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Effects of dexamethasone and cyclosporin A on the accumulation of eosinophils in acute cutaneous inflammation in the guinea-pig

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1 Eosinophils are thought to play an important role in the pathophysiology of allergic diseases and pharmacological suppression of their recruitment is considered to be of therapeutic benefit. In the present study we have assessed and compared the effects of treatment with dexamethasone and cyclosporin A on the accumulation of ^{111}In-labelled eosinophils and local oedema formation in sites of acute inflammation in guinea-pig skin.

2 When injected locally 150 min prior to i.d. administration of antigen in a passive cutaneous anaphylactic (PCA) reaction, dexamethasone (10^{-9} to 3×10^{-7} mol per site) dose-dependently inhibited oedema formation by up to 50%. Similarly, oedema formation induced by PAF and lipopolysaccharide (LPS), but not by zymosan-activated plasma (ZAP), was significantly inhibited by dexamethasone. In contrast, ^{111}In-eosinophil accumulation measured in response to i.d. injection of PAF, LPS and ZAP or in the PCA reaction was not altered.

3 Systemic treatment with dexamethasone (4 mg kg^{-1}, i.v., 150 min pretreatment period) inhibited both oedema formation and ^{111}In-eosinophil accumulation induced by PAF, ZAP, LPS and in the PCA reaction.

4 The effects of i.d. injection of cyclohexamide (2×10^{-7} mol per site) on ^{111}In-eosinophil accumulation and oedema formation induced by PAF, ZAP or in a PCA reaction were evaluated in order to assess the dependency of these responses on protein synthesis. Cycloheximide had no effect on the responses measured. In contrast, ^{111}In-eosinophil accumulation, but oedema formation, induced by LPS was inhibited by 30%.

5 Acute (10 mg kg^{-1}, i.v., 15 min pretreatment) or prolonged (10 mg kg^{-1}, s.c. daily for 3 days) systemic treatment with cyclosporin A had no effect on ^{111}In-eosinophil accumulation or oedema formation induced by PAF, ZAP, LPS or in the PCA reaction.

6 In conclusion, we demonstrate preferential inhibitory effects of dexamethasone on ^{111}In-eosinophil accumulation according to its site of administration. In addition we show that dexamethasone inhibits protein synthesis-independent acute inflammation in guinea-pig skin. Finally, our results do not support the concept that eosinophils are an important cellular site of action for the inhibitory effects of cyclosporin A in a guinea-pig model of allergic inflammation.

Keywords: Dexamethasone; glucocorticosteroids; eosinophil; cyclosporin A; oedema formation; allergic inflammation

Introduction

Eosinophils are thought to play an important role in the pathophysiology of various allergic diseases, such as asthma and atopic dermatitis (Weller, 1991; Butterfield & Leiferman, 1993). In these diseases, eosinophils accumulate in tissue where they can secrete basic proteins, oxygen radicals, lipids and cytokines which can cause damage to local cells (e.g. epithelial cells) (Weller, 1991). Thus, the pharmacological suppression of eosinophil recruitment is considered to be a valuable approach to the treatment of allergic conditions.

In guinea-pig skin, radiolabelled eosinophils rapidly accumulate in response to locally-injected mediators or in local allergic reactions (Faccioli *et al.*, 1991; Teixeira *et al.*, 1993a; Weg *et al.*, 1994). For example, after the intradermal injection of PAF and leukotriene B_4 (LTB_4), over 90% of the total accumulating radiolabelled eosinophils are recruited over the first 90 min (Weg, 1993). A similar rapid accumulation of ^{111}In-eosinophils is observed in a passive cutaneous anaphylactic (PCA) reaction (Weg *et al.*, 1994) where the release of a 5-lipoxygenase metabolite is responsible for the influx of cells (Teixeira & Hellewell, 1994). This rapid influx of eosinophils into the tissue is consistent with a direct action of the inflammatory mediators, possibly on the eosinophils themselves, causing their subsequent migration into the tissue.

Glucocorticosteroids are anti-inflammatory agents which are used in the treatment of allergic diseases (Barnes, 1995). The precise mechanism and cellular target for the action of glucocorticosteroids is not known but several actions (e.g. inhibiting eosinophil activation and accumulation in tissues) may account for their efficacy in these conditions (Schleimer, 1990; Barnes & Adcock, 1993). For example, glucocorticosteroids *in vitro* inhibit the production of various cytokines (e.g. interleukin-5) which are important for eosinophil function (Schleimer, 1990; Rolfe *et al.*, 1992). Similarly, various *in vivo* studies have demonstrated the efficacy of glucocorticosteroids in inhibiting eosinophil migration after allergen challenge of sensitized animals (e.g. Andersson *et al.*, 1988; Sanjar *et al.*, 1990; Holbrook *et al.*, 1994; Matsumoto *et al.*, 1994). However, these *in vivo* studies have in common the long time course required for eosinophil accumulation after challenge (usually occurring over 24 h) consistent with the generation and action of new mediators. The ability of glucocorticosteroids to induce or inhibit *de novo* protein synthesis may account for, or contribute to, their efficacy in such *in vivo* systems (Schleimer, 1990; Barnes & Adcock, 1993).

