British Journal of Pharmacology (1998) 123, 538–544

The role of lipocortin-1 in the inhibitory action of dexamethasone on eosinophil trafficking in cutaneous inflammatory reactions in the mouse

[1,3]Mauro M. Teixeira, [2]Anuk M. Das, [1,4]Jadwiga M. Miotla, [2]Mauro Perretti & [1,5]Paul G. Hellewell

[1]Applied Pharmacology, Imperial College School of Medicine at the National Heart and Lung Institute, Dovehouse Street, London SW3 6LY and [2]Department of Biochemical Pharmacology, The William Harvey Research Institute, Charterhouse Square, London EC1M 6BQ

1 The ability of glucocorticosteroids to inhibit tissue eosinophilia may be an important feature of their anti-inflammatory action in allergic diseases. Our previous work showed that an effect of dexamethasone on the release of eosinophils from the bone marrow could explain its inhibitory action on eosinophil accumulation in a mouse air-pouch model. Thus, it was unclear from that study whether dexamethasone could interfere with the process of eosinophil trafficking. In the present study, therefore, we used a newly developed mouse model to evaluate the effects of systemic treatment with dexamethasone on the recruitment of ^{111}In-labelled blood eosinophils to sites of cutaneous inflammation in the mouse and whether lipocortin-1 (LC-1) was involved.

2 The i.d. injection of ovalbumin (OVA) in sensitized mice induced a dose-dependent recruitment of ^{111}In-labelled blood eosinophils which peaked at 4 to 8 h after antigen challenge. Systemic treatment with dexamethasone (50 μg per mouse, 3 h after antigen) effectively inhibited ^{111}In-eosinophil recruitment in this reaction by 70 to 85%. Similarly, a 1 h pretreatment with dexamethasone significantly suppressed ^{111}In-eosinophil induced by platelet-activating factor (PAF), leukotriene B_4(LTB_4) and the chemokine macrophage inflammatory protein-1α (MIP-1α) by 40 to 70%.

3 Two experimental approaches were used to evaluate the role of LC-1: treatment with LC-1 fragment Ac2-26 and use of an anti-LC-1 antiserum. LC-1 fragment Ac2-26 (100 μg per mouse) failed to affect ^{111}In-eosinophil recruitment. Moreover, pretreatment of animals with an anti-LC-1 antiserum failed to reverse the inhibitory effects of dexamethasone on ^{111}In-eosinophil recruitment induced by MIP-1α and by antigen in sensitized mice.

4 In contrast, the LC-1 fragment significantly inhibited glycogen-induced neutrophil recruitment into the peritoneal cavity of mice. Furthermore, the anti-LC-1 antiserum reversed the inhibitory effects of dexamethasone on the glycogen-induced neutrophil recruitment.

5 Thus, our results suggest that dexamethasone can inhibit the recruitment of eosinophils in mouse skin independent of an action on the bone marrow. However, by use of two different approaches, we showed that LC-1 does not play a role in mediating the inhibitory action of dexamethasone on eosinophil migration into cutaneous inflammatory reactions in the mouse. These data add further support to a LC-1-independent action of dexamethasone on eosinophils *in vivo*.

Keywords: Eosinophils; dexamethasone; lipocortin-1; neutrophils; allergic reactions

Introduction

There is much evidence to support an important role for eosinophils in the pathophysiology of allergic diseases such as asthma and allergic dermatitis (Butterfield & Leiferman, 1993). In these diseases, activated eosinophils are thought to accumulate in the tissue where they secrete various inflammatory mediators and basic proteins leading to tissue damage and enhanced inflammation (Weller, 1991). Thus, the development of drugs which specifically inhibit the recruitment of eosinophils into tissue may be an important therapeutic development in the treatment of allergic diseases (Teixeira *et al*., 1995).

Glucocorticosteroids are drugs of choice in the treatment of allergic conditions (Barnes, 1995). However, these drugs may cause serious side-effects which hamper their use in some individuals. A useful strategy to generate new anti-allergic therapies may be the development of drugs which mimic the anti-inflammatory actions of glucocorticosteroids *in vivo* but with a safer pharmacological profile. The precise cellular targets for the anti-inflammatory effects of glucocorticosteroids *in vivo* are unknown but their striking ability to inhibit tissue eosinophilia may be an important feature of their inhibitory action (Teixeira *et al*., 1995). There are several possible mechanisms by which glucocorticosteroids inhibit tissue eosinophilia, including inhibition of the generation of chemoattractants and cytokines (Schleimer, 1990; Rolfe *et al*., 1992), direct inhibition of cell recruitment (Teixeira *et al*., 1996), inhibition of the expression of cell adhesion molecules on endothelial cells (Cronstein *et al*., 1992; Burke-Gaffney & Hellewell, 1996), inhibition of the release of eosinophils from the bone marrow (Das *et al*., 1997) and induction of apoptosis (Druilhe, *et al*., 1996).

More recently, there has been renewed interest in lipocortin-1 (LC-1), a glucocorticosteroid-inducible member of the annexin superfamily of proteins (Flower & Rothwell, 1994). It is now clear that LC-1 mediates part of the inhibitory effects of glucocorticosteroids on leucocyte recruitment in some inflammatory models *in vivo* (Flower & Rothwell, 1994). For example, in a mouse air-pouch model, systemic injection of LC-1 or LC-1 fragments effectively and dose-dependently

[3]Present address: Departmento de Farmacologia, Instituto de Ciencias Biologicas, Universidade Federal de Minas Gerais, Av. Antonio Carols 6627, Belo Horizonte, MG, Brazil 31270.901.
[4]Present address: Kennedy Institute of Rheumatology, 1 Aspenlea Road, London W6 8LH.
[5]Author for correspondence.

inhibited neutrophil migration in response to interleukin-8 (IL-8) and IL-1β (Perretti *et al*., 1993). More importantly, passive immunization of mice with an anti-LC-1 polyclonal antiserum abolished the inhibitory action of dexamethasone on neutrophil migration induced by IL-1β (Perretti & Flower, 1993). The effects of LC-1 on neutrophil recruitment *in vivo* appear to be related to the ability of this protein to inhibit neutrophil transmigration across endothelial cells (Mancuso *et al*., 1995). Despite substantial work suggesting the involvement of LC-1 in the inhibitory effects of glucocorticosteroids on neutrophil recruitment *in vivo*, much less is known about the role of this protein in modulating eosinophil recruitment.

