		All T cells

Valley Laboratories (Maryland, USA). The antibodies 198 (Smet *et al*. 1986; Wilkinson *et al*. 1993) and 2C4 (Lobel & Knight 1984; Spieker-Polet *et al*. 1990; Wilkinson *et al*. 1993) were purchased from Serotec (Oxford, UK). The antibody RTLA (Ponsard *et al*. 1986) was purchased from Cedarlane Laboratories (Ontario, Canada). Cryostat sections (8 μm-thick) were stained using an avidin-biotin complex (ABC) technique. Briefly, air-dried sections were fixed with 100% cold ethanol for 5 min, washed in phosphate-buffered saline (PBS; pH 7.4), and then incubated in a 1:20 dilution of normal goat serum (for monoclonal antibodies) and swine serum (for polyclonal antibody) for 20 min for blocking nonspecific reactivity. Overnight incubation at 4°C with primary antibodies (diluted 1:100–1:800 in 0.5% bovine serum albumin/PBS) was followed by washes in PBS, then incubation with 0.6% methanolic hydrogen peroxidase for blocking endogenous peroxidase for 15 min. The sections were incubated in biotinylated goat anti-mouse (for monoclonal antibodies) and swine anti-goat second antibodies (for polyclonal antibody) and were then incubated for 30 min with the ABC reagent. The sections were washed in PBS and developed for 3 min with 3,3′-diaminobenzidine tetorahydrochloride solution, a final wash in tap water, and counterstaining with Mayer's hematoxyline. For negative controls, the same staining procedure was performed, with the substitution of normal goat serum for RTLA or isotype-matched mouse immunoglobulin G for monoclonal antibodies respectively.

Statistical analysis

Experiment A. Results are expressed as mean values.

Experiment B. The data for joint swelling are expressed as mean values ± mean standard errors and were evaluated using Student's t-test. Macroscopical and histopathological scores are expressed as median values (ranges) and were analyzed with the Mann-Whitney U-test. The effects of dexamethasone were compared with the control group, and probability values less than 5% were considered significant.

Results

Experiment A: Induction of chronic arthritis

Joint swelling

The intra-articular injection of LPS at each dose (5, 20, and 50 μg/joint) produced rapid swelling which reached a maximum level at 1 day after the first, second, and third injections. At each dose of LPS, the persistence of joint swelling was observed even at 7 days after the third injection (Figure 2).

Macroscopic examination

The arthritis induced by intra-articular injections of LPS showed large volumes of synovial fluid with discoloration, viscousness and tissue debris, and hyperplasia of synovium with some discoloration. The macroscopic score of these lesions tended to be high with the increase of injection times and the higher dosage of LPS (Figure 3a,b, Refer to Figure 6B). However, articular bone/cartilage and capsules did not show lesions (data not shown).

Histopathological examination

The arthritis induced by intra-articular injections of LPS showed proliferation of synovial lining cells, oedema, fibrin exudation, infiltration of inflammatory cells which consisted mainly of mononuclear cells, vascularization, and fibrosis. In the articular cartilage, the loss of safranin-O staining was observed. Especially, lymphoid follicles in the synovium, the severe loss of safranin-O staining in the articular cartilage, and the irregular surface and partial loss of trabecula in the gastrocnemius sesamoid bone, the width of trabecula of which was narrow compared with that of the femoral articular bone/cartilage, were observed following three intra-articular