Like glucocorticosteroids, cyclosporin A is effective in the

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treatment of various allergic diseases (Faulds *et al.*, 1993). Cyclosporin A has also been shown to inhibit eosinophil accumulation *in vivo* in animal models of pulmonary allergic inflammation (e.g. Fukuda *et al.*, 1991; Elwood *et al.*, 1992; Lagente *et al.*, 1994). The main mechanism of the anti-inflammatory actions of cyclosporin A is thought to be the inhibition of gene induction for pro-inflammatory cytokines in T cells (Erlanger, 1992; Schreiber & Crabtree, 1992; Chapman & Mazzoni, 1994). However, cyclosporin A also inhibits basophil and mast cell function *in vitro* (Cirillo *et al.*, 1990; Stellato *et al.*, 1992) and, consistent with this, it was recently shown to inhibit tissue swelling and leukocyte accumulation in a PCA reaction in mouse skin (Wershil *et al.*, 1995). In guinea-pigs, cyclosporin A was shown to inhibit allergen-induced lung eosinophil at concentrations lower than those required to inhibit a delayed type hypersensitivity response (Chapman *et al.*, 1993; Lagente *et al.*, 1994). The suggestion was that cyclosporin A was having direct inhibitory effects on eosinophils, in addition to inhibiting T lymphocyte function (Morley, 1992; Chapman & Mazzoni, 1994).

The aims of the present study were threefold. Initial experiments were carried out to identify an inhibitory action of the steroid, dexamethasone, on eosinophil recruitment and local oedema formation in our guinea-pig skin model. Different routes of administration of dexamethasone were used to investigate the site of action of the drug. In addition, we used the protein synthesis inhibitor, cycloheximide, to assess whether the effects on inflammatory stimuli were dependent upon protein synthesis. Thirdly, we evaluated whether a known immunosuppressive dose of cyclosporin A had any inhibitory activity in the same model.

Methods

Preparation of zymosan-activated plasma

Zymosan-activated plasma (ZAP) was used as a source of guinea-pig C5a des arg. ZAP was prepared by incubating heparinized (10 iu ml^{-1}) plasma obtained from naive guinea-pigs (Harlan, Bicester, 350–400 g) with zymosan (5 mg ml^{-1}) for 30 min at 37°C. Zymosan was then removed by centrifugation (2×10 min at 3000 *g*). The activated plasma was desalted using a PD-10 Sephadex G-25M column and stored in aliquots at −20°C.

Preparation of passive cutaneous anaphylaxis (PCA) sera and reactions

Details of the preparation of IgG_1-rich sera are described elsewhere (Teixeira & Hellewell, 1994). Briefly, male guinea-pigs (Harlan, 350–400 g) were immunized with bovine gamma-globulin (BGG) in Freund's complete adjuvant (0.2 mg BGG 0.2 ml^{-1} of adjuvant s.c.). These animals received a boost of antigen in Fruend's incomplete adjuvant on day 21 and the serum was prepared on day 30. Recipient animals received an injection of 50 μl of a 1/50 dilution of the antiserum i.d., followed 16–20 h later by the i.d. injection of antigen (BGG, 0.01–1.0 μg per site).

Measurement of local oedema formation and ^{111}In-eosinophil accumulation in guinea-pig skin

Radiolabelled eosinophil infiltration and oedema formation at skin sites were measured simultaneously. ^{125}I-human serum albumin (^{125}I-HSA, ~5 μCi) was added to ^{111}In-labelled eosinophils (purified and radiolabelled as previously described: Faccioli *et al.*, 1991; Teixeira *et al.*, 1994b) and injected i.v. (2.5×10^6 cells per animal) into recipient guinea-pigs (350–400 g) sedated with Hypnorm (0.15 ml, i.m.). After 5 min, duplicate i.d. injections of inflammatory stimuli or antigen were given in 0.1 ml volumes into the shaved dorsal skin following a randomized injection plan. Inflammatory responses (^{111}In-labelled eosinophil accumulation and oedema formation) were assessed 2 h after i.d. injections of mediators or antigen. At this time, a blood sample was obtained by cardiac puncture, the animals were killed with an overdose of sodium pentobarbitone, the dorsal skin was removed, cleaned free of excess blood and the sites punched out with a 17 mm punch. The samples were counted in an automatic 5-head gamma-counter (Canberra Packard Ltd, Pangbourne, Berks, U.K.) and the counts were cross-channel corrected for the two isotyopes.

Eosinophil numbers in the skin sites were expressed as the number of ^{111}In-eosinophils per skin site and oedema formation as the ratio of ^{125}I counts of the skin sample derived by the ^{125}I counts in 1 μl of plasma.

Experimental protocol

For local treatment ($n = 8-12$), dexamethasone sulphate (10^{-9} to 3×10^{-7} mol per site) or vehicle (saline) were injected i.d. 150 min prior to the inflammatory stimuli. For systemic treatment, dexamethasone sulphate was given at a dose of 4 mg kg^{-1}, i.v., 150 min prior to the i.v. radiolabelled eosinophils and ^{125}I-HSA ($n = 6$). This dose of dexamethasone has been previously shown to inhibit eosinophil accumulation in guinea-pig lung (Whelan *et al.*, 1995). Control animals received saline ($n = 6$). The protein synthesis inhibitor, cycloheximide (2×10^{-7} mol per site), was injected i.d. alone or with inflammatory stimuli.