We have previously shown that systemic treatment with the glucocorticosteroid dexamethasone effectively inhibited the recruitment of eosinophils into air-pouches of mice following sensitization and challenge with ovalbumin (OVA) (Das *et al*., 1997). In this model, the inhibitory effects of dexamethasone could be attributed almost entirely to a reduction in circulating eosinophils and retention of these cells in the bone marrow (Das *et al*., 1997). Thus, it was unclear whether dexamethasone could actually modulate the process of eosinophil trafficking. To differentiate an effect of dexamethasone on the bone marrow from a possible effect of the drug on the process of eosinophil trafficking, we evaluated whether systemic treatment with dexamethasone would also inhibit the recruitment of exogenously administered ^{111}In-labelled eosinophils induced by direct-acting chemoattractants and in an allergic reaction in mouse skin. This is a new model that we developed to study trafficking of blood eosinophils rather than eosinophils purified from peritoneal exudates as we have previously described (Teixeira *et al*., 1994; 1996). Moreover, since this is a mouse model, we were able to use tools, previously unavailable to the guinea-pig, to assess whether LC-1 is involved in any inhibitory effect of dexamethasone. Acute and delayed-onset eosinophil recruitment in mouse skin was induced by direct-acting eosinophil chemoattractants (leukotriene B_4 (LTB_4), platelet-activating factor (PAF) and macrophage inflammatory protein-1α (MIP-1α) and by antigen in actively-sensitized animals, respectively. The potential role of LC-1 was evaluated by use of the LC-1 fragment Ac2-26 and a sheep anti-LC-1 antiserum, as previously described (Perretti & Flower, 1993; Perretti *et al*., 1993).

Methods

Sensitization procedure

Animals were immunized with ovalbumin (OVA) adsorbed to alum as we have described previously (Das *et al*., 1997). Briefly, CBA/Ca mice (18–20 g, Harlan, Bicester) were injected s.c. on days 1 and 8 with 0.2 ml of a solution containing 100 μg of OVA and 70 μg of aluminium hydroxide gel (Reheiss, Dublin, Ireland). Seven to eight days after the last immunization, the animals were anaesthetized, shaved and antigen (OVA 0.1 to 1.0 μg per site) injected i.d. This allergic reaction in mouse skin will be referred to as an active cutaneous anaphylactic (ACA) reaction.

Purification and radiolabelling of murine eosinophils

Eosinophils were purified from the blood of CBA/Ca mice over-expressing the IL-5 gene. In our transgenic mouse colony, eosinophils accounted for approximately 60% of circulating blood leucocytes (data not shown). Blood was obtained by cardiac puncture (3 to 4 donor mice per experiment) and red blood cells sedimented with Dextran T500 (1 part of blood to 4 parts of 1.25% Dextran). The leucocyte-rich supernatant was removed, centrifuged (300 *g*, 7 min) and layered onto a discontinuous 4 layer Percoll (Pharmacia) gradient (densities: 1.070, 1.075, 1.080, 1.085 g ml^{-1}). The gradients were centrifuged at 1,500 *g* for 25 min at 20°C and eosinophils and lymphocytes collected from the 1.080/1.085 interface. Lymphocytes were removed by using negative immunoselection with rat anti-mouse CD2 and B220 mAbs on a MACS BS column according to guidelines set by the manufacturers (Miltenyi Biotec Ltd, Surrey). Briefly, the eosinophil and lymphocyte pellet was resuspended in PBS/BSA (1×10^7 cells in 500 μl) and incubated with 10 μg ml^{-1} of anti-CD2 and 7.5 μg ml^{-1} of anti-B220 for 20 min on ice. The cells were washed and resuspended in PBS/BSA (80 μl of PBS/BSA per 1×10^7 cells). Twenty microlitres of goat anti-rat IgG microbeads per 1×10^7 cells were added and the cells incubated for a further 20 min at 6–8°C. The cell suspension was run through an immunomagnetic selection column and the eosinophils were collected with the column effluent. The eosinophils purified this way were over 95% pure, as determined by stained (Hema-Gurr, BDH) cytospin preparations, and greater than 98% viable, as assessed by trypan blue exclusion.

For the *in vivo* experiments, eosinophils were radiolabelled as previously described for guinea-pig cells (Teixeira *et al*., 1994). Briefly, purified mouse eosinophils were incubated with ^{111}In($\sim$ 100 μCi in 10 μl) chelated to 2-mercaptopyridine-N-oxine (40 μg in 0.1 ml of 50 mM PBS, pH 7.4) for 15 min at room temperature. Cells were then washed twice in PBS/BSA and finally resuspended at a final concentration of 1×10^7 ^{111}In-eosinophils ml^{-1}.

Measurement of eosinophil recruitment in mouse skin

Ten minutes after the i.v. injection of 1×10^6 ^{111}In-eosinophils, each animal received i.d. injections (up to 6 per mouse, each of 50 μl) of MIP-1α (10 pmol per site), PAF (50 pmol per site) or LTB_4 (150 pmol per site). The recruitment of ^{111}In-eosinophils was allowed to occur over a period of 4 h after which the animals were killed and the number of ^{111}In-eosinophils per skin site quantified on a γ-counter (Canberra Packard, Berks). For the experiments done to assess ^{111}In-eosinophil recruitment in ACA reactions, animals were injected i.d. with antigen (in 50 μl) 4 h before the i.v. injection of ^{111}In-eosinophils and ^{111}In-eosinophil recruitment was assessed over a further period of 4 h. Thus, in most experiments, ^{111}In-eosinophil recruitment in the ACA reaction was measured from 4 to 8 h after the i.d. injection of antigen. At the end of the 4 h measurement period, blood was obtained by cardiac puncture and the number of circulating ^{111}In-eosinophils calculated.

