ELSEVIER

European Journal of Pharmacology 284 (1995) 257–263

ejp

Dexamethasone inhibits the production of macrophage inflammatory protein 2 in the leukocytes in rat allergic inflammation

Jun-ichi Tanabe [a], Masako Watanabe [a,*], Suetsugu Mue [b], Kazuo Ohuchi [a]

[a] *Department of Pathophysiological Biochemistry, Faculty of Pharmaceutical Sciences, Tohoku University, Aoba Aramaki, Aoba-ku, Sendai, Miyagi 980-77, Japan*

[b] *Department of Health and Welfare Science, Faculty of Physical Education, Sendai College, Funaoka, Miyagi, Japan*

Received 23 March 1995; revised 26 May 1995; accepted 2 June 1995

Abstract

In the air pouch-type allergic inflammation model in rats, when the infiltrated leukocytes in the pouch fluid collected 4 h after antigen challenge were incubated, neutrophil chemotactic activity in the conditioned medium increased time-dependently. They produced neutrophil chemotactic factors, viz. leukocyte-derived neutrophil chemotactic factor (LDNCF)-2, a major component, and LDNCF-1, a minor component. When the infiltrated leukocytes were incubated in the presence of dexamethasone, neutrophil chemotactic activity in the conditioned medium decreased in a concentration-dependent manner, and production of LDNCF-2 and LDNCF-1 was inhibited. Because purified LDNCF-2 had been found to be identical with rat macrophage inflammatory protein 2 (MIP-2), effects of dexamethasone on the level of MIP-2 mRNA in the leukocytes were investigated. Using the reverse transcription-polymerase chain reaction technique, it was demonstrated that dexamethasone suppressed the level of MIP-2 mRNA in the leukocytes. These results indicate that dexamethasone inhibits MIP-2 production at the transcription level.

Keywords: Macrophage inflammatory protein 2; Allergic inflammation; Dexamethasone; Chemotactic factor

1. Introduction

The potent anti-inflammatory agents glucocorticoids are reported to manifest their anti-inflammatory effects by stimulating or inhibiting the transcription of a variety of genes (Beato, 1989; Burnstein and Cidlowski, 1989). While much attention has been focused on the glucocorticoid-induced gene expression, the major pharmacological actions of glucocorticoids may occur by inhibiting the production of key cytokines responsible for the initiation and coordination of inflammatory responses (Gessani et al., 1988; Lee et al., 1988).

Leukocyte infiltration in inflammatory diseases is supposed to be mediated by a variety of chemotactic factors produced in the inflammatory locus (Koch et al., 1991; Villiger et al., 1992). For example, in reperfusion-induced lung injury in rabbits, the potent neutrophil chemokine interleukin 8 is produced by alveolar macrophages, and an antibody to interleukin 8 prevents the neutrophil infiltration and lung injury (Sekido et al., 1993). In the air pouch-type allergic inflammation model in rats (Tsurufuji et al., 1982), we reported that dexamethasone treatment inhibits leukocyte infiltration into the pouch fluid (Ohuchi et al., 1982). Recently, we have reported that leukocytes infiltrated into the pouch fluid collected 4 h after injection of the antigen solution are much more activated than those in the non-immunized rats (Watanabe et al., 1994). When the infiltrated leukocytes from the immunized rats are incubated, they produce a major neutrophil chemotactic factor, viz. leukocyte-derived neutrophil chemotactic factor 2 (LDNCF-2), and a minor chemotactic factor, LDNCF-1, of which p*I* values are above 9, and 4–6, respectively (Watanabe et al., 1994). The aim of the present investigation is to clarify the mechanism of the inhibition of leukocyte infiltration by dexamethasone, focusing on the effect on the production of LDNCF-2 by infiltrated leukocytes. Our recent work on the analysis of amino acid sequence of LDNCF-2 (Tanabe et al., 1995) revealed that LDNCF-2 is identi-

* Corresponding author. Tel. 81-22-217-6859, fax 81-22-217-6859.

SSDI 0014-2999(95)00336-3

cal with rat macrophage inflammatory protein 2 (MIP-2) (Wolpe et al., 1989). Therefore, using specific primers for rat MIP-2, we examined the effect of dexamethasone on the level of MIP-2 mRNA in the infiltrated leukocytes. Here we describe that dexamethasone inhibits MIP-2 production by repressing the gene transcription for MIP-2. The significance of the inhibition of MIP-2 production by dexamethasone in rat allergic inflammation is discussed.