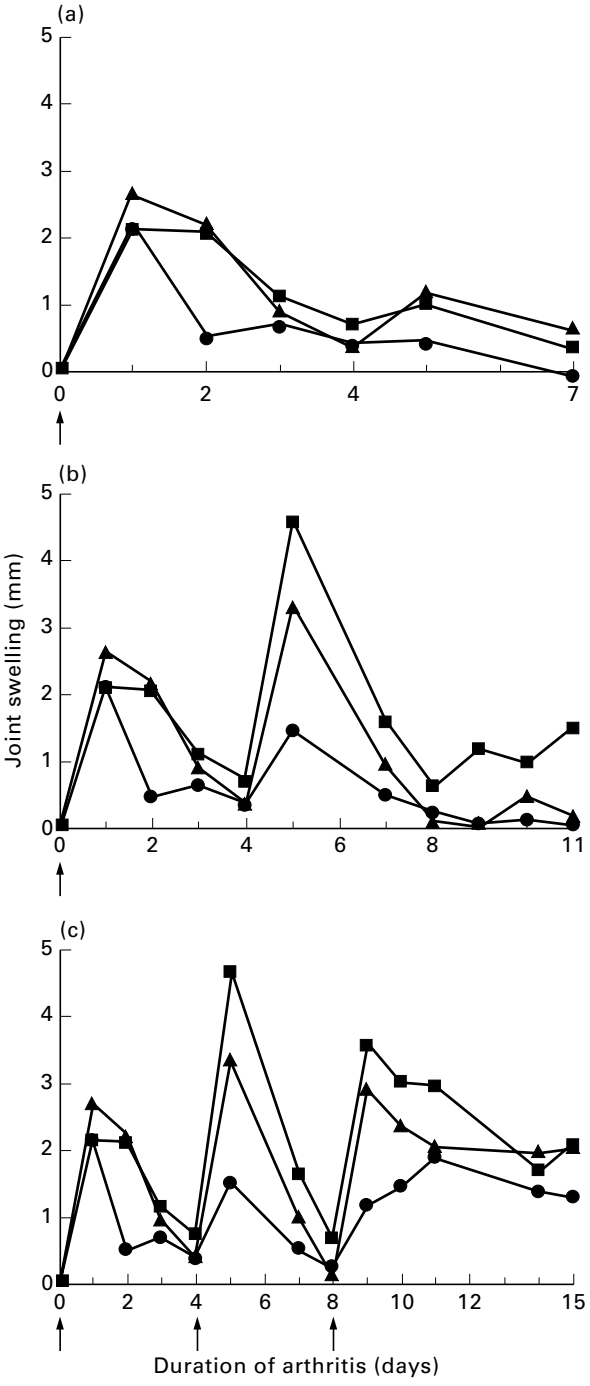

Figure 2. Knee joint swelling following single or repeated intra-articular injections of LPS (↑). a. Experiment 1; b, Experiment 2; c, Experiment 3; ● 5 μg; ▲ 20 μg; ■ 50 μg LPS. $n = 2$, each group.

injections of LPS (20 and 50 μg/joint). The histological score of these findings tended to be high with the increase of injection times and the higher dosage of LPS (Figure 3c–e, Refer to Figure 7B, 8B and 9B).

Immunohistochemical examination

We performed an immunohistochemical analysis of the cellular infiltration of the synovium which received LPS injections (50 μg/joint, three times). Unexpectedly, not only T lymphocytes but also many macrophage-like cells were stained with RTLA antibody which was prepared by immunizing goats with rabbit thymocytes. However, RTLA-reactive lymphocytes were stained only at the cell surface, showing a ring-like staining pattern, whereas the irregular macrophage-like cells showed a more diffuse staining pattern that was probably due to a predominant intracytoplasmic staining.

Therefore, we could distinguish T lymphocytes from macrophage-like cells by the staining pattern against RTLA antibody. We observed that lymphoid follicles were composed predominantly of T cells, especially $CD4^+$ T cells, most of which were positive for MHC class II antigen. In contrast, few $CD8^+$ T cells were located diffusely around lymphoid follicles. As seen in lymphoid follicles, there was also prominent infiltration of CD4 and MHC class II positive T cells, with few $CD8^+$ T cells in the area of the superficial layer and perivascular tissue of the synovium. In addition, infiltration of CD11b and Class II positive cells were also observed frequently in these areas (Figure 4A–E).

Experiment B: The effect of dexamethasone treatment

Joint swelling

In the control group, the intra-articular injections of LPS (50 μg/joint, three times at 4-day intervals) induced an increase in joint diameter. It reached a maximum level at 1 day after each injection of LPS, and joint swelling persisted even at 7 days after the last injection of LPS. Prophylactic treatment of dexamethasone (0.3 mg/joint, four times at 4-day intervals) significantly prevented such joint swelling ($P < 0.05$) as early as at 3 days after the first LPS injection, and the effect continued to the last day of the experiment (Figure 5).

