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Systemic and local dexamethasone treatments prevent allergic eosinophilia in rats via distinct mechanisms

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Abstract

We have studied the effect of local and systemic treatment with dexamethasone for prevention of the pleural eosinophilia triggered by allergen in actively sensitised Wistar rats. Parallel changes in blood and marrow eosinophil numbers were assessed for comparison. The intrapleural (i.pl.) injection of ovalbumin into ovalbumin-sensitised animals led to a long-lasting pleural fluid eosinophilia which peaked from 24 to 72 h post-challenge. At these time points, there was a significant 2- to 3-fold increase in the blood eosinophil numbers, whereas the bone marrow number of mature eosinophils remained unaltered. Systemic treatment with dexamethasone (0.05–0.5 mg/kg, i.p.) abolished the pleural and blood eosinophilia observed 24 and 48 h post-challenge, also causing a significant reduction in marrow eosinophil numbers. Despite being unable to alter blood and bone marrow eosinophil numbers, the local i.pl. administration of dexamethasone (2.5–10 μg/cavity) inhibited dose dependently the allergen-induced pleural eosinophil influx at 24 h but not at 48 h post-challenge. This treatment also shortened the time course of eosinophil accumulation in the pleural space from the 48 h time point on. We conclude that the effect of systemic but not of local treatment with dexamethasone on allergen-induced eosinophil recruitment is well correlated with the inhibition of eosinophil production in bone marrow. In contrast, low amounts of dexamethasone injected into the pleural space seem to affect locally eosinophil recruitment and survival.

Keywords: Dexamethasone; Eosinophil; Blood eosinophilia; Allergic pleurisy

1. Introduction

Isolated and synthesised in the forties, the glucocorticoid hormones have been widely used in the treatment of inflammatory disorders. However, the targets and the mechanisms of action of the beneficial effects of this class of medicaments are still not totally known (for review, see Schleimer, 1993). Among a number of mechanisms postulated for the anti-inflammatory and immunosuppressive properties of glucocorticoids their ability to exert positive and negative effects on the genic transcription process has been singled out (for review, see Barnes, 1996). Indeed, glucocorticoids can suppress the transcription of numerous cytokines which regulate cell growth, differentiation, endothelial transmigration and many other leucocyte functions (Levine et al., 1993; Wang et al., 1993; Okayama et al., 1994; Kwon et al., 1995; Wershil et al., 1995). In addition, glucocorticoids can up-regulate the expression of lipocortin-1—a Ca^{2+} and phospholipid binding protein—which has been shown to mimic glucocorticoid anti-inflammatory effects in several systems (Flower and Blackwell, 1979; Perretti and Flower, 1993).

The inflammatory eosinophilic response is largely sensitive to corticosteroid hormones, but the mechanism of action is not fully understood (Schwiebert et al., 1996). Eosinophils are polymorphonuclear leucocytes showing great affinity for tissue spaces, where they are supposed to play a very important role in allergic and parasitic conditions (Weller, 1994; Martin et al., 1996). Based on experimental model of cutaneous late-phase reaction in mice, Iwamoto et al. (1992) have reported that the allergen-induced eosinophil infiltration into the subcutaneous tissue is a biphasic phenomenon. CD^{4+} T lymphocytes and interleukin-5 were shown to be involved in the second peak, while mast cells and platelet activating factor (PAF) were

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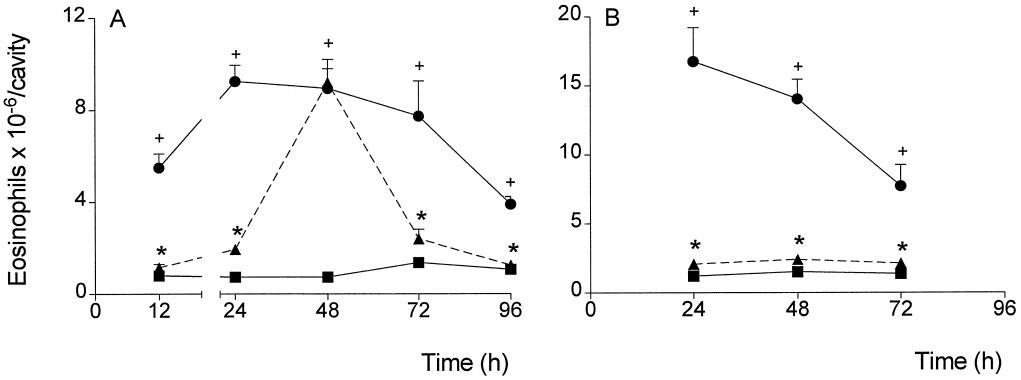