2. Materials and methods

2.1. Induction of allergic inflammation

The immunization and induction of air pouch-type allergic inflammation in rats were carried out as described previously (Tsurufuji et al., 1982). Male rats of the Sprague-Dawley strain, specific pathogen-free and weighing 120–140 g (Charles River Japan, Kanagawa, Japan) were used. They were housed four per cage on a 12 h light/dark cycle (lights on 07.00 h) at 22°C and 55% humidity, and allowed a CRF-1 diet (Charles River Japan) and tap water ad libitum. An antigen, azobenzenearsonate-conjugated acetyl bovine serum albumin, was synthesized according to the procedure described by Tabachnick and Sobotka (1962). The rats were immunized by intradermal injection of the antigen and Freund's complete adjuvant (Difco Laboratories, Detroit, MI, USA). Nine days after immunization, 8 ml of air was injected subcutaneously in the dorsum to form an ellipsoid-shape air pouch. Twenty-four hours after the injection of air, 2 mg of the antigen dissolved in 4 ml of a sterilized solution of 0.8% (w/v) sodium carboxymethylcellulose (CMC-Na, Cellogen F3H; Daiichi Kogyo Seiyaku, Niigata, Japan) in 0.9% (w/v) NaCl solution was injected into the air pouch to provoke allergic inflammation. The antigen solution was supplemented with penicillin G potassium and dihydrostreptomycin sulphate (Meiji Seika Co., Tokyo, Japan), each at a 0.1 mg/ml concentration. A group of rats that had been injected intradermally with Freund's complete adjuvant emulsion in the absence of the antigen received the antigen solution into the air pouch and served as the 'non-immunized rats'. The animal experiments were done in accordance with procedures approved by the Animal Ethics Committee of the Faculty of Pharmaceutical Sciences, Tohoku University, Japan.

2.2. Culture of leukocytes collected from the pouch fluid

Four hours after the antigen challenge into the air pouch of the immunized rats, rats were killed by cutting the carotid artery under diethylether anesthesia and the entire pouch fluid was collected. The pouch fluid was diluted 4-fold with RPMI-1640 medium (Nissui Seiyaku Co., Tokyo, Japan) and leukocytes infiltrated into the pouch fluid were precipitated by centrifugation at $350 \times g$ and 4°C for 5 min. The precipitate was washed 3 times with the medium and suspended in RPMI-1640 medium containing 0.25% (w/v) bovine serum albumin (essentially fatty acid-free; Sigma Chemical Co., St. Louis, MO, USA) at a concentration of 1×10^7 cells/ml. Four milliliters of the cell suspension (4×10^7 cells) were incubated at 37°C for various length of times. After appropriate incubation times, the cell suspension was centrifuged at $1500 \times g$ and 4°C for 5 min. The supernatant fraction was obtained, finally diluted to 20-fold with RPMI-1640 medium containing 0.25% (w/v) bovine serum albumin, and used for the measurement of neutrophil chemotactic activity. The rest of the cells were used for the differential counting after May-Grünwald-Giemsa staining.

2.3. Measurement of chemotactic activity for neutrophils

Neutrophil chemotactic activity in the diluted supernatant fraction of the conditioned medium was assayed using modified Boyden chambers as described previously (Watanabe et al., 1994). The upper and lower chambers of multiwell-type Boyden chambers were separated by a polycarbonate filter with pores 2 μm in diameter (Nuclepore Corp., Pleasanton, CA, USA). Rat peritoneal neutrophils were harvested 12–15 h after injection of 20 ml of Ca^{2+}-free Krebs-Ringer solution containing 1% (w/v) casein (casein from milk, vitamin-free, Wako Pure Chemical Ind., Osaka, Japan), that had been sterilized by autoclaving at 120°C for 15 min. The peritoneal neutrophils were washed twice and suspended in RPMI-1640 medium containing 0.25% (w/v) bovine serum albumin at 1×10^7 cells/ml; 300 μl of the cell suspension was applied into the upper chamber. In the lower chamber, 400 μl of the diluted supernatant fraction was added and incubated at 37°C for 80 min in a humidified atmosphere containing 5% CO_2. After incubation, the total number of neutrophils that migrated into the lower chamber was counted with a Coulter counter (Coulter Electronics, Luton Beds., UK). The migration rate was calculated as follows; migration rate (%) = (number of neutrophils in the lower chamber/number of neutrophils applied in the upper chamber) $\times$ 100. As a positive control, 400 μl of RPMI-1640 medium containing 0.25% (w/v) bovine serum albumin and 10 nM platelet-activating factor (PAF) (a mixture of C16 and C18 forms, Avanti Polar Lipid, Birmingham, AL, USA) was added in the lower chamber, and the migration index for each sample was obtained from the following equation and used as an index of chemotactic activity: migration index (%) = (migration rate for each sample/migration rate for 10 nM PAF) $\times$ 100.

2.4. Dexamethasone treatment

The synthetic glucocorticoid dexamethasone (Sigma Chemical Co.) was dissolved in ethanol and an aliquot of the ethanol solution was added into RPMI-1640 medium containing 0.25% (w/v) bovine serum albumin. Leukocytes infiltrated into the pouch fluid of the immunized rats were collected 4 h after the antigen challenge, and were incubated at 37°C for various lengths of times at a 1×10^7 cells/ml concentration in the medium containing various concentrations of dexamethasone. Effects of testosterone and aldosterone (Sigma Chemical Co.) also were examined in the same way. The final ethanol concentration in the medium was adjusted to 0.1% (v/v). After treatment with drugs, viability of the leukocytes was examined by trypan blue exclusion test, and no cytotoxic effect was observed.