Cyclosporin A (Sandimmune for i.v. use) or Sandimmune vehicle were diluted further in saline and given i.d. (10^{-11} to 10^{-8} mol per site) with PAF or ZAP ($n = 4$). In some experiments, cyclosporin A powder was dissolved in Tween 80 and further diluted in saline prior to i.d. injection with PAF ($n = 4$). The maximum local concentration of Tween 80 was 0.1%. For systemic treatment, cyclosporin A (Sandimmune for i.v. use) was given i.v. at the dose of 10 mg kg^{-1} 15 min prior to the radiolabelled cells ($n = 7$). A similar dose of cyclosporin A has been previously shown to inhibit eosinophil accumulation in guinea-pig lung (Francischi *et al.*, 1993). Control animals received Sandimmune vehicle diluted in saline (1:5, $n = 7$). In addition, some animals were pretreated s.c. with cyclosporin A (Sandimmune for i.v. use, 10 mg kg^{-1}) 48 h, 24 h and 1 h prior to i.v. injection of radiolabelled eosinophils. Control animals received Sandimmune vehicle only.

The choice of inflammatory stimuli used in our study (PAF, ZAP, LPS and a PCA reaction) was based on their ability to induce ^{111}In-eosinophil accumulation *in vivo*. We have previously shown that ^{111}In-eosinophil accumulation in a PCA reaction is partially due to the local release of a 5-lipoxygenase product, possibly LTB_4 (Teixeira & Hellewell, 1994). ^{111}In-eosinophil accumulation induced by LPS appears to be partially dependent on local release of tumour necrosis factor (Weg *et al.*, 1995). In addition, we chose to use ZAP and PAF to assess whether dexamethasone and cyclosporin induced a generalized (not mediator-specific) inhibitory effect on ^{111}In-eosinophil accumulation.

Reagents

The following compounds were purchased from Sigma Chemical Company (Poole, Dorset): BGG, cycloheximide, lipopolysaccharide (0111:B4) and zymosan. Hanks solutions, HEPES buffer and horse serum were purchased from Life Technologies Ltd (Paisley, Scotland). Percoll was purchased from Pharmacia (Milton Keynes, Bucks) and PAF (C16) from Bachem (Saffron Walden, Essex). ^{125}I-HSA and $^{111}InCl_3$ were obtained from Amersham International plc (Amersham, Bucks) and dexamethasone sulphate from David Bull Laboratories (Warwick, U.K.). Cyclosporin A was purchased as Sandimmune (Sandoz) solution for intravenous use. Sandimmune vehicle and cyclosporin A powder were kindly provided by Dr J. Fozard (Sandoz, Switzerland).

Statistics

Experiments were analyzed by two-way analysis of variance (ANOVA) on normally distributed data. P values were assigned using Newman-Keuls procedure and values of $P<0.05$ were considered statistically significant. Percentage inhibition was calculated after subtracting background values. Results are presented as the mean ± s.e.mean.

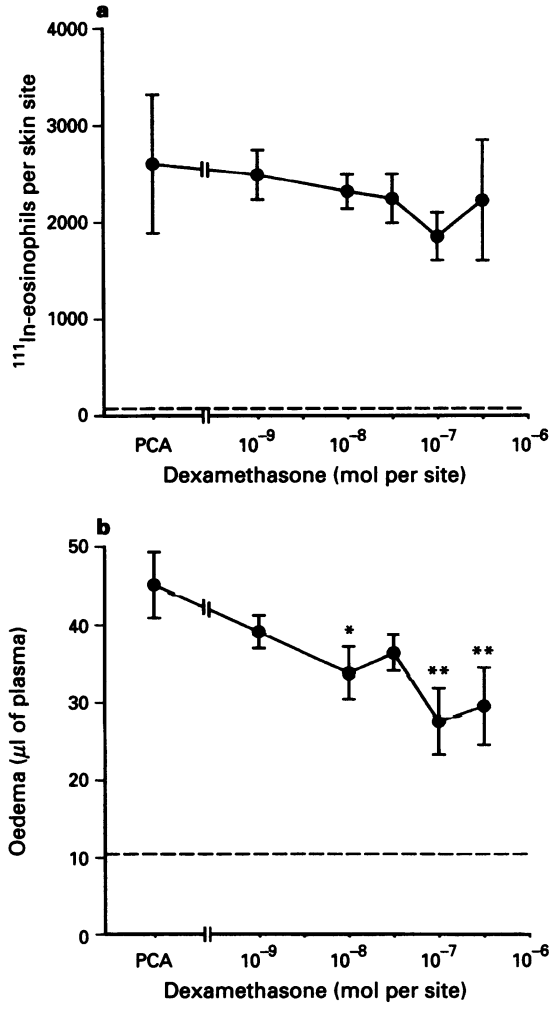

Figure 1 Effect of local pretreatment with dexamethasone on (a) ^{111}In-eosinophil accumulation and (b) oedema formation in a passive cutaneous anaphylactic (PCA) reaction. Dexamethasone (10^{-9} to 3×10^{-7} mol per site) or saline (shown as PCA) were given i.d. 150 min prior to the i.d. injection of antigen (BGG, 1 μg per site) in sites previously sensitized with anti-BGG IgG_1-rich anti-serum. Inflammatory responses were assessed after 2 h. Results are shown as the mean ± s.e.mean of 8 animals, $^*P<0.05$ and $^{**}P<0.01$, when compared to sites pretreated with saline. The line across the graphs represent background values in sensitized sites injected with saline.

