Inflammation, Vol. 16, No. 2, 1992

INDUCTION OF NEUTROPHIL INFILTRATION BY RAT CHEMOTACTIC CYTOKINE (CINC) AND ITS INHIBITION BY DEXAMETHASONE IN RATS

NORIYASU HIRASAWA,[1] MASAKO WATANABE,[1] SUETSUGU MUE,[2] KAZUYOSHI WATANABE,[3] SUSUMU TSURUFUJI,[3] and KAZUO OHUCHI[1]

[1]*Department of Biochemistry*
Faculty of Pharmaceutical Sciences, Tohoku University
Aoba Aramaki, Aoba-ku, Sendai, Miyagi 980, Japan

[2]*College of Medical Sciences, Tohoku University*
Seiryo-machi, Aoba-ku, Sendai, Miyagi 980, Japan

[3]*Institute of Cytosignal Research, Inc.*
Ohmori, Ohta-ku, Tokyo 143, Japan

Abstract—In vivo effects of cytokine-induced neutrophil chemotactic factor (CINC) derived from rats on neutrophil infiltration were investigated using an air-pouch-type inflammation model in rats, and effects of dexamethasone on neutrophil infiltration induced by CINC was also examined in order to gain further insight into the mechanism of antiinflammatory activity of glucocorticoids. Injection of CINC into the air pouch made on the dorsum of rats induced a marked infiltration of neutrophils into the pouch fluid but not mononuclear cells and eosinophils during a 30-min interval after the injection. Maximum effect was induced at a dose of 1.4 μg/pouch. Treatment with dexamethasone 3 h before the injection of CINC suppressed the neutrophil infiltration in a dose-dependent manner, but no complete inhibition was observed. CINC injection into the air pouch of rats that had been sacrificed by bleeding in order to minimize neutrophil infiltration from blood stream also stimulated neutrophil infiltration into the pouch fluid when the carcass was incubated at 37°C for 30 min, but the number of infiltrated neutrophils was about 35% of CINC-induced neutrophil infiltration in intact rats. CINC-induced neutrophil infiltration in the carcass, which is supposed to be a reflection of neutrophil migration from extravascular space in subcutaneous tissues to pouch fluid, was not inhibited by dexamethasone treatment. Therefore, the inhibition of neutrophil infiltration by dexamethasone might be due to inhibition of the extravasation of peripheral neutrophils but not due to inhibition of neutrophil chemotaxis from subcutaneous extravascular space to pouch fluid. These findings suggest that clinical effects of steroidal antiinflammatory drugs on neutrophil infiltration in inflammatory disease is partly due to inhibition of neutrophil extravasation induced by preformed neutrophil chemotactic factors in the inflammatory site.

0360-3997/92/0400-0187$06.50/0

INTRODUCTION

Neutrophil infiltration in inflammatory diseases is supposed to be mediated by a variety of chemical mediators produced in the inflammatory locus. Recently, interleukin (IL) -8 was found to be a novel chemotactic factor for neutrophils produced by mononuclear cells in humans (1, 2). Since human IL-8 shows strong chemotactic activity for neutrophils (1, 3) and T lymphocytes (4), and enhanced production of IL-8 by mononuclear cells is observed in patients with rheumatoid arthritis (5), it is possible that human IL-8 plays a significant role in inflammatory diseases.

In rats, cytokine-induced neutrophil chemotactic factor (CINC) is produced by an epithelioid cell line of normal rat kidney and has homology to the KC/*gro* protein (6, 7), which is considered to be one of the IL-8 family (8). However, there is no evidence that the rat CINC plays a significant role in neutrophil infiltration in experimental models of inflammation in rats. Although human IL-8 has the ability to induce neutrophil infiltration in rabbits (9) and rats (10), there is no report that describes in vivo effects of CINC on neutrophil infiltration in rats. The first aim of the present investigation is to clarify whether CINC has an ability to induce neutrophil infiltration in rats in vivo.

