

Dexamethasone inhibits maturation, cytokine production and FcεRI expression of human cord blood-derived mast cells

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Summary

Background Mast cells are responsible for eliciting the early phase and for contributing to the development of the late phase of allergic reactions, through the release of cytokines and other inflammatory mediators.

Objective To assess whether the glucocorticoid dexamethasone has a direct effect on mast cell progenitor maturation and on mature cord blood-derived mast cell properties.

Methods Mast cells were obtained by culturing human umbilical cord blood mononuclear cells with stem cell factor, IL-6 and prostaglandin E₂. Mast cell numbers were assessed by Toluidine Blue staining and immunocytochemistry of tryptase positive cells. The expression of FcεRI, CD49d and c-kit was assessed by flow cytometry. Histamine release was determined by a radioenzymatic assay. Cys-LT, GM-CSF and TNF-α production and release were determined by ELISA.

Results Dexamethasone (10⁻⁶ M–10⁻⁹ M) time- and dose-dependently inhibited the maturation of the mast cell progenitors. Dexamethasone did not affect the basal expression of FcεRI, CD49d and c-kit, but it inhibited the IgE-dependent enhanced expression of FcεRI. Dexamethasone (10⁻⁶ M–10⁻⁹ M) had no significant effect on FcεRI-dependent histamine release or the synthesis and release of Cys-LT from the mature mast cells. However, pre-incubation of the mast cell cultures with dexamethasone for 1 h, prior to cross-linking of FcεRI, dose-dependently inhibited the production and secretion of both GM-CSF and TNF-α.

Conclusions From these *in vitro* data we propose that glucocorticosteroids are effective drugs in the management of allergic inflammation due to their capacity to inhibit mast cell development, IgE-dependent FcεRI expression and mast cell production of GM-CSF and TNF-α.

Keywords Cys-LT, glucocorticosteroids, granulocyte macrophage colony stimulating factor, histamine, mast cells, tumour necrosis factor-α

Submitted 21 September 2001; revised 30 December 2001; accepted 4 March 2002

Introduction

Mast cells are seemingly one of the most important cell types that cause the pathogenesis of allergic disorders. Typically, in allergic reactions mast cells are activated by cross-linking of IgE, bound to its high affinity receptor (FcεRI), which triggers the early phase of allergic responses, through the release of pre-formed mediators and *de novo* synthesized phospholipid metabolites. Consequently, mast cells are viewed as the key effector cells in the immediate phase of allergic reactions [1, 2]. In addition, there is now considerable evidence that mast cells could also be involved in the late phase of allergic reactions through their capacity to produce cytokines such as granulocyte macrophage colony stimulating factor (GM-CSF) and TNF-α that have been shown to be eosinophilotropic [3–5]. For example, we have shown that mast cell-derived TNF-α enhances

eosinophil survival by inducing autocrine production of GM-CSF [6]. Mast cells also seem to play an important role in remodelling and repair stages consequent to this condition and in other inflammatory diseases, in which mast cell hyperplasia is often evident [7, 8]. Mast cells are known to originate from bone marrow progenitors that migrate into various tissues via blood circulation as immature cells and thereafter undergo complete maturation in the tissues [1]. Thus, it is conceivable that in situations of chronic inflammation, mast cells differentiate, proliferate and mature.

Glucocorticosteroids are the most effective anti-inflammatory and immunosuppressive treatment; to date [9]. They have no reported effect on the early phase of allergy, whereas they can inhibit the late-phase reaction. From this evidence it has been implied in the past that glucocorticosteroids have no activity on mast cells. Nevertheless, dexamethasone has been shown to down-regulate IL-13 production from purified human lung mast cells [10], and to inhibit the development of mast cell progenitors obtained from human fetal liver [11].

We have hypothesized that glucocorticosteroids can also decrease the magnitude of the severity of allergic inflammation by

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directly down-regulating mast cell maturation and some of the inflammatory properties of mature mast cells. In the past few years it has been possible to obtain in large numbers a high purity of human mast cells generated from umbilical cord blood-derived progenitor cells cultured in the presence of stem cell factor (SCF) [12]. Interestingly, cord blood-derived mast cells (CBMC) seem to have a phenotypic profile similar to that of human lung mast cells. They are mostly tryptase positive with variable numbers of tryptase- and chymase-positive cells [13] and they are responsive to IgE-dependent activation [13–15].

The aim of this study was to assess the effects of a standard glucocorticosteroid, dexamethasone, on human cord blood mast cell progenitor maturation, mature mast cell expression of FcεRI, CD49d and c-kit, and on IgE-dependent mediator production and release. Hereby, we report that dexamethasone has direct down-regulatory influences on both mast cell precursors and mature mast cells.