Results

After i.v. injection, ^{111}In-eosinophils circulate in the blood and accumulate in cutaneous tissue in response to endogenous or exogenous eosinophil chemoattractants (Faccioli *et al.*, 1991; Teixeira & Hellewell, 1994; Teixeira *et al.*, 1994b; Weg *et al.*, 1994). In the present experiments, we observed no significant effect on the levels of circulating radiolabelled cells at 2 h after systemic treatment with dexamethasone (control, 4.8 ± 1.5% of total injected ^{111}In-eosinophils circulating at 2 h; treated, 3.5 ± 0.8%; $n=6$ pairs of animals) or cyclosporin A (control, 6.8 ± 0.8% of the total injected ^{111}In-eosinophils circulating at 2 h; treated, 8.4 ± 0.8%; $n=7$ pairs of animals).

Effect of locally-administered dexamethasone

When administered locally 150 min prior to i.d. injection of antigen in sites previously sensitized with an IgG_1-rich antisera (i.e. PCA reaction), dexamethasone (10^{-9} to 3×10^{-7} mol per site) dose-dependently inhibited oedema formation (Figure 1b). The drug caused a maximal inhibitory response (50% inhibition) at a dose of 10^{-7} mol per site (Figure 1b). In contrast, ^{111}In-eosinophil accumulation was not affected by i.d. pretreatment with dexamethasone (Figure 1a). Similarly, oedema formation, but not ^{111}In-eosinophil accumulation, induced by PAF was significantly inhibited by local pretreatment with dexamethasone (10^{-8} and 10^{-7} mol per site, Table 1) with a maximum inhibition of 35%. ZAP-induced responses were not altered by locally-injected dexamethasone (Table 1). When dexamethasone (10^{-8} and 10^{-7} mol per site) was given i.d. prior to injection of LPS (3.0 μg per site), there was no effect on ^{111}In-eosinophil accumulation (saline, 301 ± 95 ^{111}In-eosinophils per site; LPS 3.0 μg per site, 1358 ± 404; LPS + dexamethasone 10^{-8} mol per site, 1131 ± 368; LPS + dexamethasone 10^{-7} mol per site, 1349 ± 328; $n=4$) but a significant inhibition of oedema formation was detected, although the leakage response to LPS was small when compared to other agonists (saline, 7.2 ± 0.7 μl of plasma; LPS 3.0 μg per site, 11.2 ± 1.0; LPS + dexamethasone 10^{-8} mol per site, 9.9 ± 0.7; LPS + dexamethasone 10^{-7} mol per site, 9.8 ± 0.8; $n=4$, $P<0.05$).

Effect of systemic treatment with dexamethasone

When given systemically, dexamethasone (4 mg kg^{-1}, i.v.) inhibited both ^{111}In-eosinophil accumulation and oedema formation in the PCA reaction (0.01 to 1.0 μg of BGG per site; Figure 2). For example, ^{1111}In-eosinophil accumulation and oedema formation in sites injected with 1.0 μg of BGG were inhibited by 62% and 39%, respectively. Similarly, ^{111}In-eosinophil accumulation induced by PAF (10^{-10} and 10^{-9} mol per site) and ZAP (10 to 100% in saline) was inhibited by up to 63% by systemic treatment with dexamethasone (Table 2). Oedema formation was partially suppressed in sites injected

Table 1 Effect of local treatment with dexamethasone on ^{111}In-eosinophil accumulation and oedema formation induced by PAF and zymosan-activated plasma (ZAP)

	^{111}In-eosinophils per site			*Oedema* (μl of plasma)		
		Dexamethasone			*Dexamethasone*	
	Control	10^{-8}	10^{-7}	*Control*	10^{-8}	10^{-7}
Saline	91 ± 8	115 ± 15	117 ± 6	10.0 ± 1.1	11.0 ± 1.8	9.7 ± 1.4
PAF 10^{-9}	1428 ± 106	1215 ± 109	1369 ± 165	41.4 ± 2.8	31.4 ± 2.0**	32.8 ± 3.7*
ZAP 10%	2531 ± 264	2260 ± 428	2543 ± 245	22.0 ± 3.6	20.8 ± 4.9	21.8 ± 4.0

PAF (mol per site) or ZAP (% dilution in saline) were injected i.d. alone or with dexamethasone (10^{-8} and 10^{-7} mol per site) and inflammatory responses were assessed after 2 h. Results are the mean ± s.e.mean of 8 animals for ZAP and 12 animals for PAF. $^*P<0.05$ and $^{**}P<0.01$ compared to control sites.

with 10^{-10} mol per site PAF and 10% and 30% ZAP (Table 2). Furthermore, the ^{111}In-eosinophil accumulation and oedema formation induced by lipopolysaccharide (LPS, 3.0 μg per site) were almost completely inhibited by dexamethasone (control animals: LPS, 2078±342 ^{111}In-eosinophils per site and 24.3±4.5 μl of plasma, saline 480±178 ^{111}In-eosinophils and 8.7±0.4 μl; treated animals: LPS, 521±136 ^{111}In-eosinophils and 12.8±3.1 μl, saline 178±53 ^{111}In-eosinophils and 6.9±0.6 μl; $n=4$, $P<0.01$).