Macroscopic examination

The control group showed severe changes such as large volumes of synovial fluid with discoloration, viscousness and tissue debris, and hyperplasia of synovium with

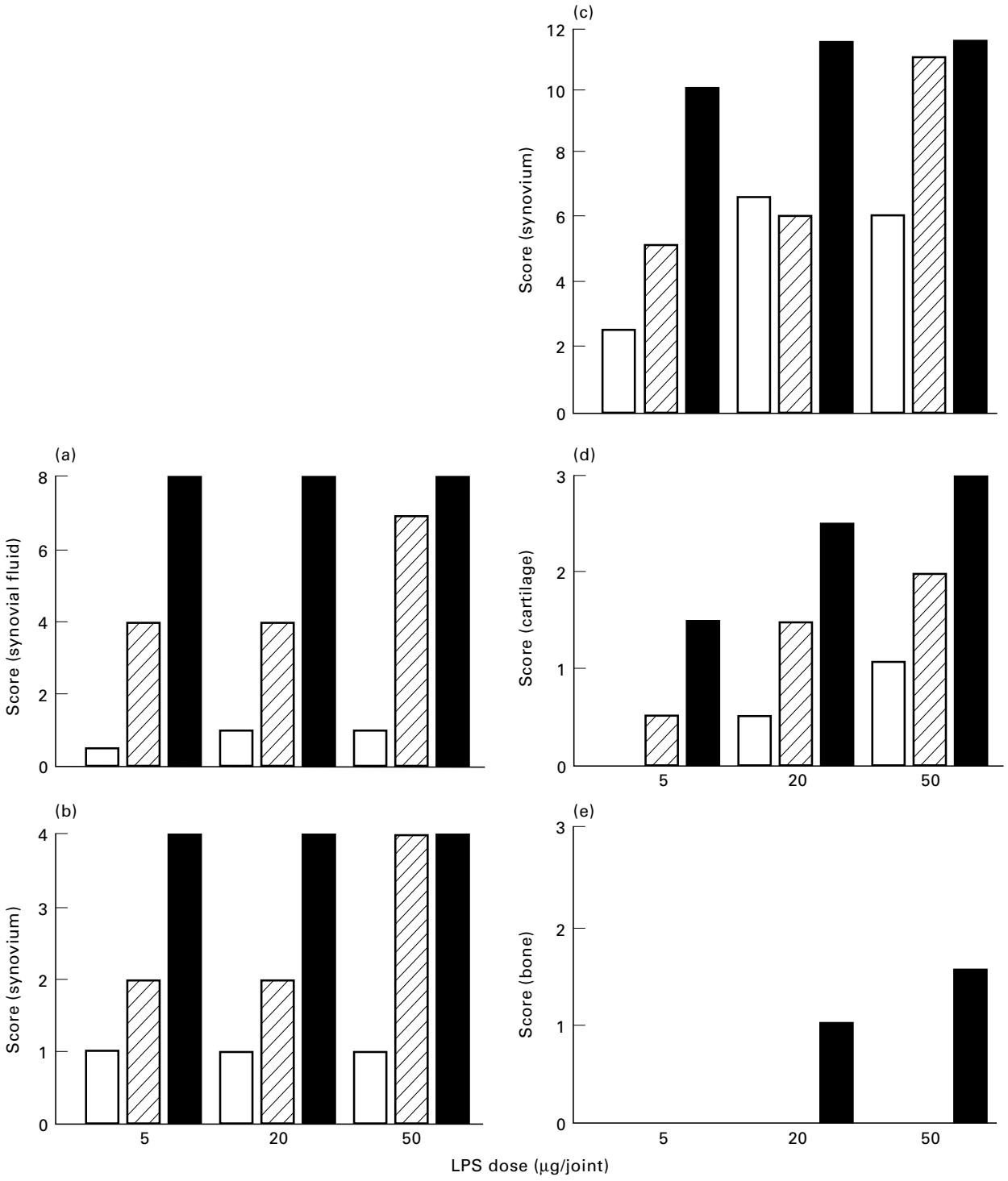

Figure 3. Macroscopic (a,b) and histopathological (c–e) examinations of LPS-induced arthritis. The rabbits were sacrificed at 7 days following the last injection of LPS. (a) Synovial fluid score; (b) synovium macroscopic score; (c) synovium histopathological score; (d) cartilage score; (e) bone score following single or repeated intra-articular injections of LPS. □ Single injection, ▨ two injections; ■ three injections. $n = 2$ in each group.

