EXACERBATION OF MDP-LYS (L18)-INDUCED ARTHRITIS BY CYCLOSPORIN A IN RATS

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Abstract: The mechanisms of MDP-Lys (L18)-induced arthritis in rats and exacerbating effect of cyclosporin A (CsA) on the arthritis were examined. Consecutive subcutaneous injections of MDP-Lys (L18) for 14 days increased tarsal joint thickness from day 7 up to day 15, with marked recovery after cessation of the treatment. Histological examination, however, revealed multilayered arrangement of synovial lining cells with vesicular cytoplasm and mild neutrophil (NEU) infiltration in the synovial membrane 6 hr after a single injection (early stage). From day 8 (late stage), the lesion progressed in association with chronic signs, such as fibroblast proliferation, infiltration of mononuclear cells and lymphocytes (LYMs), and necrosis of the synovial membrane. However, exudative changes and NEU infiltration were seen throughout the course of the treatment. Rat macrophage-conditioned medium (M ϕ -CM) stimulated with MDP-Lys (L18) produced NEU chemotactic factor (NCF), which was also detected in the synovial fluid from dogs with MDP-Lys (L18)-induced arthritis (MIA). The NCF was determined to be one of chemokines by partial purification, and cytokine-induced NEU chemoattractant [CINC, rat interleukin-8 (IL-8)] was detected in the rat M ϕ -CM. Examination of spleen T cell subsets revealed a decrease in CD8⁺ cell population in the later stage. Therefore, the chemokine, at least CINC, is thought to contribute to the early and later development of MIA, and the decreased population of CD8⁺ cells is to the later development. Cyclosporin A (CsA) markedly exacerbated MIA in the later but not early stage, and this was suggested to be caused by its additive effect to decrease CD8⁺ T cell population with MDP-Lys (L18). (J Toxicol Pathol 9: 391~406, 1996)

Key words: MDP-Lys (L18), Cyclosporin A, Arthritis, Rat

Introduction

N-Acetylmuramyl-L-alanyl-D-isoglutamine (muramyl dipeptide, MDP) is a component of bacterial cell walls and is a minimal structure having adjuvant activity^{1,2}. MDP-Lys (L18) (romurtide) is an analogue of synthetic MDP and exhibits various biological activities such as enhancement of resistance to bacterial infection in laboratory animals, specific binding to M ϕ s³, activation of humoral and cellular immune responses, production of IL-1⁴ and of colony-stimulating factor (CSF)⁵ by M ϕ s *in vitro*, increase in the number of granulocyte (G)-M ϕ progenitor cells in the bone marrow and NEUs in the blood, elevation of the serum CSF level⁶, acceleration of recovery from experimental leukopenia⁷, and stimulation of production of complement⁸ and fibronectin⁹ *in vivo*. Because MDP-Lys (L18) activated leukocyte

function and increased its number in humans, this drug has been used in patients with leukopenia caused by radiotherapy for cancers.

Freund's complete adjuvant-induced arthritis (AIA), a classic experimental model of arthritis, can be produced in rats by a single injection of *Mycobacterium butyricum* in oil emulsion. Although the mechanisms involved are not fully understood, the development of AIA is generally thought to be mediated through immunological mechanisms¹⁰; one reason is that AIA is inhibited by CsA, an immunosuppressor which suppresses T cell responses¹¹.

MDP in oil emulsion has been reported to induce a form of polyarthritis in rats that resembles AIA¹²⁻¹⁵. MDP-Lys (L18) also induced arthritis in rats¹⁶ and dogs¹⁷, but its arthritogenic profile and mechanisms were not clarified. Then, T. Sugawara and the present author began examination of the mechanisms of MIA in rats, and it was found that the arthritis is different in mechanisms from AIA, and CsA exacerbates MIA¹⁸⁻²². In the present paper, experimental data obtained so far are introduced and

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the mechanisms of the exacerbating effect of CsA on MIA discussed.

Materials and Methods

Since the materials and methods used in the present study were described in detail in separate papers¹⁸⁻²², only their outline will be written here.

Laboratory examination, tarsal joint thickness, and effect of CsA and antiinflammatory drugs

MDP-Lys (L18) was dissolved in phosphate-buffered saline (PBS) under sterile conditions and administered 4 mg/kg subcutaneously into the back of male Sprague-Dawley (S.D.) rats once daily for 14 days. CsA (10 mg/kg, p.o.) and indomethacin (2 mg/kg, p.o.) were coadministered with MDP-Lys (L18) during days 1-14, 1-5 or 11-14. Phenylbutazone (50 mg/kg, p.o.), dexamethasone (0.1 mg/kg, s.c.), D-penicillamine (100 mg/kg, p.o.), and aurothioglucose (5 mg/kg, s.c.) were also coadministered on days 1-14. Hematological examinations using the blood and bone marrow and measurement of serum IgG levels were done 2, 4, 6, 8, and 24 hr after 1-, 7- or 14-day administration of MDP-Lys (L18). The effect of CsA on these parameters was also examined. The thickness of the tarsal joint was measured with a dial thickness gauge during the experimental periods.

Histology of MIA

To examine the development and reversibility of MIA, S.D. rats were killed at 2, 4, 6, 8, and 24 hr after a single dose of MDP-Lys (L18) 4 mg/kg, and at 24 hr after each of 3-, 7- and 14-day serial administrations. An additional group was treated for 14 days, left untreated for a 7-day recovery period, and killed the next day. The tarsal joint was examined histologically.

Cytokine concentration in medium of rat peritoneal Mφ culture and dog synovial fluid

To determine specific cytokines to MIA, rat Mφs were obtained from peritoneal cavity according to the method of Ohuchi *et al.* (1985)²³ and synovial fluid was taken from the knee joint showing arthritis after s.c. injections of MDP-Lys (L18) 1 mg/kg for 14 days. Concentrations of IL-1, tumor necrosis factor (TNF), and CSF in the rat Mφ-CM stimulated with

MDP-Lys (L18) in the presence or absence of CsA for 24 hr and in the dog synovial fluid were measured by bioassays. NCF was determined as the chemotaxis of NEUs collected from rats and dogs²⁴, using modified Boyden chamber method. Furthermore, rat CINC (IL-8 related protein in rodents) in the rat Mφ-CM was measured by a sandwich enzyme immunoassay kit. Rat Mφ-CM stimulated with 1 μg/ml of MDP-Lys (L18) for 24 hr and the dog synovial fluid were fractionated by the addition of (NH₄)₂SO₄, the resultant precipitate then dialyzed against 0.05 M tris-HCl buffer, and the fraction applied to a heparin-Sepharose column. Aliquots of each fraction were withdrawn, and the protein concentration and NEU chemotactic activity were measured.