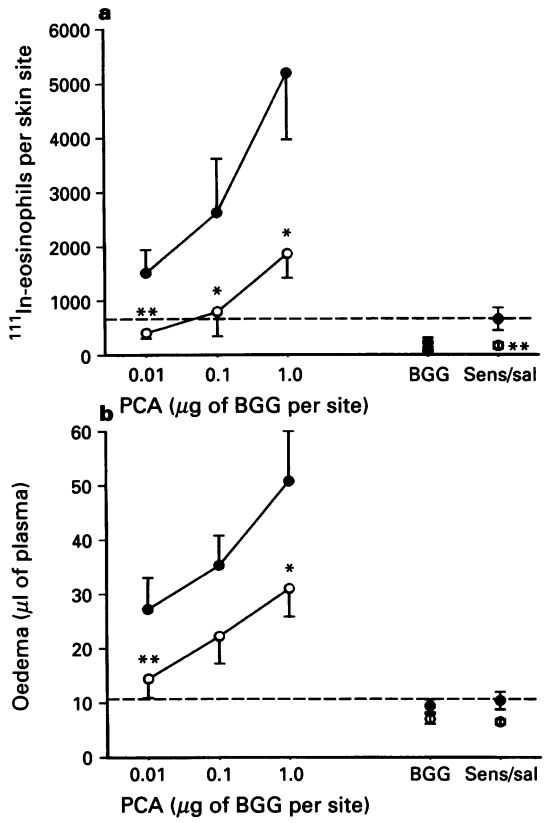

Figure 2 Effect of systemic treatment with dexamethasone on (a) ^{111}In-eosinophil accumulation and (b) oedema formation in a passive cutaneous anaphylactic (PCA) reaction. Dexamethasone (○, 4 mg kg^{-1}) or saline (●, 1 ml kg^{-1}) were given i.v. 150 min prior to the radiolabelled cells. Antigen (BGG, 0.01 to 1 μg per site; shown as PCA) or saline (Sens/sal) were injected i.d. in sites previously sensitized with anti-BGG IgG_1-rich anti-serum and inflammatory responses assessed after 2 h. The injection of antigen (1 μg of BGG) in non-sensitized sites is shown as BGG. Results are shown as the mean±s.e.mean of 6 animals; *$P<0.05$ and **$P<0.01$. The line across the graphs represent background values in sensitized sites injected with saline.

Effect of cycloheximide

The protein synthesis inhibitor, cycloheximide, has been previously shown to inhibit by approximately 80% IL-1-induced neutrophil accumulation in rabbit skin when administered i.d. at a dose of 2×10^{-7} mol per site (Rampart & Williams, 1988). In guinea-pig skin, the same dose of cycloheximide had no effect on responses induced by PAF (10^{-9} mol per site), ZAP (10% in saline) or in the PCA reaction (1.0 μg of BGG per site) (Table 3). In contrast, cycloheximide significantly inhibited by approximately 30% ^{111}In-eosinophil accumulation induced by i.d. injection of LPS (LPS 3.0 μg per site, 3708±292 ^{111}In-eosinophils per site; LPS+cycloheximide, 2908±291; saline, 432±36; cycloheximide, 504±96; $P<0.05$, $n=4$). Oedema formation induced by LPS was unaltered by co-injection with cycloheximide (LPS 3.0 μg per site, 21.8±5.4 μl of plasma; LPS+cycloheximide, 19.4±2.2; saline, 13.6±1.1; cycloheximide, 15.0±1.8; $n=4$).

Effect of cyclosporin A

Both Tween 80 and Sandimmune vehicle induced significant inflammation (i.e. ^{111}In-eosinophil accumulation and oedema formation) when injected i.d. in the guinea-pig (data not shown). In addition, i.d. injection of cyclosporin A in either solution induced further ^{111}In-eosinophil accumulation and oedema formation when compared with vehicle alone. Thus, no further experiments were carried out with i.d. injection of the drug.

The effect of systemic treatment with cyclosporin A (10 mg kg^{-1}, i.v.) on ^{111}In-eosinophil accumulation and oedema formation in the PCA reaction and in sites injected with PAF and ZAP is shown in Table 4. Cyclosporin A had no inhibitory effect on the responses induced by any of the stimuli tested. Similarly, inflammatory responses induced by 3.0 μg per site of LPS were not significantly altered by i.v. injection of cyclosporin A (control: 1944±243 ^{111}In-eosinophils per site and 11.7±1.7 μl of plasma; treated: 2356±604 ^{111}In-eosinophils and 9.0±1.0 μl, $n=4$). In order to ascertain that the lack of effect of cyclosporin was not due to insufficient dosing, animals received a 3 day treatment with cyclosporin A (10 mg kg^{-1}, s.c., daily) before skin experiments were carried out. However, prolonged treatment with the drug was not effective in inhibiting ^{111}In-eosinophil accumulation (control animals; saline 154±23 ^{111}In-eosinophils per site, PAF 10^{-9} mol per site 1378±63, PCA reaction 1.0 μg of antigen 3608±842, ZAP 10% in saline 1118±180, LPS 3.0 μg per site 1704±260: treated animals; saline 209±23, PAF 1904±693, PCA reaction 4303±1003, ZAP 1083±172, LPS 1434±260; $n=4$ sites in 2 pairs of animals) or oedema formation (data not shown).