Table 2. Effects of dexamethasone on macroscopic and histopathological assessment of LPS-induced arthritis in rabbits

			Histopathological assessment		
Treatment	*n*	Macroscopic assessment	Synovium	Cartilage	Bone
Control	5	19 (19–20)	12 (11–13)	2 (2–3)	2 (2)
Dexamethasone	5	4 (3–17)*	6 (5–7)*	1 (1)*	0 (0)*

Macroscopic assessment was done using a 0–3 rating for the following 12 parameters: Synovial fluid (volume, viscosity, discolouration and tissue debris), Synovium (petechiae, hyperplasia, vascularization and discolouration), Cartilage and bone (pitting, erosion and new bone formation), Capsule (fibrosis). Histopathological assessment was done using a 0–3 rating for the following 9 parameters: Synovium (proliferation of the lining layer, oedema, fibrin, fibrosis, inflammatory cell infiltration, vascularization and lymphoid follicles), Cartilage (loss of safranin-O staining), Bone (trabecular destruction). Median values are shown, with ranges given in parentheses. * $P < 0.05$ compared with control group.

some discoloration. In the dexamethasone-treated group, these changes were mild and a significantly lower score ($P < 0.05$) was observed compared with the control group (Figure 6A–C and Table 2).

Histopathological examination

The synovium in the control group showed proliferation of synovial lining cells, edema, fibrin exudation, infiltration of inflammatory cells which consisted mainly of mononuclear cells, vascularization, fibrosis, and lymphoid follicles. These changes were observed extensively from the surface area to the deep area of the synovium. In contrast, these changes existed only in the surface area of synovium in the dexamethasone-treated group, and the score was significantly lower ($P < 0.05$) as compared with the control group (Figure 7A–C and Table 2). In the cartilage, the control group joints showed severe loss of safranin-O staining. In contrast, the dexamethasone-treated group joints showed a mild loss of safranin-O staining which was located only in the surface area of articular cartilage, and the score was significantly lower ($P < 0.05$) as compared with the control group (Figure 8A–C and Table 2). Concerning the bone, in the control group, the irregular surfaces and partial loss of the trabecula in the gastrocnemius sesamoid bone were observed with proliferation of fibrous connective tissue which derived

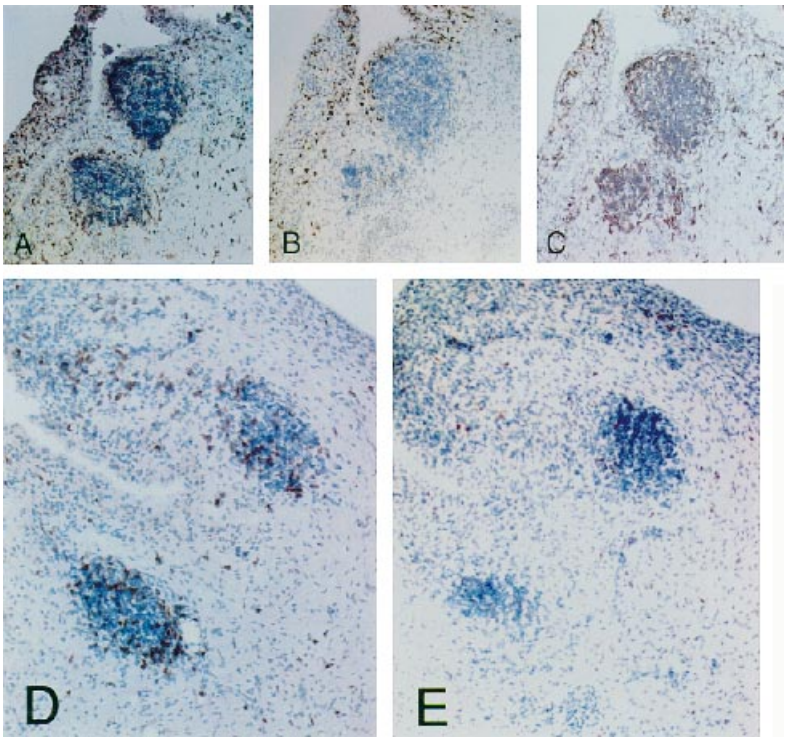

Figure 4. Immunohistochemical staining pattern of frozen sections of synovium from rabbits which received three injections of LPS at 50 μg/joint, stained for RTLA (A), 198 (B), 2C4 (C), KEN-4 (D), and 12.C7 (E). A, Lymphoid follicles consisted predominantly of T cells; B, $CD11b^+$ cells, most of which are mononuclear, are present in the superficial layers of the synovium; C, Most cells within lymphoid follicles are MHC class II^+; D, Many $CD4^+$ T cells are observed within lymphoid follicles; E, Few $CD8^+$ T cells are diffusely present around lymphoid follicles; A, B and C, Original magnification $\times 50$; D and E, Original magnification $\times 80$.