Fig. 1. Time course of ovalbumin-evoked eosinophil accumulation in the pleural cavity of non-sensitized (■), sensitised untreated (●) and sensitised rats pretreated with dexamethasone (▲). Panels A and B show results from local (10 μg/cavity) and systemic dexamethasone (0.5 mg/kg, i.p.) administration respectively. Each point represents the mean ± S.E.M. from at least eight animals; $^{+}P < 0.05$ as compared to the non-sensitised group; $^{*}P < 0.05$ as compared to the sensitised untreated group.

implicated in the first peak of allergen-induced eosinophil recruitment. Accordingly, we have confirmed, using a pleurisy test system in actively sensitised rats, that the allergen challenge leads to long-lasting pleural eosinophil accumulation (Pasquale et al., 1992; Silva et al., 1992). The phenomenon appeared to have a single peak, being first noted within 8 h, reaching a plateau from 24 to 72 h and returning to baseline levels 120 h post-challenge. This apparent monophasic response of eosinophil accumulation was paralleled by an increase in the number of mononuclear cells and preceded by mast cell degranulation, plasma leakage and intense neutrophil infiltration (Lima et al., 1990, 1991; Pasquale et al., 1992).

Considering that the long-term pleural eosinophilia triggered by allergen challenge could be produced by an overlapping sequence of independent mechanisms, we now examined the putative effect of either local or systemic treatment with dexamethasone on the kinetics of eosinophil accumulation evoked by allergen challenge. Parallel changes in the number of blood and bone marrow eosinophils, as well as other parameters, including plasma leakage, mast cell degranulation and neutrophil recruitment, were also investigated in an attempt to clarify the mechanism of blockade by the steroid.

2. Materials and methods

2.1. Animals and allergic pleurisy

Wistar rats of both sexes (150–200 g) were obtained from the Oswaldo Cruz Foundation breeding farm (Rio de Janeiro, Brazil). Sensitisation was achieved by a subcutaneous (s.c.) injection (0.2 ml) of a mixture of ovalbumin (50 μg) and aluminium hydroxide (5 mg) in 0.9% NaCl solution (saline). Ovalbumin dissolved in saline was administered intrapleurally (i.pl.) (12 μg/cavity) to 14-day-sensitised rats as well as to non-sensitised rats (negative controls). The challenge was performed in ether-anaesthetised animals in a final volume of 0.1 ml, using a 27.5-gauge needle about 3 mm in length. At several time points ranging from 12 to 96 h post-challenge, the rats were killed with an overdose of ether and the pleural cavity was rinsed with 3 ml of heparinized saline (10 IU/ml). The pleural effluent was collected and its volume was measured with a graduated syringe. Total leucocytes were counted in a Coulter Counter ZM (Coulter Eletronic®) after red blood cell lysis using zap-oglobin® II (Coulter

