Glucocorticoids inhibit chemokine generation by human eosinophils

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Recent identification of eosinophils as a cellular source of various cytokines suggests that eosinophil-derived cytokines contribute to allergic inflammation through either an autocrine or a paracrine fashion. The profound inhibitory effects of glucocorticoids (GCCs) on the production of various cytokines have been well recognized, however, there has been no definitive evidence that GCCs in fact inhibit cytokine generation by eosinophils. To verify the inhibitory ability of GCCs on eosinophil cytokine generation, we studied the effect of GCCs by determination of IL-8 and monocyte chemoattractant protein-1 (MCP-1) as parameters. Dexamethasone (DEX) inhibited both generation and secretion of IL-8 in a dose-dependent fashion. DEX also dampened formyl-methionyl-leucyl-phenylalanine– or ionomycin-induced eosinophil IL-8 production. Furthermore, MCP-1 production was also inhibited by DEX. The slope and the shape of the dose-response curve of DEX were similar irrespective of either the input stimuli or the output cytokines; half-maximal inhibition was observed at 10^{-8} mol/L, and nearly complete abolishment was observed at 10^{-7} mol/L. The competitive polymerase chain reaction for IL-8 mRNA and semiquantitative polymerase chain reaction for MCP-1 mRNA revealed that the inhibition occurred at a level of pretranslation. These results indicate that the beneficial effect of GCCs in allergic inflammation might be related, at least in part, to a direct effect of the drugs on eosinophil cytokine synthesis.
(J Allergy Clin Immunol 1998;101:75-83.)

Key words: *Eosinophil, cytokine, chemokine, glucocorticoid, C5a, FMLP, IL-8, MCP-1, IL-5*

Eosinophils play active pathogenic roles in the inflammation associated with allergic disorders.[1] In allergic inflammation, pivotal roles of cytokines have also been established; cytokines regulate recruitment and function of inflammatory cells, such as eosinophils[2-5] and basophils.[6-8] In addition, recent identification of eosinophils as a cellular source of various cytokines suggests that eosinophils play not only an effector role but also a regulatory role within the allergic inflammatory cell network.[9] On a single-cell basis, the production of cytokines by eosinophils seems to be much less than that by other types of cells such as monocytes. Nevertheless, the overall contribution of eosinophils to the local production of cytokines at the sites of allergic inflammation is potentially of significance because of the quantitative predominance of eosinophils over monocytes and lymphocytes in these sites. Thus the cytokines secreted by eosinophils potentially contribute to regulating the maintenance and progression of allergic inflammation through either an autocrine or a paracrine mechanism.

Glucocorticoids (GCCs) have long been extensively used therapeutically because of their potent antiinflammatory effects in inflammatory disease. This is also the case in allergic disorders; GCCs represent a mainstay of therapy for bronchial asthma, allergic rhinoconjunctivitis and atopic dermatitis. Topical usage of GCCs has become a first line of controller medication for patients with mild, moderate, and severe asthma. Although the therapeutic efficacy of GCCs in inflammation associated with allergic disease has been widely recognized, the mechanisms underlying the efficacy of GCCs in chronic allergic disorders have not been fully elucidated. However, the efficacy of GCCs in allergic inflammations results at least in part from their profound effects on the cytokine network.[10] GCCs downregulate the production of various cytokines by lymphocytes and other types of cells. Although eosinophils have been shown to possess high-affinity GCC binding sites,[11] there has been no definitive evidence that GCCs in fact inhibit cytokine generation by eosinophils. Moreover, detailed studies of the regulation of eosinophil cytokine generation by GCCs have not yet been performed. Given the potential importance of eosinophil-derived cytokines in allergic inflammation together with the therapeutic efficacy of GCCs in allergic disorders, we decided to conduct an analysis of the effect of GCCs on eosinophil cytokine generation. This study was undertaken to verify the

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Supported in part by a grant from the Manabe Medical Foundation, a Hokuriku Allergy Research Award, a Grant-in-Aid for young researchers from the Kitasato University Alumni association, and a Grant-in-Aid (07670523) from the Ministry of Education, Science, Sports, and Culture of Japan.

Received for publication Feb. 3, 1997; revised June 26, 1997; accepted for publication Aug. 20, 1997.
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0091-6749/98 $5.00 + 0 **1/1/85687**

Abbreviations used
CB: Cytochalasin B
DEX: Dexamethasone
FMLP: Formyl-methionyl-leucyl-phenylalanine
GCC: Glucocorticoid
MCP-1: Monocyte chemoattractant protein-1
PCR: Polymerase chain reaction

inhibitory ability of GCCs on eosinophil cytokine generation by determination of two chemokines (i.e., IL-8 and monocyte chemoattractant protein-1 [MCP-1]) as parameters.

METHODS

Reagents

Reagents used in the experiments were exactly the same as previously described.[12] Steroids were obtained from Wako (Osaka, Japan) and dissolved in ethanol to yield a stock solution of 2×10^{-5} mol/L.