Both the production of human IL-8 and rat CINC are inhibited by steroidal antiinflammatory drugs (11, 12). These drugs also inhibit production of chemotactic factors such as leukotriene B_4 and platelet-activating factor via suppressing phospholipase A_2 activity (13). Therefore, inhibition of chemotactic factor production might be a primary mechanism for the inhibition of leukocyte infiltration by steroidal antiinflammatory drugs. On the other hand, we recently demonstrated that dexamethasone, a steroidal antiinflammatory drug, inhibits adherence of neutrophils to cultured vascular endothelial cells (14). Inhibition of neutrophil chemotaxis by dexamethasone using Boyden chambers is also reported (15). These observations suggest another possibility: that the inhibition of neutrophil infiltration by steroidal antiinflammatory drugs is due to inhibition of neutrophil responses to chemotactic factors. The second aim of the present investigation is to prove this possibility, viz., the effects of dexamethasone on CINC-induced neutrophil infiltration were examined using an air-pouch-type inflammation model in rats. The mechanism of action of dexamethasone, especially on inhibition of neutrophil infiltration, is also described.

MATERIALS AND METHODS

Preparation of CINC Solution. Glass instruments were heated at 250°C for 2 h to destroy lipopolysaccharide (LPS) before use. Preparation of CINC solution was performed under aseptic conditions. Chemically synthesized rat CINC (LPS-free, Peptide Institute, Inc., Osaka, Japan) was

dissolved in saline (isotonic sodium chloride solution, Otsuka Pharmaceutical Co., Osaka, Japan) containing 0.1% (w/v) human serum albumin (LPS-free, Fuji Rebio, Tokyo, Japan). A 1% (w/v) solution of sodium carboxymethylcellulose (CMC-Na; Cellogen F3H, Daiichi Kogyo Seiyaku, Niigata, Japan) in saline was autoclaved at 120°C for 15 min, and after cooling to room temperature, an aliquot of the CINC solution and LPS-free human serum albumin were added. Final concentration of human serum albumin was adjusted to 0.1% (w/v).

Induction of Air-Pouch-Type Inflammation in Rats. Male rats of the Sprague-Dawley strain, specific pathogen-free, weighing 160–180 g (Charles River Japan, Inc., Kanagawa, Japan) were used. Eight milliliters of air were injected subcutaneously on the dorsum to make an air pouch in the shape of oval, and 24 h later 4 ml of a 1% (w/v) solution of the CMC-Na solution containing various amounts of rat CINC was injected into the air pouch. Thirty minutes later, the rats were sacrificed by cutting the carotid artery under diethylether anesthesia and the entire volume of the pouch fluid was collected and measured.

In some experiments, in order to minimize participation of peripheral leukocytes in neutrophil infiltration into the pouch fluid, 4 ml of the CMC-Na solution containing 1.4 μg CINC was injected into the air pouch of rats that had been sacrificed by cutting the carotid artery under diethylether anesthesia just before the injection of CINC. The carcasses were then incubated at 37°C for 0.5 h, and the pouch fluid was collected and measured. One group of rats termed "0 h control" was prepared, viz., the rats were sacrificed, then 4 ml of the CMC-Na solution without CINC was injected into the air pouch, and the entire pouch fluid was collected immediately.

Measurement of Number of Neutrophils in Pouch Fluid. The number of total leukocytes in the pouch fluid was counted in a hemacytometer. Differential counts were performed microscopically after May-Gruenwald and Giemsa stain. The number of total neutrophils in the pouch fluid was obtained by multiplying the total number of leukocytes in the pouch fluid by the proportion of the number of neutrophils to leukocytes.

Measurement of Chemotactic Activity for Neutrophils in Pouch Fluid. The obtained pouch fluid was diluted to twofold with RPMI 1640 medium and centrifuged at 10,000*g* for 10 min at 4°C. The supernatant fraction was then diluted to fourfold with RPMI 1640 medium, and the chemotactic activity for neutrophils was measured in a modified Boyden chamber (16). Chemotactic activity is expressed as follows: (number of neutrophils in the lower chamber/number of neutrophils applied in the upper chamber) × 100 (%).

Dexamethasone Treatment. Dexamethasone (Sigma Chemical Co., St. Louis, Missouri) was dissolved in ethanol, then diluted with saline. The final concentration of ethanol in saline was adjusted to 10% (v/v). An aliquot of the drug solution was injected subcutaneously in the abdomen 3 h before the injection of CINC. Control rats received the same amount of the vehicle.

Statistical Analysis. The results are presented as means ± *SEM* from six to seven rats in each group. Comparisons were done with Student's unpaired *t* test.