Methods

Isolation of human cord blood-derived mononuclear cells

Written informed consent was obtained from all of the volunteers according to the guidelines established by the Hadassah-Hebrew University Human Experimentation Helsinki Committee. Mononuclear cells were isolated from freshly obtained (less than 12 h from the Caesarian section) heparin-treated human umbilical cord blood according to methodology previously described [13]. Briefly, the blood was diluted in Hank's Balanced Salt Solution (1 : 1) and layered on Histopaque®-1077 (Sigma Chemicals, St Louis, MO, USA), and centrifuged at 400 *g* for 20 min at room temperature. The mononuclear cell layer was obtained and washed twice with MEM-Eagle (Biological Industries, Beith Haemek, Israel). Total cell numbers were determined by Kimura's staining.

Cell cultures

Cord blood-derived mononuclear cells were cultured in MEM-α medium ($1-2 \times 10^6$ cells/mL) supplemented with 10% v/v heat inactivated fetal calf serum (FCS) and containing penicillin (100 U/mL), streptomycin (100 µg/mL), L-Glutamine (2 mM), deoxy and ribonucleosides (10 µg/mL) (Biological Industries), human recombinant stem cell factor (rhSCF) (80 ng/mL) (a generous gift from Amgen, Thousand Oaks, CA, USA), rhIL-6 (10 ng/mL) (PeproTech, Rocky Hill, NJ, USA) and PGE₂ (3×10^{-7} M) (Sigma Chemicals). Cells were cultured at 37 °C in a 5% CO₂ humidified incubator, in standing NUNC™ 200 mL culture flasks (Nalge Nunc International, Roskilde, Denmark) similar to conditions previously described [13]. Medium was changed every week by gently aspirating half of the culture medium over the bottom of the flask and replacing it with the same volume of fresh medium. After 8 weeks of culture > 85% of the cells were identified as CBMC. For some experiments where high mast cell purity was required, CBMC were purified by affinity positive selection as previously described [16].

Cell viability and mast cell characterization

Cell cultures were stained with Trypan blue (0.4% in saline) (Sigma Chemicals) to assess cell viability. Viable cells were identified as those excluding the Trypan blue dye. Results are expressed as percentage viability. Toluidine Blue (Sigma

Chemicals) (0.70 mg/mL in a 60% ethanolic solution, pH 3.5) was used to stain metachromatically cord blood-derived mast cell cultures, in order to assess the purity and numbers of mast cells. For all counting-methods 20 µL of cell suspension were diluted with an equal volume of the stain and counted after 2 min under a light microscope (Olympus, MH-2). A minimum of 250 cells was counted.

In addition to Toluidine Blue staining, tryptase protein expression was analysed by immunostaining as previously described [17]. Briefly, cells (100 µL of 10^6 mL) were cytocentrifuged onto poly-L-lysine slides (Merck Eurolabs, Lutterworth, UK). The cells were fixed in 4% paraformaldehyde in phosphate buffered saline (PBS), washed and incubated with mouse monoclonal anti-tryptase antibody (2 µg/mL) (clone AA1, Dako, Cambridge, UK) for 1 h. After washings, detection was made using the alkaline phosphatase anti-alkaline phosphatase (APAAP) method (Dako) and counter-stained with haematoxylin. Negative controls consisted of slides incubated with an isotype matched (IgG1) mouse monoclonal antibody instead of the primary antibody. Positive cells stained red after development with Fast Red (Sigma Chemicals).

Effect of glucocorticoids on the maturation of mast cell progenitors from cord blood-derived mononuclear cells

To analyse the effect of glucocorticosteroids on differentiation of mast cell progenitors, dexamethasone (Dexacort® 4 mg/mL) (TEVA Pharmaceutical Industries, Petah-Tikva, Israel) (10^{-6} M– 10^{-9} M) was added to cord blood-derived mononuclear cells that had been cultured *in vitro* for 3 weeks. For this set of experiments, testosterone (Sigma Chemicals) was used as control. Solvent control cultures contained 0.1% dimethyl sulphoxide (DMSO) in the absence of any compound. Every 3 days, for the subsequent 12 days, the following assays were performed: total cell numbers, cell viability, mast cell numbers, tryptase expression and histamine content (see below).

Effects of glucocorticosteroids on human CBMC mediator release

CBMC cultured for at least 10 weeks were incubated either in culture media, or in the presence of dexamethasone (10^{-6} M– 10^{-9} M) or of testosterone (10^{-6} M– 10^{-9} M) for either 1 or 24 h. Afterwards, the cells were activated by addition of the mouse monoclonal anti-human FcεRI-α chain antibody, 22E7 (2 µg/mL) (a generous gift from Dr J. P. Kochan, Hoffman La Roche, Nutley, NJ, USA). The cells were stimulated for 1 h at 37 °C for histamine and cysteinyl-leukotrienes (Cys-LT) release. For TNF-α and GM-CSF release the cells were incubated for 18 h under the same conditions. After activation, cell sonicates were obtained by 1 min of continuous sonication (W-380 sonicator Heat System Ultrasonics, 50% duty cycle, output 5, 0 °C). Sonicates were centrifuged (12 000 *g*, 5 min, 4 °C) and debris-free supernatants were collected for histamine, GM-CSF and TNF-α assessment.

