

Dexamethasone inhibits basophil migration

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Glucocorticoids have been shown to inhibit the local accumulation of basophils during the allergen-induced late-phase reaction (LPR). Since migration is an essential step in the recruitment of basophils from the circulation, we examined whether the widely used glucocorticoid, dexamethasone (DEX), directly acts on basophils to inhibit the migration caused by C5a, interleukin (IL)-3, and IL-8. When purified basophils were preincubated with various concentrations of DEX, a dose-dependent inhibition was observed; DEX at concentrations as low as 1 nM reduced the number of migrated basophils by 30–40%; at higher concentrations, it showed a slightly stronger inhibitory effect. There was no significant difference in the effect of DEX on the migration caused by the three chemoattractants. The action of DEX took place rapidly; apparent inhibition was observed even when migration was initiated without preincubation. Although the inhibitory effect of this agent was not reversed when DEX was removed by washing, the inhibition was not mediated by the toxicity as measured by the trypan-blue exclusion test. These results indicate that the *in vivo* blocking effect of glucocorticoids on basophil accumulation during LPR is mediated in part by direct action to inhibit the migration of basophils.

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Antigen challenge of the skin and upper and lower airways induces biphasic responses; an immediate-phase reaction (IPR) followed by a late-phase reaction (LPR) 4–8 h after antigen exposure (9, 20). These two phenomena are different from each other; LPR is often more severe, and prolonged than IPR. LPR, but not IPR, is sensitive to glucocorticoids. LPR has attracted research in recent years as it more accurately reflects clinical disease than IPR.

Several studies have stated that biopsy specimens from skin or upper airways show the accumulation of both eosinophils and basophils in LPR (2, 5, 6). In addition, it has been recently reported that histamine-containing cells which appear in nasal lavage during LPR have functional and phenotypic characteristics of basophils (15). It has also been shown that the late-phase nasal symptoms are accompanied by a local increase in histamine and LTC₄, but not by a rise in PGD₂ or tryptase. The mediator profile suggests the involvement of basophils, but not mast cells, in LPR (6, 26). These facts strongly indicate that infiltrating basophils play a critical role in the clinical manifestation and pathogenesis of LPR.

Under normal conditions, basophils exist in circulation. Therefore, the local accumulation of basophils at sites of LPR indicates the presence of mecha-

nisms for the recruitment of basophils to these areas from the circulating blood. Actually, basophils are motile cells, and have been reported to respond *in vitro* to various chemoattractants such as C5a, IL-8, or lymphocyte-derived factors (17, 22, 23). We have shown that IL-3 and GM-CSF, which have been shown to be produced at the sites of LPR (18, 24), induce basophil migration *in vitro* at picomolar concentrations by increasing the chemokinetic activity of these cells (33).

Glucocorticoids are among the most potent and effective drugs used in the treatment of allergic diseases. In particular, the efficacy of glucocorticoids in LPR after antigen challenge is well known. However, their precise mechanisms of action are incompletely characterized. Many studies have shown that glucocorticoids inhibit various biologic functions of many types of inflammatory cells including basophils (reviewed in ref. 31). Oral and topical administration of glucocorticoids has been shown to inhibit the influx of basophils and eosinophils into skin blisters and nasal washings, respectively, during LPR to antigen challenge in man (2, 7). However, the effects of glucocorticoids on basophil migration, an essential step in the recruitment of basophils from the circulation to inflammatory sites, remain unclear. Thus, the present study was undertaken to deter-

mine whether or not glucocorticoids inhibit basophil migration.