RESULTS

Effects of CINC on Neutrophil Infiltration into Pouch Fluid. Injection of various doses of CINC into the air pouch induced significant increases in the total number of leukocytes in the pouch fluid at doses of 0.4 and 1.4 μg when examined 0.5 h after the injection (Table 1). However, differential cell counts revealed that the number of neutrophils was increased significantly at doses of 0.14 μg and over, and the increase was dose-dependent. By increasing the doses of CINC up to 4 and 14 μg/pouch, no further increase in the number of neu-

trophils in the pouch fluid was induced (data not shown). No significant changes in the number of mononuclear cells and eosinophils were induced by CINC at doses examined (Table 1). Injection of the CMC-Na solution containing no CINC also induced significant increases in the number of total leukocytes, neutrophils, and mononuclear cells during the 30-min interval in comparison with those of the 0 h control (Table 1). However, the effect of CINC on neutrophil infiltration was much more potent than that of the vehicle, CMC-Na solution.

Effects of Dexamethasone on CINC-Induced Neutrophil Infiltration into Pouch Fluid. Administration of dexamethasone 3 h before the injection of CINC solution into the air pouch inhibited neutrophil infiltration into the pouch fluid during the 30-min interval in a dose-dependent manner (Figure 1). Maximum inhibition by dexamethasone was attained at 3 mg/kg, but complete inhibition was not obtained; the inhibition was about 55% at a dose of 3 mg/kg (Figure 1).

Effects of Dexamethasone on Chemotactic Activity for Neutrophils in Pouch Fluid. The pouch fluid obtained from rats receiving the CMC-Na solution containing no CINC showed very little chemotactic activity for neutrophils (Figure 2). However, the pouch fluid collected from rats that received the CMC-Na solution containing CINC showed strong chemotactic activity (Figure 2). There was no statistically significant difference in chemotactic activity in the pouch fluid between dexamethasone-treated and corresponding control rats (Figure 2).

Effects of Dexamethasone on CINC-Induced Neutrophil Infiltration into Pouch Fluid from Extravascular Space in Subcutaneous Tissues. Injection of

Table 1. Effects of Various Doses of CINC on Number of Leukocytes in Pouch Fluid[a]

Treatment	No. of Cells ($\times 10^6$/pouch)			
	Total leukocytes	Neutrophils	Mononuclear cells	Eosinophils
0 h control	2.38 ± 0.45**	0.11 ± 0.02**	2.18 ± 0.40**	0.06 ± 0.02
CINC (μg/pouch)				
0	5.62 ± 0.30	0.58 ± 0.10	4.89 ± 0.30	0.08 ± 0.02
0.14	7.05 ± 0.90	1.44 ± 0.23*	5.59 ± 0.80	0.09 ± 0.01
0.4	8.22 ± 0.61*	1.98 ± 0.26**	6.25 ± 0.49	0.09 ± 0.02
1.4	7.40 ± 0.50*	2.39 ± 0.25**	4.72 ± 0.47	0.12 ± 0.03

[a]Four milliliters of a 1% (w/v) CMC-Na solution containing the indicated amounts of CINC and 0.1% (w/v) human serum albumin were injected into the air pouch. The entire volume of pouch fluid was collected 0.5 h later, and total numbers of leukocytes, neutrophils, mononuclear cells and eosinophils were measured. "0 h control" means that rats were sacrificed, then injected with 4 ml of a 1% (w/v) CMC-Na solution containing 0.1% (w/v) human serum albumin into the air pouch, and the pouch fluid was collected immediately. Values are the means ± *SEM* of seven rats. Statistical significance: $*P < 0.01$, $**P < 0.001$ vs. CINC control (CINC, 0 μg/pouch).