Differentiation of MIA from AIA

Male Lewis, Fischer and athymic nude rats were used. MDP-Lys (L18) 4 mg/kg was administered subcutaneously for 14 days. To make AIA, 0.1 ml of a suspension of heat killed and dried *Mycobacterium butyricum* in paraffin oil (adjuvant) was intradermally injected once into the footpad of the hind paw. In addition, CsA was administered for 14 days concurrently with MDP-Lys (L18) or after single administration of adjuvant (only to Lewis rats), and control groups of respective strains were included. Passive transfer of arthritis was performed by the method of Taurog *et al.* (1983)²⁵ with a minor modification. The thickness of the tarsal joint was measured during the course of treatments and histological examination of the joint performed after the dosing periods.

T lymphocyte subset

The alteration of spleen lymphocyte subset was analyzed by flow cytometry in Lewis rats which were treated with MDP-Lys (L18), MDP-Lys (L18) and CsA, adjuvant (only on day 1), adjuvant and CsA, CsA or PBS alone for 1, 7, or 14 days, and killed on the day after the last dose.

Results

Laboratory examination, tarsal joint thickness, and effect of CsA and antiinflammatory drugs

NEU counts began to increase from 4 hr, reached a peak at 8 hr, and returned to the control level by 24

hr after the 1st administration of MDP-Lys (L18). There were similar changes in the NEU counts after the 7th and 14th administrations. The number of myelocytes in the bone marrow was transiently decreased after each dosing of MDP-Lys (L18) and these changes were mirrored by contrary changes in the NEU counts in the blood. A single administration of the drug markedly decreased the LYM counts 2 to 8 hr later, and the counts recovered to the control level at 24 hr. A slight decrease and a significant increase in the LYM counts were observed 24 hr after 7th (day 8) and 14th (day 15) administrations, respectively. Monocyte (MON) counts tended to show decreases after the 1st administration, but increases after both the 7th and 14th administrations. Serum IgG level determined on day 15 was markedly increased. On these MDP-Lys (L18)-induced changes, CsA elicited increased NEU and MON counts without any effects on LYM counts, but it

significantly lowered the serum IgG level (Table 1).

In animals treated with MDP-Lys (L18) alone, tarsal joint thickness began to increase from day 7, and the increase was gradually augmented up to day 15 (Fig. 1). After 7-day recovery period, however, the thickness was markedly decreased. Coadministration of CsA markedly exacerbated the joint swelling from day 7 to day 15 without any effect during days 1-6. Additionally, CsA coadministered during days 1-5 and 11-14 also exacerbated the joint swelling from days 6 and 12, respectively. Furthermore, the exacerbating effect of CsA was shown to be dose-dependent in the other experiment. Coadministration of indomethacin or phenylbutazone caused 60% or more decrease in the MDP-Lys (L18)-induced swelling of the tarsal joint. In animals given indomethacin on days 1-5, the initial swelling was delayed up to day 10 and the swelling then gradually increased, and the compound applied on days 11-14

Table 1. Effect of Cyclosporin A (CsA) on Lymphocytes, Neutrophils, Monocytes, and Serum Immunoglobulin G (IgG) Levels of Rats with MDP-Lys(L18)-induced Arthritis

Treatment	Neutrophils ($10^3/\mu\text{l}$)	Lymphocytes ($10^3/\mu\text{l}$)	Monocytes ($10^3/\mu\text{l}$)	Serum IgG (mg/ml)
Vehicle	1.09 ± 0.23^a	9.75 ± 0.35	0.12 ± 0.02	1.98 ± 0.19
MDP-Lys(L18)	0.95 ± 0.15	13.58 ± 0.75^b	0.65 ± 0.11^b	5.58 ± 5.53^b
MDP-Lys(L18)+CsA	1.59 ± 0.14^c	14.66 ± 0.95^b	1.11 ± 0.27^b	2.95 ± 0.49^c

^a, mean $\pm$ S.E.M. from 5 rats; ^b, $p < 0.05$ vs. vehicle control; ^c, $p < 0.05$ vs. MDP-Lys(L18).

Fig. 1. Time-course effects on the thickness of the tarsal joint of rats receiving 14-day treatment of MDP-Lys (L18) 4 mg/kg (s.c.) alone (●) or in combination with cyclosporin A (CsA) 10 mg/kg (p.o.) during days 1-5 (◆), days 11-14 (▲), and days 1-14 (■). Vehicle control (○) was subcutaneously treated with PBS alone.

reduced tarsal joint swelling after day 12. Treatment with dexamethasone resulted in a markedly smaller joint thickness than the vehicle control because of an antiinflammatory effect and a partly depressed body weight gain. D-Penicillamine and aurothioglucose, antirheumatic drugs, had no effect on MDP-Lys (L18)-induced tarsal joint swelling.

Histology of MIA

Histological changes in the synovial membrane of the tarsal joint from rats receiving MDP-Lys (L18) were as follows:

2 hr after a single dose (day 1). No change was observed.

4 hr. Synovial lining cells appeared to be arranged in layers in a few areas, but not to a great extent. There were a few NEUs attached to the capillary walls just beneath the lining cells.

6 hr (Fig. 2). The synovial membrane was slightly congested and edematous. Synovial lining cells with vesicular cytoplasm showed a partially multilayered arrangement, with, in some areas, destruction and desquamation of cytoplasm into the joint space, in association with infiltration of NEUs. At this sampling point, the reaction of the synovial lining cells was more prominent than of NEUs.

8 hr. Increased number of synovial lining cells, rarely with mitotic figures, and increased infiltration of NEUs were seen compared with the earlier sam-

pling times. Synovial lining cells showed nuclei of various shapes, eosinophilic cytoplasm with somewhat angular contours, and small intercellular spaces at the base of the multilayered area. Together with these findings, more marked vesiculation of the cytoplasm of the lining cells suggested functional activation of the cells.

Day 2 (Fig. 3). Markedly expanded joint spaces contained numerous desquamated synovial lining cells, NEUs and amorphous substances. The synovial membrane showed partial edema, significant infiltration of NEUs throughout the membrane and some surface areas without lining cells. The synovial lining cells also revealed significant reactions: partial proliferation, a more marked multilayer arrangement with infiltration of cells, enlargement with vesicular cytoplasm, and occasional necrosis.

Day 4. Desquamated and infiltrated cells were markedly decreased in number in the joint space, which contained only a small amount of eosinophilic amorphous substance and erythrocytes. The synovial membrane showed considerable reduction of cell infiltration and appeared to be fibrotic. The synovial lining cells, in parts arranged in layers, were smaller in size because vesiculation was absent.