Table 2 Effect of systemic treatment with dexamethasone on ^{111}In-eosinophil accumulation and oedema formation induced by PAF and zymosan-activated plasma (ZAP)

	^{111}In-eosinophils per site		*Oedema (μl of plasma)*	
	Control	Dexamethasone	Control	Dexamethasone
Saline	480±178	178±53	8.7±0.4	6.9±0.6
PAF 10^{-10}	1856±400	696±175**	49.9±3.8	37.9±4.1*
10^{-9}	3319±905	1511±350**	82.3±6.1	66.8±4.3
ZAP 10%	1778±486	734±193**	39.1±6.6	22.8±4.6*
30%	3362±1003	1385±398**	41.8±5.6	24.8±7.9*
100%	5514±1788	2417±560**	56.5±9.9	35.9±9.0

PAF (mol per site) and ZAP (% dilution in saline) were injected i.d. after the radiolabelled eosinophils and inflammatory responses were assessed after 2 h. Dexamethasone (4 mg kg^{-1}) was given i.v. 150 min prior to the radiolabelled cells. Results are the mean±s.e.mean of 6 pairs of animals; *$P<0.05$ and *$P<0.01$.

Table 3 Effect of local injection of cycloheximide on ^{111}In-eosinophil accumulation and oedema formation induced by PAF, ZAP and in a passive cutaneous anaphylactic (PCA) reaction

	^{111}In-eosinophils per site		*Oedema* (μl of plasma)	
	Control	*Cycloheximide*	*Control*	*Cycloheximide*
Saline	206±44	176±34	22.6±3.6	25.2±4.9
PAF 10^{-9}	700±83	580±104	64.5±3.9	66.9±5.9
ZAP 10%	1016±152	1044±181	50.5±5.3	50.2±4.2
PCA 1.0	1701±156	1754±238	64.5±5.7	65.1±6.4

PAF (mol per site), ZAP (% dilution in saline) or antigen (BGG, μg per site) in sites previously sensitized with anti-BGG IgG_1-rich antiserum (PCA) were injected i.d. alone or with cycloheximide (2×10^{-7} mol per site) and inflammatory responses were assessed after 2 h. Results are the mean±s.e.mean of 8 animals.

Table 4 Effect of systemic treatment with cyclosporin A on ^{111}In-eosinophil accumulation and oedema formation induced by PAF, zymosan-activated plasma (ZAP), lipopolysaccharide (LPS) and in a passive cutaneous anaphylactic (PCA) reaction

	^{111}In-eosinophils per site		*Oedema* (μl of plasma)	
	Vehicle	*Cyclosporin*	*Vehicle*	*Cyclosporin*
Saline	195±24	264±85	8.8±1.2	8.7±1.2
PAF 10^{-10}	654±71	498±81	29.8±2.7	33.8±4.4
10^{-9}	2341±605	1975±553	60.3±7.3	66.9±6.9
ZAP 10%	862±214	946±374	21.7±3.0	20.4±2.8
30%	2519±587	2428±766	24.5±3.8	23.0±2.9
100%	8293±1369	7194±1810	29.4±6.2	25.0±5.7
LPS 3.0	1944±243	2356±604	11.7±1.7	9.0±1.0
PCA 0.1	1627±578	1926±616	30.0±6.7	31.1±5.2
1.0	2573+513	2690±1091	34.2±5.8	41.6±5.5

PAF (mol per site), ZAP (% dilution in saline), LPS (μg per site) and antigen (μg of BGG per site) in sites previously sensitized with anti-BGG IgG_1-rich antiserum (PCA) were injected i.d. after the radiolabelled eosinophils and inflammatory responses were assessed after 2 h. Cyclosporin A (10 mg kg^{-1}) was given i.v. 15 min prior to the radiolabelled cells. Results are the mean±s.e.mean of 7 pairs of animals, with the exception of LPS (n = 4).

Discussion

In the guinea-pig, the i.d. injection of inflammatory mediators (e.g. PAF and C5a des-arg) or induction of a PCA reaction induces a rapid accumulation of ^{111}In-eosinophils in the injected skin sites (Faccioli *et al.*, 1991; Teixeira *et al.*, 1993a; Weg *et al.*, 1994). Time course studies have demonstrated that the majority of radiolabelled cells accumulate over the first 90 min with few cells accumulating at later times (Weg *et al.*, 1994). In this system, local or systemic administration of drugs which elevate cyclic AMP (e.g. E-series prostaglandins, phosphodiesterase inhibitors and β-adrenoceptor agonists) are effective inhibitors of ^{111}In-eosinophil accumulation induced by various inflammatory stimuli (Teixeira *et al.*, 1993b; 1994b; 1995). In the present study, we found that systemic, but not local treatment with dexamethasone effectively inhibited ^{111}In-eosinophil accumulation; oedema formation measured in the same sites was inhibited by dexamethasone given by either route of administration, with the exception of ZAP-induced oedema formation. In contrast, cyclosporin A had no effect on the inflammatory responses even when administered chronically. We also found that ^{111}In-eosinophil accumulation and oedema formation induced by ZAP, PAF and in a PCA reaction were independent of protein synthesis as assessed by the use of cycloheximide.