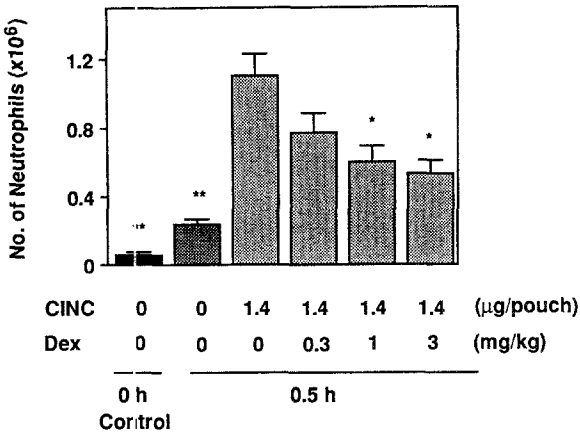

Fig. 1. Effects of various doses of dexamethasone on CINC-induced neutrophil infiltration. Dexamethasone (Dex) at indicated doses was administered subcutaneously 3 h before the injection of CINC. Four milliliters of a 1% (w/v) CMC-Na solution containing 1.4 μg of CINC and 0.1% (w/v) human serum albumin were injected into the air pouch. The pouch fluid was collected 0.5 h later and the total number of neutrophils in the pouch fluid was measured. "0 h control" (the left-hand column) means that rats were sacrificed, then injected with 4 ml of a 1% (w/v) CMC-Na solution containing 0.1% (w/v) human serum albumin into the air pouch, and the pouch fluid was collected immediately. Vertical bars represent *SEM* of six to seven rats. Statistical significance: $*P < 0.01$, $**P < 0.001$ vs. CINC control.

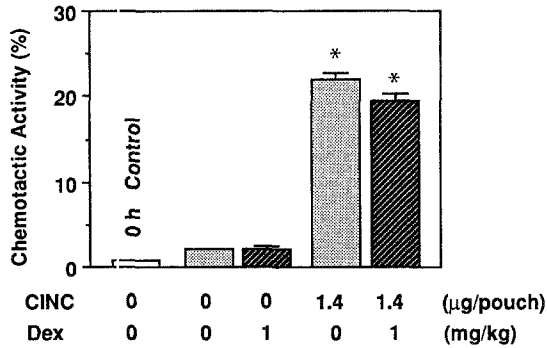

Fig. 2. Effects of dexamethasone on chemotactic activity for neutrophils in the pouch fluids. Dexamethasone (1 mg/kg) was administered subcutaneously 3 h before the injection of CINC. Four milliliters of a 1% (w/v) CMC-Na solution containing 1.4 μg of CINC and 0.1% (w/v) human serum albumin were injected into the air pouch. Rats were sacrificed 0.5 h after the injection of CINC, then the pouch fluid was collected. The pouch fluid was centrifuged, and the supernatant was diluted to eightfold with RPMI 1640 medium. Chemotactic activity for neutrophils was measured by a modified Boyden's chamber method. Each sample was assayed using four chambers. Vertical bars represent *SEM* of six to seven rats. Statistical significance: $*P < 0.001$ vs. corresponding control group.

the CMC-Na solution containing no CINC into the air pouch of rats that had been sacrificed by cutting the carotid artery did not induce a significant increase in neutrophil infiltration during the incubation of the carcasses at 37°C for 30 min (Figure 3B). On the other hand, injection of the CMC-Na solution containing CINC into the air pouch of the carcasses followed by incubation at 37°C for 30 min induced a significantly higher neutrophil infiltration into the pouch fluid (Figure 3B). However, the number of neutrophils was only 35% of that obtained 30 min after the injection of the CMC-Na solution containing CINC into the air pouch of intact rats (Figure 3A). Administration of dexamethasone 3 h before the injection of the CMC-Na solution with or without CINC failed to inhibit neutrophil infiltration into the pouch fluid during the 30-min incubation of the carcasses at 37°C (Figure 3B).

DISCUSSION

This paper is the first to report that chemically synthesized rat CINC has an ability to stimulate neutrophil infiltration in vivo and that dexamethasone directly inhibits the CINC-induced neutrophil infiltration. CINC (6, 7) is a