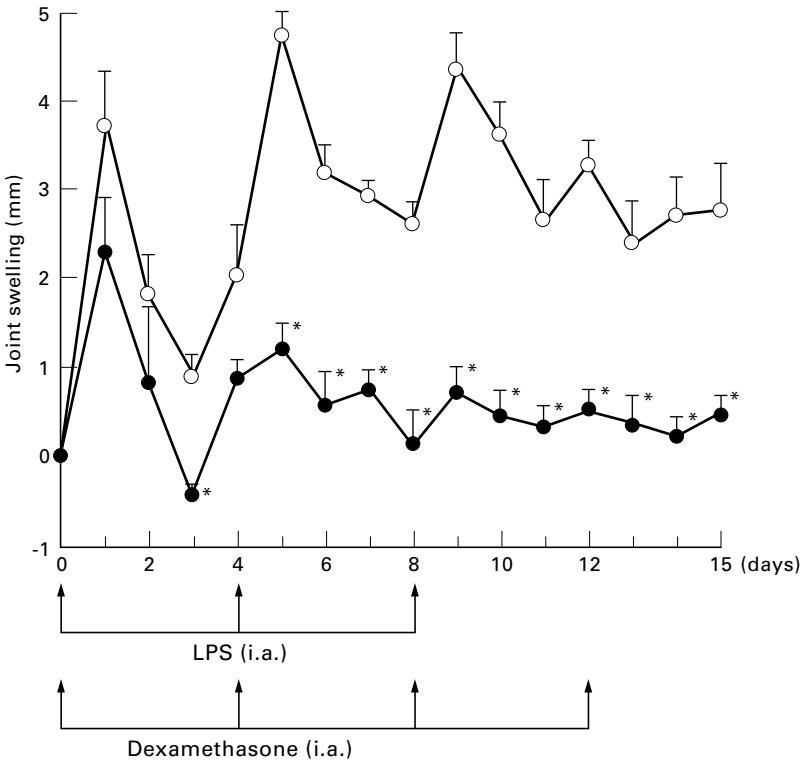

Figure 5. Effect of dexamethasone on knee joint swelling of arthritis. ○ Control; ● dexamethasone. Bar,s.e. $n=5$, each group. *$P<0.05$ compared with control group.

from synovium and periosteum, and activation of osteoclasts. The dexamethasone-treated group showed a smooth surface of the trabecula in the gastrocnemius sesamoid bone, and the score was significantly lower ($P<0.05$) as compared with the control group (Figure 9A–C and Table 2).

Discussion

As a representative rabbit model of chronic inflammatory arthritis, antigen-induced arthritis (AIA) has been used for the evaluation of anti-rheumatic drugs (Blackham 1978; Davis 1971). The articular lesions of AIA are

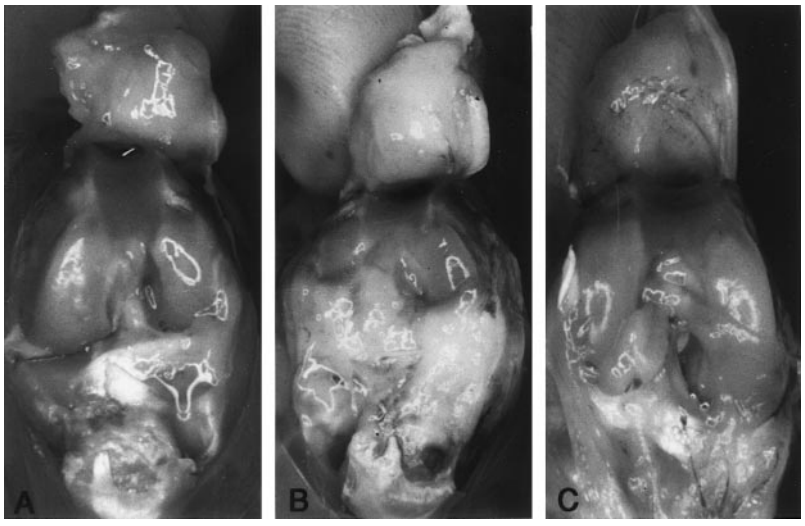

Figure 6. Effect of dexamethasone on macroscopic changes of arthritis. A, No remarkable changes in a normal knee joint are seen; B, Hyperplasia of synovium with severe discolouration in a control group joint; C, Mild discolouration of synovium in a dexamethasone treatment group joint.