For time-course experiments, sensitized animals were challenged with antigen 20 h, 4 h and immediately (time 0) before the i.v. injection of radiolabelled cells and ^{111}In-eosinophil recruitment was assessed over a period of 4 h. Thus, the following measurement periods were considered in the time course: 0 to 4 h, 4 to 8 h and 20 to 24 h.

Treatment with dexamethasone, LC-1 analogue and anti-LC-1 antiserum

Dexamethasone (5 to 50 μg per mouse in 0.1 ml saline) was administered s.c. 1 h before to the i.v. administration of ^{111}In-eosinophils (Figure 1). Thus, in the ACA reaction, dexamethasone was given 3 h after antigen challenge of skin sites. This is in contrast with our previous studies where dexamethasone was given 1 h before antigen challenge (Das *et al*., 1997). The

LC-1 fragment Ac2- 26 (200 μg per mouse in 0.1 ml saline) was administered i.v. 30 min before the i.v. administration of ^{111}In-eosinophils (Figure 1). The treatment schedule with the LC-1 analogue has been previously shown to inhibit neutrophil recruitment in the mouse (Perretti *et al*., 1993). The anti-LC-1 antiserum (named LCS-3, 50 μl per mouse) or control, non-immune sheep serum (NSS, 50 μl per mouse) were administered s.c. 24 h before the administration of dexamethasone. This dose and pretreatment time of antiserum was chosen based on the ability of the antiserum to reverse the inhibitory effects of dexamethasone on neutrophil recruitment in the mouse (Perretti & Flower, 1993).

Induction of peritonitis

Naive CBA/Ca mice received an i.p. injection of glycogen (0.1%, 2 ml per mouse) and the number of neutrophils migrating into the peritoneal cavity assessed after 4 h. Total leucocytes were assessed with Kimura's stain on a Neubauer chamber and differential counts on a cytospin slide stained with DiffQuik (BDH, Dorset). Dexamethasone (50 μg per mouse) was administered s.c. 1 h before the i.p. administration of glycogen. The LC-1 analogue Ac2 – 26 was administered s.c. as a 30 min pretreatment and LCS-3 or NSS were administered s.c. 24 h before the administration of dexamethasone, as described above.

Reagents

The following compounds were purchased from Sigma Chemical Company (Poole, Dorset): glycogen, bovine serum albumin, ovalbumin, zymosan, 2-mercaptopyridine-N-oxine. Hank's and PBS solutions and HEPES buffer were purchased from Life Technologies Ltd (Paisley, Scotland). Percoll was purchased from Pharmacia (Milton Keynes, Bucks) and PAF (C16) from Bachem (Saffron Walden, Essex). $^{111}InCl_3$ was obtained from Amersham International plc (Amersham, Bucks) and dexamethasone sulphate from David Bull Laboratories (Warwick). MIP-1α was from Cambridge Biosciences Ltd (Cambridge).

Statistical analysis

All results are presented as the mean $\pm$ s.e.mean. Normalized data were analysed by one way ANOVA and differences between groups assessed by use of Student-Newman-Keuls *post*-test. A P value < 0.05 was considered significant. % inhibition was calculated by subtracting background values obtained in response to i.d. injection of PBS.

Results

Effect of dexamethasone on ^{111}In-eosinophil recruitment in mouse skin

The effects of dexamethasone on mediator-induced ^{111}In-eosinophil recruitment is shown in Figure 2. Dexamethasone suppressed ^{111}In-eosinophil recruitment induced by PAF, LTB_4 and MIP-1α by 62%, 42% and 68%, respectively. In contrast to the effects on the recruitment of ^{111}In-eosinophils, dexamethasone had no effect on the numbers of circulating ^{111}In-eosinophils (data not shown).

The i.d. injection of OVA in actively sensitized animals induced a dose- and time-dependent ^{111}In-eosinophil recruitment that peaked at 4 to 8 h after antigen challenge (Figure 3). Pretreatment of animals with a dose of dexamethasone (50 μg per mouse, s.c., 1 h pretreatment) previously shown to induce maximal inhibition of eosinophil recruitment into the mouse air pouch (Das *et al*., 1997), effectively inhibited ^{111}In-eosinophil recruitment induced by 0.1, 0.3 and 1.0 μg of OVA by 71%, 73% and 79%, respectively (Figure 3a). Similarly, dexamethasone inhibited by up to 84% acute (0 to 4 h) and delayed-onset (4 to 8 h and 20 to 24 h) ^{111}In-eosinophil recruitment induced by antigen in actively sensitized animals (Figure 3b). Because MIP-1α was more effectively inhibited by dexamethasone and maximal ^{111}In-eosinophil recruitment and inhibition by dexamethasone occurred at 4 to 8 h in the ACA reaction, these inflammatory stimuli were used in all further experiments.

Effect of the LC-1 fragment Ac2-26 on ^{111}In-eosinophil recruitment in mouse skin

We have previously shown that the systemic administration of LC-1 or LC-1 fragments inhibits the recruitment of neutrophils into air-pouches in response to IL-1β or IL-8 (Perretti *et al*., 1993). In the present series of experiments, we evaluated the effects of the LC-1 fragment Ac2-26 on ^{111}In-eosinophil recruitment induced by MIP-1α and in the ACA reaction with a dose of the peptide fragment shown to inhibit maximally