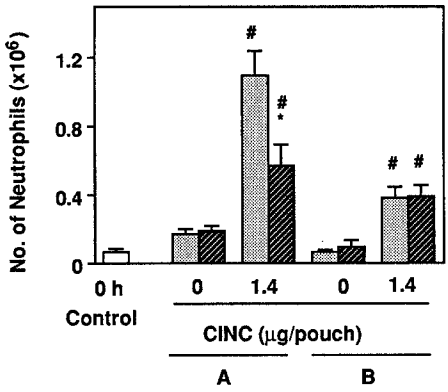

Fig. 3. Effects of dexamethasone on CINC-induced neutrophil infiltration in the sacrificed rats. Dexamethasone (1 mg/kg) was administered subcutaneously 3 h before the injection of CINC. Four milliliters of a 1% (w/v) CMC-Na solution containing 1.4 μg of CINC and 0.1% (w/v) human serum albumin were injected into the air pouch of rats (A) or rats that had been sacrificed just before the injection of CINC (B). (A) Rats were sacrificed 0.5 h after the injection of CINC; then the pouch fluid was collected, and the total number of neutrophils in the pouch fluid was measured. (B) The carcasses were incubated for 0.5 h at 37°C, then the pouch fluid was collected. "0 h control" (the left-hand column) means that rats were sacrificed, then injected with 4 ml of a 1% (w/v) CMC-Na solution containing 0.1% (w/v) human serum albumin into the air pouch, and the pouch fluid was collected immediately. Vertical bars represent *SEM* of six to seven rats. ▒, control rats; ▓, dexamethasone-treated rats. Statistical significance: $^{\#}P < 0.001$ vs. corresponding group received no CINC. $^{*}P < 0.01$ vs. corresponding control group.

protein related to mouse KC protein with 91% sequence homology and human *gro* gene product. IL-8 (8), CINC/KC/*gro* (7), and platelet factor 4 (17) belong to a family of 8-kDa peptides and have strong chemotactic activity for neutrophils.

Formation of an air pouch on the dorsum of rats induced a slight infiltration of mononuclear cells into the air pouch (Table 1; 0 h control), but the injection of the vehicle (CMC-Na) alone into the air pouch elicited a significant increase in the number of mononuclear cells and neutrophils in the pouch fluid (Table 1). The increase in these cells might be due to random migration of neutrophils and mononuclear cells from the surrounding tissues because the injection of the vehicle alone induced very little increase in chemotactic activity in the pouch fluid (Figure 2). In contrast, injection of CINC solution into the air pouch induced a marked infiltration of neutrophils into the pouch fluid in a dose-dependent manner (Table 1), but the number of mononuclear leukocytes and eosinophils in the pouch fluid was almost equal to those of rats injected the vehicle alone (Table 1), indicating that the effect of CINC is specific to neutrophils. Maximum response of CINC was induced at a concentration of 350 ng/ml (5×10^{-8} M, 1.4 μg/pouch), which is close to the concentration of CINC (6) and human IL-8 (3, 18) that exerts maximum chemotactic activity for neutrophils in vitro.

Steroidal antiinflammatory drugs inhibit neutrophil infiltration in several experimental models of inflammation (19, 20). The mechanism of the inhibitory action of the drugs is generally considered to be the inhibition of chemotactic factor production because the drugs have an ability to inhibit production of leukotriene B_4 (13), platelet-activating factor (13), human IL-8 (11), and CINC (12) in vitro, and in fact chemotactic activity in the inflammatory sites is reduced by dexamethasone treatment (21). On the other hand, effects of dexamethasone on chemotactic factor-induced leukocyte infiltration in vivo have not been analyzed precisely. The present investigation demonstrated that dexamethasone treatment does inhibit CINC-induced neutrophil infiltration into the pouch fluid (Figure 1). Since IL-8 has an ability to produce leukotriene B_4 in human neutrophils (22), CINC also might produce leukotriene B_4 when injected into the air pouch. If so, dexamethasone should inhibit CINC-induced production of leukotriene B_4. However, as shown in Figure 2, chemotactic activity for neutrophils in the pouch fluid was not suppressed by dexamethasone treatment. Consequently, inhibition by dexamethasone of CINC-induced neutrophil infiltration can not be ascribed to inhibition of chemotactic factor production that might be induced by CINC injection. Our findings strongly indicate that dexamethasone inhibits CINC-induced neutrophil infiltration directly, not by inhibiting production of chemoattractants.

