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Glucocorticoids inhibit granulocyte–macrophage colony-stimulating factor-1 and interleukin-5 enhanced *in vitro* survival of human eosinophils

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SUMMARY

The effects of three corticosteroids, hydrocortisone, dexamethasone and methylprednisolone, on eosinophil survival enhanced by recombinant human granulocyte–macrophage colony-stimulating factor (rhGM-CSF) and recombinant murine interleukin-5 (rmIL-5) have been studied. Eosinophils were incubated at a concentration of 5×10^5 cells/ml in the presence of different concentrations of the three steroids with either rhGM-CSF (1 ng/ml) or rmIL-5 (50 U/ml). The eosinophils were cultured in the presence of the same concentrations of rhGM-CSF and rmIL-5 alone as a positive control and medium alone as a negative control. Viability was assessed after 7 days by trypan blue exclusion. All three steroids inhibited rhGM-CSF-enhanced eosinophil survival in a dose-dependent manner; the dose of these drugs producing 50% inhibition (IC_{50}) was $> 1{\cdot}0 \times 10^{-4}$ M, $6{\cdot}5 \times 10^{-6}$ M and $1{\cdot}8 \times 10^{-6}$ M for hydrocortisone, dexamethasone and methylprednisolone, respectively. When eosinophils were cultured with the same concentration of rhGM-CSF in the presence of two non-glucocorticoids, β-oestradiol and testosterone, neither of these steroids inhibited eosinophil survival over the concentration range 1×10^{-10} M to 1×10^{-4} M ($n = 5$). Dexamethasone and methylprednisolone, but not hydrocortisone, also inhibited eosinophil survival induced by rmIL-5 in a dose-dependent manner. These results suggest one mechanism for the efficacy of corticosteroids against eosinophil-related disorders.

The eosinophil is an important effector cell in eliciting allergic inflammation. The maturation and functional activities of eosinophils are regulated by a variety of cytokines which include GM-CSF, IL-5 and IL-3. GM-CSF has been shown to stimulate eosinophil colony formation from bone marrow progenitors.[1] It can activate eosinophils by priming them for enhanced functions, for example increased leukotriene C_4 (LTC_4) generation following stimulation with a calcium ionophore,[2] and it has been shown to enhance eosinophil survival *in vitro*.[3,4] We have previously demonstrated that the production of GM-CSF by alveolar macrophages and peripheral blood mononuclear cells of asthmatic and allergic individuals is elevated compared with normal subjects.[2,5] IL-5 also plays a role in the induction and maintenance of eosinophilia. Recombinant human IL-5 has been demonstrated to induce a marked eosinophilia when injected into mice,[6] and IL-5 has been implicated in parasite-induced eosinophilia in both humans and animal models.[7,8] Eosinophils maintained in culture have demonstrated enhanced survival in the presence of IL-5.[9] Purified murine IL-5 has been shown to support the terminal differentiation and proliferation of murine eosinophilic precursors,[10] stimulate eosinophil function and prolong *in vitro* survival.[11] These observations suggest that GM-CSF and IL-5 may be important in the induction and maintenance of the eosinophilic inflammatory state characteristic of allergic disorders.

Glucocorticoids are steroid hormones with demonstrated anti-inflammatory effects,[12] and their administration to man results in a peripheral blood eosinopenia.[13] They are used in the treatment of asthma and other inflammatory disorders.[14] However, the mechanisms by which corticosteroids reduce eosinophil numbers remain unclear, although they may include inhibition of eosinophil colony formation from bone marrow, inhibition of eosinophil migration and adherence, or inhibition of eosinophil survival.

We have investigated the effects of three glucocorticoids, hydrocortisone, dexamethasone and methylprednisolone, on eosinophil survival induced by GM-CSF and IL-5 using an eosinophil culture assay we have already established.[5]

Eosinophils were isolated from 100 ml of ethylenediamine tetra-acetic acid (EDTA) (0·2 M) (Sigma, Poole, U.K.) anticoagulated peripheral blood from patients with an eosinophilia of > 5% who had bronchial asthma, allergic rhinitis, parasite infection or idiopathic hypereosinophilic syndrome. After sedimentation of the red cells with 0·2 vol. of 6% Dextran 110 in saline (Fisons, Loughborough, U.K.) for 40 min at room temperature, the leucocyte-rich plasma was aspirated and the