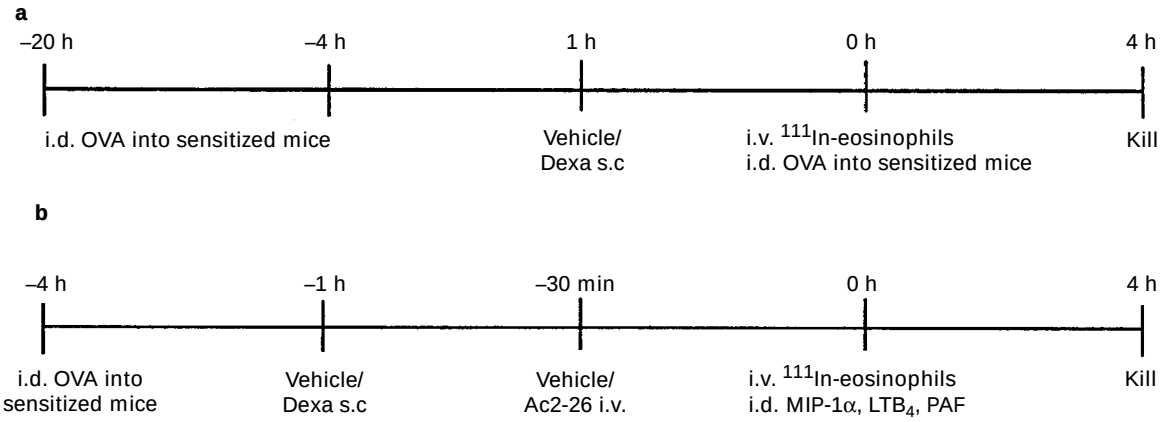


Figure. 1 Protocols used to test (a) the effect of dexamethasone on the kinetics of ^{111}In-eosinophil trafficking in the ACA reaction and (b) the effect of dexamethasone or Ac2-26 on the trafficking or ^{111}In-eosinophils into skin sites which received injections of MIP-1α, LTB_4 or PAF. Dexamethasone (Dexa, 50 μg per mouse), Ac2-6 (200 μg per mouse) or vehicle (100 μl per mouse) were administered at the times and by the routes indicated.

neutrophil recruitment (200 μg per mouse i.v.). In contrast to its marked effects on neutrophil recruitment, Ac2-26 failed to affect ^{111}In-eosinophil recruitment induced by the chemoattractant MIP-1α or in the ACA reaction (Figure 4), or in response to PAF (control, 3623±395; Ac2-26, 4128±178 ^{111}In-eosinophils per site).

Effect of the anti-LC-1 antiserum LCS-3 on the inhibitory effects of dexamethasone

In order to find the least effective inhibitory concentration of dexamethasone to test against LCS-3, dose-dependent inhibition curves of dexamethasone on ^{111}In-eosinophil recruitment induced by MIP-1α and in ACA reactions were constructed. As seen in Figure 5, dexamethasone failed to inhibit ^{111}In-eosinophil recruitment when used at 5 μg per mouse and, at 15 μg per mouse, it only inhibited ^{111}In-eosinophil recruitment in the ACA reaction induced by 0.1 μg of OVA. Thus, because dexamethasone consistently inhibited ^{111}In-eosinophil recruitment only when used at 50 μg per mouse, this dose was chosen to be tested against LCS-3.

When compared to animals which received non-immune sheep serum, LCS-3 failed to reverse significantly the inhibitory effects of dexamethasone on ^{111}In-eosinophil recruitment induced by MIP-1α and in the ACA reaction (Figure 6). Thus, in these experiments, MIP-1α-induced ^{111}In-eosinophil recruitment was inhibited by 73% and 71% in dexamethasone-treated-mice which received NSS or LCS-3, respectively. Similarly, ^{111}In-eosinophil recruitment in an ACA reaction was inhibited by up to 62% and 66% in NSS- and LCS-3-treated mice, respectively (Figure 6).

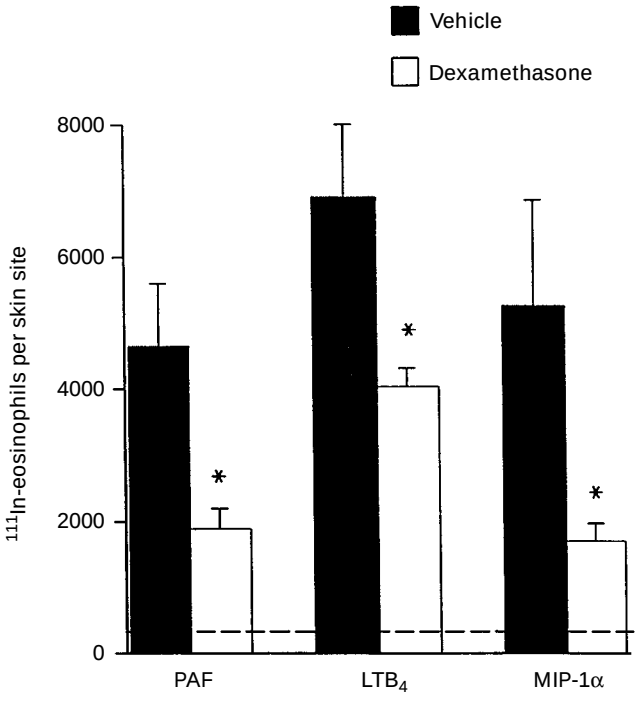
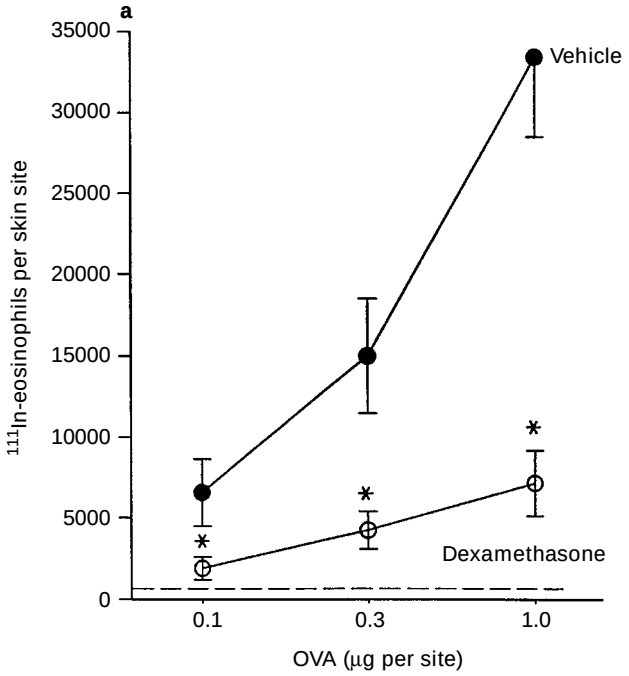