When CINC was injected into the air pouch of rats that had been sacrificed by bleeding, and the carcass was incubated at 37°C for 30 min, neutrophil

infiltration into the pouch fluid was induced, although it was about 35% of intact rats (Figure 3). In this case, since the rats had been sacrificed by bleeding, neutrophils in the bloodstream could not participate in the CINC-induced increase in the number of neutrophils. Therefore, it is likely that CINC-induced neutrophil infiltration in the sacrificed rats is a reflection of neutrophil chemotaxis into the pouch fluid from the extravascular space in subcutaneous tissues. Histological observations revealed that neutrophils and mononuclear cells do exist in the extravascular space in the subcutaneous tissue when examined 24 h after injection of 8 ml air, which is a proinflammatory stimulus. The observation that dexamethasone inhibits CINC-induced neutrophil infiltration in intact rats but not in the sacrificed rats strongly indicates that dexamethasone inhibits the process of extravasation of neutrophils, viz., adherence to microvascular endothelial cells and diapedesis into the extravascular space through gaps of microvascular endothelial cells. The result that CINC-induced neutrophil infiltration was not inhibited completely by dexamethasone treatment (Figure 1) could be explained by the finding that chemotaxis of neutrophils in the extravascular space of subcutaneous tissues into the pouch fluid is not inhibited by dexamethasone treatment (Figure 3).

IL-8 has an ability to stimulate neutrophil adherence to endothelial cells in vitro (23). We recently reported that dexamethasone inhibits thrombin- or histamine-induced neutrophil adherence to cultured vascular endothelial cells (14). Consequently, it might be possible that dexamethasone inhibits neutrophil adherence to microvascular endothelial cells stimulated by CINC. In conclusion, inhibitory effects of steroidal antiinflammatory drugs on neutrophil infiltration are due to not only inhibition of chemotactic factor production but also inhibition of chemotactic factor-induced extravasation of neutrophils.

REFERENCES

1. YOSHIMURA, T., K. MATUSHIMA, J. J. OPPENHEIM, and E. J. LEONARD. 1987. Neutrophil chemotactic factor produced by lipopolysaccharide (LPS) -stimulated human blood mononuclear leukocytes: Partial characterization and separation from interleukin 1 (IL 1). *J. Immunol.* **139**:788–793.
2. WALZ, A., P. PEVERI, H. ASCHAUER, and M. BAGGIOLLINI. 1987. Purification and amino acid sequencing of NAF, a novel neutrophil-activating factor produced by monocytes. *Biochem. Biophys. Res. Commun.* **149**:755–761.
3. TANAKA, S., E. A. ROBINSON, T. YOSHIMURA, K. MATSUSHIMA, E. J. LEONARD, and E. APPELLA. 1988. Synthesis and biological characterization of monocyte-derived neutrophil-chemotactic factor. *FEBS Lett.* **236**:467–470.
4. LARSEN, C. G., A. O. ANDERSON, E. APPELLA, J. J. OPPENHEIM, and K. MATSUSHIMA. 1989. The neutrophil-activating protein (NAP-1) is also chemotactic for lymphocytes. *Science* **243**:1464–1466.