Day 8. The joint space was expanded again, and there were many NEUs, a small number of desquamated synovial lining cells and a little eosinophilic amorphous substance. The synovial membrane became very congested and edematous, and this

Fig. 2. Synovial membrane of the tarsal joint of a Sprague-Dawley rat treated with a single dose of MDP-Lys (L18) 4 mg/kg and killed 6 hr later. Synovial lining cells with vesicular cytoplasm are arranged in layers. HE $\times 333$.

Fig. 3. Synovial membrane of the tarsal joint of a Sprague-Dawley rat treated with a single dose of MDP-Lys (L18) 4 mg/kg and killed 24 hr later. Many synovial lining cells are desquamated into the joint space, which contains amorphous substance. HE $\times 141$.

was associated with proliferation of fibroblasts. Multilayered synovial lining cells again showed cytoplasmic vesiculation and desquamation. There were sometimes small necrotic foci with marginal infiltration of NEUs in the synovial membrane and superficial focal destruction of the membrane. Beneath the lining cells, many NEUs and a moderate number of mononuclear cells had infiltrated. The number of infiltrating cells was larger than that after 1st administration.

Day 15 (Fig. 4). The synovial membrane showed moderate to severe edematous thickening with

a large number of mononuclear cells, M ϕ s, and fibroblasts and a small number of LYMs. NEUs were seen mainly in the marginal areas of necrotic foci. Superficial necrotic areas and destruction were more frequent and larger than those observed on day 8.

After 7-day recovery period. Edema and cell infiltration were greatly decreased in the synovial membrane, which appeared to be shrunken and fibrotic and included a small number of mononuclear cells and a few small granulomatous foci. The surface of the synovial membrane was covered with the

Fig. 4. Synovial membrane of the tarsal joint of a Sprague-Dawley rat treated with a single dose of MDP-Lys (L18) 4 mg/kg for 14 days and killed 24 hr later. Marked cell infiltration and a necrotic area are seen. HE $\times 81$.

lining cells of normal arrangement and appearance.

Cytokine concentration in medium of rat peritoneal $M\phi$ culture and dog synovial fluid

IL-1 level tended to increase at 0.1–1 $\mu\text{g/ml}$ of MDP-Lys (L18) and was significantly increased at 10 $\mu\text{g/ml}$. TNF level was increased at 0.1 $\mu\text{g/ml}$ of MDP-Lys (L18) or more. CSF level was significantly increased at 0.01 $\mu\text{g/ml}$ of MDP-Lys (L18) and upward, reaching a peak at 0.1 $\mu\text{g/ml}$. NEU chemotaxis (NCF) tended to increase at 0.01 $\mu\text{g/ml}$ of MDP-Lys (L18), reached a peak at 0.1 $\mu\text{g/ml}$, and then gradually decreased at 1.0 $\mu\text{g/ml}$ and

more. CsA showed no effect on the MDP-Lys (L18)-induced increase in cytokine levels (Fig. 5).

Coculture of cycloheximide inhibited the MDP-Lys (L18)-stimulated production of NCF in a dose-dependent manner, while AA-861 did not.

CINC levels in the rat $M\phi$ -CM were significantly increased in a dose-dependent manner in the presence of MDP-Lys (L18) at 0.01 to 10 $\mu\text{g/ml}$ (Fig. 6).

NCF in the rat $M\phi$ -CM stimulated with 0.1 $\mu\text{g/ml}$ of MDP-Lys (L18) was partially purified by a series of ammonium sulfate precipitation, dialysis and heparin-affinity chromatography. As shown in

Fig. 5. Neutrophil chemotaxis of the rat macrophage conditioned medium stimulated by MDP-Lys (L18) with or without cyclosporin A (CsA). Vertical bars show the S.D. from quadruplicate wells. *, $p < 0.05$ vs. control.

Fig. 6. Cytokine-induced neutrophil chemoattractant (CINC) in the rat macrophage conditioned medium stimulated with various concentrations of MDP-Lys (L18) for 24 hr. Vertical bars show the S.D. from triplicate wells. **, $p < 0.01$ vs. control.

Table 2. Partial Purification of the Neutrophil Chemotactic Factor in the Rat Macrophage Conditioned Medium Treated with MDP-Lys (L18)

	Volume (ml)	Total protein (mg)	Chemotaxis ^a	Total chemotaxis ^b	Specific activity ^c
Crude supernatant ^d	300	1071.6	34.5	10350	9.66
Ammonium sulfate precipitate and dialysis	65	1030.4	266.5	17322	16.81
Heparin-Sepharose column flow-through fraction	65	949.1	1.0	65	0.07
fraction eluted with 0.05M tris-HCL	80	46.7	0	—	—
fraction eluted with 0.05M tris-HCL+0.1M NaCl	80	30.3	0	—	—
fraction eluted with 0.05M tris-HCL+0.4M NaCl	60	27.3	184	11040	404

^a, mean number of migrated neutrophils against 1/100 diluted sample.

^b, volume (ml) × chemotaxis.

^c, total chemotaxis/total protein (mg)

^d, rat peritoneal macrophages were incubated in a medium containing 0.1 mg/ml of MDP-Lys(L18) and 10% of fetal bovine serum for 24 hr.

Table 2, the chemotactic activity was not decreased after ammonium sulfate precipitation and dialysis. The dialyzed sample was chromatographed on a heparin-affinity column, and chemotactic activity mostly remained in the heparin-binding fraction. By these purification procedures, a total amount of protein decreased from 1071.6 mg to 27.3 mg; hence the specific activity of NCF increased by some forty times.

In the synovial fluid from dogs with MIA, IL-1, TNF or CSF was not detected by bioassays. However, NCF was demonstrated in the synovial fluid,

and its chemotactic activity also remained in the dialyzed synovial fluid sample.

Differentiation of MIA from AIA

Consecutive treatment with MDP-Lys (L18) for 14 days increased the thickness of the tarsal joint in Lewis rats. Coadministration of CsA with MDP-Lys (L18) remarkably enhanced the increase. Adjuvant also increased the thickness in Lewis rats on day 15 after administration, and this was completely inhibited by CsA. In Fischer and nude rats, com-

Fig. 7. Tibio-tarsal joint of a Lewis rat receiving MDP-Lys (L18) for 14 days. Moderate synovitis is seen. HE × 18.

bined treatment with CsA and MDP-Lys (L18) increased tarsal joint thickness, whereas MDP-Lys (L18) alone did not. On the other hand, adjuvant increased the thickness in Fischer rats but not in nude rats.

In Lewis rats, MDP-Lys (L18) induced moderate synovitis (Fig. 7) which is similar to that on day 15 in S.D. rats. In addition, periosteal bone proliferation and invasion of granulation tissue into epiphyseal bone were also observed to very mild degrees. In spite of no change in the tarsal joint thickness, there was synovitis in Fischer and nude rats as well, with similar severity which was lower than that in

Lewis rats. Changes were located in the surface and sometimes deeper in the synovial membrane. The synovial lining cell layer was thickened, invaded by NEUs and partially desquamated (Fig. 8), being similar to the early change observed in S.D. rats (Fig. 2). Inflammatory cells appeared more packed in deeper areas of the synovial membrane, and necrotic cells were seen in that area in nude rats.