Histamine was assessed in supernatants and sonicated cells using a radioenzymatic assay (REA) with [¹⁴C]-labelled methyl-S-adenosyl-L-methionine and a crude preparation of rat kidney histamine methyl transferase [18]. The concentrations of histamine were calculated using histamine standards (Sigma Chemicals). The concentrations of Cys-LT were measured with a commercially available enzyme immunoassay (EIA) kit

and according to manufacturer's instructions (Amersham Pharmacia Biotech, Buckinghamshire, UK). Percentages of cross-reactivity are 100% for LTC₄ and LTD₄ and 30% for LTE₄.

The concentrations of GM-CSF and TNF- α in mast cell culture supernatants and cell sonicates were measured by ELISA, using matched antibody pairs according to the manufacturer's instructions. The anti-cytokine antibodies and recombinant human cytokines were purchased from R & D Systems (Abingdon, Oxfordshire, UK).

Effects of glucocorticosteroids on the expression of Fc ϵ RI, c-kit (CD117) and CD49d by human CBMC

IgE (5 μ g/mL) (Serotec, Raleigh, NC, USA) was added to the CBMC cultures for 24 h prior to the assessment of Fc ϵ RI- α expression, in order to enhance Fc ϵ RI- α expression [15]. IgE was cultured with the CBMC in medium alone or containing dexamethasone or testosterone. The mast cells were washed in PBS containing bovine serum albumin (BSA) (0.5%) and sodium azide (PAB) (0.1%) and then resuspended at a concentration of 2×10^6 mL. Mast cell suspension (80 μ L) was incubated for 1 h on ice with 20 μ L of either the control mouse IgG1 (Sigma Chemicals), mouse monoclonal anti-human c-kit (clone YB5.B8, Pharmingen, Franklin Lakes, NJ, USA), 22E7 (anti-Fc ϵ RI α) and mouse monoclonal anti-human CD49d (clone 9F10, Pharmingen), all at 50 μ g/mL. The cells were washed twice with PAB and bodipyTM-labelled goat anti-mouse IgG (20 μ g/mL) (Molecular Probes, Leiden, the Netherlands) was incubated with the cells for 30 min on ice. The cells were washed with PAB and resuspended in 200 μ L of PBS prior to analysis, within 1 h of staining, on a Becton Dickinson FACScan (Becton Dickinson, Cowley, Oxfordshire, UK). Five thousand events were collected and the fluorescence staining of the mast cells was assessed at 525 nm (FL1). Analysis of the cells was performed using 'Lysis II' software and the mean fluorescence intensity (m.f.i.) values were obtained. The results are represented as the ratio of the m.f.i. of the cells incubated with the specific antibody (anti-c-kit, anti-Fc ϵ RI- α or anti-CD49d) compared with the m.f.i. of the cells incubated with the isotype control antibody.

Statistical analysis

Results are expressed as mean $\pm$ SEM. Statistical analysis was performed by the Student's paired *t*-test. A *P*-value of <0.05 was considered statistically significant.

Results

Effect of glucocorticoids on the maturation of mast cell progenitors from human cord blood-derived mononuclear cells

The mean percentage of mast cells following culture of cord blood-derived mononuclear cells with SCF, IL-6 and PGE₂ for 21 days was $27.7 \pm 12.4\%$ ($n=5$). When dexamethasone was added to such cord blood-derived mononuclear cell cultures it was found to dose-dependently inhibit the development of mast cells compared with cells cultured in medium alone (Fig. 1). There were no significant effects of the control steroid, testosterone, over the same concentration range and time period.