5. SEITZ, M., B. DEWALD, N. GERBER, and M. BAGGIOLINI. 1991. Enhancing production of neutrophil-activating peptide-1/interleukin-8 in rheumatoid arthritis. *J. Clin. Invest.* **87**:463–469.
6. WATANABE, K., S. KINOSHITA, and H. NAKAGAWA. 1989. 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.
7. WATANABE, K., K. KONISHI, M. FUJIOKA, S. KINOSHITA, and H. NAKAGAWA. 1989. The neutrophil chemoattractant produced by the rat kidney epithelioid cell line NRK-52E is a protein related to the KC/*gro* protein. *J. Biol. Chem.* **264**:19559–19563.
8. MATSUSHIMA, T., K. MORISHITA, T. YOSHIMURA, S. LEVU, Y. KOBAYASHI, W. LEE, E. APPELLA, H. F. KUNG, E. J. LEONARD, and J. J. OPPENHEIM. 1988. Molecular cloning of a human monocyte-derived neutrophil chemotactic factor (MDNCF) and the induction of MDNCF mRNA by interleukin 1 and tumor necrosis factor. *J. Exp. Med.* **167**:1883–1893.
9. RAMPART, M., J. VAN DAMME, L. ZONNEKEYN, and A. G. HERMAN. 1989. Granulocyte chemotactic protein/interleukin-8 induces plasma leakage and neutrophil accumulation in rabbit skin. *Am. J. Pathol.* **135**:21–25.
10. LARSEN, C. G., A. O. ANDERSON, J. J. OPPENHEIM, and K. MATSUSHIMA. 1989. Production of interleukin-8 by human dermal fibroblasts and keratinocytes in response to interleukin-1 or tumor necrosis factor. *Immunology* **68**:31–36.
11. MUKAIDA, N., M. SHIROO, and K. MATSUSHIMA. 1989. Genomic structure of the human monocyte-derived neutrophil chemotactic factor IL-8. *J. Immunol.* **143**:1366–1371.
12. WATANABE, K., and H. NAKAGAWA. 1990. Cytokines enhance the production of a chemotactic factor for polymorphonuclear leukocytes by rat renal glomerular epithelioid cells. *Nephron* **54**:169–175.
13. FRADIN, A., B. ROTHHUT, B. POINCELOT-CANTON, M. ERRASFA, and F. RUSSO-MARIE. 1988. Inhibition of eicosanoid and PAF formation by dexamethasone in rat inflammatory polymorphonuclear neutrophils may implicate lipocortin's'. *Biochim. Biophys. Acta* **963**:248–257.
14. WATANABE, M., M. YAGI, M. OMATA, N. HIRASAWA, S. MUE, S. TSURUFUJI, and K. OHUCHI. 1991. Stimulation of neutrophil adherence to vascular endothelial cells by histamine and thrombin and its inhibition by PAF antagonists and dexamethasone. *Br. J. Pharmacol.* **102**:239–245.
15. KURIHARA, A., F. OJIMA, and S. TSURUFUJI. 1984. Analysis of the effects of an antiinflammatory steroid, dexamethasone, on neutrophil chemotaxis in the Boyden chamber with a modified ^{51}Cr-labeling method. *J. Pharmacobio. Dyn.* **7**:742–754.
16. WATANABE, K., H. NAKAGAWA, and S. TSURUFUJI. 1985. A new simple plastic chemotaxis device of the Boyden chamber type utilizing an immunoassay plate. *Jpn. J. Pharmacol.* **39**:102–104.
17. DEUEL, T. F., R. M. SENIOR, D. CHANG, G. L. GRIFFIN, R. L. HEINRIKSON, and E. T. KAISER. 1981. Platelet factor 4 is chemotactic for neutrophils and monocytes. *Proc. Natl. Acad. Sci. U.S.A.* **78**:4584–4587.
18. LINDLEY, I., H. ASCHAUER, J.-M. SEIFERT, C. LAM, W. BRUNOWSKY, E. KOWNATZKI, M. THELEN, P. PEVERI, B. DEWALD, V. VON TSCHARNER, A. WALZ, and M. BAGGIOLINI. 1988. Synthesis and expression in *Escherichia coli* of the gene encoding monocyte-derived neutrophil-activating factor: Biological equivalence between natural and recombinant neutrophil-activating factor. *Proc. Natl. Acad. Sci. U.S.A.* **85**:9199–9203.
19. TSURUFUJI, S., A. KURIHARA, and F. OJIMA. 1984. Mechanisms of anti-inflammatory action of dexamethasone: Blockade by hydrocortisone mesylate and actinomycin D of the inhibitory effect of dexamethasone on leukocyte infiltration in inflammatory sites. *J. Pharmacol. Exp. Ther.* **229**:237–243.

20. MIYASAKA, K., and T. MIKAMI. 1982. Comparison of the antiinflammatory effects of dexamethasone, indomethacin and BW 755c on carrageenin-induced pleurisy in rats. *Eur. J. Pharmacol.* **77**:229–236.
21. TSURUFUJI, S., A. KURIHARA, S. KISO, Y. SUZUKI, and K. OHUCHI. 1984. Dexamethasone inhibits generation in inflammatory sites of the chemotactic activity attributable to leukotriene B_4. *Biochem. Biophys. Res. Commun.* **119**:884–890.
22. SCHRODER, J.-M. 1989. The monocyte-derived neutrophil activating peptide (NAP/interleukin 8) stimulates human neutrophil arachidonate-5-lipoxygenase, but not the release of cellular arachidonate. *J. Exp. Med.* **170**:847–863.
23. CARVETH, H. J., J. F. BOHNSACK, T. M. MCINTYRE, M. BAGGIOLINI, S. M. PRESCOTT, and G. A. ZIMMERMAN. 1989. Neutrophil activating factor (NAF) induces polymorphonuclear leukocyte adherence to endothelial cells and to subendothelial matrix protein. *Biochem. Biophys. Res. Commun.* **162**:387–393.