We chose a pretreatment period of 150 min for the studies with dexamethasone. The delayed action of glucocorticosteroids is a well described phenomenon and probably reflects the need for the synthesis of protein for the action of the drug (Schleimer, 1990; Barnes & Adcock, 1993). Consistent with this, systemic pretreatment with cycloheximide effectively reversed the inhibitory activity of various glucocorticosteroids on mediator-induced oedema formation in guinea-pig skin (Hashimoto *et al.*, 1993). In our studies, when co-administered locally with PAF, LPS and antigen in a PCA reaction, dexamethasone had no inhibitory effect on ^{111}In-eosinophil accumulation while partially inhibiting oedema formation measured in the same sites. This is similar to our previous findings in rabbit skin where local pretreatment with dexamethasone inhibited mediator-induced oedema formation but not ^{111}In-neutrophil accumulation (Yarwood *et al.*, 1993). When given locally, dexamethasone presumably inhibited oedema formation by an action on endothelial cells (Williams & Yarwood, 1990; Peers & Flower, 1991) or by inhibiting the release of local inflammatory mediators (e.g. inhibition of histamine release in the PCA reaction). Glucocorticosteroids have been previously shown to inhibit mediator release from guinea-pig mast cells (Schleimer *et al.*, 1987; Schleimer, 1990). An alternative explanation for the local effects of dexamethasone is the ability of the drug to cause vasoconstriction (Williams & Yarwood, 1990). However, this possibility is less likely inasmuch as one would except inhibition of both oedema formation and cell accumulation if vasoconstriction were present (Teixeira *et al.*, 1993c).

As shown in Table 1, locally-administered dexamethasone failed to alter oedema formation induced by ZAP. These results contrast with the effects of the drug when co-injected with PAF, LPS or antigen in the PCA reaction. The reason for this discrepancy is unknown but the variability of ZAP-induced oedema responses (compare Tables 1 and 2, for example) may partially account for the differences observed. In addition, it is possible that the mechanisms underlying ZAP-induced oedema formation may differ in part from those involved in inducing oedema formation in a PCA reaction or after i.d. injection of PAF. However, we failed to note any significant neutrophil-dependent activity in responses induced by ZAP, PAF or in the PCA reaction (Teixeira *et al.*, 1994a).

There are several potential sites of action for the inhibitory effects of systemically-administered dexamethasone. First, dexamethasone may act directly on eosinophils to inhibit their accumulation *in vivo*. Although still a controversial issue (Schleimer, 1990), some studies, but not all (Altman *et al.*, 1981; Lamas *et al.*, 1991), have failed to demonstrate an inhibitory effect of steroids on eosinophil function *in vitro* (Kita *et al.*, 1991a) even though these cells do possess glucocorticosteroid receptors (Peterson *et al.*, 1981). There is also the

possibility that dexamethasone induces *de novo* synthesis of proteins *in vivo* (e.g. lipocortin 1) which may suppress ^{111}In-eosinophil accumulation. In this regard, it has been carefully demonstrated that lipocortin accounts for some of the inhibitory effects of steroids on neutrophil accumulation in an air pouch model in the mouse (Perretti & Flower, 1993). Second, another possible cellular site of action of dexamethasone is the endothelial cell, which would account not only for inhibition of cell influx, through altered expression of cell adhesion molecules (Cronstein *et al.*, 1992; Burke-Gaffney & Hellewell, 1996), but also for the dimished oedema formation (Williams & Yarwood, 1990). Interestingly, a recent *in vivo* study has reported that dexamethasone, although not affecting mediator-induced leukocyte rolling, significantly enhanced the number of cells which detached from endothelial cells after stimulation (Mancuso *et al.*, 1995). Thirdly, direct or indirect (through proteins such as lipocortin) suppression of local mediator release from mast cells or macrophages may also explain some of the inhibitory effects of dexamethasone in the PCA reaction but would not explain inhibition of the actions of directly acting mediators such as PAF.

Since glucocorticosteroids can inhibit protein synthesis which may account for some of their anti-inflammatory activity, we tested whether ^{111}In-eosinophil accumulation and oedema formation were dependent on protein synthesis in guinea-pig skin. Initial experiments were carried out using LPS, a potent stimulator of protein synthesis (Manthous *et al.*, 1993). At a dose (2×10^{-7} mol per site) previously shown to cause maximal inhibition of IL-1-induced neutrophil accumulation in rabbit skin (Rampart & Williams, 1988), cycloheximide inhibited LPS-induced ^{111}In-eosinophil accumulation by about 30%. In contrast cycloheximide had no effect on inflammatory responses induced by PAF, ZAP or in a PCA reaction. Similarly, systemic treatment with cycloheximide at a dose (5 mg kg^{-1}, s.c. 1 h prior to i.v. injection of ^{111}In-eosinophils, $n=3$ pairs) shown to be effective in the guinea-pig (Hashimoto *et al.*, 1993) also failed to alter ^{111}In-eosinophil accumulation and oedema formation induced by these same mediators (data not shown). Interestingly, systemic treatment with dexamethasone effectively inhibited ^{111}In-eosinophil accumulation induced by i.d. injection of LPS; it is possible, therefore, that at least part of the inhibitory effect of dexamethasone on LPS-induced responses was due to inhibition of protein synthesis. However, a similar degree of inhibition of ^{111}In-eosinophil accumulation was observed in response to i.d. injection of low dose PAF and antigen in a PCA reaction but these responses were protein synthesis-independent. Thus, although a number of the effects of glucocorticosteroids *in vivo* depend on the synthesis of protein (Hashimoto *et al.*, 1993), dexamethasone inhibits protein synthesis-independent acute inflammation in guinea-pig skin.