Concurrent treatment with CsA and MDP-Lys (L18) exacerbated the synovitis in extent and severity of inflammation, and synovial lining cells were mostly desquamated in many areas (Fig. 9). Periosteal bone proliferation and invasion of bone by granula-

Fig. 8. Synovial membrane in the tibio-tarsal joint of a nude rat receiving MDP-Lys (L18) for 14 days. The layer of synovial lining cells is thickened, invaded by infiltrating cells, and partially desquamated. HE $\times 142$.

Fig. 9. Tibio-tarsal joint of a Lewis rat receiving MDP-Lys (L18) and cyclosporin A for 14 days. Synovitis is exacerbated compared to that induced by MDP-Lys (L18) alone (Fig. 7.). HE $\times 18$.

tion tissue were also increased in severity. The exacerbation was observed in all strains, being more pronounced in Lewis and Fischer rats than in nude rats. In an additional experiment, however, CsA showed no modification of the early synovitis induced by a single administration of MDP-Lys (L18).

Adjuvant induced the most severe arthritis characterized by LYM infiltration and accumulation in synovial membrane and accelerated bone destruction and formation in Lewis and Fischer rats. This was more severe than in Lewis and Fischer rats receiving MDP-Lys (L18) plus CsA. Concomitant administration of CsA completely blocked the development of AIA in Lewis rats. Furthermore, adjuvant never induced arthritis in nude rats.

As shown in Fig. 10, the recipient rats injected with spleen cells from AIA Lewis rats showed a significant increase in tarsal joint thickness from 7 days after cell transfer onwards. However, transfer of spleen cells from MIA rats did not induce arthritis in the recipient rats by day 28.

T lymphocyte subset

Subpopulations of CD5⁺ (thymocyte/peripheral T) and CD4⁺ (helper/inducer T) cells were not changed by MDP-Lys (L18) or adjuvant either with or without CsA on day 2, but the CD8⁺ (suppressor/

cytotoxic T) populations in all these groups were slightly increased as compared with that of the control. The consecutive treatment with MDP-Lys (L18) thereafter showed a tendency to decrease the CD5⁺ and CD8⁺ populations, but significantly increased that of CD4⁺ from day 8 (Fig. 11). The percentages of CD5⁺, CD4⁺, and CD8⁺ cells in the MDP-Lys (L18) group were 82.5%, 133.3%, and 78.1%, respectively, of those of the control on day 15. CsA alone tended to decrease the CD5⁺ and CD8⁺ populations and significantly accelerated both the MDP-Lys (L18)-induced decrease in those populations and the MDP-Lys (L18)-induced increase in the CD4⁺ on day 15. The CD5⁺, CD4⁺, and CD8⁺ populations of the coadministered group were 66.0%, 156.8%, and 57.7%, respectively, of those of the control on day 15. Adjuvant did not affect the LYM subpopulation until day 8, but significantly increased the CD4⁺ population by 20% of that of the control on day 15. CsA significantly blocked the increase in the CD4⁺ induced by adjuvant. The CD4⁺/CD8⁺ ratio in the MDP-Lys (L18) group was increased from day 8 until day 15, and this increase was markedly accelerated by CsA coadministration. However, adjuvant showed no effect on the CD4⁺/CD8⁺ ratio throughout the experimental period.

Fig. 10. Transfer of MDP-Lys (L18)- and adjuvant-induced arthritis to normal Lewis rats. Spleen cells from donor Lewis rats treated with PBS (○) or MDP-Lys (L18) 4 mg/kg (■) daily for 10 days, or with a single injection of Freund's complete adjuvant (FCA) (▲) were cultured with Con A for 48 hr, then injected intravenously into recipient Lewis rats. The thickness of the tarsal joint of the recipients was measured after cell transfer. Each mark shows group mean value and vertical bars show the S.D. from 5 rats. *, $p < 0.05$ vs. control.

Fig. 11. Percentages of CD4⁺ and CD8⁺ lymphocytes among the spleen cells of rats receiving MDP-Lys (L18) 4 mg/kg and adjuvant alone or combination with cyclosporin A (CsA) on days 2, 8, and 15. Vertical bars show S.D. from 3 rats. *, $p < 0.05$ vs. control; #, $p < 0.05$ vs. MDP-Lys (L18) and adjuvant alone.

Fig. 12. Mechanisms of MDP-Lys (L18)-induced arthritis and exacerbating effect of cyclosporin A (CsA) on it.

Discussion

Laboratory examination

MDP-Lys (L18) increased NEU counts in the blood of rats, with recovery to the control level by 24 hr, after 1-, 7- and 14-day administration, and also increased the counts of MON and LYM in the present study. MDP and MDP-Lys (L18) have been reported to stimulate the production of G-CSF in mouse M ϕ cultures^{5,26,27}. The G-CSF is also known to move Gs from the bone marrow into the peripheral circulation. Therefore, the increases in the peripheral NEU count in rats treated with MDP-Lys (L18) are thought to be induced by an enhanced delivery of NEUs from the bone marrow to the peripheral circulation. Moreover, the recovery of the NEU counts 24 hr after each dosage is speculated to be due to G-CSF production induced by the drug. The number of M ϕ progenitor cells in the bone marrow and of MONs in the peripheral blood was reported to be increased in mice receiving MDP²⁸, indicating a movement of MONs from the bone marrow to the circulation. MDP-Lys (L18) has also been reported to increase the number of G-M ϕ progenitor cells⁶ and stimulate *in vitro* production of IL-1, which can induce proliferation of LYMs⁴, and these data may explain the increase in MON and LYM counts which was seen in MIA rats.

CsA caused significant augmentation of MDP-Lys (L18)-induced increase in NEU counts without affecting LYM counts, but serum IgG level was decreased. CsA was reported to bind to NEUs as well as LYMs²⁹, and to enhance the mobilization of NEUs to an inflammatory focus³⁰. In view of decreased serum IgG levels seen with CsA, there was no positive relationship between serum IgG levels and the process of arthritis because CsA enhanced MIA without increasing the serum IgG level, although the serum IgG measured was nonspecific polyclonal IgG. These results suggest that humoral immunity is not included in the development of MIA. Then, the increased serum IgG levels induced by MDP-Lys (L18) seem to have been induced by the adjuvant actions of the drug⁴.