2.5. Separation of neutrophil chemotactic activity in the conditioned medium by isoelectric focusing

Leukocytes infiltrated into the pouch fluid of the immunized rats were collected 4 h after the antigen challenge, and were incubated for 4 h at 37°C in RPMI-1640 medium containing 0.25% (w/v) bovine serum albumin at a concentration of 1×10^7 cells/ml with or without drugs. After incubation, the cell suspension was centrifuged at $1500 \times g$ and 4°C for 5 min. The supernatant fraction (49 ml) was dialyzed against 10 mM NaCl and mixed with 1 ml of Bio-Lyte ampholytes (pH range 3–10, Bio-Rad Lab., Richmond, CA, USA). The mixture was loaded into the focusing chamber of the Rotofor cell (Bio-Rad Lab.), and carried out isoelectric focusing at 12 W constant power for 3 h at 4°C (Watanabe et al., 1994). Twenty fractions were harvested and their pH values measured. Each fraction (2.5 ml) was dialyzed against 1 M NaCl to remove ampholytes, and further dialyzed against phosphate-buffered saline (pH 7.4) at 4°C. Chemotactic activity in each fraction was then determined after a 10-fold dilution with RPMI-1640 medium containing 0.25% (w/v) bovine serum albumin.

2.6. Semi-quantitation of MIP-2 mRNA by reverse transcription-polymerase chain reaction (RT-PCR)

Leukocytes (2×10^7 cells) collected from the pouch fluid 4 h after the antigen challenge were incubated at 37°C for 4 h in 2 ml of RPMI-1640 medium containing 0.25% (w/v) bovine serum albumin with or without dexamethasone. Total RNA in the leukocytes was then prepared by acid guanidinium thiocyanate-phenol-chloroform extraction (Chomczynski and Sacchi, 1987), and the yield of RNA isolated was determined by spectrophotometry. One microgram RNA from each sample was reverse transcribed at 37°C for 1 h in 40 μl of PCR buffer (Sigma Chemical Co.) containing 5 μM of random hexamer oligonucleotides, 200 U of the reverse transcriptase from moloney murine leukemia virus (Gibco, Gaithersburg, MD, USA), 0.5 μM deoxynucleotide triphosphate, 10 mM dithiothreitol, and 80 U RNA guard (Toyobo Co., Osaka, Japan). Because LD-NCF-2 is identical with rat macrophage inflammatory protein 2 (MIP-2) (Tanabe et al., 1995), PCR primers for MIP-2 were designed from rat MIP-2 cDNA sequences obtained from the EMBL/Genbank/DDBJ database. The sequences of primers used were; (former) 5′-GCCTAGCGCCATGGCCCCTCCCACT-3′, and (reverse) 5′-GGCACATCAGGTACGATCCAGGCTT-3′, which amplify a 413 MIP-2 base pair (bp) fragment. PCR reactions consisted of 5 μl reverse transcribed RNA, and 45 μl PCR buffer containing 0.25 μM each primer, 125 μM dNTP, and 0.5 U Taq polymerase (Applied Biosystem). PCR was performed for 30 cycles; 2 min denaturation at 94°C, 2.5 min annealing at 55°C, and 3 min extension at 70°C. The rat glyceraldehyde phosphate dehydrogenase (GAPDH) was used as an internal control. PCR primers for rat GAPDH primers used were; (former) 5′-TGATGACATCAAGAAGGTGGTGAAG-3′, and (reverse) 5′-TCCTTGGAGGCCATGTAGGCCAT-3′, which amplify a 249 GAPDH bp fragment, and PCR was performed as described by Robbins and McKinney (1992). After the PCR performance, 7.5 μl of the PCR reaction mixture were loaded onto a 2% agarose minigel and the PCR products were visualized by ethidium bromide staining after electrophoresis.

2.7. Statistical analysis

Results were analyzed for statistical significance by Dunnett's test for multiple comparisons and Student's *t*-test for unpaired observations.

3. Results

3.1. Effects of dexamethasone on the neutrophil chemotactic factor production by the infiltrated leukocytes

When the leukocytes in the pouch fluid collected 4 h after injection of the antigen solution into the air pouch of the immunized rats were incubated for 4 h, neutrophil chemotactic activity in the conditioned medium increased time-dependently (Table 1), indicating that the infiltrated leukocytes produce neutrophil chemotactic factors during the incubation period. Differential cell count for the infiltrated leukocytes showed that 86% of the leukocytes were neutrophils, 10% were mononuclear cells, and 4% were eosinophils. When the infiltrated leukocytes were incubated for 4 h in the medium containing various concentrations of dexa-

Table 1
Effects of dexamethasone on neutrophil chemotactic factor production by the infiltrated leukocytes