Figure. 2 Effect of dexamethasone on ^{111}In-eosinophil recruitment induced by PAF, LTB_4 and MIP-1α. Dexamethasone (50 μg per mouse) or vehicle (100 μl per mouse) were administered s.c. 1 h before the i.v. injection of radiolabelled eosinophils. PAF (50 pmol per site), LTB_4 (150 pmol per site) and MIP-1α (10 pmol per site) were administered i.d. and ^{111}In-eosinophil recruitment assessed over a period of 4 h. The line across the graph represents background values in response to i.d. injection of PBS. Results are mean±s.e. mean of 4 to 7 animals in each group and * represents $P<0.01$ when compared to control groups.

Effects of the LC-1 fragment Ac2-26 and the anti-LC-1 antiserum LCS-3 on neutrophil recruitment

In order to validate the doses and treatment times of the LC-1 fragment and the anti-LC-1 antiserum, we assessed the effects



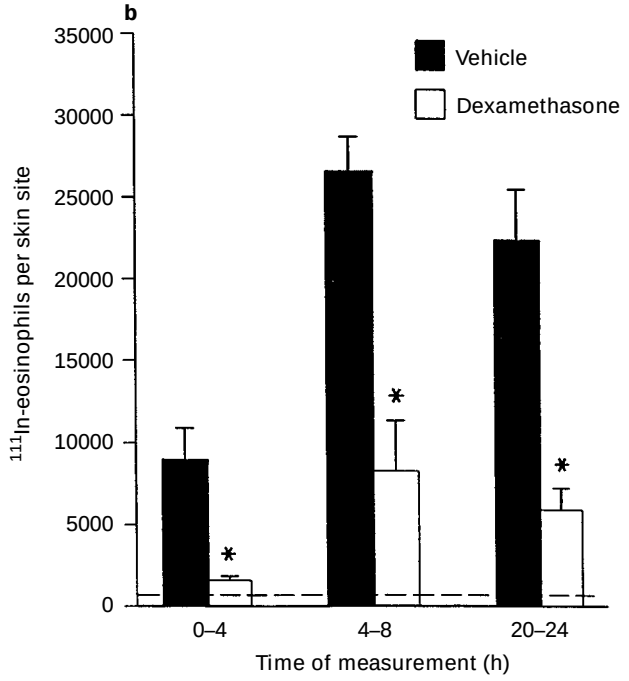


Figure. 3 Effect of dexamethasone on ^{111}In-eosinophil recruitment in an active cutaneous anaphylactic (ACA) reaction. Dexamethasone (50 μg per mouse) or vehicle (100 μl per mouse) were administered s.c. 1 h before the i.v. injection of radiolabelled eosinophils. In (a) increasing concentrations of ovalbumin (OVA, 0.1 to 1.0 μg per site) were given i.d. 4 h before the cells and ^{111}In-eosinophil recruitment assessed over a period of 4 h; in (b) OVA (1.0 μg per site) was given i.d. just before (time 0), 4 h and 20 h before the radiolabelled cells and ^{111}In-eosinophil recruitment assessed over a period of 4 h. The lines across the graphs represent background values in response to i.d. injection of PBS. Results are mean±s.e.mean (vertical lines) of 4 animals in each group and * represents $P<0.01$ when compared to control groups.

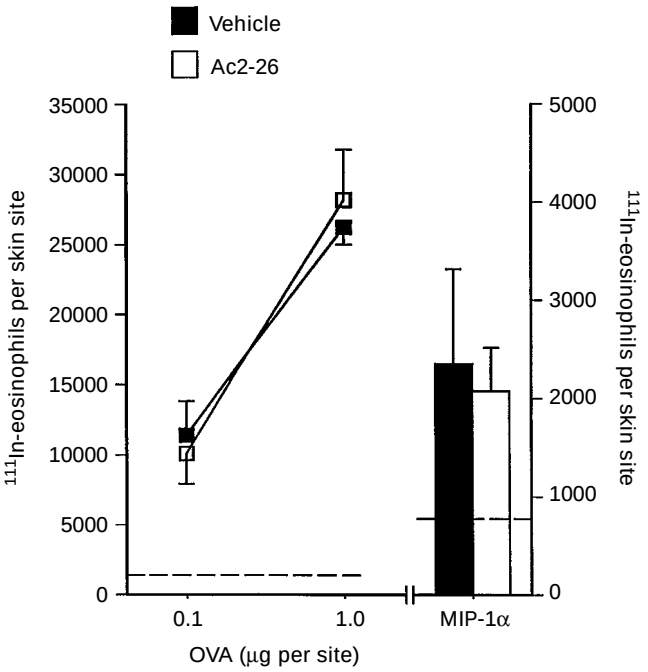


Figure. 4 Effect of the LC-1 fragment Ac2-26 on ^{111}In-eosinophil recruitment induced by MIP-1α and in an active cutaneous anaphylactic (ACA) reaction. The LC-1 fragment Ac2-26 (200 μg per mouse) or vehicle (100 μl per mouse) were administered s.c. 30 min before the i.v. injection of radiolabelled eosinophils. Ovalbumin (OVA, 0.1 and 1.0 μg per site) was injected i.d. 4 h before the cells, MIP-1α (10 pmol per site) was injected i.d. 10 min after the cells and ^{111}In-eosinophil recruitment assessed over a period of 4 h. The lines across the graph represent background values in response to i.d. injection of PBS. Results are mean ± s.e.mean (vertical lines) of 5 animals in each group.