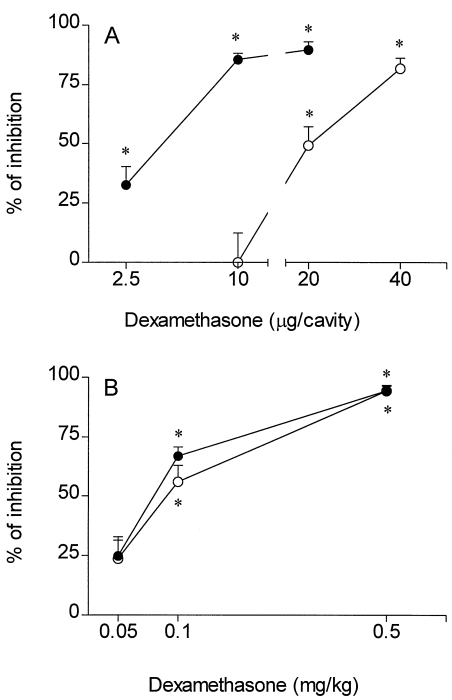

Fig. 2. Effect of local (2.5–40 μg/cavity) (Panel A) or systemic (0.05–0.5 mg/kg, i.p.) (Panel B) dexamethasone pretreatment on the pleural eosinophil infiltration observed 24 h (●) and 48 h (○) after antigen challenge in sensitised rats. Results are expressed as percent inhibition and the values are the means ± S.E.M. from at least six animals. $^{*}P < 0.05$ as compared to the control untreated group.

Diagnostics). Differential leucocyte analysis was performed under an oil immersion objective on cytocentrifuged smears stained with May–Grünwald–Giemsa dye.

2.2. Blood and bone marrow cell counts

Peripheral blood samples were obtained from the tail vein at 24 and 48 h after the i.pl. injection of ovalbumin. For the marrow analysis, we used a procedure in common use (Collins et al., 1995). The animals were killed as described above, the right femur was removed and the bone marrow was gently eluted with 5 ml of minimum essential medium (MEM) (pH 7.2) containing 30 mM HEPES buffer and 2 mg/ml sodium bicarbonate plus heparin (40 IU/ml). Total leucocytes were counted as described above and differential cell analyses for blood and bone marrow were performed in blood smears and cytocentrifuged marrow smears, respectively, stained with May–Grünwald–Giemsa dye.

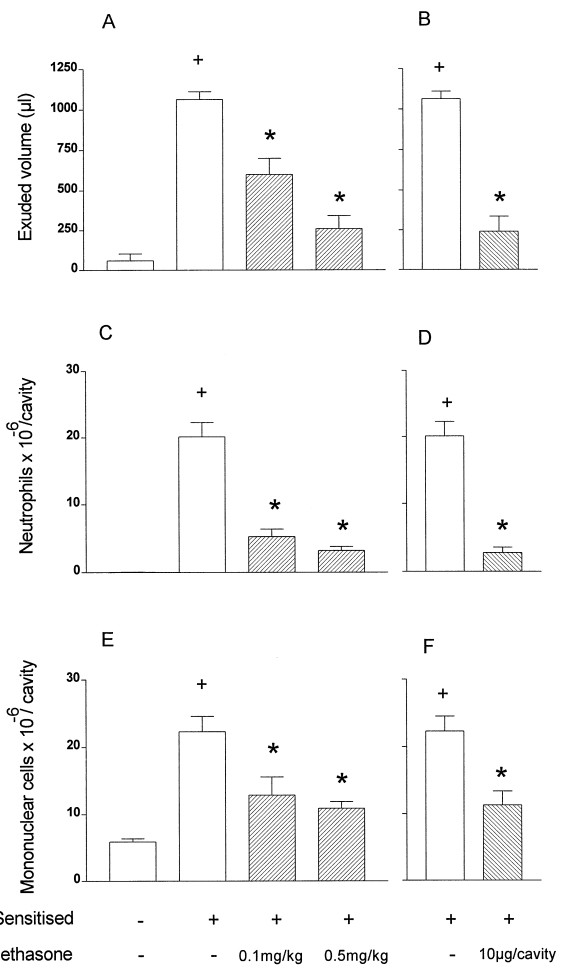

Fig. 3. Effect of local (left-hatched columns, 10 μg/cavity) or systemic (right-hatched columns, 0.1 and 0.5 mg/kg, i.p.) dexamethasone pretreatment on allergen-evoked exudation (Panels A, B), neutrophil (Panels C, D) and mononuclear cell infiltration (Panels E, F) noted 4 h post-challenge. Columns represent the means ± S.E.M. from at least six animals. $^{+}P < 0.05$ as compared to the non-sensitised group; $^{*}P < 0.05$ as compared to the sensitised untreated group.