Eosinophil purification

Eosinophils were purified from healthy consenting volunteers by Percoll density centrifugation followed by negative selection by using anti-CD16-bound micromagnetic beads (Miltenyi, Bergisch-Gladbach, Germany) and a magnetic-activated cell sorter column (Miltenyi) as previously described.[12] The mean percentage of eosinophil purity was 98.6% ± 0.2% (n = 28), and viability was consistently greater than 95%. Neutrophils were the most frequent contaminating cells, and the preparations contained virtually no monocytes (< 0.2%).

Culture of eosinophils

The complete medium employed for eosinophil culture consisted of RPMI 1640 medium (GIBCO, Grand Island, N.Y.) supplemented with 5% (vol/vol) heat-inactivated fetal calf serum. Eosinophils (0.5 to 1×10^6/ml) were cultured at 37° C in 5% CO_2 (total volume = 200 ml) in flat-bottomed 96-well culture plates. Combined supernatants and cell lysates were obtained by addition of 0.5% Nonidet P-40 (NP-40) to cultures. In some experiments, supernatants and cell lysates were separately harvested by centrifugation and addition of 0.5% NP-40 to cell pellets, respectively.

Measurement of apoptosis

Apoptosis was assessed by determination of DNA fragmentation. DNA fragmentation was measured by quantitation of cytosolic oligonucleosome-bound DNA with a Cell Death Detection ELISA^{PLUS} kit (Boehringer Mannheim, Mannheim, Germany).

Measurement of chemokine generation

Immunoreactive IL-8 and MCP-1 were quantitated as previously described,[13, 14] with slight modification. The ELISA methods detected IL-8 and MCP-1 concentrations of above 30 pg/ml and 10 pg/ml, respectively.

The amounts of cytokine generation were calculated on the basis of the total cytokine content in cultures. The spontaneous IL-8 (62 ± 16 pg/10^6 eosinophils, n = 29) and MCP-1 (6 ± 4 pg/10^6 eosinophils, n = 20) production in the absence of exogenous stimuli was subtracted from all values.

RNA isolation and reverse transcription

Total RNA was extracted with an RNeasy total RNA kit (Qiagen, Hilden, Germany). The first-strand cDNA was synthesized from the total RNA by using M-MLV reverse transcriptase and random hexamer primers (Takara, Ohtsu, Japan).

Competitive PCR for IL-8 mRNA

Competitive polymerase chain reaction (PCR) for IL-8 mRNA was performed as previously described,[15] and cDNA and varying amounts of competitor cDNA were used as templates. Direct and reverse oligo primers for IL-8 (5'TAAACATGACTTCCAAGCTGGC3' for sense, NH_2-5'CACTGATTCTTGGATACCACAG3' for anti-sense) were used.

Quantification of the amplified polymerase chain reaction products was performed by ELISA as previously described.[15, 16] In brief, the amplified products were immobilized on carboxylated-surface plates, and nonaminated DNA strands were removed by treating the plates with 0.1 N NaOH. The resultant covalently-bound single-strand DNAs were then hybridized with digoxigenin-labeled oligonucleotide probes (5'GATTGAGAGTGGACCACACTG3' for IL-8 and 5'ACGTACTCAGAACTGCTCTGA3' for competitor). The plates were then developed with peroxidase-conjugated anti-digoxigenin antibodies (Boeringer Mannheim, Mannheim, Germany), and signals were finally visualized with tetramethyl benzidine as the substrate. The optical absorbance data read at 450/630 nm were plotted on a logarithmic scale. Because the ratio of the target and competitor templates remained constant during amplification, the quantity of competitor DNA in the PCR templates, which yields an equal amount of the two PCR products, shows the initial amount of the target gene.

Semiquantitative PCR ELISA for MCP-1 mRNA

Semiquantitative PCR ELISA for MCP-1 mRNA was performed as previously described.[17] Direct and reverse oligo primers for MCP-1 (5'TCAAACTGAAGCTCGCACTCTCG3' for sense and NH_2-5'AGCTGCAGATTCTTGGGTTGTGG3' for anti-sense) and β-actin (5'GGTCAGAAGGATTCCTATGTG3' for sense and NH_2-5'ATTGCCAATGGTGATGACCTG3' for anti-sense) were used. Amplification was performed for 30 cycles. These cycles did not give plateau levels of PCR products (data not shown). The amplified cDNA bands from controls (activated mononuclear cells) were confirmed to hybridize with specific oligonucleotide probes on Southern blotting (data not shown). Quantification of the amplified products was performed essentially as previously described above, with the exception that β-actin mRNA was coamplified as the internal control in place of competitors. Digoxigenin-labeled oligonucleotide probes (5'CTCAGTGCAGAGGCTCGCGAGC3' for MCP-1 and 5'CCAACTGGGACGACATGGAG3' for β-actin) were used. The amounts of MCP-1 mRNA were standardized relative to the amount of β-actin mRNA.

Data presentation

The results are expressed as the mean ± SEM of n experiments.