Histology of MIA

During the course of MDP-Lys (L18) treatment,

tarsal joint thickness began to increase from day 7 up to day 15. However, the earliest histological changes were already induced in the synovial membrane on day 1. The early synovitic changes induced by MDP-Lys (L18) are thought to be due to an activation of the synovial lining (M ϕ -like) cells, which were observed as vesiculation and enlargement of the cells, M ϕ s in synovial membrane, or both because MDP-Lys (L18) has been reported to stimulate M ϕ s to release cytokines. Moreover, neutrophilic infiltration was seen at the same time in the synovial membrane. Acute exudative synovitis caused by MDP-Lys (L18) was transiently decreased in severity on day 4. There is no clear explanation for the significance of this decrease, but it may indicate an intermediate stage between an acute inflammation and a chronic one, or a stage preceding an immunological reaction. From day 8, the arthritis progressed in association with chronic signs, such as fibroblast proliferation, infiltration of mononuclear cells and LYMs, and necrosis or destruction of the synovial membrane. However, exudative changes and neutrophilic infiltration were seen throughout the course of MDP-Lys (L18) dosing, and the synovitis readily decreased in degree after the cessation of dosing. This may mean that the repeated daily acute inflammation caused by MDP-Lys (L18) induced the chronic appearance of the synovitis, and that marked recovery was seen because the drug could induce only acute synovitis in rats.

Cytokines

MDP-Lys (L18)-induced tarsal joint swelling was markedly suppressed by indomethacin and phenylbutazone which are well known to inhibit the activity of cyclooxygenase and the production of prostaglandins (PGs)³¹. The same suppressive action was observed with dexamethasone, a steroidal antiinflammatory drug that is reported to reduce PGs through the blocking of phospholipase A₂^{32,33}. In addition, indomethacin showed the suppression in any coadministration regimen with MDP-Lys (L18) during days 1-14, 1-5 and 11-14. These results suggest that PGs may be involved throughout the developmental course of MIA.

MDP-Lys (L18) is a potent inducer of several cytokines from murine cell lines of M ϕ , or human and monkey MONs *in vitro*, including IL-1⁴, IL-

^{63,35}, G-CSF³⁶, and M ϕ -CSF³⁷. The present study has demonstrated that IL-1, TNF, CSF, and NCF were also produced from rat M ϕ s stimulated with MDP-Lys (L18). On the other hand, in the dog synovial fluid, NCF, but not IL-1, TNF or CSF, activity was detected. These suggest that NCF is the most important factor for MIA. CsA, however, did not modify the IL-1, TNF, CSF, and NCF levels elevated by MDP-Lys (L18) in rat M ϕ -CM, suggesting no contribution of the cytokines to the exacerbating effect of CsA on MIA in rats.

The production of NCF by rat M ϕ s was inhibited by cycloheximide in a dose-dependent manner, but not by AA-861. These results suggest that NCF in the rat M ϕ -CM was a protein rather than leukotriene B₄, which is a potent chemotactic substance derived from arachidonic acid by lipooxygenase³⁸. The partial purification of NCF revealed that the chemotactic activity was not decreased by dialysis and was detected in the heparin-binding fraction. From these, NCF in the rat M ϕ -CM is thought to be a member of the chemokine supergene family, such as IL-8, gro, M ϕ chemotactic and activating factor, and M ϕ inflammatory peptides³⁹. Moreover, the present immunoassay showed that CINC (rat IL-8)⁴⁰ was produced by rat M ϕ s stimulated with MDP-Lys (L18) in a dose-dependent manner, suggesting that CINC could be one of NCFs in the rat M ϕ -CM. Chemotactic activity in the synovial fluid from arthritic dogs was not lost by dialysis. Therefore, the high molecular weight chemotactic factors are also thought to be a chemokine. Although a further characterization is necessary to confirm the chemokine in the dog synovial fluid, this is practically impossible to dogs because of shortage of sample and absence of a specific antibody against the dog chemokines.

A significantly high level of IL-8 has been reported in the synovial fluid from patients with rheumatoid arthritis^{41,42}, and intra-articular administration of IL-8 to rabbits caused arthritis^{43,44}. Chemokines were shown to induce NEU infiltration into the synovial membrane and lysosomal enzyme release from the activated NEUs⁴⁵⁻⁴⁸. From these, the chemokines are considered to play an important role in the pathogenesis of MIA in rats and dogs.

MIA and AIA

MIA was induced in S.D., Lewis and Fischer

rats and mild synovitis even in athymic nude rats. MIA was also induced in Wister and Donryu rats (data not shown), showing no strain dependent difference. The susceptibility, however, was lower in Fischer and nude rats than in Lewis rats. A similar susceptibility in the former 2 strains is thought to depend on a genetic relationship because the nude rats were produced by back cross of the *rnu* gene into Fischer rats. The low susceptibility of Fischer rats has also been reported in other experimental models such as type II collagen- and streptococcal cell wall-induced arthritis⁴⁹⁻⁵¹. Fischer rats were shown to have high serum levels of corticotrophin releasing hormone, adrenocorticotrophic hormone and corticosterone in response to inflammatory stimuli, and to be relatively resistant to experimental arthritis^{52,53}. The low susceptibility of Fischer rats to MIA may be related to high levels of these hormones. However, combined treatment of CsA with MDP-Lys (L18) exacerbated MIA in Fischer rats in comparable degrees to that in Lewis rats. Therefore, the exacerbation of MIA by CsA is concluded not to be under hormonal control.

The present study clearly differentiated MIA in the mechanism from AIA. Although AIA has generally been induced by a single injection of *Mycobacterium butyricum*, MIA required repeated treatment with MDP-Lys (L18) to arise and recover after cessation of the treatment. Histologically, MIA showed NEU infiltration into the synovial membrane in its early phase, whereas AIA was reported to be characterized by LYM infiltration⁵⁴ and showed LYM accumulation in the synovial membrane in this study. MIA was produced in rats of all 3 strains, Lewis, Fischer, and athymic nude, while AIA was not produced in nude rats. CsA showed contrary effects on MIA and AIA; the former was exacerbated whereas the latter completely blocked. Furthermore, in a transfer experiment, AIA was successfully transferred to recipient Lewis rats through spleen cells (mostly LYMs) of the donors with AIA, but attempts to transfer MIA failed. It has been widely accepted that AIA is induced by a mechanism involving helper T cell dependent delayed type hypersensitivity (DTH)^{11,14,55-58}. On the contrary, the present results have demonstrated that the pathogenesis of MIA is not helper T cell dependent, and that no DTH reactions take place in the pathogenesis of MIA.