Table 1
Effect of compound 48/80 or polymyxin B on pleural mast cell population

Treatment	Intact mast cell $\times 10^{-3}$/cavity
Saline	470 ± 3
Compound 48/80	5 ± 3[a]
Polymyxin B	6 ± 1[a]

Analyses were performed 5 days or 24 h after i.pl. injection of compound 48/80 (12 μg/cavity) or polymyxin B (10 μg/cavity), respectively. Results are expressed as means ± S.E.M. from at least eight animals.
[a] $P < 0.001$ as compared to the control group.

2.3. Localised mast cell granule depletion and mast cell enumeration

Local mast cell granule depletion was obtained with compound 48/80 (12 μg/cavity) or polymyxin B (10 μg/cavity) injected intrapleurally 5 days and 24 h before ovalbumin challenge respectively. Mast cell counts for pleural fluid were performed in a Neubauer chamber under light microscopy after dilution in toluidine blue solution (Mota, 1966).

2.4. Drug treatments

Dexamethasone was administered either intrapleurally (2.5–40 μg/cavity) or intraperitoneally (0.05–0.5 mg/kg) 1 h before allergen administration. The steroid was dissolved in saline immediately before use.

2.5. Materials

Ovalbumin was purchased from Biochemika Fluka (Switzerland). HEPES, MEM Eagle's medium, compound 48/80 and polymyxin B were obtained from Sigma (USA); dexamethasone (Decadron®) was from Merck, Sharp and Dohme (Brazil).

2.6. Statistical analysis

All data are presented as means ± S.E.M. Statistical analysis involving two groups was done with Student's *t*-test, whereas the analysis of variance (ANOVA) and Newman–Keuls–Student's test were used to compare more than two groups. *P*-values of 0.05 or less were considered significant.

3. Results

3.1. Effect of local or systemic dexamethasone pretreatment on allergen-induced pleural eosinophilia

Fig. 1A shows the kinetics of allergen-evoked eosinophil accumulation in the pleural cavity of actively sensitised rats, and the influence of local pretreatment with dexa-

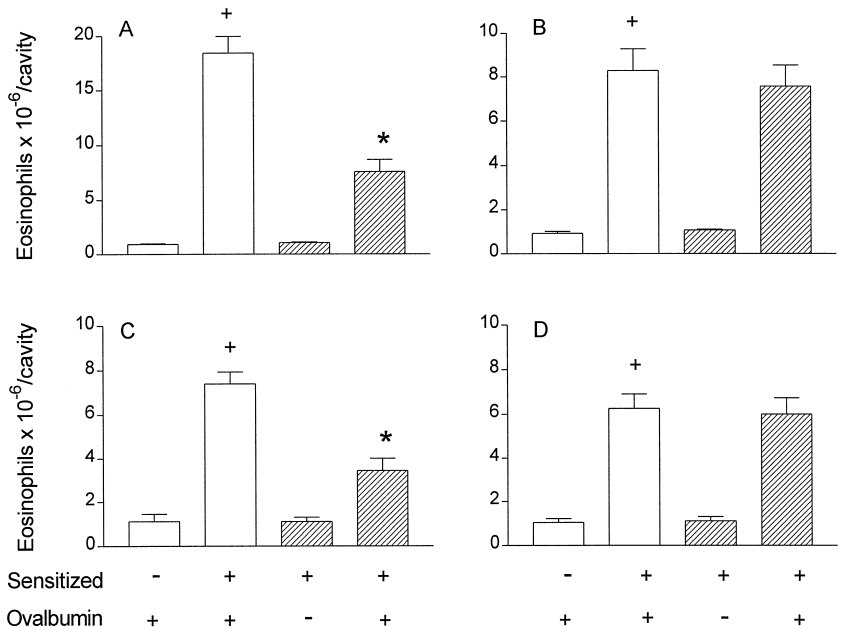

Fig. 4. Effect of mast cell granule depletion triggered by compound 48/80 (12 μg/cavity, A–B) or polymyxin B (10 μg/cavity, C–D) on allergen-induced pleural eosinophilia noted within 24 h (left panels) or 48 h (right panels). Hatched and open columns represent depleted and non-depleted groups respectively. Columns represent the means ± S.E.M. from at least six animals. $^{+}P < 0.05$ as compared to the non-sensitised group; $^{*}P < 0.05$ as compared to the sensitised non depleted group.