RESULTS

Inhibition of eosinophil IL-8 and MCP-1 production by dexamethasone (DEX)

We have previously reported that chemotactic agonists, such as C5a and formyl-methionyl-leucyl-phenylalanine (FMLP), are important physiologic stimuli for

TABLE I. Effect of DEX on apoptosis of eosinophils

Time (hrs)	Treatment	Apoptosis (OD, 405 nm) Experiment 1	Experiment 2
0		0.006	0.041
2	Nil	0.030	0.031
	DEX (10^{-8} mol/L)	0.034	0.027
	DEX (10^{-7} mol/L)	0.040	0.049
18	Nil	0.304	0.102
	DEX (10^{-8} mol/L)	0.259	0.043
	DEX (10^{-7} mol/L)	0.313	0.047
42	Nil	0.446	0.859
	DEX (10^{-8} mol/L)	0.722	1.325
	DEX (10^{-7} mol/L)	0.864	2.289

Eosinophils (10^5/300 μl) were incubated with or without DEX for the indicated times. Cells were then lysed and apoptotic cells were determined by determination of DNA fragmentation as described in Methods.
OD, Optical density.

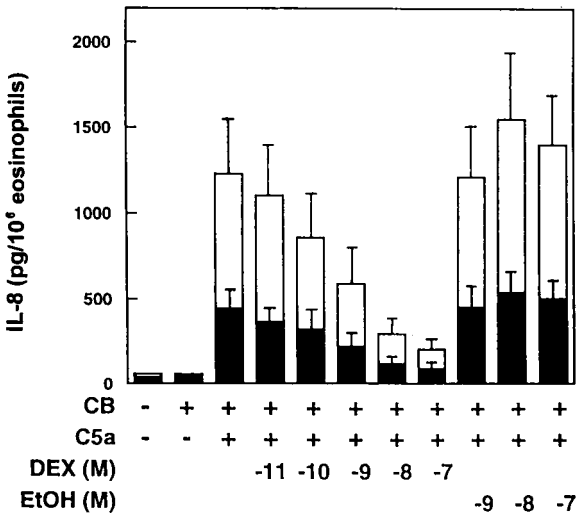

FIG. 1. Effect of DEX on released or cell-associated IL-8 of C5a-stimulated eosinophils. Eosinophils (n = 6) were incubated with DEX or control vehicle for 5 minutes. Cells were then stimulated with C5a (10^{-7} mol/L) plus CB (5 μg/ml) for 18 hours. Supernatants *(open columns)* were harvested by centrifugation and cell lysates *(closed columns)* were obtained by the addition of 0.5% Nonidet P-40 to the pellets. Immunoreactive IL-8 was measured by ELISA. Bars represent SEM. *EtOH,* Ethanol.

eosinophil cytokine generation.[12] As in the case of chemotactic agent-induced degranulation,[18] C5a-elicited eosinophil cytokine generation is absolutely dependent on the pretreatment with cytochalasin B (CB).[12] In the first series of experiments, eosinophils were preincubated with a wide range of concentrations of DEX for 5 minutes and then stimulated with C5a plus CB. After 18 hours, the secreted and cell-associated IL-8 levels were separately measured by ELISA. Pretreatment with DEX inhibited C5a-induced eosinophil IL-8 generation in a dose-dependent manner, and treatment with DEX at 10^{-7} mol/L resulted in nearly complete abolishment of IL-8 generation (Fig. 1). On the other hand, the ethanol vehicle control did not exert any significant effects. It should be noted that IL-8 in both supernatants and cell lysates was inhibited by DEX by a similar magnitude, indicating that IL-8 secretion, as well as generation, was inhibited by DEX (Fig. 1).

To exclude the potential contribution of DEX-induced apoptosis in the experiments, eosinophils were treated with DEX at 10^{-8} mol/L, 10^{-7} mol/L, or the control vehicle, and DNA fragmentation was measured after 2, 18, and 42 hours. As shown in Table I, no significant difference in apoptosis was found between cells treated with the control vehicle or DEX for 18 hours.

We next studied the effect of DEX on FMLP–and ionomycin-induced eosinophil IL-8 generation in comparison with that of C5a-induced production. In this case on the basis of the aforementioned results, we measured the net IL-8 production of cultures instead of released or cell-associated IL-8; combined supernatants and cell lysates were obtained by addition of NP-40. DEX inhibited the IL-8 generation induced by FMLP and ionomycin, as well as that of C5a. The inhibition occurred at similar concentrations irrespective of stimuli: half-maximal inhibition was observed at a concentration of approximately 10^{-8} mol/L in a dose-dependent fashion between 10^{-10} and 10^{-7} mol/L (Fig. 2, *A*).

Eosinophils can generate an array of cytokines, and we recently observed that eosinophils represent an important source of MCP-1.[17] To evaluate whether DEX-induced inhibition is limited only to IL-8 generation, we studied the effect of DEX on the production of MCP-1 instead of IL-8. Pretreatment with DEX also inhibited eosinophil MCP-1 generation initiated by C5a, FMLP, and ionomycin. Again, half-maximal inhibition was observed at a concentration of approximately 10^{-8} mol/L in a dose-dependent fashion between 10^{-10} and 10^{-7} mol/L (Fig. 2, *B*).

Effects of delayed addition of DEX on C5a-induced eosinophil chemokine generation

To determine the time frame of DEX-mediated inhibition of chemokine generation, eosinophils were treated with DEX (10^{-7} mol/L) for 5 minutes before or 1, 2, 4, or 8 hours after the addition of C5a plus CB. As shown in Fig. 3, the inhibitory effect of DEX was apparent even when DEX was added after eosinophils were stimulated with C5a; statistically significant inhibition was observed up to 4 hours after stimulation.