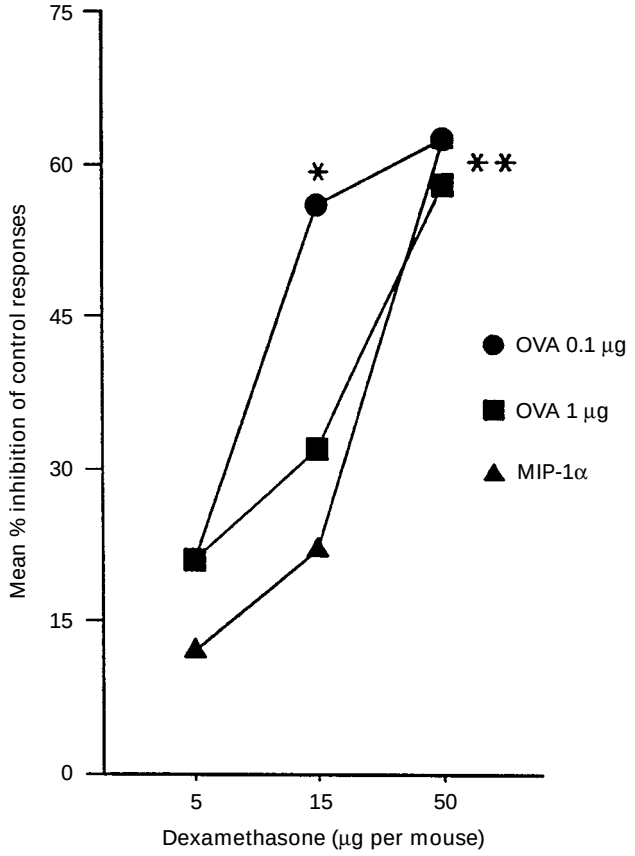


Figure. 5 Dose-dependent inhibitory effects of dexamethasone on ^{111}In-eosinophil recruitment induced by MIP-1α and in an active cutaneous anaphylactic (ACA) reaction. Dexamethasone (5 to 50 μg per mouse) or vehicle (100 μl per mouse) were administered s.c. 1 h before the i.v. injection of radiolabelled eosinophils. Ovalbumin (OVA, 0.1 and 1.0 μg per site) was injected i.d. 4 h before the cells, MIP-1α (10 pmol per site) was injected i.d. 10 min after the cells and ^{111}In-eosinophil recruitment assessed over a period of 4 h. Results are shown as the mean percentage inhibition of control responses. Error bars were omitted for clarity but there was less than 15% variation of the mean. There were 4 to 5 animals in each group and * and ** represent $P<0.05$ and $P<0.01$, respectively, when compared to vehicle-treated groups.

of the same batches of these pharmacological tools on the recruitment of neutrophils into the peritoneal cavity of glycogen-treated mice. As seen in Table 1, pretreatment of animals with dexamethasone (50 μg per mouse) 1 h before the i.p. injection of glycogen virtually abolished the recruitment of neutrophils into the peritoneal cavity. Similarly, 30 min pretreatment with Ac2-26 (200 μg, s.c.) inhibited neutrophil recruitment by 80% (Table 1). Moreover, pretreatment with LCS-3, but not NSS, reversed the inhibitory effects of dexamethasone on neutrophil recruitment induced by glycogen (Table 1).

Discussion

In our previous work, we failed to observe a role for LC-1 in mediating the inhibitory actions of dexamethasone on the accumulation of eosinophils in an air pouch model of allergic inflammation in the mouse (Das *et al*., 1997). It was thus important to test the role of LC-1 in a more simple model of eosinophil recruitment before the hypothesis that this mediator is involved in the emigration process of eosinophils is discarded. Moreover, the inhibitory effects of dexamethasone on eosinophil accumulation in the air-pouch model could be attributed entirely to an effect on bone marrow release and the circulating levels of eosinophils (Das *et al*., 1997). Thus, an effect of dexamethasone on the process of trafficking of blood eosinophils to sites of inflammation had not been tested before.

Using a new method in which eosinophils were purified from the blood of transgenic mice over-expressing the IL-5 gene, we assessed the recruitment of these cells in response to the direct acting chemoattractants PAF, LTB_4 and MIP-1α and in sites of allergic inflammation in OVA sensitized animals. Our findings can be summarized as follows: (1) systemic treatment with dexamethasone effectively and dose-dependently inhibited the acute (mediator-induced) and delayed-onset (4 to 8 h and 20 to 24 h ACA reaction) recruitment of ^{111}In-eosinophils in mouse skin; (2) systemic treatment with the LC-1 fragment Ac2-26 had no effect on the recruitment of ^{111}In-eosinophils; (3) pretreatment of mice with an anti-lipocortin antiserum, LCS-3, failed to modify the inhibitory actions of dexamethasone on ^{111}In-eosinophil recruitment.

In the guinea-pig, systemic treatment with dexamethasone inhibited the recruitment of ^{111}In-eosinophils induced by PAF, C5a, lipopolysaccharide and in a passive cutaneous anaphylactic reaction (Teixeira *et al*., 1996). ^{111}In-eosinophil recruitment was only inhibited when dexamethasone was given as a 150 min pretreatment, suggesting a role for newly synthesized proteins in the inhibitory actions of the drug, although due to lack of tools it was not possible to assess the role of LC-1 (Teixeira *et al*., 1996). In the present study, we used a dose of dexamethasone previously shown to inhibit maximally the recruitment of eosinophils in air-pouches of antigen-challenged mice (Das *et al*., 1997). Dexamethasone inhibited not only