Incubation time (h)	Migration index (%)		
	Dexamethasone (nM)		
	0	10	100
0.5	5.02±0.21	2.71±0.01 [a]	1.55±0.10 [a]
1	12.85±0.18	9.14±0.29 [a]	3.51±0.11 [a]
2	60.52±1.31	32.14±0.58 [a]	10.29±0.22 [a]
4	107.69±3.57	64.03±2.05 [a]	14.42±0.33 [a]

Infiltrated leukocytes (4×10^7 cells) in the pouch fluid collected 4 h after antigen challenge were incubated for the indicated periods in 4 ml of the medium containing 0, 10, or 100 nM dexamethasone. Neutrophil chemotactic activity in the conditioned medium was determined and expressed as migration index. Values are the means ±S.E. of four samples. Statistical significance: [a] $P < 0.001$ vs. corresponding control.

methasone, neutrophil chemotactic activity in the conditioned medium decreased in a concentration-dependent manner (Fig. 1). Significant inhibition by dexamethasone was observed even 30 min after incubation (Table 1). At concentrations of 1–100 nM, dexamethasone showed no direct effect on neutrophil chemotaxis toward the conditioned medium (Fig. 2). These results suggested that dexamethasone inhibits neutrophil chemotactic factor production by the infiltrated leukocytes. Testosterone and aldosterone at 10 and 100 nM concentrations did not inhibit the chemotactic factor production at 4 h (data not shown).

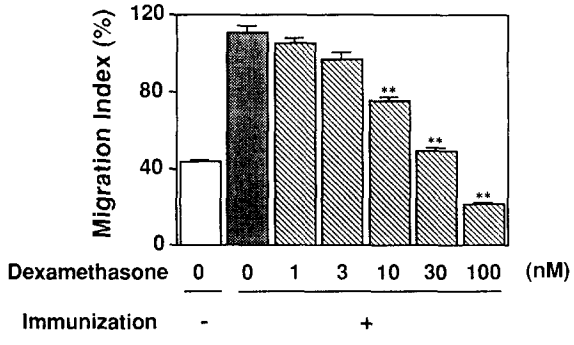

Fig. 1. Effects of dexamethasone on neutrophil chemotactic factor production by the infiltrated leukocytes. Leukocytes (4×10^7 cells) in the pouch fluid of the immunized rats collected 4 h after the antigen challenge were incubated for 4 h in 4 ml of the medium, and neutrophil chemotactic activity in the conditioned medium was determined. For the reference, the infiltrated leukocytes (4×10^7 cells) in the pouch fluid of the non-immunized rats collected 4 h after injection of the antigen solution were also incubated in 4 ml of the medium. Vertical bars represent S.E. of four rats. Statistical significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. control in the immunized rats. The results were confirmed by three separate experiments.

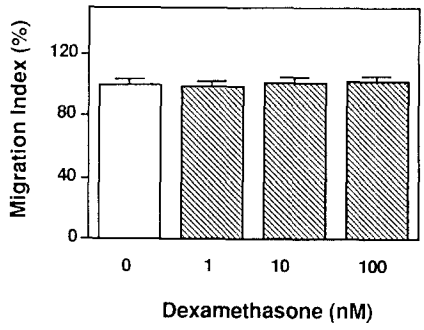

Fig. 2. Effects of dexamethasone on the conditioned medium-induced neutrophil chemotaxis. Infiltrated leukocytes (4×10^7 cells) from the immunized rats were incubated for 4 h in 4 ml of the medium, and the conditioned medium diluted 4-fold was applied into the lower chamber. Peritoneal neutrophils (3×10^6 cells) in 300 μl of the medium containing the indicated concentrations of dexamethasone were applied into the upper chamber. The chambers were then incubated at 37°C for 80 min, and the migration index was determined. Vertical bars represent S.E. of four rats.

3.2. Effects of dexamethasone on the production of LDNCF-1 and LDNCF-2 by the infiltrated leukocytes

After 4 h incubation of the infiltrated leukocytes in the medium, conditioned medium was collected, and chemotactic activity for neutrophils in the conditioned