Synovitis appeared from day 1, recovered once on day 4, and thereafter increased in severity as the treatment with MDP-Lys (L18) progressed up to day 14. No involvement of helper T cells in the pathogenesis of MIA was shown as mentioned above, but there is a possibility that T cells of other type may play a role in the later, but not early, stage of MIA because MDP-Lys (L18) induced only mild synovitis in athymic nude rats, which is similar to the early change seen in S.D. rats, and CsA markedly exacerbated the later development of MIA in euthymic Fischer, Lewis, and S.D. rats without any effect on the histology of early synovitis induced by MDP-Lys (L18). Kohashi *et al.* (1986)¹⁶ observed that MDP-Lys (L18) induced acute polyarthritis in both euthymic and athymic rats, the former becoming chronic but not the latter. Accordingly, they suggested that T cells play a role in transformation from acute to chronic arthritis. Then, the suppressor T cells may play some roles in both the later development of MIA and its exacerbation by CsA although there is no information about the effect of CsA on the suppressor T cells in rats.

T lymphocyte subset

The change in the T cell subpopulations of CD4⁺ and CD8⁺ was shown to be involved from day 8 in the development of MIA in rats, of which tarsal joint thickness began to increase. Furthermore, the increase in the CD4⁺ population was common on the later stage to both MIA and AIA, with the difference between them owing to the populations of CD8⁺, which decreased in the former, thus leading to a higher CD4⁺/CD8⁺ ratio, but not in the latter. CsA was shown to enhance the MDP-Lys (L18)-induced effects on both CD4⁺ and CD8⁺, but to inhibit completely the adjuvant-induced increase in the CD4⁺ population. These findings are thought to correlate with the development of MIA and AIA and the enhancing and inhibiting effects of CsA on them. It is evident that CsA inhibits both the induction and the effector phases of DTH responses *in vivo*⁵⁹, suggesting that CsA completely inhibited AIA development by the reduction of the CD4⁺ (helper/inducer) cell population. On the contrary, MIA was not caused by DTH reactions and was exacerbated by CsA. Taking these together, the decreased CD8⁺ (suppressor/cytotoxic) rather than the increased CD4⁺ cells is

thought to play an important role in the later development of MIA. However, it is impossible to determine which type of LYM, suppressor or cytotoxic, changed in such a way as to cause the decrease in the CD8⁺ cell population in MIA. AIA has been reported to be more severe in suppressor-T-cell-depleted rats created by thymectomy, low-dose whole-body irradiation⁶⁰, or treatment with low doses of cyclophosphamide^{61,62}. Suppressor T cells, therefore, seem to exert a regulatory effect on the development of AIA in rats, and a regulatory effect of CD8⁺ cells has also been suggested in rats with collagen- and proteoglycan-induced arthritis^{63,64} and in MRL/l mice⁶⁵. From the reference data, it is possible that decreased number of suppressor T cells contributed to the later development of MIA, and CsA exacerbated the arthritis by further decrease in the cell population.

Mechanisms

From the present results, the mechanisms of MIA and its exacerbation by CsA in rats are considered to be as follows (Fig. 12). In the early stage, MDP-Lys (L18) stimulates synovial lining (Mφ-like) cells and/or Mφ in the synovial membrane to release IL-1, TNF, CSF, and NCF (particularly CINC). The first two of them induce PGs to produce edema and the last one does NEU infiltration, leading to acute synovitis in the tarsal joint. In the later stage, repetition of the synovitis and reduced number of suppressor T cells make the lesion more severe, associated with joint swelling. CsA exacerbated the later development of MIA by the additive effect to decrease suppressor T cells with MDP-Lys (L18) and, in part, by enhancing mobilization of NEU to the inflammatory focus.

References

1. Ellouz, F., Adam, A., Ciorbaru, R., and Lederer, E.: Minimal structural requirements for adjuvant activity of bacterial peptidoglycan derivatives. *Biochem Biophys Res Commun* **59**: 1317-1325, 1974.
2. Merser, C., Sinay, P., and Adam, A.: Total synthesis and adjuvant activity of bacterial peptidoglycan derivatives. *Biochem Biophys Res Commun* **66**: 1316-1322, 1975.
3. Akasaki, M., Masayasu, H., Tsukada, W., and Takegoshi, T.: Specific binding sites of murectasin on murine macrophages. *Arzneim-Forsch/Drug Res* **38(II)**: 978-980, 1988.
4. Akasaki, M., Takashi, T., Kita, Y., and Tsukada, W.:

- Augmentation of immune responses by a muramyl dipeptide analog, MDP-Lys (L18). *Agents Actions* **22**: 144-150, 1987.
5. Yamaguchi, F, Akahane, K, Takashi, T, and Tsukada, W: Production of colony-stimulating factor from macrophages by murectasin. *Arzneim-Forsch/Drug Res* **38(II)**: 983-986, 1988.
6. Yamaguchi, F, Akasaki, M, and Tsukada, W: Induction of colony-stimulating factor and stimulation of stem cell proliferation by injection of murectasin. *Arzneim-Forsch/Drug Res* **38(II)**: 980-983, 1988.
7. Nakajima, R, Ishida, Y, Yamaguchi, F, Otani, T, Ono, Y, Nomura, M, Une, T, and Osada, Y: Beneficial effect of murectasin on experimental leukopenia induced by cyclophosphamide or irradiation in mice. *Arzneim-Forsch/Drug Res* **38(II)**: 986-992, 1988.
8. Endo, N: *In vitro* and *in vivo* augmentation by murectasin of the production of the third component of complement by murine macrophages. *Arzneim-Forsch/Drug Res* **38(II)**: 993-996, 1988.
9. Endo, N, Okuda, T, Osada, Y, and Zen-Yoji, H: Stimulation of plasma fibronectin production in mice by murectasin. *Arzneim-Forsch/Drug Res* **38(II)**: 997-998, 1988.
10. Chang, Y-H: Immunopathogenesis of adjuvant arthritis in rats and Reiter's syndrome in humans. *Adv Inflamm Res* **7**: 149-160, 1984.
11. Borel, JF, Feurer, C, Gubler, HU, and Stahelin, H: Biological effects of cyclosporin A: a new antilymphocytic agent. *Agents Actions* **6**: 468-475, 1976.
12. Nagao, S, and Tanaka, A: Muramyl dipeptide-induced adjuvant arthritis. *Infect Immun* **28**: 624-626, 1980.
13. Kohashi, O, Kohashi, Y, Kotani, S, and Osawa, A: A new model of experimental arthritis induced by an aqueous form of synthetic adjuvant in immunodeficient rats (SHR and nude rats). *Ryumachi* **21**: 149-156, 1981.
14. Kohashi, O, Aihara, K, Ozawa, A, Kotani, S, and Azuma, I: New model of a synthetic adjuvant, N-acetylmuramyl-L-alanyl-D-isoglutamine-induced arthritis. Clinical and histologic studies in athymic nude and euthymic rats. *Lab Invest* **47**: 27-36, 1982.
15. Zidek, Z, Masek, K, and Jiricka, Z: Arthritogenic activity of a synthetic immunoadjuvant, muramyl dipeptide. *Infect Immun* **35**: 674-679, 1982.
16. Kohashi, O, Kohashi, Y, Shigematsu, N, Ozawa, A, and Kotani, S: Acute and chronic polyarthritis induced by an aqueous form of 6-O-acyl and N-acyl derivatives of N-acetylmuramyl-L-alanyl-D-isoglutamine in euthymic rats and athymic nude rats. *Lab Invest* **55**: 337-346, 1986.
17. Ono, Y, Iwasaki, T, Sekiguchi, M, and Onodera, T: Subacute toxicity of murectasin in mice and dogs. *Arzneim-Forsch/Drug Res* **38(II)**: 1024-1027, 1988.
18. Sugawara, T, Kato, M, and Takayama, S: Arthritis and hematological changes induced by an analogue of muramyl dipeptide in rats. *Fundam Appl Toxicol* **18**: 395-404, 1992.
19. Sugawara, T, Kato, M, Furuhashi, K, Takada, S, and Takayama, S: Pharmacological aspects of arthritis induced by a muramyl dipeptide analogue in rats. *Eur J Pharmacol* **228**: 147-153.
20. Sugawara, T, Takada, S, Furuhashi, K, Takayama, S, Nomura, M, Kato, M: Different effect of cyclosporin A on arthritides induced by a muramyl dipeptide analogue or the complete adjuvant in rats. *Int J Exp Pathol* **76**: 191-199, 1995.
21. Sugawara, T, Miyamoto, M, Takada, S, and Kato, M: Alteration of spleen lymphocyte populations in rats with arthritis induced by muramyl dipeptide analogue or complete adjuvant. *Int J Tissue React* **17**: 5-13, 1995.
22. Sugawara, T, Takada, S, Miyamoto, M, Nomura, M, Kato, M: Inflammatory cytokine production induced by an analogue of muramyl dipeptide MDP-Lys (L18) in the rat macrophage culture and the dog synovial fluid. *Inflammation* **20**: 43-56, 1996.
23. Ohuchi, K, Watanabe, M, Yoshizawa, K, Tsurufuji, S, Fujiki, H, Suganuma, M, Sugimura, T, and Levine, L: Stimulation of prostaglandin E₂ production by 12-O-tetra-decanoylphorbol 13-acetate (TPA)-type and non-TPA type tumor promoters in macrophages and its inhibition by cycloheximide. *Biochim Biophys Acta* **834**: 42-47, 1985.
24. Sugawara, T, Miyamoto, M, Takayama, S, and Kato, M: Separation of neutrophils from blood in human and laboratory animals and comparison of the chemotaxis. *J Pharmacol Toxicol Method* **33**: 91-100, 1995.
25. Taurig, JD, Sandberg, GP, and Mahowald, ML: The cellular basis of adjuvant arthritis. I. Enhancement of cell-mediated passive transfer by concanavalin A and by immunosuppressive pretreatment of the recipient. *Cell Immunol* **75**: 271-282, 1983.
26. Staber, FG, Gisler, RH, Schumann, G, Tarcsay, L, Schlaffli, E, and Dukor, P: Modulation of myelopoiesis by different bacterial cell-wall components: Induction of colony-stimulating activity (by pure preparations, low-molecular-weight degradation products, and a synthetic low-molecular analog of bacterial cell-wall components) in vitro. *Cell Immunol* **37**: 174-187, 1978.
27. Galelli, A, Lefrancier, P, and Chedid, L: Colony-stimulating activity induced by synthetic muramyl peptides: variation with chemical structure and association with anti-infectious activity. *Infect Immun* **46**: 495-500, 1984.
28. Wuest, B, and Wachsmuth, ED: Stimulatory effect of N-acetyl muramyl dipeptide *in vivo*: Proliferation of bone marrow progenitor cells in mice. *Infect Immun* **37**: 452-462, 1982.
29. Kharazmi, A, Svenson, M, Nielsen, H, and Birgens, HS: Effect of cyclosporin A on human neutrophil and monocyte function. *Scand J Immunol* **21**: 585-591, 1985.
30. Ormrod, DJ, Cawley, S, and Miller, TE: Cyclosporin A modulation of the acute inflammatory response: an explanation for the effect of CsA on host defences in infection. *J Exp Pathol* **71**: 69-82, 1990.
31. Vane, J, and Botting, R: Inflammation and the mechanism of action of anti-inflammatory drugs. *FASEB J* **1**: 89-96, 1987.
32. Flower, RJ, and Blackwell, GJ: The importance of phospholipase-A₂ in prostaglandin biosynthesis. *Biochem Pharmacol* **25**: 285-291, 1976.