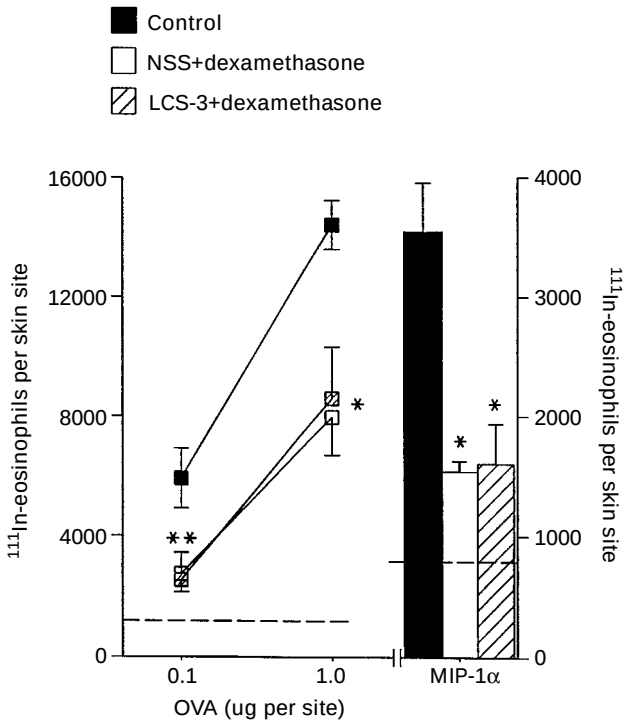


Figure. 6 Effect of the anti-LC-1 antiserum LCS-3 on the inhibitory effects of dexamethasone on ^{111}In-eosinophil recruitment induced by MIP-1α and in an active cutaneous anaphylactic (ACA) reaction. Mice were pretreated with normal sheep serum (NSS, 50 μl) or anti-LC-1 antiserum (LCS-3, 50 μl) 24 h before the s.c. administration of dexamethasone (50 μg per mouse, s.c., 1 h pretreatment). Control animals received vehicle (100 μl per mouse) 1 h before the i.v. administration of radiolabelled cells. Ovalbumin (OVA, 0.1 and 1.0 μg per site) was injected i.d. 4 h before the cells, MIP-1α (10 pmol per site) was injected i.d. 10 min after the cells and ^{111}In-eosinophil recruitment assessed over a period of 4 h. Results are mean$\pm$s.e. mean (vertical lines) of 5 animals in each group and * and ** represent $P<0.05$ and $P<0.01$, respectively, when compared to the control group.

Table 1 Effects of dexamethasone, the LC-1 fragment Ac2-26 and the anti-LC-1 antiserum LCS-3 on glycogen-induced neutrophil recruitment into the mouse peritoneal cavity

Treatment	*Neutrophils per cavity* ($\times 10^5$)
Glycogen 0.1%	9.2$\pm$2.6
+ dexamethasone + NSS	0.6$\pm$0.2**
+ dexamethasone + LCS-3	9.9$\pm$5.4
+ LC-1 fragment Ac2-26	1.8$\pm$0.7*

Mice were treated with glycogen and neutrophil recruitment into the peritoneal cavity assessed after 4 h. Dexamethasone (50 μg) was administered s.c. 1 h before glycogen. Normal sheep serum (NSS, 50 μl) or anti-LC-1 antiserum (LCS-3, 50 μl) were administered s.c. 24 h before dexamathesone. The LC-1 fragment Ac2-26 (200 μg) was administered s.c. 30 min before glycogen. Results are mean$\pm$s.e.mean of 4 animals in each group and * and ** represent $P<0.05$ and $P<0.01$, respectively, when compared to the glycogen only group.

^{111}In-eosinophil recruitment induced by the lipid mediators PAF and LTB_4 but also ^{111}In-eosinophil recruitment induced by the eosinophil-active chemokine MIP-1α (Rot *et al*., 1992; Kita & Gleich, 1996). In an ACA reaction in mouse skin, there was early accumulation of ^{111}In-eosinophils during the first 4 h after antigen challenge, recruitment peaked at 4 to 8 h but there was still significant migration of cells from 20 to 24 h following antigen challenge. Dexamethasone, when administered 1 h before the 4 h measurement period, markedly inhibited both the acute (0 to 4 h) and delayed-onset (4 to 8 h and 20 to 24 h) ^{111}In-eosinophil recruitment in this ACA reaction, even when administered hours after antigen challenge. Thus, in addition to the well documented action of dexamethasone on the release of mediators from mast cells and other leucocytes (Schleimer, 1990), our results suggest an action of dexamethasone on the chemoattractant effects of inflammatory mediators, including chemokines, which were released or injected locally. Moreover, because we measured the recruitment of exogenously administered ^{111}In-eosinophils and dexamethasone had no significant effect on the numbers of circulating ^{111}In-eosinophils (data not shown), an action of the drug on the release of eosinophils from the bone marrow cannot explain any inhibition observed (Das *et al*., 1997).

Because ^{111}In-eosinophil recruitment induced by MIP-1α and in an ACA reaction was more effectively inhibited by dexamethasone, we chose to use these inflammatory stimuli to investigate the role of LC-1 in mediating the inhibitory actions of the glucocorticosteroid. Two experimental approaches were used: systemic administration of the LC-1 fragment Ac2-26 (Perretti *et al*., 1993) and administration of the anti-LC-1 antiserum LCS-3 before dexamethasone (Perretti & Flower, 1993). At the dose used (200 μg per mouse), the LC-1 fragment Ac2-26 had comparable efficacy to an anti-CD11b monoclonal antibody at inhibiting neutrophil-dependent oedema formation and the recruitment of neutrophils in the mouse (Perretti *et al*., 1993). Similarly, we observed a 80% inhibition of neutrophils recruited to the peritoneal cavity of glycogen-treated mice. In contrast, Ac2-26 failed to modify ^{111}In-eosinophil recruitment in the mouse skin. LCS-3 was unable to affect the inhibitory effects of dexamethasone on ^{111}In-eosinophil recruitment in mouse skin. However, when used against a model of glycogen-induced peritonitis, LCS-3 reversed the inhibitory effects of dexamethasone, suggesting that the batch of antiserum and experimental conditions used were adequate for complete inhibition of LC-1. Thus, our results provide strong evidence against a role for LC-1 in mediating the inhibitory actions of dexamethasone on eosinophil migration in mouse skin. Interestingly, we have previously shown that eosinophils obtained from the blood of IL-5 transgenic mice express similar amounts of LC-1 before and after treatment with dexamethasone (Das *et al*., 1997). Thus, in contrast to blood neutrophils (Perretti *et al*., 1996), blood eosinophils appear not to upregulate the intracellular levels of LC-1 following glucocorticosteroid treatment. However, eosinophils are tissue-dwelling cells (Weller, 1991) and we have recently shown that eosinophils which had migrated to the pleural cavity of IL-5 transgenic mice contained greater levels of LC-1 than blood eosinophils (Das *et al*., 1997). Thus, it is possible that LC-1 may play a role in the modulation of eosinophil activation following their migration into the tissue and this deserves further investigation.