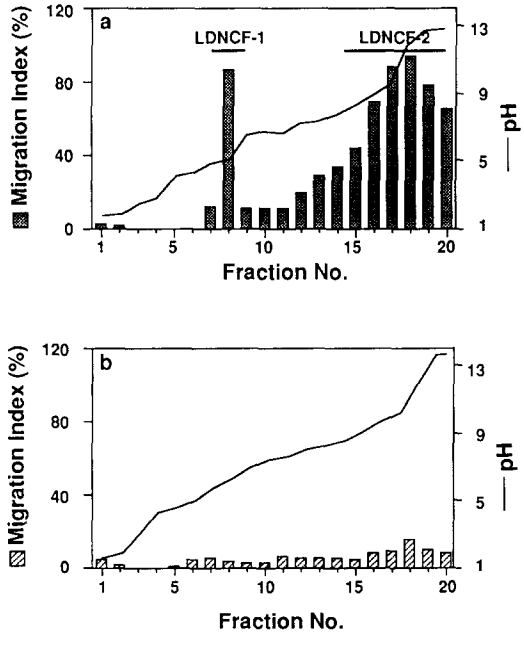

Fig. 3. Effects of dexamethasone on the production of LDNCF-1 and LDNCF-2. Leukocytes (5×10^8 cells) in the pouch fluid from the immunized rats were incubated for 4 h in 50 ml of the medium with (b) or without (a) dexamethasone (100 nM). After isoelectric focusing of the conditioned medium, chemotactic activity in each fraction was determined. Values are the means of four assays. S.E. for each value was less than 5% of the mean value. The results were confirmed by three separate experiments.

medium was separated by isoelectric focusing. As shown in Fig. 3a, neutrophil chemotactic activity was separated into two peaks; the first one is a minor factor (leukocyte-derived neutrophil chemotactic factor 1, LDNCF-1) with a p*I* value around 6, and the second one is a major factor, LDNCF-2, with a p*I* value above 8. When the infiltrated leukocytes were incubated in the presence of dexamethasone at 10 or 100 nM, both the production of LDNCF-1 and LDNCF-2 were inhibited in a concentration-dependent manner in parallel with the decrease in neutrophil chemotactic activity in the conditioned medium. At 100 nM dexamethasone, both the production of LDNCF-1 and LDNCF-2 was inhibited almost completely (Fig. 3b).

3.3. Effects of dexamethasone on the level of MIP-2 mRNA in the infiltrated leukocytes

The mechanism of the inhibition by dexamethasone of LDNCF-2 production was investigated. The infiltrated leukocytes collected 4 h after antigen challenge were incubated for 4 h in the medium containing various concentrations of dexamethasone. Using RT-PCR, it was demonstrated that the level of MIP-2 mRNA in the infiltrated leukocytes was lowered by dexamethasone at 10 and 100 nM. In contrast, the level of GAPDH mRNA was not affected by dexamethasone treatment. Fig. 4 shows that dexamethasone at 100 nM decreased the level of MIP-2 mRNA, but did not affect the level of GAPDH mRNA. Densitometric analysis indicated that when the ratio (MIP-2 mRNA/GAPDH mRNA) for control was expressed as 1.0, it went down to 0.04 for dexamethasone at 10 nM and to less than 0.01 for dexamethasone at 100 nM. These results suggested that dexamethasone inhibits the production of MIP-2 by repressing the gene transcription for MIP-2 in the infiltrated leukocytes.

4. Discussion

Dexamethasone inhibited the production of LDNCF-1 and LDNCF-2 by leukocytes infiltrated into the pouch fluid of the immunized rats 4 h after the antigen challenge (Fig. 3). No inhibition was induced by the other steroids such as testosterone and aldosterone (data not shown), indicating that the inhibition of the chemotactic factor production is specific to glucocorticoids. Because more than 75% of neutrophil chemotactic activity in the conditioned medium is due to LDNCF-2 (MIP-2), the effect of dexamethasone on the level of MIP-2 mRNA in the leukocytes was examined, and it was found that the level of MIP-2 mRNA was lowered by dexamethasone (Fig. 4). These results suggest that the inhibition of LDNCF-2 production by dexamethasone is due to the repression of gene transcription for MIP-2.

Dexamethasone binds to its receptor protein in cytosol, moves into nucleus, and the activated receptor-dexamethasone complex acts as a transcriptional factor, and finally shows its biological effects by inducing certain protein synthesis (Sherratt et al., 1989; Robbins and McKinney, 1992), or by suppressing the expression of genes for pro-inflammatory proteins (Lew et al., 1988; Zuckerman et al., 1989; Tobler et al., 1992). In the present investigation, because inhibition by dexamethasone of the neutrophil chemotactic factor production was observed within a short period of incubation (30 min after incubation) (Table 1), the effect by dexamethasone might not be mediated by inducing certain protein synthesis, but by suppressing the expression of MIP-2 mRNA. The transcriptional interference by glucocorticoids is mediated by direct interaction between activator protein 1 (Jonat et al., 1990; Schule et al., 1990; Yang-Yen et al., 1990) or nuclear-factor κB (Mukaida et al., 1994), and glucocorticoid-re-