33. Hong, SL, and Levine, L : Inhibition of arachidonic acid release from cells as the biochemical action of anti-inflammatory corticosteroids. *Proc Natl Acad Sci USA* **73** : 1730-1734, 1976.
34. Shimoda, K, Okamura, S, Harada, N, Omori, F, and Niho, Y : Production of interleukin-6 from macrophages by MDP-Lys (L18), romurtide. *Res Commun Chem Pathol Pharmacol* **70** : 289-296, 1990.
35. Nakajima, R, Ishida, Y, Akahane, K, Sekiguchi, M, and Osada, Y : Stimulatory effect of romurtide on hematopoiesis in monkeys. *Arzneim-Forsch/Drug Res* **41(I)** : 60-65, 1991.
36. Shimoda, K, Okamura, S, Kawasaki, C, Omori, F, Matsuguchi, T, and Niho, Y : Muroctasin [MDP-Lys (L18)] augments the production of granulocyte colony-stimulating factor (G-CSF) from human peripheral blood mononuclear cells in vitro. *Int J Immunopharmac* **12** : 729-735, 1990.
37. Akahane, K, Yamaguchi, F, Kita, Y, Une, T, and Osada, Y : Stimulation of macrophages by muroctasin to produce colony-stimulating factors. *Arzneim-Forsch/Drug Res* **40(I)** : 179-183, 1990.
38. Ford-Hutchinson, AW, Bray, MA, Doig, MV, Shipley, ME, and Smith, MJH : Leukotriene B, a potent chemokinetic and aggregating substance released from polymorphonuclear leukocytes. *Nature* **286** : 264-265, 1980.
39. Oppenheim, JJ, Zachariae, COC, Mukaida, N, and Matsushima, K : Properties of the novel proinflammatory supergene "intercrine" cytokine family. *Ann Rev Immunol* **9** : 617-648, 1991.
40. Watanabe, K, Kinoshita, S, and Nakagawa, H : Purification and characterization of cytokine-induced neutrophil chemoattractant produced by epithelioid cell line of normal rat kidney (NRK-52E cell). *Biochem Biophys Res Commun* **161** : 1093-1099, 1989.
41. Brennan, FM, Zachariae, COC, Chantry, D, Larsen, CG, Turner, M, Maini, RN, Matsushima, K, and Feldmann, M : Detection of interleukin 8 biological activity in synovial fluids from patients with rheumatoid arthritis and production of interleukin 8 mRNA by isolated synovial cells. *Eur J Immunol* **20** : 2141-2144, 1990.
42. Koch, AE, Kunkel, SL, Burrows, JC, Evanoff, HL, Haines, GK, Pope, RM, and Strieter, RM : Synovial tissue macrophage as a source of the chemotactic cytokine IL-8. *J Immunol* **147** : 2187-2195, 1991.
43. Forrest, MJ, Eiermann, GJ, Meurer, R, Walakovits, LA, and MacIntyre, DE : The role of CD18 in IL-8 induced dermal and synovial inflammation. *Br J Pharmacol* **106** : 287-294, 1992.
44. Endo, H, Akahoshi, T, Nishimura, A, Tonegawa, M, Takagishi, K, Kashiwazaki, S, Matsushima, K, and Kondo, H : Experimental arthritis induced by continuous infusion of IL-8 into rabbit knee joints. *Clin Exp Immunol* **96** : 31-35, 1994.
45. Peveri, P, Walz, A, Dewald, B, and Baggiolini, M : A novel neutrophil-activating factor produced by human mononuclear phagocytes. *J Exp Med* **167** : 1547-1559, 1988.
46. Djeu, JY, Matsushima, K, Oppenheim, JJ, Shiotsuki, K, and Blanchard, DK : Functional activation of human neutrophils by recombinant monocyte-derived neutrophil chemotactic factor/IL-8. *J Immunol* **144** : 2205-2210, 1990.
47. Schröder, J-M, Persoon, NLM, and Christophers, E : Lipopolysaccharide-stimulated human monocytes secrete, apart from neutrophil-activating peptide 1/interleukin 8, a second neutrophil-activating protein. NH₂-terminal amino acid sequence identity with melanoma growth stimulatory activity. *J Exp Med* **171** : 1091-1100, 1990.
48. Wolpe, SD, and Cerami, A : Macrophage inflammatory proteins 1 and 2: members of a novel superfamily of cytokines. *FASEB J* **3** : 2565-2573, 1989.
49. Griffiths, MM, Eichwald, EJ, Martin, JH, Smith, CB, and DeWitt, CW : Immunogenetic control of experimental type II collagen-induced arthritis. *Arthritis Rheum* **24** : 781-789, 1981.
50. Griffiths, MM, and DeWitt, CW : Genetic control of collagen-induced arthritis in rats: the immune response to type II collagen among susceptible and resistant strains and evidence for multiple gene control. *J Immunol* **132** : 2830-2836, 1984.
51. Wilder, RL, Calandra, GB, Garvin, AJ, Wright, KD, and Hansen, CT : Strain and sex variation in the susceptibility to streptococcal cell wall-induced polyarthritis in the rat. *Arthritis Rheum* **25** : 1064-1072, 1982.
52. Sternberg, EM, Hill, JM, Chrousos, GP, Kamilaris, T, Listwak, SJ, Gold, PW, and Wilder, RL : Inflammatory mediator-induced hypothalamic-pituitary-adrenal axis activation is defective in streptococcal cell wall arthritis-susceptible Lewis rats. *Proc Natl Acad Sci USA* **86** : 2374-2378, 1989.
53. Sternberg, EM, Young III, WS, Bernardini, R, Calogero, AE, Chrousos, GP, Gold, PW, and Wilder, RL : A central nervous system defect in biosynthesis of corticotropin-releasing hormone is associated with susceptibility to streptococcal cell wall-induced arthritis in Lewis rats. *Proc Natl Acad Sci USA* **86** : 4771-4775, 1989.
54. Pearson, CM : Experimental joint disease. Observations on adjuvant-induced arthritis. *J Chron Dis* **16** : 863-874, 1963.
55. Waksman BH, Pearson CM, and Sharp JT : Studies of arthritis and other lesions induced in rats by injection of Mycobacterial adjuvant. II. Evidence that the disease is a disseminated immunologic response to exogenous antigen. *J Immunol* **85** : 403-417, 1960.
56. Pearson CM, and Wood FD : Passive transfer of adjuvant arthritis to lymph node or spleen cells. *J Exp Med* **120** : 547-560, 1964.
57. Van Eden, W, Holoshitz, J, Nevo, Z, Frenkel, A, Klajman, A, and Cohen, IR : Arthritis induced by a T-lymphocyte clone that responds to *Mycobacterium tuberculosis* and to cartilage proteoglycans. *Proc Natl Acad Sci USA* **82** : 5117-5120, 1985.
58. Borel JF, Feurer, C, Magnee, C, and Stahelin, H : Effects of the new anti-lymphocytic peptide cyclosporin A in animals. *Immunology* **32** : 1017-1025, 1977.
59. Thomson, AW, Moon, D, Inoue, Y, Geczy, C, and Nelson, DS : Modification of delayed-type hypersensitivity reactions to ovalbumin in cyclosporin A-treated

- guinea-pigs. *Immunology* **48** : 301-308, 1983.
60. Kayashima, K, Koga, T, and Onoue, K : Role of T lymphocytes in adjuvant arthritis. I. Evidence for the regulatory function of thymus-derived cells in the induction of the disease. *J Immunol* **117** : 1878-1882, 1976.
61. Kayashima, K, Koga, T, and Onoue, K : Role of T lymphocytes in adjuvant arthritis. II. Different subpopulations of T lymphocytes functioning in the development of the disease. *J Immunol* **120** : 1127-1131, 1978.
62. Bersani-Amado, CA, Duarte, AJS, Tanji, MM, Cianga, M, and Jancar, S : Comparative study of adjuvant induced arthritis in susceptible and resistant strains of rats. III. Analysis of lymphocyte subpopulations. *J Rheumatol* **17** : 153-158, 1990.
63. Chiocchia, G, Boissier, M-C, Manoury, B, and Fourier, C : T cell regulation of collagen-induced arthritis in mice. II. Immunomodulation of arthritis by cytotoxic T cell hybridomas specific for type II collagen. *Eur J Immunol* **23** : 327-332, 1993.
64. Banerjee, S, Webber, C, and Poole, R : The induction of arthritis in mice by the cartilage proteoglycan aggrecan : Roles of CD4⁺ and CD8⁺ T cells. *Cell Immunol* **144** : 347-357, 1992.
65. Lewis, DE, Giorgi, JV, and Warner, NL : Flow cytometry analysis of T cells and continuous T-cell lines from autoimmune MRL/l mice. *Nature* **289** : 298-300, 1981.