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cells were washed twice in modified minimal essential medium (MEM, Gibco, Uxbridge, U.K.), pH 7·4, containing Earle's salts, 20 mM *N*-2-hydroxyethylpiperizine-*N*-2-ethanesulphonic acid (HEPES), 2% heat-inactivated foetal bovine serum (Globepharm, Esher, U.K.) and 30 mg/ml deoxyribonuclease I (Sigma) by centrifugation at 100 ***g*** for 10 min at 20°. The washed leucocyte suspension was then layered on a discontinuous density gradient made up of 18%, 20%, 22%, 23% and 24% (w/v) metrizamide (Sigma) in 15-ml Falcon conical tubes (Marathon Laboratory Supplies, London, U.K.) as described by Vadas *et al.*[15] The tubes were centrifuged at $1200 \times g$ for 45 min at 18°. Eosinophils that sedimented at the 22/23% and the 23/24% interfaces were aspirated and combined. Preparations of greater than 90% purity were used.

Freshly isolated eosinophils were resuspended at a concentration of 2×10^6 cells/ml in RPMI-1640 (Gibco) supplemented with 25 mM HEPES, 32 mM L-glutamine (Gibco), penicillin-streptomycin (100 U/ml and 100 μg/ml) (Gibco) and 10% foetal bovine serum (supplemented RPMI). Twenty-five microlitres of the cell suspension was cultured in Nunclon 96-well plastic tissue culture plates (Gibco) in the presence of 75 μl of supplemented RPMI containing 1 ng/ml recombinant human granulocyte–macrophage colony-stimulating factor (rhGM-CSF) (kindly donated by Schering-Plough, Bury-St-Edmunds, U.K.) or 50 U/ml recombinant murine interleukin-5 (rmIL-5) (Genzyme/New Brunswick Scientific, Hatfield, U.K.), with different concentrations of three corticosteroids: hydrocortisone (10^{-10} to 10^{-4} M), dexamethasone (10^{-10} to 10^{-4} M) and methylprednisolone (10^{-10} to 10^{-4} M) (Sigma), and two sex steroids: β-oestradiol (10^{-10} to 10^{-4} M) and testosterone (10^{-10} to 10^{-4} M) (Sigma). The cells were cultured for a period of 7 days, the culture medium being changed on the fourth day. In each experiment eosinophils were cultured in the presence of 1 ng/ml rhGM-CSF or 50 U/ml rmIL-5 alone as a positive control and supplemented RPMI alone as a negative control. Eosinophil viability was assessed by trypan blue exclusion.

In four of the GM-CSF and steroid cultures, when the medium was changed the plates were centrifuged at 200 ***g*** for 10 min at room temperature, 90 μl of the culture medium was aspirated and fresh medium was added without the inclusion of hydrocortisone, dexamethasone or methylprednisolone. The eosinophils from these cultures were compared for viability with corresponding eosinophils that had been cultured in the presence of the three steroids for the entire 7-day period.

Statistics were performed on untransformed data points, and inhibition was determined by a paired *t*-test. The Bonferroni correction was used where appropriate. A value of $P < 0{\cdot}05$ was considered significant.

Hydrocortisone, dexamethasone and methylprednisolone inhibited eosinophil survival induced by GM-CSF from a positive control value of $74 \pm 4\%$ viability (mean ± SEM, $n = 10$) in a dose-related manner (Fig. 1). The concentrations producing 50% inhibition (IC_{50}) were $> 1 \times 10^{-4}$ M, $6{\cdot}5 \times 10^{-6}$ M and $1{\cdot}8 \times 10^{-6}$ M for hydrocortisone, dexamethasone and methylprednisolone, respectively. Inhibition was significant at concentrations of 10^{-6} M and greater for hydrocortisone ($P < 0{\cdot}05$) and methylprednisolone ($P < 0{\cdot}03$), and at concentrations of 10^{-8} M and greater for dexamethasone ($P < 0{\cdot}03$).

When the steroids were removed from culture at the change of media after 3 days and were not replaced, there was no statistically significant decrease in the degree of inhibition of eosinophil survival as compared with eosinophils that were cultured in the presence of steroids throughout the 7-day period ($n = 4$, data not shown).

Both dexamethasone and methylprednisolone inhibited eosinophil survival induced by IL-5 from a positive control of $75 \pm 3\%$ viability (mean ± SEM, $n = 5$) in a dose-related manner (Fig. 1). The IC_{50} was $1{\cdot}3 \times 10^{-5}$ M and $1{\cdot}6 \times 10^{-5}$ M for dexamethasone and methylprednisolone, respectively. Hydrocortisone only inhibited eosinophil survival at concentrations higher than 1×10^{-6} M and did not reach an IC_{50} value over the concentrations tested. Inhibition was significant only at 10^{-4} M for hydrocortisone ($P < 0{\cdot}05$), but at concentrations of 10^{-6} M and greater dexamethasone ($P < 0{\cdot}005$) and methylprednisolone ($P < 0{\cdot}05$).