Material and methods

Purification of basophils

Basophils were purified from normal human peripheral blood by density gradient centrifugation followed by negative panning selections (34). Briefly, after informed consent, venous blood was drawn from normal volunteers and anticoagulated with EDTA. The blood was overlaid on Percoll (Pharmacia Fine Chemicals, Uppsala, Sweden) solutions of different densities (1.079 and 1.070), and after centrifugation at 700 *g* for 15 min at room temperature, cell bands formed between the two Percoll solutions were collected and washed with phosphate-buffered saline (PBS) (GIBCO, Grand Island, NY, USA). Cell pellets were resuspended in 1 ml of PBS containing mouse monoclonal antibodies (0.35 μ g anti-CD2, -CD14, and -CD19 (Becton Dickinson, Mountain View, CA, USA), and 1.4 μ g anti-CD16 (Immunotech, Marseille, France)). After incubation at 4°C for 1 h, the cell suspension was washed twice with PBS, resuspended in 3.5 ml of PBS containing 2% FCS, and added to a plastic Petri dish previously coated with 100 μ g goat antimouse IgG (affinity purified, Tago, Burlingame, CA, USA). After incubation at 4°C for 1 h, the dish was gently swirled and nonadherent cells were collected. Cells were washed twice and resuspended in RPMI 1640 medium containing 0.03% human serum albumin which was used to stabilize chemoattractants throughout the migration assay procedure. The purity of basophils determined by May-Grünwald-Giemsa stain was 88.6% (range: 79–96%; *n* = 10), and the overall recovery rate assessed by Alcian blue stain was 23.6% (range: 14.3–38.9%; *n* = 10).

Chemoattractants for basophils

Recombinant (r) human C5a was purchased from Sigma Chemical Co (St Louis, MO, USA). Human rIL-8, produced by Dai-Nippon Pharmaceutical Co, was kindly donated by Dr K. Matsushima (Kanazawa University, Ishikawa, Japan) (25). Human rIL-3 was donated by Dr Gordon G. Wong (Genetics Institute, Cambridge, MA, USA) (35).

Migration assays

The migration of basophils was assayed in modified Boyden chambers (Neuro Probe, Bethesda, MD, USA) with 10- μ m-thick polycarbonate membrane filters with 5- μ m pores (Nuclepore, Pleasanton, CA, USA), as previously described (33).

Dexamethasone (DEX) (Wako Pure Chemical

Industries, Osaka, Japan) was dissolved in ethanol and added to the cell suspensions. After preincubation of basophils with various concentrations of DEX at 37°C for 30 min, a volume of 50 μ l cell suspension containing 1×10^4 basophils was added to the upper compartment of the chamber. Human rC5a at 10^{-10} M, rIL-3 at 3×10^{-10} M, and rIL-8 at 10^{-8} M were dissolved in RPMI 1640 medium containing 0.03% human serum albumin, and placed in the lower compartment. A concentration of ethanol was adjusted to 0.05% in both the upper and lower compartments. The presence of this percentage of ethanol had no migratory or migration-inhibitory effects on basophils (data not shown). Chambers were incubated for 2 h at 37°C in humidified air with 5% CO₂, and then filters were removed, dried, and stained with Alcian blue. Basophils on the lower surface of membranes were identified easily as cells with Alcian-blue-positive granules in the cytoplasm. Migration was determined by totaling the number of basophils that migrated completely through the filter in 15 microscopic fields ($\times 400$) (Olympus, Lake Success, NY, USA). Measurements were made in triplicate wells. The effect of DEX was expressed as percent inhibition of basophil migration, as calculated by the following formula:

$$\% \text{ inhibition} = 100 \times$$

(no. of nontreated migrated basophils
toward chemoattractant)

– (no. of DEX-treated migrated basophils
toward chemoattractant)

(no. of nontreated migrated basophils
toward chemoattractant

– (no. of nontreated migrated basophils
in medium alone)

Results

The effect of DEX on basophil migration was analyzed with purified human basophils. Since preliminary experiments showed that the number of migrated basophils toward attractants did not increase after 90 min, the chambers were incubated for 2 h in the subsequent experiments.

Purified basophils did not migrate toward the control medium without exogenous stimuli; only 2–5 cells per 15 high-power fields migrated. C5a, IL-3, and IL-8, which are reportedly potent basophil chemoattractants, induced basophil locomotion. The number of migrated basophils toward the factors was more than four or five times that at their optimal concentrations; 20–90 cells for both C5a and IL-3, and 20–40 cells for IL-8 per 15 microscopic fields. When basophils were preincubated with 0.01–

1000 nM DEX, a dose-dependent inhibition of chemotactic response was observed (Fig. 1); at concentrations as low as 1 nM, DEX reduced the basophil migration by 30–40%, and at higher concentrations, DEX showed a slightly stronger inhibitory effect. There was no significant difference in the inhibitory effect of DEX among C5a-, IL-3-, and IL-8-induced basophil migration.