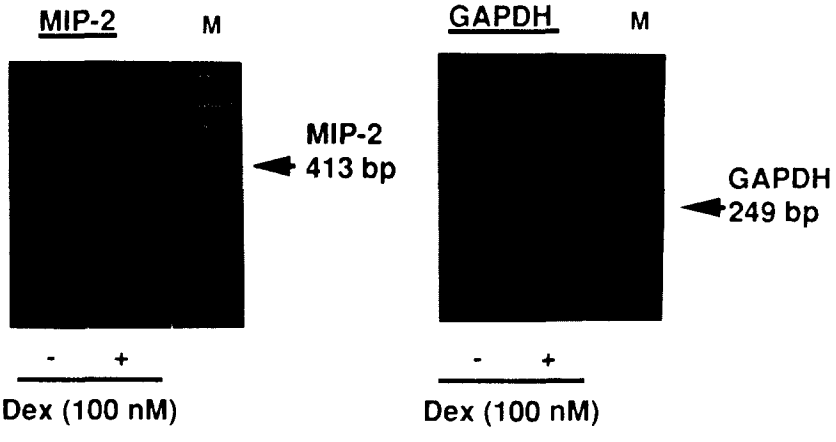

Fig. 4. Effects of dexamethasone on the levels of mRNA for MIP-2 and GAPDH in the leukocytes. Infiltrated leukocytes (2×10^7 cells) in the pouch fluid from the immunized rats were incubated for 4 h in 2 ml of the medium with (+) or without (−) dexamethasone (Dex) (100 nM). The level of mRNA was determined by RT-PCR technique. Lane M: size markers of HincII-digested ϕX174 DNA. The results were confirmed by three separate experiments.

ceptor complex, or by binding of the glucocorticoid-receptor complex to the DNA regulatory sequence termed negative glucocorticoid response element (Sakai et al., 1988). The MIP-2 promoter region in rats has not yet been cloned, but it is reported that the nuclear-factor κB site and activator protein 3 site are conserved in the murine MIP-2 promoter region (Widmer et al., 1993). As these regulatory *cis* elements are also conserved in the promoter regions of melanoma growth-stimulating activity (GRO)-α, GRO-β, and GRO-γ that are human homologs of murine MIP-2 (Anisowicz et al., 1991), these *cis* elements might be conserved in the rat MIP-2 promoter region. Furthermore, activator protein 1 and nuclear-factor κB are activated by inflammatory stimuli, such as interleukin 1 (Montgomery et al., 1991; Williams et al., 1992), tumor necrosis factor α (Montgomery et al., 1991; Sato and Seiki, 1993), and lipopolysaccharide (Montgomery et al., 1991). Consequently, the inhibitory effect of dexamethasone on LDNCF-2 production by infiltrated leukocytes might be mediated by the interference of the function of activator protein 1 or nuclear-factor κB.

Steroidal anti-inflammatory drugs inhibit neutrophil infiltration in several models of inflammation (Miyasaka and Mikami, 1982; Tsurufuji et al., 1984). In the air pouch-type allergic inflammation model in rats, infiltration of leukocytes into the pouch fluid is also inhibited by dexamethasone (Ohuchi et al., 1982). Leukocyte infiltration in inflammatory diseases is supposed to be mediated by a variety of chemotactic factors produced in the inflammatory locus (Koch et al., 1991; Villiger et al., 1992). In fact, in several experimental animal models, the potent neutrophil chemokine interleukin 8 mediates neutrophil infiltration, and an antibody to interleukin 8 prevents the neutrophil infiltration and protects from the tissue damage caused by infiltrated neutrophils (Akahoshi et al., 1994; Wada et al., 1994). Dexamethasone has an ability to inhibit the production of chemokines, such as interleukin 8 (Koch et al., 1991), mouse beta chemokine, a JE gene product (Kawahara et al., 1991), and monocyte chemoattractant protein 1 (Villiger et al., 1992) in vitro. As shown in Fig. 3, the production of LDNCF-2 is also inhibited by dexamethasone. Therefore, it is possible that the inhibition of neutrophil infiltration by dexamethasone in the air pouch-type allergic inflammation model (Ohuchi et al., 1982) is partly due to the inhibition of the production of LDNCF-2 by infiltrated leukocytes. Further purification and characterization of the acidic neutrophil chemotactic factor LDNCF-1 are under investigation in our laboratories. Because the production of LDNCF-1 was also inhibited by dexamethasone in vitro (Fig. 3), the inhibition of LDNCF-1 might also account for the inhibition of neutrophil infiltration by dexamethasone in vivo. Recently, we found (Tanabe et al., 1994) that the production of LDNCF-1 and LDNCF-2 by the infiltrated leukocytes is inhibited by the protein kinase C inhibitor and by the tyrosine kinase inhibitor. Therefore, dexamethasone might contribute to the inhibition of these kinases. It is also possible that dexamethasone inhibits neutrophil infiltration by inhibiting the leukocyte-endothelial cell interactions (Watanabe et al., 1991) by inhibiting the expression of the intercellular adhesion molecule, such as intercellular adhesion molecule 1 (Cronstein et al., 1992). For the expression of the potent anti-inflammatory effects, it is reasonable that glucocorticoids have pluripotential mechanisms for the inhibition of neutrophil infiltration in vivo.