Effects of various steroids on C5a-induced eosinophil chemokine production

In the next series of experiments, we investigated whether other steroids also inhibited eosinophil chemokine generation. As illustrated in Fig. 4, other GCCs, such as methylprednisolone and hydrocortisone, showed

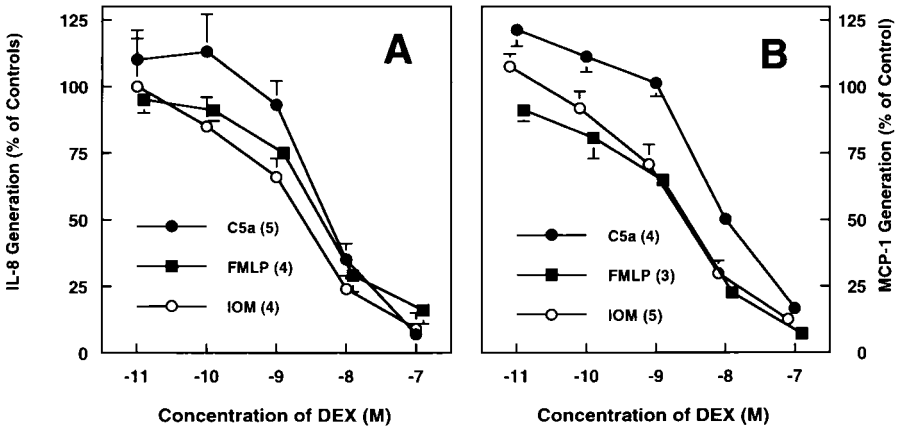

FIG. 2. Effect of DEX on IL-8 or MCP-1 production by eosinophils. Eosinophils were incubated with DEX for 5 minutes and then stimulated with C5a (*filled circles,* 10^{-7} mol/L) plus CB (5 μg/ml), FMLP (*filled squares,* 10^{-6} mol/L) plus CB or ionomycin (*open circles,* 1 μmol/L) for 18 hours. Immunoreactive IL-8 **(A)** or MCP-1 **(B)** in cultures were assayed by ELISA. Value is expressed as a percentage of control production initiated by each stimulus in absence of DEX. Control IL-8 production by C5a, FMLP, or ionomycin was 2553 ± 1304 pg, 1229 ± 220 pg, and 3504 ± 1972 pg/10^6 eosinophils, respectively. Control MCP-1 production induced by C5a, FMLP, or ionomycin was 789 ± 237 pg, 523 ± 90 pg and 1858 ± 820 pg/10^6 eosinophils, respectively. Values in parentheses indicate number of experiments. *IOM,* Ionomycin.

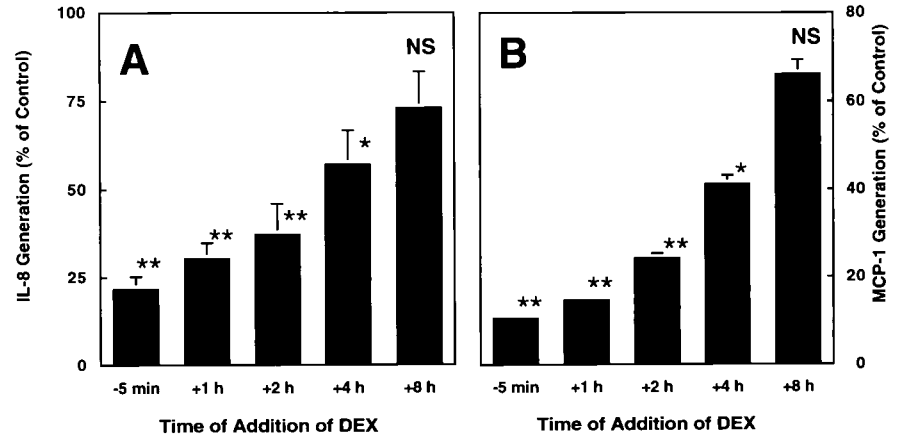

FIG. 3. Effects of delayed addition of DEX on C5a-induced eosinophil chemokine generation. Eosinophils (n = 4) were treated with DEX (10^{-7} mol/L) for the indicated times before or after the addition of C5a (10^{-7} mol/L) plus CB (5 μg/ml). After 18 hours of stimulation, IL-8 **(A)** and MCP-1 **(B)** were assayed in cultures by ELISA. Values are expressed as percentage of control production (1307 ± 435 pg and 1516 ± 568 pg/10^6 eosinophils for IL-8 and MCP-1, respectively) induced by C5a in the absence of DEX. *NS,* Not significant. *$p < 0.05$, **$p < 0.01$.

dose-dependent inhibition of both IL-8 and MCP-1 generation evoked by C5a. The order of inhibitory potency, as determined by the 50% inhibitory dose values, was DEX greater than methylprednisolone greater than hydrocortisone. In contrast, the generation of these chemokines was not affected by the presence of the sex steroids (i.e., testosterone, estradiol, or progesterone).