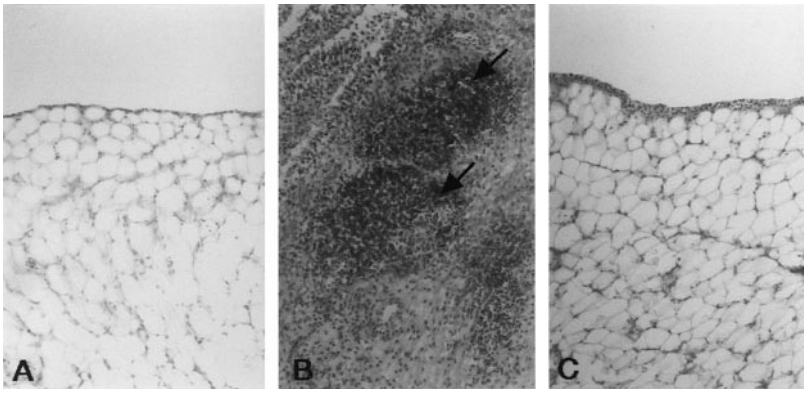

Figure 7. Effect of dexamethasone on histopathological changes in synovium after staining with HE. A, No remarkable changes in the synovium are seen in this normal joint; B, Marked proliferation of synovial lining cells and fibroblasts, vascularization, and mononuclear cells infiltration with lymphoid follicles (arrow) (Control joint); C, Mild proliferation of fibroblasts and infiltration of leucocytes in the surface area of synovium (Dexamethasone treatment joint). Original magnification ×70. The upper sides represent the joint cavity sides of synovium.

similar to RA in that lymphoid follicles in the synovium and pannus formation with destruction of articular bone/cartilage are observed (Dumonde & Glynn 1962). However, AIA models cannot easily be used to screen anti-rheumatic drugs, because the AIA takes a few months to induce. We therefore attempted here to develop a novel monoarthritis model in rabbits, which induced in a short time chronic inflammation with lymphoid follicles in the synovium, and articular bone/cartilage destruction. Since immunohistological analysis of lymphocyte subpopulations and microenvironment in the synovial lesions were not reported in arthritis in rabbits, we attempted to examine the synovial lesions with the use of immunohistological analysis. We further studied the prophylactic effect of dexamethasone (steroids), which has been clinically used in the treatment of rheumatoid arthritis (American College of Rheumatology 1996; Bensen *et al.* 1990), by intra-articular administration, to examine whether this model can be utilized to screen anti-rheumatic drugs in a short period of time.

There are few reports concerning histopathological examinations of arthritis induced by intra-articular injection of LPS. For instance, in the synovium of rabbits, a single intra-articular injection of high-dose or low-dose

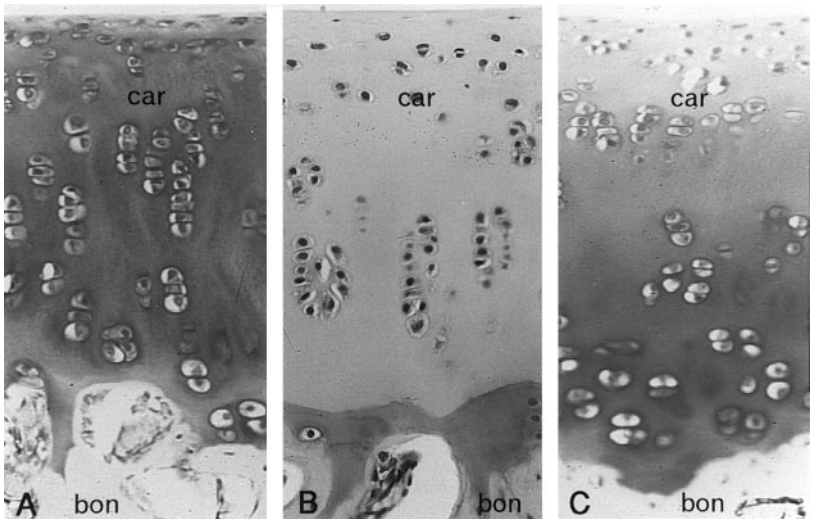

Figure 8. Effect of dexamethasone on histopathological changes in articular cartilage after staining with safranin-O and fast green. A, An intense staining of safranin-O (Normal joint); B, Severe loss of staining of safranin-O (Control joint); C, Mild loss of staining of safranin-O (Dexamethasone treatment joint). Original magnification ×230. bon, bone; car, cartilage.