In conclusion, this study provides strong evidence to suggest that dexamethasone does interfere with the process of eosinophil trafficking in addition to its previously demonstrated effects on the bone marrow. However, our data show, by use of two pharmacological approaches, that LC-1 does not play a role in mediating the inhibitory action of dexamethasone on eosinophil migration into cutaneous inflammatory reactions in the mouse.

References

BARNES, P.J. (1995). Inhaled glucocorticoids for asthma. *New Engl.J.Med.,* **332,** 868–875.

BURKE-GAFFNEY, A. & HELLEWELL, P.G. (1996). Regulation of ICAM-1 by dexamethasone in a human vascular endothelial cell line EAhy926. *Am.J.Physiol.,* **270,** C552–C561.

BUTTERFIELD, J.H. & LEIFERMAN, K.M. (1993). Eosinophil-associated diseases. In *Immunopharmacology of Eosinophils*. ed. Smith, H. & Cook, R.M. pp.152–192. London: Academic Press.

CRONSTEIN, B.N., KIMMEL, S.C., LEVIN, R.I., MARTINIUK, F. & WEISSMANN, G. (1992). A mechanism for the anti-inflammatory effects of corticosteroids: the glucocorticoid receptor regulates leukocyte adhesion to endothelial cells and expression of endothelial-leukocyte adhesion molecule 1 and intercellular adhesion molecule 1. *Proc.Natl.Acad.Sci.U.S.A.,* **89,** 9991–9995.

DAS, A.M., FLOWER, R.J., HELLEWELL, P.G., TEIXEIRA, M.M. & PERRETTI, M. (1997). A novel murine model of allergic inflammation to study the effect of dexamethasone on eosinophil recruitment. *Br.J. Pharmacol.,* **121,** 97–104.

DRUILHE, A., CAI, Z., HAILE, S., CHOUAIB, S. & PRETOLANI, M. (1996). Fas-mediated apoptosis in cultured human eosinophils. *Blood,* **87,** 2822–2830.

FLOWER, R.J. & ROTHWELL, N.J. (1994). Lipocortin-1: cellular mechanisms and clinical relevance. *Trends Pharmacol. Sci.* **15,** 71–76.

KITA, H. & GLEICH, G.J. (1996). Chemokines active on eosinophils: Potential roles in allergic inflammation. *J. Exp. Med.,* **183,** 2421–2426.

MANCUSO, F., FLOWER, R.J. & PERRETTI, M. (1995). Leukocyte transmigration, but not rolling or adhesion, is selectively inhibited by dexamethasone in the hamster post-capillary venule. *J. Immunol.,* **155,** 377–386.

PERRETTI, M., AHLUWALIA, A., HARRIS, J.G., GOULDING, N.J. & FLOWER, R.J. (1993). Lipocortin-1 fragments inhibit neutrophil accumulation and neutrophil-dependent oedema in the mouse. A qualitative comparison with an anti-CD11b monoclonal antibody. *J. Immunol.,* **151,** 4306–4314.

PERRETTI, M., CROXTALL, J.D., WHELLER, S.K., GOULDING, N.J., HANNON, R. & FLOWER, R.J. (1996). Mobilization of lipocortin 1 in adherent human leukocytes negatively modulates their transmigration. *Nature Med.,* **2,** 1259–1262.

PERRETTI, M. & FLOWER, R.J. (1993). Modulation of IL-1-induced neutrophil migration by dexamethasone and lipocortin 1. *J.Immunol.,* **150,** 992–999.

ROLFE, F.G., HUGHES, J.M., ARMOUR, C.L. & SEWELL, W.A. (1992). Inhibition of interleukin-5 gene expression by dexamethasone. *Immunology,* **77,** 494–499.

ROT, A., KRIEGER, M., BRUNNER, T., BISCHOFF, S.C., SCHALL, T.J. & DAHINDEN, C.A. (1992). RANTES and macrophage inflammatory protein 1α induce the migration and activation of normal human eosinophil granulocytes. *J. Exp.Med.,* **176,** 1489–1495.

SCHLEIMER, R.P. (1990). Effects of glucocorticosteroids on inflammatory cells relevant to their therapeutic applications in asthma. *Am.Rev.Respir.Dis.,* **141,** S59–S69.

TEIXERIA, M.M., ROSSI, A.G., WILLIAMS, T.J. & HELLEWELL, P.G. (1994). Effects of phosphodiesterase isoenzyme inhibitors on cutaneous inflammation in the guinea-pig. *Br.J.Pharmacol.,* **112,** 332–340.

TEIXEIRA, M.M., WILLIAMS, T.J. & HELLEWELL, P.G. (1995). Mechanisms and pharmacological manipulation of eosinophil accumulation in vivo. *Trends Pharmacol.Sci.,* **16,** 418–423.

TEIXEIRA, M.M., WILLIAMS, T.J. & HELLEWELL, P.G. (1996). Effects of dexamethasone and cyclosporin A on the accumulation of eosinophils in acute cutaneous inflammation in the guinea pig. *Br.J.Pharmacol.,* **118,** 317–324.

WELLER, P.F. (1991). The immunobiology of eosinophils. *New Engl.J.Med.,* **324,** 1110–1118.

(Received June 9, 1997
Revised October 17, 1997
Accepted October 21, 1997)