Effects of DEX on C5a-induced chemokine generation from IL-5-primed eosinophils

It has been well documented that eosinophilopoietic factors such as IL-5 enhance various functions of eosinophils.[19] These factors prolong the in vitro life-span of eosinophils by rescuing them from apoptosis. GCCs have been shown to inhibit the enhanced survival of eosinophils induced by submaximal doses of these cytokines. However, when eosinophils are maintained in the presence of higher concentrations of these factors, the inhibitory effect of GCCs is completely overcome.[20, 21] We recently observed that both IL-8 and MCP-1 generation by eosinophils was markedly enhanced by pretreatment with IL-5.[15, 17] Half-maximal amplification is observed at concentrations of 30 pmol/L, and the enhancement reaches plateau levels at concentrations of 1 nmol/L.[15] In the next series of experiments, we determined whether high concentrations of IL-5 can antago-

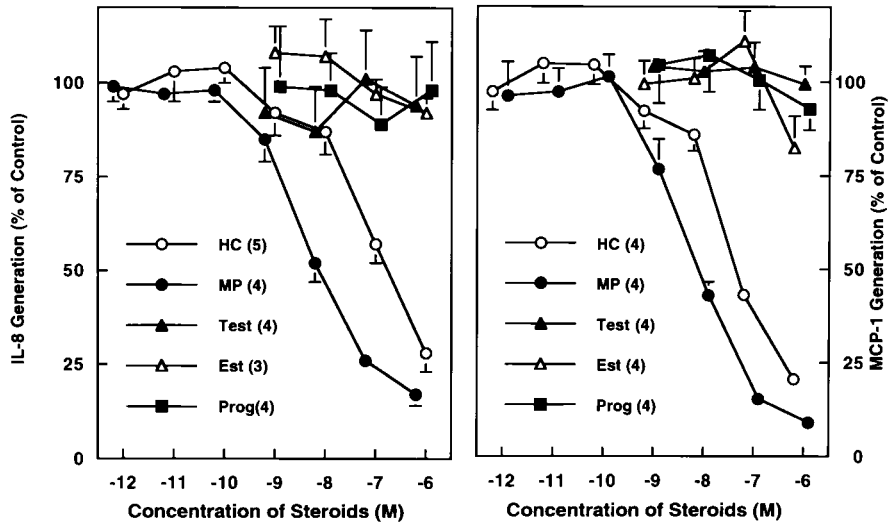

FIG. 4. Effect of various steroids on C5a-induced chemokine generation by eosinophils. Eosinophils were incubated with hydrocortisone *(open circles)*, methylprednisolone *(filled circles)*, testosterone *(filled triangles)*, estradiol *(open triangles)*, or progesterone *(filled squares)* for 5 minutes. Cells were then stimulated with C5a (10^{-7} mol/L) plus CB (5 μg/ml) for 18 hours and IL-8 **(A)** or MCP-1 **(B)** in cultures were assayed by ELISA. Value is expressed as percentage of control production induced by C5a in absence of steroids. Values in parentheses indicate number of experiments. *HC,* Hydrocortisone; *MP,* methylprednisolone; *Test,* testosterone; *Est,* estradiol; *Prog,* progesterone.

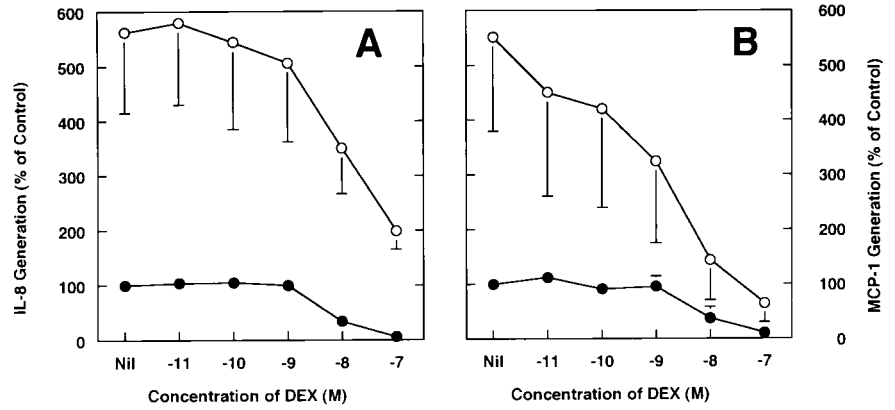

FIG. 5. Effect of DEX on C5a-induced chemokine generation by IL-5-primed eosinophils. Eosinophils were preincubated with *(open circles)* or without *(filled circles)* 10 nmol/L IL-5 for 30 minutes, after which the cells were incubated with DEX for 5 minutes. Cells were then stimulated with C5a (10^{-7} mol/L) plus CB (5 μg/ml) for 18 hours. IL-8 (**A**, $n = 3$) or MCP-1 (**B**, $n = 3$) in cultures were assayed. IL-8 production induced by C5a from nonprimed and primed eosinophils was 625 ± 287 pg and 2438 ± 811 pg/10^6 eosinophils, respectively. MCP-1 production induced by C5a from nonprimed and primed eosinophils was 415 ± 85 pg and 2186 ± 872 pg/10^6 eosinophils, respectively. Values are expressed as percentage of control production from nonprimed and non-drug-treated eosinophils stimulated with C5a plus CB.