Neither β-oestradiol nor testosterone showed any statistically significant inhibition of GM-CSF-induced eosinophil survival over the concentration range 10^{-10} M to 10^{-4} M ($n = 5$) (Fig. 2).

Administration of corticosteroids produces a peripheral blood eosinopenia *in vivo*.[16,17] Eosinophils have been shown to possess high-affinity steroid binding sites,[18] with a dissociation constant of $15{\cdot}3 \pm 0{\cdot}6$ nM dexamethasone, indicating that the receptor is capable of mediating biological effects at physiological hormonal concentrations. Few *in vitro* studies, however, have been able to demonstrate any effect on eosinophil function with physiologically relevant concentrations of glucocorticoids. Some effects have been observed using relatively high concentrations of steroids, such as inhibition of the chemotactic response of guinea-pig eosinophils to eosinophil chemotactic factor of anaphylaxis and C5a ($1{\cdot}4 \times 10^{-4}$ to $4{\cdot}1 \times 10^{-3}$ M for hydrocortisone and methylprednisolone)[19] and the direct inhibition of the antibody-dependent cellular cytotoxicity of rat eosinophils by methylprednisolone over the concentration range 10^{-7} to 10^{-3} M.[20] More recently it has been demonstrated that glucocorticoids inhibit the chemotactic response of human eosinophils *in vitro*.[21] Similarly there has been limited work investigating the effects of corticosteroids on cellular events induced by various cytokines, such as GM-CSF, IL-5 and IL-3, although dexamethasone inhibits the augmented survival of eosinophils co-cultured with supernatants derived from cultured human vascular endothelial cells.[22] Recent work has also demonstrated that dexamethasone (10^{-6} M) can inhibit eosinophil survival prolonged by GM-CSF.[23]

We now demonstrate that glucocorticoids inhibit the effects of GM-CSF and IL-5 in prolonging eosinophil survival *in vitro*. Relatively high concentrations of glucocorticoids are required to cause these effects, however there is evidence to show that these concentrations can be achieved during steroid therapy. Plasma cortisol levels have been shown to rise to 4×10^{-6} mol/l[24] and $1{\cdot}4 \times 10^{-5}$ mol/l[25] in normal subjects following intravenous injection of 100 mg and 300 mg of hydrocortisone, respectively. In the treatment of patients with asthma, where higher doses of steroid may be used, plasma levels of at least 10 times these values could be reached. We conclude, therefore, that the effects of glucocorticoids we have observed occur at concentrations that could be achieved *in vivo*.

The mechanisms by which the inhibition of eosinophil survival may occur have not been defined. Removal of the steroids from the culture system after 3 days did not significantly alter the inhibition subsequently observed at 7 days, indicating that they induce biochemical events during those first 3 days and