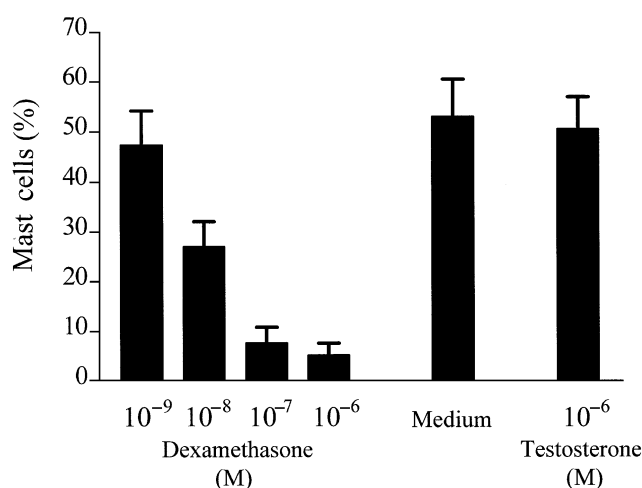


Fig. 1. Dose-dependent effect of dexamethasone on the percentage of mast cells derived from cord blood-derived mononuclear cells. Cord blood-derived mononuclear cells were incubated with SCF, IL-6 and PGE₂ for 21 days. The resulting CBMC-containing cultures were incubated further for 12 days either in the presence of the same medium or with dexamethasone (10^{-6} M– 10^{-9} M) ($n=5$) or with testosterone tested over the same concentration range (10^{-6} M– 10^{-9} M) ($n=2$). For simplicity, only the results of 10^{-6} M testosterone are shown. Mast cell numbers were assessed by Toluidine blue staining and the total cell numbers by Kimura's staining after 12 days of incubation with the steroids. Results are expressed as percentage of mast cells $\pm$ SEM.

The effects of dexamethasone on the percentage of mast cells became more pronounced with time. The percentage of mast cells cultured in medium alone increased with time and after a further 12 days of culture the percentage of mast cells increased by 1.88-fold, to $52.3 \pm 13.3\%$ ($n=5$). When dexamethasone was added to the culture medium the numbers and percentages of mast cells were significantly reduced. After 3 days incubation of cord blood-derived mononuclear cells with dexamethasone (10^{-6} M) the percentage of mast cells was $14.8 \pm 8.7\%$ compared with $32.3 \pm 15.9\%$ in cultures in medium alone ($P=0.040$) ($n=5$). After 12 days of culture with dexamethasone the percentage of mast cells was $4.2 \pm 4.8\%$ compared with $52.3 \pm 13.3\%$ in medium alone ($P<0.001$) ($n=5$). The numbers, as well as the percentage of mast cells, increased with time when cultured in medium alone, whereas the numbers of other cells showed a slight decrease in cell number (Table 1). When dexamethasone was included in the cultures for 12 days mast cell numbers were reduced by $98.0 \pm 4.2\%$ compared with a $63.7 \pm 5.2\%$ reduction of other cells ($n=3$) (Table 1). There were no significant effects of the control steroid, testosterone, over the same concentration range and time period.

In contrast, the histamine content of the mast cells that remained throughout the 12 days of culture with dexamethasone was not affected. For example, the histamine content of mast cells cultured for 12 days in medium alone was $3.1 \mu\text{g}/10^6$ cells and of mast cells cultured with dexamethasone (10^{-8} M) was $2.9 \mu\text{g}/10^6$ cells ($n=5$) ($P=0.843$).

Immunocytochemistry of tryptase-positive cells cultured for 36 days in medium alone (Fig. 2a) was compared with cells incubated in medium alone for 27 days and subsequently cultured in either testosterone (10^{-6} M) (Fig. 2b) or dexamethasone (10^{-6} M) (Fig. 2c) for a further 9 days. In these experiments, mast cells stained bright red. Nuclei stained dark blue

Table 1. Mast cells and non-mast cells obtained from cord blood-derived mononuclear cell cultures cultured for 12 days with dexamethasone. Cord blood-derived mononuclear cells were incubated with SCF, IL-6 and PGE₂ for 21 days. The resulting CBMC-containing cultures were incubated further for 12 days, either in the presence of the same medium or with dexamethasone (10^{-6} M). Mast cell numbers were assessed by Toluidine blue staining and the total cell numbers by Kimura staining. Results are expressed as numbers (10^6) of mast cells and non-mast cells

Donor	Cell type	Number of treatment days								
		0	3		6		9		12	
			Medium	Dex	Medium	Dex	Medium	Dex	Medium	Dex
I	Non-mast cells (10 ⁶)	2.57	2.35	2.15	2.40	1.72	2.38	1.22	2.28	0.64
	Mast cells (10 ⁶)	1.30	1.56	0.51	1.69	0.28	2.06	0.18	2.62	0.02
II	Non-mast cells (10 ⁶)	2.49	2.50	1.69	2.68	1.62	2.49	1.51	2.38	0.90
	Mast cells (10 ⁶)	0.96	1.04	0.49	1.41	0.23	2.01	0.16	2.78	0.06
III	Non-mast cells (10 ⁶)	2.34	2.67	2.14	2.69	2.10	2.41	1.56	2.28	0.98
	Mast cells (10 ⁶)	1.01	1.49	0.53	1.89	0.21	2.03	0.15	2.23	0.07