nize the inhibitory effects of GCCs on chemokine generation. Eosinophils were preincubated with or without 10 nmol/L of IL-5 for 30 minutes before addition of DEX, and chemokine generation was induced by addition of C5a plus CB. As compared with nonprimed eosinophils, IL-5-primed eosinophils showed increased chemokine generation in response to C5a (Fig. 5). Treatment with DEX resulted in inhibition of chemokine generation by IL-5-primed eosinophils and nonprimed eosinophils, indicating that IL-5 does not antagonize the inhibitory effects of DEX on eosinophil chemokine generation (Fig. 5).

Effects of DEX on expression of IL-8 or MCP-1 mRNA in eosinophils

To determine whether the decrease in IL-8 or MCP-1 protein resulted from a decrease in the translation of each mRNA into the protein product in DEX-treated eosinophils, we examined the expression of IL-8 and MCP-1 mRNAs. Eosinophils were treated with DEX or

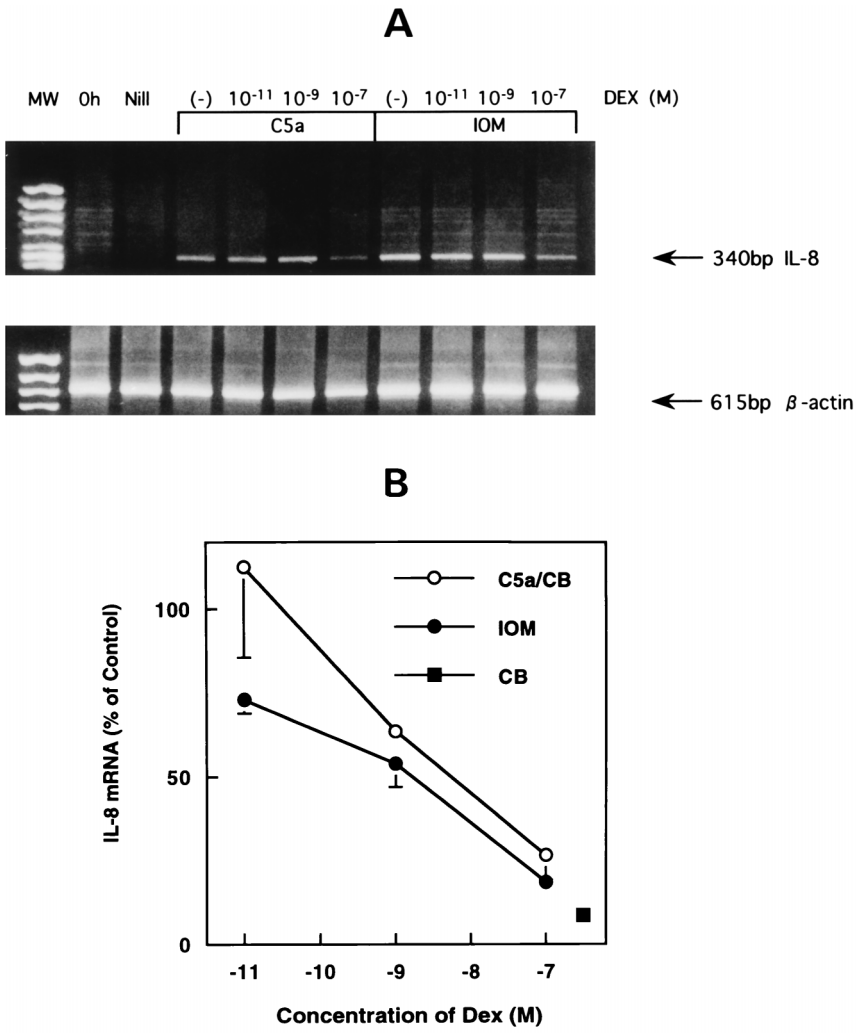

FIG. 6. Inhibition of C5a- or ionomycin-induced IL-8 mRNA accumulation by DEX. Eosinophils were preincubated with or without DEX for 5 minutes and then stimulated with C5a (*open circles,* 10^{-7} mol/L) plus CB (5 μg/ml) or ionomycin (*filled circles,* 1 μmol/L) for 2 hours. The RNA was reverse-transcribed to cDNA. **A,** A 40-cycle PCR was performed, and products were electrophoresed on agarose gel followed by staining with ethidium bromide. Representative experiment is shown. **B,** IL-8 mRNA level was determined by competitive PCR as described in Methods. Value is expressed as percentage of control (i.e., non-drug-treated eosinophils stimulated with the corresponding stimulus). Blank value (i.e., IL-8 mRNA level of eosinophils cultured with CB) is also shown *(filled squares).* Each point represents the mean ± SEM of three different experiments. *IOM,* Ionomycin.

controls, followed by stimulation with C5a or ionomycin. The total RNA was isolated for 2 hours after stimulation, which was determined to be the optimum time for IL-8 and MCP-1 message expression by preliminary experiments. IL-8 and MCP-1 mRNAs were determined by competitive PCR ELISA and semiquantitative PCR ELISA, respectively. Stimulation with both ionomycin and C5a caused dramatic increases in IL-8 (Fig. 6) and MCP-1 (Fig. 7) mRNAs compared with those of nonstimulated cells. Nevertheless, these accumulations of mRNA were effectively inhibited in a dose-dependent fashion by the treatment with DEX; treatment with 10^{-7} mol/L of DEX resulted in nearly complete abrogation of the expression of both IL-8 and MCP-1 mRNAs, indicating that DEX inhibits eosinophil chemokine generation at the pretranslational level.