methasone. Intrapleural injection of ovalbumin (12 μg/cavity) led to a long-lasting pleural eosinophil accumulation, which peaked from 24 to 72 h, persisting for at least 96 h post-challenge. Intrapleural injection of dexamethasone (10 μg/cavity) 1 h before challenge abolished the eosinophil accumulation noted between 12 and 24 h but failed to do so at 48 h post-challenge, despite being able to shorten the duration of the eosinophilic response

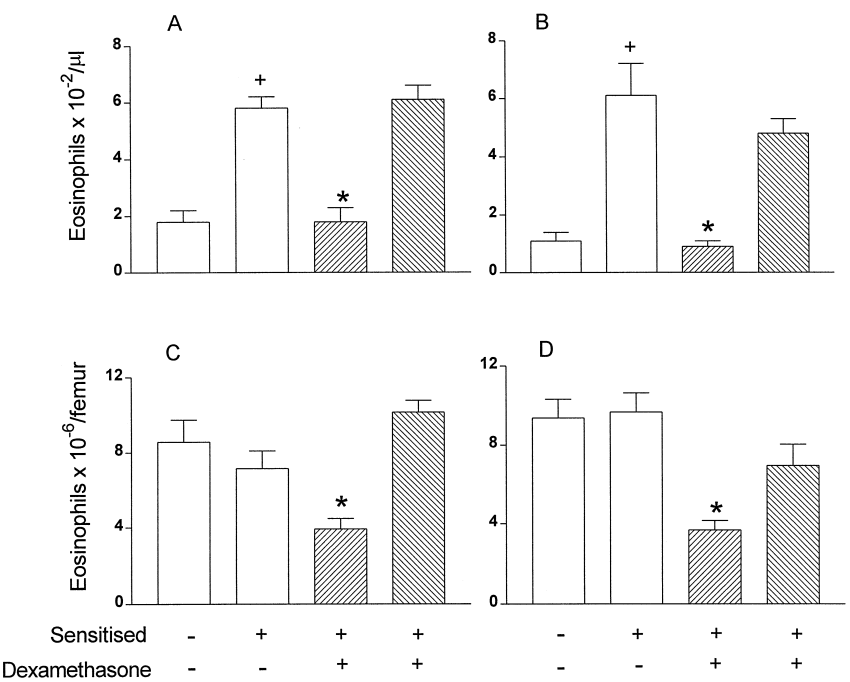

Fig. 5. Effect of systemic (right-hatched columns, 0.5 mg/kg, i.p.) or local (left-hatched columns, 10 μg/cavity, i.pl.) pretreatment with dexamethasone on the blood (A–B) and bone marrow (C–D) changes in eosinophil numbers observed 24 h (left panels) and 48 h (right panels) post-allergen challenge. Columns represent the means ± S.E.M. from at least six animals. $^{+}P < 0.05$ as compared to the non-sensitised group; $^{*}P < 0.05$ as compared to the sensitised untreated group.

Table 2
Effect of systemic dexamethasone on bone marrow eosinophils from unchallenged rats

Conditions	Treatment	Eosinophils $\times 10^{-6}$/femur
Non-sensitised	None	11.43 ± 0.99
	Dexamethasone	13.98 ± 1.61
Sensitised	None	14.66 ± 1.46
	Dexamethasone	13.85 ± 1.75

Non-sensitised and sensitised rats without ovalbumin challenge were treated with dexamethasone (0.5 mg/kg, i.p.).
The analysis was performed within 24 h and the values represent the means ± S.E.M. from at least eight animals.

thereafter. In contrast, as shown in Fig. 1B, systemic administration of dexamethasone (0.5 mg/kg, i.p.), 1 h before challenge, prevented the animals from reacting with pleural fluid eosinophilia in all time points analysed.