In the next series of experiments, the kinetics of the DEX effect on basophil migration was examined by altering the preincubation time of cells with DEX (10 nM). The inhibition by DEX was a rapid process; apparent inhibition was observed even when migration was initiated without preincubation (Fig. 2). We assessed the migration of basophils after a longer period of pretreatment of DEX. When basophils were preincubated with 10 nM of DEX for 12 h, about 70% inhibition of basophil migration toward C5a 10^{-10} M was observed ($n = 2$). However, in one case, cell viability declined to 77% after pretreatment.

In the next set of experiments, we examined the reversibility of the DEX effect on basophil migration. After a 30-min incubation, DEX was removed by washing, and the migration of basophils was tested. As shown in Fig. 3, inhibition of basophil migration by DEX was still observed after removal of DEX, indicating that the inhibitory effect of DEX was not reversible.

To rule out the possibility that the decrease in basophil migrating activity was due to a decrease in

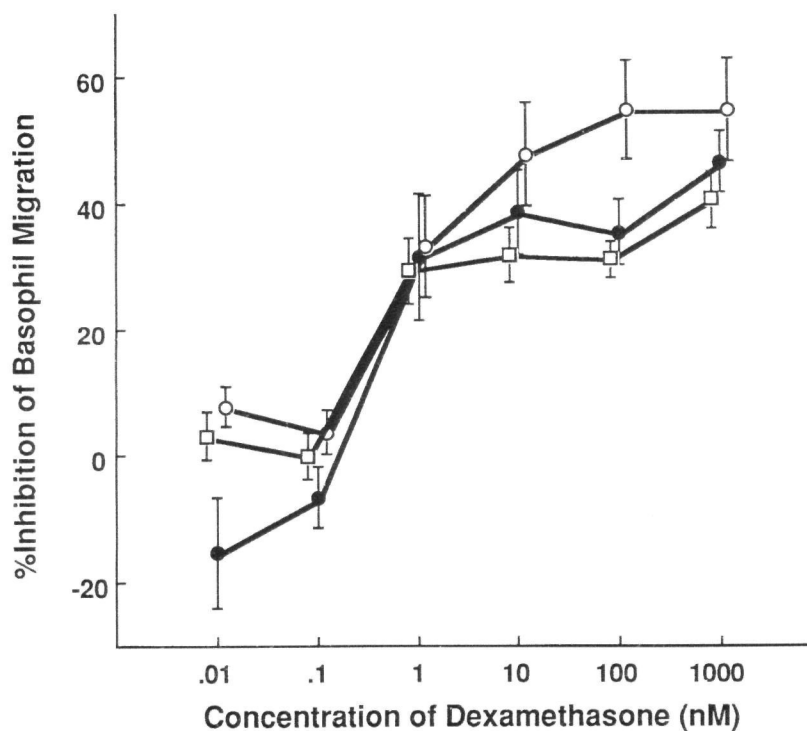


Fig. 1. Inhibition of basophil migration by different concentrations of DEX. After pretreatment of purified basophils with serially diluted DEX, migration toward C5a, 10^{-10} M ($\bullet$, $n = 6$); IL-3, 3×10^{-10} M ($\square$, $n = 6$); or IL-8, 10^{-8} M ($\circ$, $n = 4$) was measured. Data are average of separate experiments from different donors. Bars indicate SEM. Three curves were analyzed by testing homogeneity among three regression coefficients which showed no significant difference.