DISCUSSION

To verify the inhibitory ability of GCCs on eosinophil cytokine generation, we employed the determination of two chemokines (IL-8 and MCP-1) as parameters of eosinophil cytokine production. IL-8 and MCP-1 are prototypes of CXC chemokines and CC chemokines, respectively. Both chemokines have been shown to be potent chemotaxins and secretagogues for eosinophils and basophils,[22-24] suggesting their in vivo pathogenic roles in allergic inflammation.

The clinical efficacy of GCCs in allergic inflammation

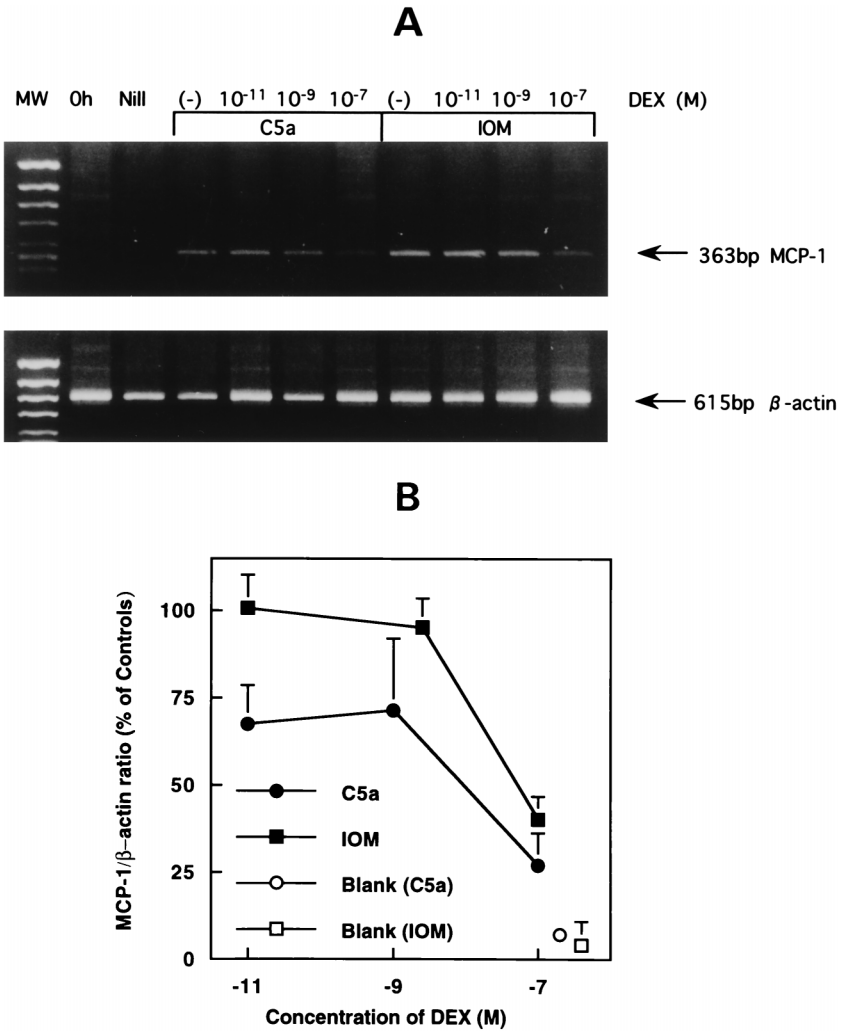

FIG. 7. Inhibition of C5a- or ionomycin-induced MCP-1 mRNA accumulation by DEX. Eosinophils were preincubated with or without DEX for 5 minutes and then stimulated with C5a (*filled circles,* 10^{-7} mol/L, $n = 4$) plus CB (5 μg/ml) or ionomycin (*filled squares,* 1 μmol/L, $n = 3$) for 2 hours. RNA was reverse-transcribed to cDNA and amplified with primers for β-actin or human MCP-1. **A,** 30-cycle PCR was performed, and products were electrophoresed on agarose gel followed by staining with ethidium bromide. Representative experiment is shown. **B,** Ratios of MCP-1 to β-actin were determined by ELISA as described in Methods. Value is expressed as percentage of control (i.e., non-drug-treated eosinophils stimulated with the corresponding stimulus). *Open symbols* indicate value of eosinophils exposed to control vehicle *(blank). IOM,* Ionomycin.