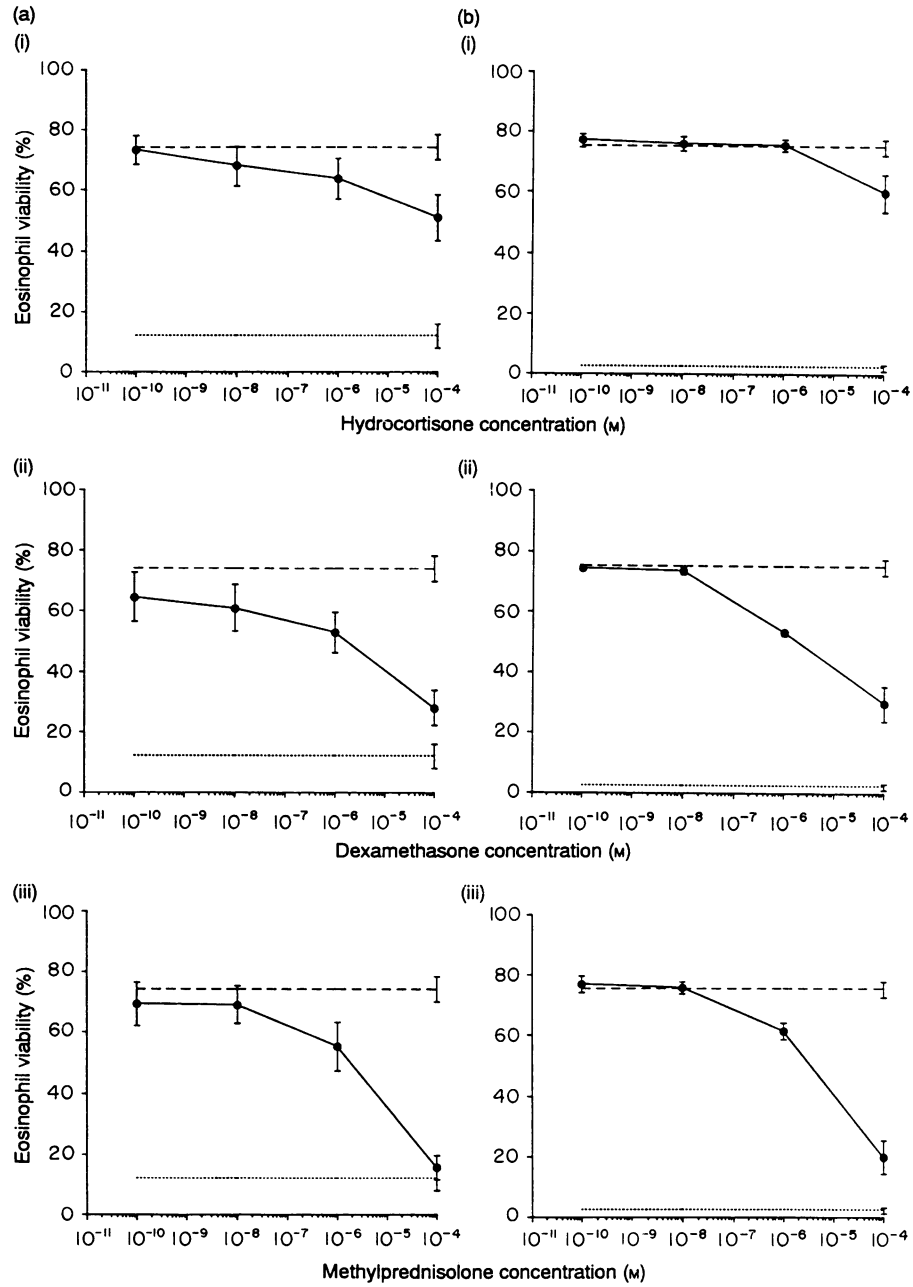

Figure 1. The dose-dependent inhibition of (a) GM-CSF-enhanced and (b) IL-5-enhanced eosinophil survival by (i) hydrocortisone, (ii) dexamethasone and (iii) methylprednisolone. The (·····) and (------) represent the negative and positive controls, respectively. The positive control was incubation with (a) 1 ng/ml GM-CSF or (b) 50 U/ml IL-5, the negative control was incubation with medium alone. Each point is the mean ± SEM of (a) 10 experiments and (b) five experiments.

need not be present thereafter. This, together with the fact that two non-glucocorticosteroids did not produce any inhibition, suggests that either the effects of the glucocorticoids may be receptor mediated, influencing intracellular second messenger events, or that they may exert their effects by blocking or modifying cytokine receptors on the eosinophil. Previous studies have shown that corticosteroids can inhibit the production of factors that modulate eosinophil function by other cell types, such as a factor derived from cultured vascular endothelial cells that prolongs eosinophil survival[22] and IL-3 production by cloned murine T lymphocytes.[26] In this study we have demonstrated that glucocorticoids can also exert a direct effect on eosinophils. This mechanism may be responsible in part for the potent anti-inflammatory effects of corticosteroids in allergic diseases.

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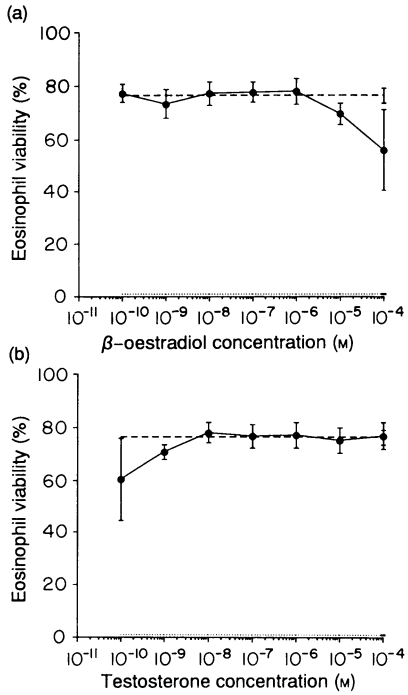

Figure 2. The effect of (a) β-oestradiol and (b) testosterone on eosinophil survival enhanced by GM-CSF. The (· · · · · ·) and (- - - - - -) represent the negative and positive controls, respectively. The positive control was incubation with 1 ng/ml GM-CSF and the negative control was incubation with medium alone. Each point is the mean ± SEM of five experiments.

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