Fig. 2 illustrates the resistance to local dexamethasone pretreatment of allergen-evoked eosinophilia at 48 h compared to the eosinophilia at 24 h post-challenge. As shown in Fig. 2A, only doses of 20 and 40 μg/cavity were active within 48 h, whereas 2.5 μg/cavity dexamethasone was enough to significantly inhibit the eosinophil accumulation observed within 24 h. In contrast, the systemic treatment with dexamethasone (0.05–0.5 mg/kg, i.p.) inhibited equally both the 24 h and the 48 h eosinophilia (Fig. 2B).

3.2. Effect of dexamethasone on allergen-induced plasma leakage, mast cell degranulation, neutrophil and mononuclear cell recruitment

As we have found earlier, the allergen-induced eosinophilic response in the rat pleurisy model is preceded by mast cell degranulation, plasma leakage, neutrophil and mononuclear cell recruitment (Lima et al., 1991, 1997). As shown in Fig. 3, dexamethasone injected either systemically (0.1 and 0.5 mg/kg, i.p.) or locally (10 μg/cavity) significantly inhibited plasma exudation (Fig. 3A,B) and the influx of both neutrophils (Fig. 3C,D) and mononuclear cells (Fig. 3E,F) noted 4 h post-challenge. Dexamethasone (0.5 mg/kg, i.p.) also prevented the allergen-evoked mast cell degranulation, as attested to by toluidine blue dye staining of cytoplasmatic mast cell granules. The numbers of intact mast cells per cavity (mean ± S.E.M.) for ovalbumin-injected sensitised rats in the absence and presence of dexamethasone were $14 \pm 3 \times 10^3$ ($n = 6$) and $374 \pm 19 \times 10^3$ ($n = 6$) ($P < 0.001$), respectively. The value for ovalbumin-injected normal rats was $540 \pm 60 \times 10^3$ ($n = 6$), all the analyses being performed 4 h post-allergen challenge. It is noteworthy that the local treatment with dexamethasone (10 μg/cavity) failed to modify the profile of mast cell degranulation caused by allergen challenge (data not shown).

The effects of two recognised mast cell degranulating agents, compound 48/80 (12 μg/cavity) and polymyxin B (10 μg/cavity), on mast cells recovered from the pleural cavity were also investigated. As indicated in Table 1, the number of intact mast cells recovered from the pleural cavity was reduced by 95 and 98% ($n = 8$, $P < 0.001$) following compound 48/80 and polymyxin B, respectively. Under these conditions, the pleural eosinophil infiltration noted 24 h after ovalbumin was significantly reduced. The reduction was by 65% ($n = 8$, $P < 0.001$) and 67% ($n = 8$, $P < 0.001$) for pretreatment with compound 48/80 (Fig. 4A) and polymyxin B (Fig. 4C), respectively. In contrast, both methods of mast cell granule depletion failed to modify the eosinophil accumulation in the pleural cavity 48 h post-challenge (Fig. 4B and D).

3.3. Effect of dexamethasone on the number of blood and bone marrow eosinophils following allergen challenge

Sensitised rats had a 3-fold increase in blood eosinophil numbers at 24 h (Fig. 5A) and 48 h (Fig. 5B) after ovalbumin pleural challenge, under conditions where bone marrow eosinophil counts remained unaltered (Fig. 5C and D). The effect of dexamethasone on allergen-induced blood eosinophil accumulation is also illustrated in this figure. While the systemic pretreatment (0.5 mg/kg, i.p.) abolished the blood eosinophilia at 24 h (Fig. 5A) and 48 h (Fig. 5B), the intrapleural injection of dexamethasone (10 μg/cavity) was inactive in both cases. As shown in Fig. 5C and D, local administration (10 μg/cavity) also failed to alter the number of eosinophils in the bone marrow. Interestingly, the blockade of allergen-induced blood eosinophilia following systemic administration of corticosteroid was accompanied by a marked bone marrow eosinopenia 24 and 48 h post-challenge. It is noteworthy that a higher dose of dexamethasone given intrapleurally (20 μg/cavity) clearly abolished the blood eosinophilia noted 48 h post-challenge. The values decreased from $0.74 \pm 0.06 \times 10^3$ eosinophils/μl (mean ± S.E.M., $n = 8$) to $0.16 \pm 0.05 \times 10^3$ eosinophils/μl (mean ± S.E.M., $n = 8$) ($P < 0.001$) in untreated and treated animals, respectively.