is at least in part mediated by their potent inhibitory effects on the elaboration of proinflammatory and eosinophil-active cytokines.[10] However, a substantial body of evidence has demonstrated that GCCs act directly on eosinophils to inhibit their biologic functions. For example, GCCs inhibit the antibody-dependent cellular cytotoxicity of rat eosinophils.[25] In vivo administration of GCC results in reduction of the migratory and adhesive abilities of eosinophils after 24 hours,[26] although in vitro treatment with GCCs at physiologic concentrations for 24 hours fails to inhibit eosinophil adhesion to endothelial cells.[27] An experiment with rat eosinophils showed that the degranulation of eosinophils is inhibited by GCCs.[25] Another study, however, was unable to demonstrate any inhibitory effects of GCCs on Ig-dependent degranulation of human eosinophils.[28] On the other hand, several lines of evidence have revealed that GCCs antagonize the survival-enhancing effects of eosinophil-directed cytokines through apoptosis.[20, 21]

Despite its likelihood, there has been no definitive evidence that GCCs in fact inhibit cytokine generation by eosinophils. In this study, we provide the first evidence that GCCs represent potent inhibitors of chemokine synthesis by human eosinophils. The eosinophil preparations contain contaminating neutrophils (approximately 2%), which are capable of synthesizing IL-8 and MCP-1. However, the contribution of neutrophil-derived chemokines to the data is only marginal, because the ability of neutrophils to generate IL-8 or MCP-1 does not exceed that of eosinophils.[17] DEX dampened

eosinophil IL-8 protein generation induced by receptor-mediated stimuli (i.e., C5a and FMLP), as well as by a nonreceptor-mediated stimulus, ionomycin (Figs. 1 and 2, *A*). Eosinophil MCP-1 generation induced by these stimuli was also inhibited by DEX (Fig. 2, *B*). The slope and the shape of the dose-response curve of DEX were similar irrespective of both the input stimuli and the output cytokine; half-maximal inhibition was observed at concentrations of approximately 10^{-8} mol/L, which are easily achieved in the clinical setting (Fig. 3). Furthermore, steroid inhibition of eosinophil chemokine production is a specific GCC effect. Sex steroids did not inhibit the generation of either IL-8 or MCP-1 by eosinophils (Fig. 4). The order of the inhibitory potencies of the GCCs was DEX greater than methylprednisolone greater than hydrocortisone, an order that is similar to that of their binding to GCC receptors.

Meagher et al.[29] have demonstrated that eosinophils treated with DEX showed increased apoptosis as determined by microscopic examination. However, the inhibitory effect of GCCs on eosinophil chemokine generation can not be explained solely by the apoptosis-inducing activity of GCCs because of the following reasons. First, DEX inhibited the accumulation of mRNA of chemokines in eosinophils which were stimulated for only 2 hours (Figs. 6 and 7). Second, DEX also inhibited chemokine generation by eosinophils primed with IL-5 at 10 nmol/L (Fig. 5), concentrations sufficient to antagonize the apoptosis-inducing effect of DEX.[20, 21] Finally and most importantly, the concentration of DEX required for inducing eosinophil apoptosis seems to be higher than that for inhibition of cytokine production; significant apoptosis-inducing activity of DEX was observed at a rather high concentration (10^{-6} mol/L).[29] Wallen et al.[20] demonstrated that DEX at 10^{-8} mol/L did not inhibit eosinophil survival. After 18 hours of incubation, we found no significant increase in apoptosis in eosinophils treated with DEX at concentrations of 10^{-8} mol/L and 10^{-7} mol/L, which induce half-maximal inhibition and complete abrogation of chemokine generation by eosinophils, respectively (Table I). These results indicate that specific effects other than apoptosis are involved in the inhibition of eosinophil chemokine generation by DEX.

Most of the biologic effects of GCCs are expressed through specific binding of GCCs to intracellular GCC receptors. The GCC-receptor complex induces gene repression through direct binding to various transcriptional factors. In astrocytoma cell-line cells, downregulation of IL-8 production by GCCs has been demonstrated to be mediated by antagonism of the action of nuclear factor-κB.[30] The molecular mechanisms underlying GCC-mediated inhibition of MCP-1 expression has not been investigated. However, the nuclear factor-κB response element has also been identified in the 5′-flanking region of the MCP-1 gene.[31] Although the inhibitory effect of GCCs on eosinophil IL-8 and MCP-1 synthesis are likely to be exerted through binding to transactivating factor or factors, further investigation such as gel shift assays is required.

In summary, we, for the first time, have demonstrated that GCCs are capable of inhibiting eosinophil chemokine generation. Although it remains unclear whether GCCs in fact inhibit generation of various cytokines other than IL-8 and MCP-1 by eosinophils, our results suggest that the beneficial effect of GCCs in allergic inflammation might be related to the direct effect of the drug on eosinophil cytokine synthesis. Because eosinophil-derived cytokines potentially contribute to allergic inflammation either through an autocrine or paracrine mechanism, the efficacy of GCCs in the treatment of allergic diseases might be explained at least in part by the inhibitory effect of GCCs on cytokine generation by these cells.

We thank Yasuko Asada, Kayoko Maema, and Sayaka Jibiki for their fine technical assistance. Thanks are also extended to Dr. K. Ito for his encouragement throughout this work.

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