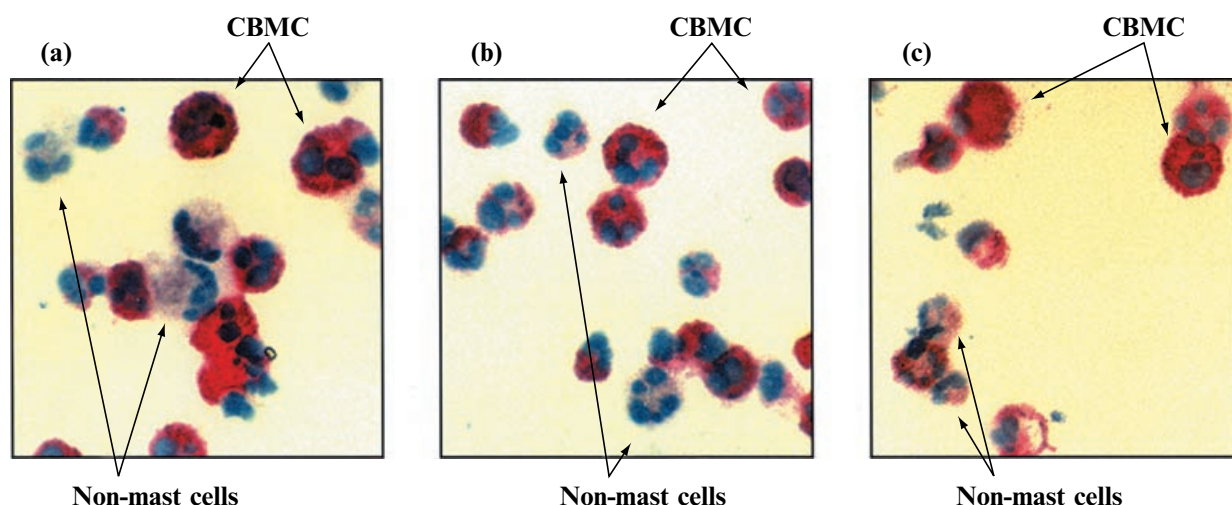


Fig. 2. Immunocytochemical identification of the effect of dexamethasone on tryptase expression by cord blood-derived mast cells. Cord blood-derived mononuclear cells were incubated with SCF, IL-6 and PGE₂ for 27 days and then cultured in the presence of either the same medium (a), or testosterone (10^{-6} M) (b), or dexamethasone (10^{-6} M) (c), for a further 9 days. All cell nuclei stain dark blue with haematoxylin. Tryptase-positive cells were identified by their bright red staining after development with Fast Red. Non-mast cells have a pale blue/pink cytoplasm. (Magnification $40\times$).

with haematoxylin. Cultures that included dexamethasone exhibited considerably fewer mast cells per field (Fig. 2c). However, the mast cells obtained from cord blood-derived mononuclear cells cultured in medium alone for 36 days (Fig. 2a), compared with those obtained from cord blood-derived mononuclear cells incubated in medium for 27 days and subsequently cultured in either testosterone (10^{-6} M) (Fig. 2b) or dexamethasone (10^{-6} M) (Fig. 2c) for a further 9 days, displayed a similar morphology and size as evidenced by the antitryptase antibody staining.

Effect of glucocorticoids on human CBMC activation

Human CBMC released $1.3 \pm 0.3 \mu\text{g}$ histamine/ 10^6 mast cells when activated by cross-linking of Fc ϵ RI- α . The percentage of histamine release was $24.3 \pm 5.8\%$ ($n=6$). No significant effect on Fc ϵ RI-dependent histamine release was observed when dexamethasone or testosterone was added to mast cell cultures, for either 1 h ($n=4$) or 24 h ($n=4$) prior to Fc ϵ RI-cross-linking compared with cultures in medium alone (data not shown).

Similarly to histamine release, Cys-LT release was not influenced by dexamethasone. In fact, mast cells activated for 1 h with an anti-Fc ϵ RI- α antibody were found to release $89.3 \pm 12.4 \text{ pg Cys-LT}/10^6$ mast cells and mast cells incubated with dexamethasone released $94.2 \pm 16.7 \text{ pg Cys-LT}/10^6$ mast cells ($n=4$) ($P=0.451$). Under similar incubation conditions testosterone (10^{-6} M– 10^{-9} M) had no effect on Cys-LT production. In addition, there was no significant effect on Fc ϵ RI-dependent Cys-LT release from mast cells when dexamethasone or testosterone (10^{-6} M– 10^{-9} M) was added for 24 h prior to Fc ϵ RI-cross-linking ($< 10 \text{ pg/mL}$) ($n=3$).

Mast cells were found to produce $585 \pm 84 \text{ pg GM-CSF}/10^6$ mast cells, 22 h after Fc ϵ RI-cross-linking ($n=5$). No detectable GM-CSF was found in mast cell sonicates, thereby demonstrating the *de novo* production of GM-CSF following Fc ϵ RI-dependent mast cell activation.