In conclusion, the inhibitory effect by dexamethasone on chemotactic factor production was induced within a short period of incubation. Therefore, the inhibition by dexamethasone might not be mediated by inducing the production of some anti-inflammatory protein(s) that inhibits the production of chemotactic factor. Dexamethasone inhibits the production of the neutrophil chemotactic factor LDNCF-2 (MIP-2) by repressing the gene transcription for MIP-2. However, glucocorticoids may also increase the instability of MIP-2 mRNA, resulting in a decreased MIP-2 production. Therefore, to clarify this point, nuclear run-off transcription experiments and mRNA half-life experiments remain to be performed.

Acknowledgements

This work was supported in part by a Grant-in-Aid for General Scientific Research (07807200) from the Ministry of Education, Science and Culture of Japan.

References

Akahoshi, T., H. Endo, H. Kondo, S. Kashiwazaki, T. Kasahara, N. Mukaida, A. Harada and K. Matsushima, 1994, Essential involvement of interleukin-8 in neutrophil recruitment in rabbits induced by lipopolysaccharide and interleukin-1, Lymphokine Cytokine Res. 13, 113.

Anisowicz, A., M. Messineo, S.W. Lee and R. Sager, 1991, An NF-kappa B-like transcription factor mediates IL-1/TNF-alpha induction of gro in human fibroblasts, J. Immunol. 147, 520.

Beato, M., 1989, Gene regulation by steroid hormones, Cell 56, 335.

Burnstein, K.L. and J.A. Cidlowski, 1989, Regulation of gene expression by glucocorticoids, Annu. Rev. Physiol. 51, 683.

Chomczynski, P. and N. Sacchi, 1987, Single-step method of RNA isolation by acid guanidinium-thiocyanate-phenol-chloroform extraction, Anal. Biochem. 162, 156.

Cronstein, B.N., S.C. Kimmel, R.I. Levin, F. Martiniuk and G. Weissmann, 1992, A mechanism for the antiinflammatory effects of corticosteroids: the expression of endothelial-leukocyte adhesion molecule 1 and intercellular adhesion molecule 1, Proc. Natl. Acad. Sci. USA 89, 9991.

Gessani, S., S. McCandless and C. Baglioni, 1988, The glucocorticoid

dexamethasone inhibits synthesis of interferon by decreasing the level of its mRNA, J. Biol. Chem. 263, 7454.

Jonat, C., H.J. Rahmsdorf, K.-K. Park, A.C. Cato, S. Gebel, H. Ponta and P. Herrlich, 1990, Antitumor promotion and antiinflammation: down-modulation of AP-1 (Fos/Jun) activity by glucocorticoid hormone, Cell 62, 1189.

Kawahara, R.S., Z.-W. Deng and T.F. Deuel, 1991, Glucocorticoids inhibit the transcriptional induction of JE, a platelet-derived growth factor-inducible gene, J. Biol. Chem. 266, 13261.

Koch, A.E., S.L. Kunkel, J.C. Burrowa, H.L. Evanoff, G.K. Haines, R.M. Pope and R.M. Strieter, 1991, Synovial tissue macrophage as a source of the chemotactic cytokine IL-8, J. Immunol. 147, 2187.

Lee, S.W., A.P. Tsou, H. Chan, J. Thomas, K. Petrie, E.M. Eugui and A.C. Allison, 1988, Glucocorticoids selectively inhibit the transcription of the interleukin 1β mRNA, Proc. Natl. Acad. Sci. USA 85, 1204.

Lew, W., J.J. Oppenheim and K. Matsushima, 1988, Analysis of the suppression of IL-1α and IL-1β production in human peripheral blood mononuclear adherent cells by a glucocorticoid hormone, J. Immunol. 140, 1895.

Miyasaka, K. and T. Mikami, 1982, Comparison of the anti-inflammatory effects of dexamethasone, indomethacin and BW 755C on carrageenin-induced pleurisy in rats, Eur. J. Pharmacol. 77, 229.

Montgomery, K.F., L. Osborn, C. Hession, R. Tizard, D. Goff, C. Vassallo, P.I. Tarr, K. Bomsztyk, R. Lobb and J.M. Harlan, 1991, Activation of endothelial-leukocyte adhesion molecule 1 (ELAM-1) gene transcription, Proc. Natl. Acad. Sci. USA 88, 6523.

Mukaida, N., M. Morita, Y. Ishikawa, N. Rice, S. Okamoto, T. Kasahara and K. Matsushima, 1994, Novel mechanism of glucocorticoid-mediated gene repression. Nuclear factor-κB is target for glucocorticoid-mediated interleukin 8 gene repression, J. Biol. Chem. 269, 13289.

Ohuchi, K., S. Yoshino, K. Kanaoka, S. Tsurufuji and L. Levine, 1982, A possible role of arachidonate metabolism in allergic air pouch inflammation in rats, Anti-inflammatory effect of indomethacin and dexamethasone and the level of prostaglandin E_2 in the exudate, Int. Arch. Allergy Appl. Immunol. 68, 326.

Robbins, M. and M. McKinney, 1992, Transcriptional regulation of neuromodulin (GAP-43) in mouse neuroblastoma clone N1E-115 as evaluated by the RT/PCR method, Mol. Brain Res. 13, 83.