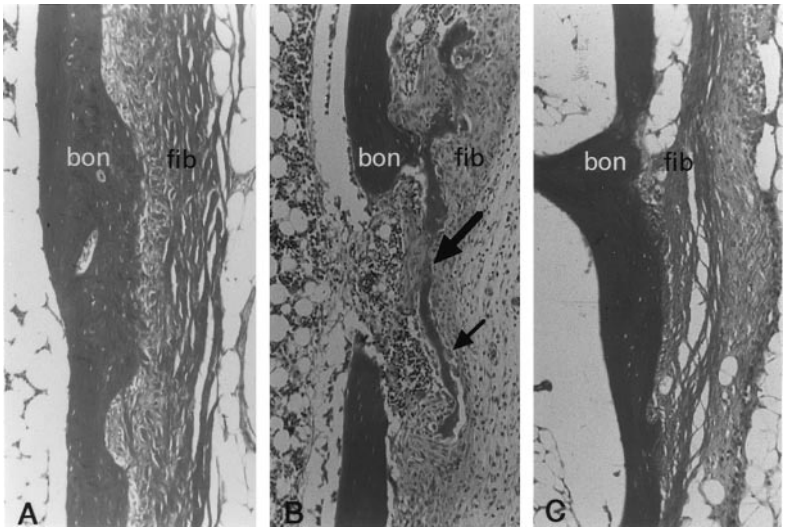

Figure 9. Effect of dexamethasone on histopathological changes of the trabecula in the gastrocnemius sesamoid bone after staining with HE. A, No remarkable changes in the trabecula of the gastrocnemius sesamoid bone (Normal joint); B, Severe trabecular destruction (large arrow) by proliferation of synovium and periosteum, and activation of osteoclasts (small arrow) (Control joint); C, No remarkable changes of the trabecula in the gastrocnemius sesamoid bond (Dexamethasone treatment joint). Original magnification $\times 70$. bon, bone; fib, fibrous connective tissue.

LPS (*E. coli*, 10 ng/joint or *Neisseria gonorrhaeae*, $1 \sim 2\,\mu$g/joint) caused an acute, polymorphonuclear synovitis in rabbits from a few hours to a few days after LPS injection (Goldenberg *et al*. 1984; Matsukawa *et al*. 1993a,b). A chronic synovitis with lymphoid follicles was present at 1–2 weeks after a high-dose LPS (*Neisseria gonorrhaeae*, 5–20 μg/joint) injection, but the low dosage of LPS (*Neisserie gonorrhaeae*, below 3 μg/joint) did not cause chronic persistent synovitis (Goldenberg *et al*. 1984). It was also reported that a single intra-articular injection of LPS (*Salmonella minneosota*, 1 μg/joint) led to severe loss of safranin-O staining in the articular cartilage in hamster within several days (Otterness *et al*. 1994). However, these studies have been done using only a single injection of LPS, and the subsequent arthritic destruction was mild compared with that of AIA. We attempted to induce severe arthritis in rabbits in the present study using repeated injections of high-dose LPS.

The present results showed that the destruction of joints tended to be severe with the increase of injection times and the higher dosages of LPS; chronic synovitis with lymphoid follicles, the trabecular destruction of gastrocnemius sesamoid bone, and the severe loss of safranin-O staining in the articular cartilage were observed by three intra-articular injection of LPS at 20 and 50 μg/joint. In addition, analysis of the infiltration of inflammatory cells and lymphoid follicles in the synovial lesions, which were induced by three intra-articular injections of LPS (50 μg/joint) revealed that the synovial membrane consisted predominantly of $CD4^+$ T cells with few $CD8^+$ T cells. Thus, flare-ups of arthritis by repeated LPS administration appeared to lead the severe articular destruction and inflammatory response progressed chronically.