Dose-dependent inhibition of GM-CSF release was observed when dexamethasone was added to mast cell cultures (> 10 weeks old) for either 1 h ($n=4$) (Fig. 3) or 24 h ($n=4$)

(data not shown) prior to FcεRI-cross-linking. There was no significant difference in the level of inhibition between 1 h and 24 h pre-incubation with dexamethasone. Dexamethasone also inhibited TNF-α release from mast cells activated by FcεRI-α. Mast cells that were found to produce 152.3 ± 26.4 pg TNF-α/ 10^6 mast cells 22 h after FcεRI-cross-linking released negligible amounts of TNF-α 1 h after activation. Furthermore, negligible amounts of TNF-α were detected in mast cell sonicates (< 8.0 pg/ 10^6 mast cells). Dose-dependent inhibition of TNF-α release was observed when dexamethasone was added to mast cells for either 1 h ($n = 2$) or 24 h ($n = 2$) prior to FcεRI-cross-linking.

To exclude the possibility that contaminating FcεRI-bearing cells other than mast cells are the producers of the two assessed cytokines, mast cells were purified by positive selection ($> 99.5\%$). Also, these highly purified mast cells were found to produce comparable concentrations of both GM-CSF and TNF-α to the non-purified cells ($> 97\%$ mast cells) and were inhibited by dexamethasone similarly to non-purified mast cells (data not shown).

Mast cell viability after treatment with dexamethasone (10^{-6} M) for 24 h was always higher than 90% as assessed by mast cell ability to exclude Trypan Blue.

Effects of glucocorticosteroids on the expression of FcεRI, c-kit (CD117) and CD49d by human CBMC

A significant inhibition of IgE-enhanced FcεRI-α expression on CBMC was induced by dexamethasone ($n = 3$) (78.1% inhibition compared with medium, $P = 0.036$) when the cells were simultaneously incubated with IgE (5 µg/mL) and dexamethasone (10^{-6} M) for 24 h prior to analysis by flow cytometry (Fig. 4). However, no difference in the expression of either CD49d or c-kit was detected under the same conditions. Testosterone (10^{-6} M) did not influence the expression of FcεRI-α, CD49d or c-kit (Fig. 4). The expression of FcεRI-α, CD49d and c-kit was also not affected when mast cells were incubated in the absence of IgE ($n = 2$) (data not shown).

Discussion

Glucocorticosteroids are the most effective drugs in down-modulating allergic inflammation. They inhibit cell differentiation, proliferation, migration and activation. However, there are limited reports on the direct effects of steroids on mast cells, which are the initiators of these responses [10, 11, 19]. Recently, mast cells have been postulated to have a role in late/chronic stages of allergic inflammation by their capacity to produce cytokines and hence influence the infiltrated inflammatory cells, notably the eosinophils [20].

In the present study, by using cord blood-derived mononuclear cells, we have shown that the maturation of mast cell progenitors is inhibited by dexamethasone. Likewise, it has previously been shown that development of human fetal liver-derived mast cells is inhibited by dexamethasone [11]. In contrast glucocorticosteroids had no effect on the *in vitro* maturation of murine bone marrow-derived mast cells [21, 22], whose maturation depends on IL-3 as well as on SCF. Nevertheless, the number of mature murine mast cells in the skin is decreased by glucocorticosteroids through an indirect activity of this drug. In fact, glucocorticosteroids inhibit SCF production by fibroblasts, with consequent mast cell apoptosis [23]. Also administration of glucocorticosteroids *in vivo*, in humans, has been shown to profoundly reduce mast cell numbers localized in mucosa and submucosa of bronchial biopsies [24, 25] where the putative mechanism was the inhibition of SCF production by human fibroblasts [23].

In the present study we have demonstrated that dexamethasone is able to hinder human mast cell development even when the level of SCF is constant. This finding points to a direct effect of dexamethasone on human mast cell progenitors that have shown an increased sensitivity to these drugs in comparison with mature mast cells. This observation could suggest that glucocorticoids can modulate the expression of either anti- or pro-apoptotic molecules in mast cell progenitors and mature mast cells. Indeed, it has been recently demonstrated that