Evidence that dexamethasone-induced bone marrow eosinopenia was clearly associated with the immunological challenge is presented in Table 2. Dexamethasone (0.5 mg/kg, i.p.) failed to change bone marrow eosinophil levels in either naive or 14-day actively sensitised unchallenged rats.

4. Discussion

The current study has provided evidence for a two-phase increase of pleural eosinophil influx following allergen challenge of sensitised rats. The response peaked from 24 to 72 h and decreased thereafter. Local treatment with small amounts of dexamethasone (2.5–10 μg/cavity) clearly suppressed the allergen-induced eosinophil accu-

mulation noted within 24 h, without an effect 48 h post-challenge. In contrast, systemic treatment with dexamethasone inhibited both eosinophilia components. In addition, we found that the effect of systemic but not of local treatment with dexamethasone appeared clearly associated with a reduction of eosinophil availability in the bone marrow and blood.

Glucocorticoids are a very effective class of drugs to impair allergen-induced tissue eosinophil recruitment, whether administered locally or systemically. The steroid effect on eosinophil distribution and function seems to result from a combination of direct and indirect mechanisms. Potential targets at which glucocorticoids can act to prevent eosinophil accumulation and activation include eosinophil generation, priming and recruitment of eosinophils by eosinophil-activating agents and the production/release of eosinophil chemoattractants by distinct cellular sources including mast cells, endothelial cells, epithelial cells, fibroblasts and others (for review, see Barnes and Adcock, 1993; Schwiebert et al., 1996). In the present study, pleurisy triggered by allergen challenge in actively sensitised rats was used to compare the results of local and systemic treatment with dexamethasone for prevention of pleural eosinophil accumulation, and for inhibition of the potential concurrent changes in the number of blood and bone marrow eosinophils.

As found earlier (Lima et al., 1991; Pasquale et al., 1992), a long-lasting pleural eosinophil accumulation was obtained following intrapleural injection of ovalbumin (12 μg/cavity). The effect reached a plateau at 24 to 72 h, and was still apparent 96 h post-challenge. Confirming previous findings, it was shown that the eosinophilic response was preceded by marked changes in other key inflammatory parameters, including mast cell degranulation, plasma leakage, neutrophil and mononuclear cell infiltration (Lima et al., 1991, 1997).

The pleural eosinophil enrichment was accompanied by a 3-fold increase in the number of circulating eosinophils, seen from blood leucocyte counts 24 and 48 h after ovalbumin. At these times, no alterations in bone marrow eosinophil numbers were detected. It is worth noting that, there was a clear, but non-significant, trend to a reduction in eosinophil numbers in the bone marrow pool (approximately 1×10^6 cells/femur). This decrease would have been sufficient to account for the eosinophil number increase in the blood compartment. Results of studies involving quantification of bone marrow and blood eosinophils support the concept that the bone marrow has the potential to replace, by eosinophilopoesis, eosinophils recruited into the tissue after allergen challenge (Gibson et al., 1990; Kung et al., 1994; Woolley et al., 1994). Therefore, antigen-induced bone marrow eosinophilopoesis seems to be a crucial target for the anti-allergic effect of glucocorticoids. This interpretation is in line with results of early studies showing that eosinophil proliferation in bone marrow colony assays is clearly impaired by the presence of glucocorticoids in culture, through a mechanism presumably related to the blockade of generation and/or function of eosinophilopoietic cytokines (Bjornson et al., 1985; Butterfield et al., 1986). In the current study, the systemic administration of dexamethasone (0.5 mg/kg) did not alter eosinophil numbers in the bone marrow of non-sensitised or sensitised non-challenged rats. In contrast, animals with allergic pleurisy reacted to the glucocorticoid systemic treatment with a 60% reduction in the number of bone marrow eosinophils 24 and 48 h post-challenge, suggesting that sensitivity to the steroid can be markedly improved following allergen provocation. Moreover, the impairment of bone marrow eosinophil availability correlated with the inhibition of blood and pleural eosinophilia. It must be emphasised that the blockade of pleural eosinophil accumulation was dose-dependent and of the same magnitude at 24 and 48 h post-challenge. The results are consistent with the interpretation that inhibition of bone marrow eosinophilopoiesis by glucocorticoids would be decisive for the impairment of blood and pleural eosinophil accumulation following intrapleural injection of allergen.