Sakai, D.D., S. Helms, J. Carlstedt-Duke, J.-A. Gustafsson, F.M. Rottman and K.R. Yamamoto, 1988, Hormone-mediated repression: a negative glucocorticoid response element from the bovine prolactin gene, Genes Dev. 2, 1144.

Sato, H. and M. Seiki, 1993, Regulatory mechanism of 92 kDa type IV collagenase gene expression which is associated with invasiveness of tumor cells, Oncogene 8, 395.

Schule, R., P. Rangarajan, S. Kliewer, L.J. Ransone, J. Bolado, N. Yang, I.M. Verma and R.M. Evans, 1990, Functional antagonism between oncoprotein c-Jun and the glucocorticoid receptor, Cell 62, 1217.

Sekido, N., N. Mukaida, A. Harada, I. Nakanishi, Y. Watanabe and K. Matsushita, 1993, Prevention of lung reperfusion injury in rabbits by a monoclonal antibody against interleukin-8, Nature 365, 654.

Sherratt, A.J., D.E. Banet, M.W. Linder and R.A. Prough, 1989, Potentiation of 3-methylchoranthrene induction of rat hepatic cytochrome P450IA1 by dexamethasone in vivo, J. Pharmacol. Exp. Ther. 249, 667.

Tabachnick, M. and H. Sobotka, 1962, Azoprotein. II. A spectrophotometric study of the coupling on diazotized arsanilic acid with proteins, J. Biol. Chem. 235, 1051.

Tanabe, J., M. Watanabe, S. Kondoh, S. Mue and K. Ohuchi, 1994, Possible roles of protein kinases in neutrophil chemotactic factor production by leucocytes in allergic inflammation in rats, Br. J. Pharmacol. 113, 1480.

Tanabe, J., M. Watanabe, N. Fujimoto, M. Mue and K. Ohuchi, 1995, Characterization of leukocyte-derived neutrophil chemotactic factor-2 and its possible roles in neutrophil infiltration in allergic inflammation in rats, Int. Arch. Allergy Appl. Immunol. (in press).

Tobler, A., R. Meier, M. Seitz, B. Dewald, M. Baggiolini and M.F. Fey, 1992, Glucocorticoids downregulate gene expression of GM-CSF, NAP-1/IL-8, and IL-6, but not of M-CSF in human fibroblasts, Blood 79, 45.

Tsurufuji, S., S. Yoshino and K. Ohuchi, 1982, Induction of an allergic air-pouch inflammation in rats, Int. Arch. Allergy Appl. Immunol. 69, 189.

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.

Villiger, P.M., R. Terkeltaub and M. Lotz, 1992, Production of monocyte chemoattractant protein-1 by inflamed synovial tissue and cultured synoviocytes, J. Immunol. 149, 722.

Wada, T., N. Tomosugi, T. Naito, H. Yokoyama, K. Kobayashi, A. Harada, N. Mukaida and K. Matsushita, 1994, Prevention of proteinuria by the administration of anti-interleukin 8 antibody in experimental acute immune complex-induced glomerulonephritis, J. Exp. Med. 180, 1135.

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.

Watanabe, M., Y. Arakida, J. Tanabe, A. Sugidachi, N. Hirasawa, S. Mue and K. Ohuchi, 1994, Pharmacological analysis of neutrophil chemotactic factor production by leucocytes and roles of PAF in allergic inflammation in rats, Br. J. Pharmacol. 111, 123.

Widmer, U., K.R. Manogue, A. Cerami and B. Sherry, 1993, Genomic cloning and promoter analysis of macrophage inflammatory protein (MIP)-2, MIP-1α, and MIP-1β, members of the chemokine superfamily of proinflammatory cytokines, J. Immunol. 150, 4996.

Williams, D.H., L.J. Jeffery and E.J. Murray, 1992, Aurothioglucose inhibits induced NF-κB and AP-1 activity by acting as an IL-1 functional antagonist, Biochim. Biophys. Acta 1180, 9.

Wolpe S.D., B. Sherry, D. Juers, G. Davatelis, R.W. Yurt and A. Cerami, 1989, Identification of macrophage inflammatory protein 2, Proc. Natl. Acad. Sci. USA 86, 612.

Yang-Yen, H.-F., J.-C. Chambard, Y.-L. Sun, T. Smeal, T.J. Schmidt, J. Drouin and M. Karin, 1990, Transcriptional interference between c-Jun and the glucocorticoid receptor: mutual inhibition of DNA binding due to direct protein-protein interaction, Cell 62, 1205.

Zuckerman, S.H., J. Shellhaas and L.D. Bulter, 1989, Differential regulation of lipopolysaccharide-induced interleukin 1 and tumor necrosis factor synthesis: effects of endogenous and exogenous glucocorticoids and the role of the pituitary-adrenal axis, Eur. J. Immunol. 19, 301.