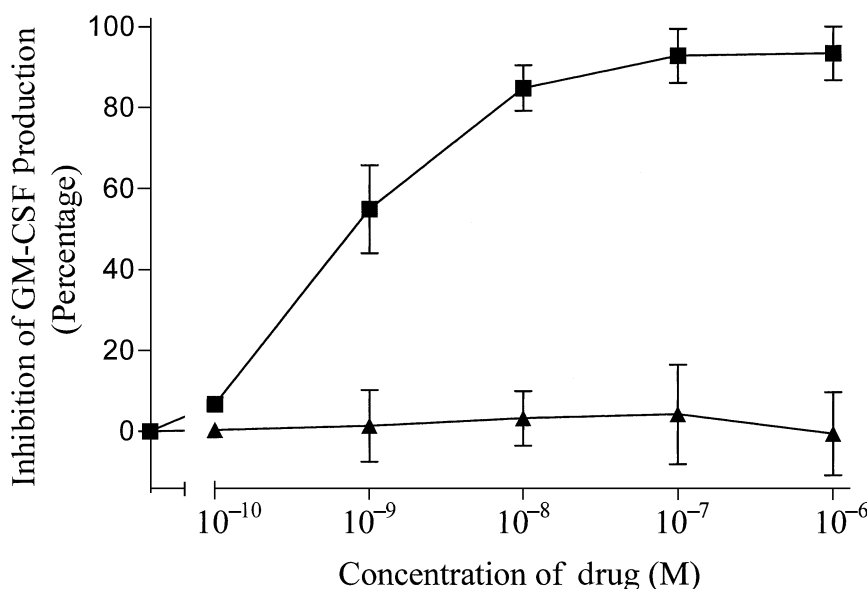


Fig. 3. Dose-dependent inhibition of GM-CSF production by dexamethasone of FcεRI-dependent activated CBMC. Cord blood-derived mononuclear cells were cultured for at least 10 weeks with SCF, IL-6 and PGE₂. The resulting CBMC were incubated either in the same medium alone, or in the presence of dexamethasone (10^{-6} M– 10^{-10} M) (■) or of testosterone (10^{-6} M– 10^{-10} M) (▲) for 1 h. A monoclonal anti-FcεRI antibody was incubated with the cells for a further 22 h prior to the determination of the cytokines in the culture supernatants. Results are expressed as percentage of cytokine inhibition, compared with medium alone $\pm$ SEM.

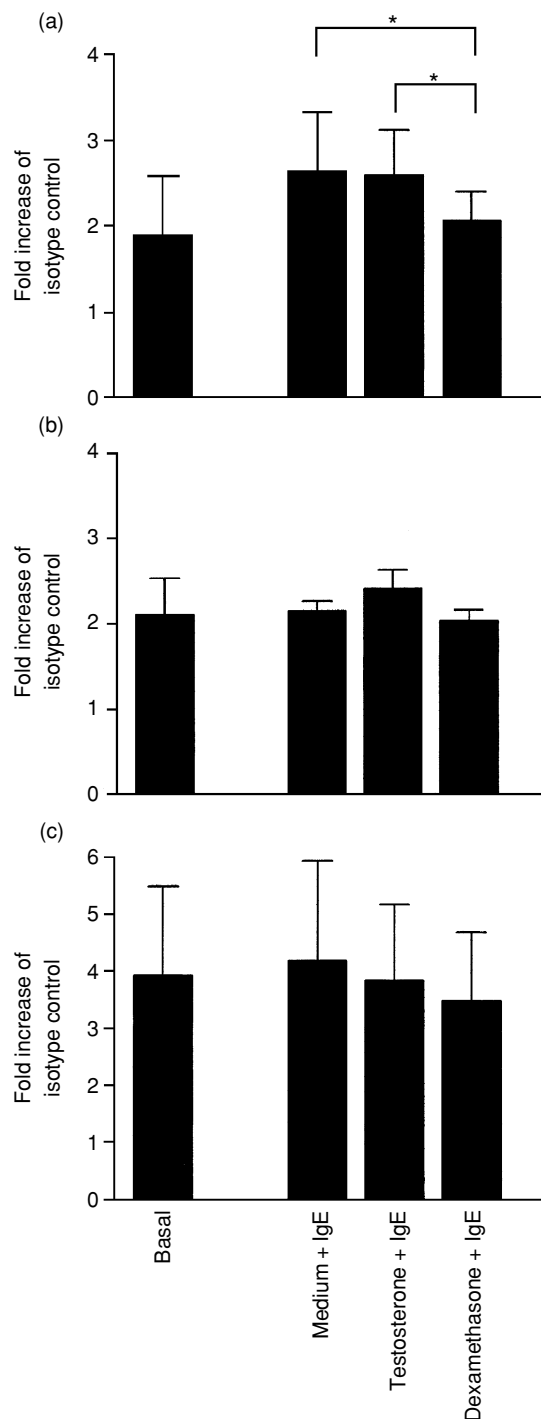


Fig. 4. Effect of dexamethasone on FcεRI-α, c-kit, and CD49d expression by CBMC. Cord blood mononuclear cells were cultured for at least 10 weeks with SCF, IL-6 and PGE₂. Expression of FcεRI-α (a), c-kit (b) and CD49d (c) was measured by labelling with monoclonal antibodies and detected with bodipy-labelled goat anti-mouse IgG antibodies. Human IgE and dexamethasone were simultaneously added to the cord blood-derived mast cell cultures ($n = 3$). Results are expressed as fold increase of isotype control $\pm$ SEM. *Denotes significance $P < 0.05$.