RA is a chronic inflammatory disease, the main feature of which is the development of a destructive arthropathy that may involve all synovial joints in the body. The synovial lesions of RA vary from patient to patient at different stages of the disease, and synovial membrane hypertrophy, mononuclear cell infiltration, fibrinoid deposits, and pannus are observed in histopathological examination. Since these lesions are also observed in gout, septic arthritis, systemic lupus erythematosus, Reiter's syndrome, and osteoarthritis, the changes listed above are not characteristic of only RA. The features that are unique to rheumatoid synovitis are the presence of lymphoid follicles (Cush & Lipsky 1991; Goldenberg & Cohen 1978; Zvaifler & Firestein 1994).

Previous immunohistochemical analyses of rheumatoid synovitis have indicated that the primary follicles consisted mainly of T cells with few B cells; the germinal centers of the secondary follicles were composed predominantly of B cells, and the mantel zone around these germinal centers was formed mainly by T cells (Young *et al*. 1984). Although differences in synovial lesions have been observed among samples of RA, both primary and secondary lymphoid follicels have shown that there were many T cells and that the majority of the T cells

were $CD4^+$ T cells, whereas very few $CD8^+$ T cells were present around follicles (Duke *et al*. 1982; Førre Ø *et al*. 1982). In addition, these $CD4^+$ T cells were found in close proximity to Class II^+ macrophage-like cells (containing dendritic or interdigitating cells), a finding which has been postulated to show an ongoing presentation of antigen to $CD4^+$ T cells (Førre Ø *et al*. 1982; Janossy *et al*. 1981; Lindblad *et al*. 1983). Of the representative rodent models of chronic inflammatory arthritis which closely resemble RA, the follicular formation of lymphocytes has been observed in only AIA in rabbits. Though $CD4^+$ T cells are more common than $CD8^+$ T cells in the synovial lesion of adjuvant arthritis (Larsson *et al*. 1985) and collagen arthritis (Holmdahl *et al*. 1985, 1988; Klareskog *et al*. 1983), there was an absence of lymphoid follicles. In contrast, with our LPS-induced rabbit arthritis model, although the articular bone/cartilage destruction was weak compared with RA, the synovitis of this model is closely similar to that of RA because of the presence of primary lymphoid follicles and the distribution of inflammatory cells as seen by immunohistochemical analysis. Thus, these findings suggest that this arthritis model can be useful for study of the pathogenesis of RA.

We studied the prophylactic effects of dexamethasone (0.3 mg/joint) by intra-articular administration in this arthritis model, with the consequence that dexamethasone markedly reduced the articular destruction. The effect of steroids by intra-articular administration on articular destruction was previously reported in AIA in rabbits (Wollheim *et al*. 1994). In that experiment, Wollheim *et al*. (1994) showed that prophylactic triamcinolone hexacetonide (1 mg/joint) treatment, which was done at 1-week interval for 3 weeks starting at one day after induction of the arthritis, markedly prevented knee joint destruction, but it took a few months to evaluate the effect. Since we observed a definite effect of a steroid in our present model, in accordance with the above result in AIA, we speculated that our model could be utilized to screen anti-rheumatic drugs in a short time. It is generally accepted that steroids have various antiinflammatory and immune suppressive effects. For instance, Jafari *et al*. (1993) have shown that treatment with dexamethasone (1 mg/kg, i.v.) markedly reduced the synovial fluid concentration of TNF-α and IL-1β in a rabbit model of *Haemophilus influenza* type b-induced arthritis. Using monoclonal anti-rabbit TNF-α antibody and IL-1 receptor antagonist, Matsukawa *et al*. (1993a, b) have also shown that TNF-α and IL-1β acted as key mediators in LPS (*E. coli*, 10 ng/joint)-induced arthritis in rabbits. Therefore, the marked prophylactic effect of dexamethasone in the present arthritis model may be involved in the inhibition of TNF-α and IL-1β production and that from a clinical viewpoint, intra-articular injection of high-dose steroid may be useful in the treatment of a fluminant arthritis.

We concluded that chronic synovitis with lymphoid follicles and articular bone/cartilage destruction were induced by repeated intra-articular injections of LPS (50 μg/joint, three times at 4-day intervals). The immunohistochemical analysis of the synovial lesions revealed that inflammatory cells and lymphoid follicles consisted predominantly of $CD4^+$ T cells with few $CD8^+$ T cells, which were similar to rheumatoid synovitis. Thus, this arthritis model may be useful for study of the pathogenesis of RA. In addition, since dexamethasone markedly reduced the articular destruction, this model could be utilized to screen anti-rheumatic drugs in a short time.

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