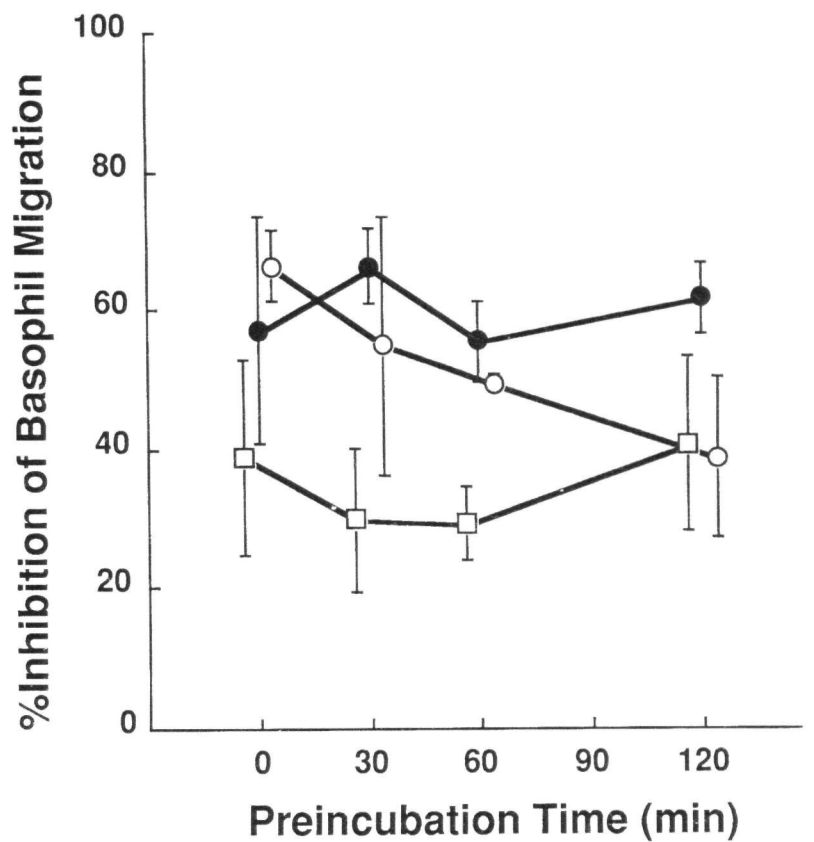


Fig. 2. Kinetics of DEX effect on basophil migration. Basophil preparations were preincubated with DEX 10^{-8} M for various periods from 0 to 120 min. After pretreatment, basophil migration was measured toward C5a, 10^{-10} M ($\bullet$, $n = 3$); IL-3, 3×10^{-10} M ($\square$, $n = 2$); or IL-8, 10^{-8} M ($\circ$, $n = 3$). Data are indicated as mean $\pm$ SEM of separate experiments from different donors.

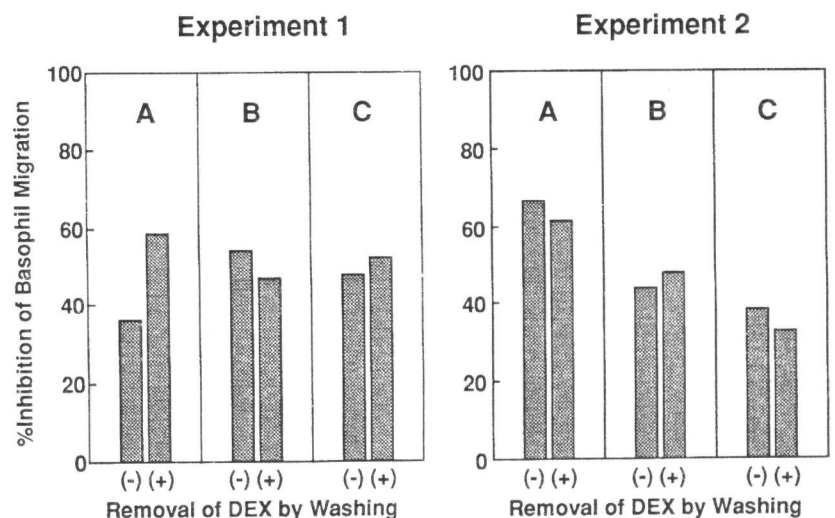


Fig. 3. Irreversible effect on basophil migration after removal of DEX by washing. After pretreatment with DEX 10 nM for 30 min, purified basophil preparations were washed twice with RPMI 1640 medium containing 0.03% human serum albumin with or without DEX, and resuspended with same medium. Control, non-pretreated cells were also washed twice with medium without DEX. Migration was induced by C5a (A), IL-3 (B), and IL-8 (C). Experiments were performed with two different donors. Data are averages of triplicate determinations.

the number of viable cells, we assessed the viability of cells after incubating with DEX for 3 h. We found that $> 90\%$ cells were viable, as shown by the trypan-blue exclusion test after incubation in the presence of DEX in concentrations ranging from 0.01 to 1000 nM, indicating that the inhibitory effect of DEX was not the result of its cytotoxic effect.