Sensitivity of local cell recruitment mechanisms to glucocorticoids has been well demonstrated (Oda and Katori, 1992; Mancuso et al., 1995). In the current work, although local administration of dexamethasone did not diminish the bone marrow or the circulating levels of eosinophils, it did abolish the pleural eosinophil accumulation observed 24 h post-challenge. Systemic and local treatment with dexamethasone also reduced pleural oedema, neutrophil and mononuclear cell recruitment, suggesting that some of these effects may also contribute to the subsequent blockade of the eosinophil accumulation noted within 24 h. Surprisingly, local treatment with dexamethasone failed to alter the eosinophil influx noted 48 h after ovalbumin, despite being able to shorten, thereafter, the duration of the eosinophilic response. Although the reason for this phenomenon is not completely clear, a plausible explanation is that the long-term pleural eosinophilia in response to allergen challenge results from an overlapping sequence of independent mechanisms. We postulate that there are two concurrent components of eosinophil recruitment only the first being sensitive to local dexamethasone injection, and a further sustaining component closely associated with eosinophil survival, also sensitive to local glucocorticoid administration. On the other hand, it is noteworthy that the 48-h eosinophilia was reduced about 50 and 80% following local injection of dexamethasone, when doses 2- and 4-fold as high as that required to abolish the 24-h eosinophilia were employed, respectively. Since these doses were also able to prevent circulating blood eosinophilia, it is tempting to speculate that the blockade noted within 48 h indeed results from a systemic effect of the glucocorticoid.

The hypothesis that the tissue eosinophilia triggered by allergen may be brought about by distinct components of eosinophil influx is supported by results reported by

Iwamoto et al. (1992). They demonstrated that s.c. injection of antigen to sensitised mice led to a biphasic eosinophilic infiltration, which reached a first peak within 6 h and a second one from 24 to 48 h post-challenge. While mast cells and PAF were shown to play a critical role in the first peak, CD^{4+} T cells and interleukin-5 were implicated in the late eosinophilic response. It is of interest to note that dexamethasone injected systemically did prevent the massive mast cell degranulation evoked by allergen challenge in the pleural cavity, as shown by toluidine blue dye analysis under light microscopy, suggesting that mast cell-stabilising properties may also be relevant in this context. We had reported that the local mast cell granule depletion significantly inhibited both pleural oedema noted within 4 h (Lima et al., 1996) and eosinophil accumulation observed 24 h post-challenge (Diaz et al., 1996). In contrast, as shown here, the number of eosinophils found 48 h post-challenge remained unchanged, supporting the interpretation that the eosinophil accumulation evoked by allergen stimulation in the current system can, indeed, be accounted for by distinct waves of influx of these cells.

In conclusion, the present data suggest that allergic pleurisy in sensitised rats is marked by two independent phases of eosinophil influx. Both phases are sensitive to systemic dexamethasone treatment, through a mechanism associated with impairment of eosinophil availability from bone marrow. The local administration of low doses of dexamethasone clearly impaired the early phase and shortened the time course of the eosinophilic response, probably by interfering exclusively with local mechanisms of eosinophil enrichment, including chemotaxis and survival.

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