thymocytes exposed to dexamethasone express high levels of the pro-apoptotic molecule Bad that affects the T cell developmental and selection processes that occur in the thymus [26]. In addition, mature T cells in their proliferation phase were found to express high levels of Bcl-2, an anti-apoptotic molecule that attenuates T cell susceptibility to dexamethasone-induced apoptosis [27]. In the present study, we have found that the histamine content of mature mast cells that developed from cord blood-derived mononuclear cells cultured in the presence of dexamethasone was not influenced. Furthermore, these mast cells also expressed tryptase with a pattern similar to untreated cells. Also in bone marrow-derived mast cells the transcript levels for MCP-5 and MCP-6 were not affected [19]. These observations would indicate that dexamethasone does not interfere with the enzymes involved in the synthesis of these two mast cell mediators and that this steroid preferentially affects mast cell progenitors rather than mature mast cells.

The activity of dexamethasone on mature mast cell histamine release was evaluated. In accordance with previous reports on the lack of effects of dexamethasone on mediator release from lung and skin mast cells [28], we found that the FcεRI-dependent release of histamine and *de novo* synthesis and release of Cys-LT from mast cells was unaffected by dexamethasone. In contrast to these results, it has been shown in the past that dexamethasone is able to inhibit histamine and LTC₄ release from mouse peritoneal and mouse bone marrow-derived mast cells. This observation points out the heterogeneity between mast cells from different species in relation to their sensitivity to steroids and secretion of pre-formed and newly formed mediators [22].

However, mast cells seem not to be heterogeneous concerning the effect of dexamethasone on their cytokine production. In the present study we have shown that dexamethasone potently inhibited both GM-CSF and TNF-α production following FcεRI-dependent mast cell activation. It is interesting to point out that cord blood-derived mast cells were found to synthesize the majority of TNF-α *de novo*. These data are in accordance with others who have shown that TNF-α release induced by anti-IgE was maximal 24 h after stimulation [29].

Dexamethasone has also been shown to inhibit production of IL-4, IL-8 and GM-CSF from the human mast cell line (HMC-1) [30] and IL-13 from isolated human lung mast cells [10]. Furthermore, glucocorticosteroids, including dexamethasone, have been shown to inhibit IgE-dependent release of cytokines, namely TNF-α and IL-4, from the rat basophil leukaemia cell line (RBL), which are similar to rat mucosal type mast cells [31, 32].

We next evaluated the effects of dexamethasone on FcεRI-α, CD49d and c-kit expression on mast cells and found that dexamethasone inhibited the IgE-dependent up-regulation of FcεRI-α expression on mast cells, without affecting CD49d and c-kit expression. This observation suggests that dexamethasone selectively inhibits the IgE-dependent expression of FcεRI-α. In addition, we have found in preliminary experiments that this reduction in FcεRI-α expression is correlated with a decrease in the percentages of histamine release (80.0% inhibition, $n = 2$) after IgE-dependent activation (data not shown). Interestingly, the expression of FcεRI-α was not affected when mast cells were incubated with IgE previous to dexamethasone addition. This observation would suggest that IgE prevented the down-regulating effects of dexamethasone on

FcεRI-α expression. Similar results have been observed in mouse bone marrow-derived mast cells where pre-incubation with dexamethasone has been shown to reduce FcεRI expression [22, 33] and also inhibit IgE-dependent LTC₄ and histamine secretion [22]. This evidence suggests a new mechanism by which glucocorticosteroids are able to inhibit the release of pre-formed and newly formed mediators from mast cells in the absence of IgE or low levels of this antibody.

In summary, we have demonstrated that *in vitro* dexamethasone has direct inhibitory effects on human mast cell maturation and on mature mast cell function. Consequently, we can speculate that the inhibition of mast cell development by glucocorticosteroids would lead to fewer mast cells in tissues and this could lead to a reduced allergic inflammatory reaction. In addition, the capacity of glucocorticosteroids to inhibit cytokine production in mature mast cells suggests that they might reduce the recruitment, activation and survival of cells involved in the late phase of allergic reactions or in airway remodelling, such as eosinophils. This combined evidence points to a wider activity of glucocorticosteroids in allergic inflammation.

Acknowledgements

This work was supported in part by the Aimwell Charitable Trust (UK). S. J. Smith is a recipient of the Isaac and Myrna Kaye Travelling Fellowship between the Imperial College School of Medicine, London, UK, and The Hebrew University of Jerusalem, Israel. The authors gratefully acknowledge the generous gifts of human recombinant stem cell factor from AMGEN Corporation, Thousand Oaks, CA, USA and the monoclonal anti-FcεRI-α (22E7) from Dr J. Kochan, Hoffman La Roche, Nutley, NJ, USA.

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