Discussion

The present results provide the first confirmation that glucocorticoids inhibit basophil migration. DEX decreased basophil migration induced by C5a, IL-8, and IL-3 in a dose-dependent fashion. The inhibitory effects of DEX were not the result of its toxicity for basophils; no decrease in viable cells was observed 3 h after the exposure of basophils to the agent. DEX acted directly on basophils and not indirectly via other cell types, because our experimental system employed highly purified basophil populations (purity about 90%). Irrespective of the chemoattractants used, the inhibitory effect occurred with similar concentrations of DEX. The half maximal inhibition was observed at concentrations of 10^{-9} – 10^{-10} M, which can be achieved easily in a clinical setting. The effect of DEX took place rapidly; inhibition reached plateau levels within 30 min of pretreatment. In addition, the DEX effect was not reversed after the removal of DEX by washing.

Glucocorticoids are potent anti-inflammatory agents which inhibit various biologic functions in various cell types. It has been reported that *in vitro* and *in vivo* administration of glucocorticoids inhibits the migration of eosinophils as well as neutrophils (1, 8, 27). The agents act also on basophils; systemic administration of glucocorticoids reduces the number of peripheral basophils (10, 28), and culturing of basophils with glucocorticoids decreases IgE-mediated release of histamine and LTC₄ (29, 30), but the effect on basophil migration has been unclear. Our novel finding that DEX inhibits *in vitro* basophil migration extends the repertoire of anti-inflammatory actions of glucocorticoids.

Schleimer et al. found that basophils exposed to 1000 U/ml of IL-3 for 24 h were insensitive to the inhibitory effect of DEX on mediator release (30). In this study, however, we used a much lower concentration of IL-3 (3×10^{-10} M) as a chemoattractant. This may explain the different effects of DEX in these two studies.

Although many of the actions of glucocorticoids are mediated via synthesis of a protein or a family of proteins such as lipocortin (4), the action of DEX observed in this study cannot feasibly be exerted by newly synthesized protein(s) because of its rapidity. It appears that another, unidentified mechanism is involved in the inhibitory action of DEX on basophil migration.

Local accumulation of basophils is an important aspect of LPR (2, 5, 6), and basophils, as well as lymphocytes and eosinophils, are thought to be important proinflammatory participants in LPR. Systemic and topical glucocorticoid treatment has been shown to inhibit basophil influx during LPR at antigen-challenge sites (2, 7). The recruitment of

blood cells from the circulation into the sites of tissue inflammation consists of two essential steps; adherence to vascular endothelial cells and migration to inflammatory sites after diapedesis. Our finding that DEX inhibits this second step indicates that the *in vivo* blocking effect of basophil accumulation by glucocorticoids in LPR is mediated in part by its direct action on basophil migration. However, we did not observe complete blocking of basophil migration by DEX; 40% of migrated basophils were observed even at higher concentrations of DEX (1000 nM). The incomplete inhibition of basophil migration might be consistent with the report that some populations of basophils are relatively resistant to corticosteroids (21).

Adherence of inflammatory cells to the vessel wall is an essential first step in the development of inflammatory cell infiltrates. Recently, it has been demonstrated that glucocorticoids do not inhibit either basophil adhesion to, or the expression of adhesion molecules of, endothelial cells (3, 16). Thus, the inhibitory effect on basophil accumulation during LPR might be explained by the effect mainly on migration, and not on adhesion to endothelium.

Several lines of evidence implicate the basophil active cytokines, IL-3, GM-CSF, IL-5, and insulin-like growth factors as primary effectors of basophil "priming" (11–14, 19, 32). These factors are capable of enhancing their functions and prolonging their life span *in vitro* (34). Although the clinical effect of glucocorticoids on LPR could be mediated in part by inhibiting the synthesis and release of these factors from lymphocytes or other cell types, our results collectively suggest that inhibition of cytokine-induced migration by glucocorticoids is one of the mechanisms whereby this agent alleviates LPR.

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