In addition to the well-described inhibitory effects on cytokine production by lymphocytes (Erlanger, 1992; Schreiber & Crabtree, 1992), cyclosporin A can also inhibit mast cells, basophils and eosinophils *in vitro* (Cirillo *et al.*, 1990; Kita *et al.*, 1991b; Stellato *et al.*, 1992). A recent study has shown that treatment with cyclosporin A (10 mg kg^{-1}, s.c., -15 h and -1 h) inhibits tissue swelling, leukocyte influx and TNF levels in a PCA reaction in mouse ear, suggesting that mast cell function in the mouse is inhibited by this drug (Wershil *et al.*, 1995). In guinea-pig, lung eosinophilia after antigen challenge was inhibited at doses of cyclosporin A lower than those necessary for inhibition of a delayed type hypersensitivity in the lung (Chapman *et al.*, 1993; Lagente *et al.*, 1994). In contrast, cutaneous oedema formation as assessed by tissue thickening was not altered (Chapman & Mazzoni, 1994). The suggestion was that an effect of cyclosporin A, possibly on the eosinophil, was responsible for the greater sensitivity of lung eosinophilia to inhibition by this drug (Morley, 1992; Chapman & Mazzoni, 1994). In our studies, systemic treatment with cyclosporin A was without any effect on ^{111}In-eosinophil accumulation induced by several mediators, LPS or in the PCA reaction. The dose of cyclosporin A used in our study was based on immunosuppressive doses in human subjects (Faulds *et al.*, 1993). We chose to use the i.v. route because of the greater bioavailablity of the drug via this route (or when given s.c.) compared to the oral route (Backman *et al.*, 1988; Faulds *et al.*, 1993). In addition, similar doses (5 mg kg^{-1}) have been shown to be inhibitory for eosinophil accumulation in guinea-pig lung (Francischi *et al.*, 1993). Moreover, prolonged treatment over three days with cyclosporin A was also without effect on ^{111}In-eosinophil accumulation. Thus, using two treatment schedules which should achieve effect immunosuppression *in vivo*, we were unable to detect any significant inhibitory effect of cyclosporin A on ^{111}In-eosinophil accumulation in the guinea-pig skin. Furthermore, ^{111}In-eosinophil accumulation and oedema formation were unaltered in PCA reaction sites suggesting that, at least in this species, mast cell degranulation was not inhibited *in vivo*. The possibility remains, however, that the elicited eosinophils used in our studies are somehow less sensitive to the effects of cyclosporin A when compared with peripheral blood eosinophils. We hope to resolve this issue in future studies.

In conclusion, we have assessed the effects of two anti-inflammatory agents in ^{111}In-eosinophil accumulation and oedema formation induced by different inflammatory mediators and in a PCA reaction in the guinea-pig skin. Whereas a glucocorticosteroid inhibited both parameters measured, cyclosporin A was without any inhibitory effects. Keeping in mind the possible differences in the microcirculation of the skin and the lung and the fact that we have studied elicited eosinophils, our data do not support the concept that eosinophils are an important site of action for the inhibitory effects of cyclosporin A in a guinea-pig model of acute allergic inflammation.

References

ALTMAN, L.C., HILL, J.S. & HAIRFIELD, W.M. (1981). Effects of corticosteroids on esoinophil chemotaxis and adherence. *J. Clin. Invest.*, **67**, 28–36.

ANDERSSON, P., BRANGE, C., VON KOGERER, B., SONMARK, B. & STAHRE, G. (1988). Effect of glucocorticosteroids on ovalbumin-induced IgE-mediated immediate and late allergic response in guinea pig. *Int. Arch. Allergy Appl. Immunol.*, **87**, 32–39.

BACKMAN, L., BRANDT, I., DALLNER, D. & RINGDEN, O. (1988). Tissue distribution of [^{3}H]cyclosporine a in mice. *Transplant. Proc.*, **XX**, 684–691.

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

BARNES, P.J. & ADCOCK, I. (1993). Anti-inflammatory actions of steroids: molecular mechanisms. *Trends Pharmacol. Sci.*, **14**, 436–441.

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**, (in press).

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.

CHAPMAN, I.D., HOSHIKO, K., MAZZONI, L., PFANNKUCHE, H.J. & MORLEY, J. (1993). Cyclosporin A and the acute allergic reaction in the guinea-pig and rat. *Br. J. Pharmacol.*, **108**, 209P.

CHAPMAN, I. & MAZZONI, L. (1994). Mechanisms of inhibition of cyclosporin A on pulmonary leukocyte accumulation. *Trends Pharmacol. Sci.*, **15**, 99–101.

CIRILLO, R., TRIGGIANO, M., SIRI, L., CICCARELLI, A., PETTIT, G.R., CONDORELLI, M. & MARONE G. (1990). Cyclosporin A rapidly inhibits mediator release from human basophils presumably by interacting with cyclophilin. *J. Immunol.*, **144**, 3891–3897.

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.

ELWOOD, W., LOTVALL, J.O., BARNES, P.J., & CHUNG, K.F. (1992). Effect of dexamethasone and cyclosporin A on allergen-induced airway inflammatory cell responses in sensitized Brown-Norway rats. *Am. Rev. Respir. Dis.*, **145**, 1289–1294.

ERLANGER, B.F. (1992). Do we know the site of action of cyclosporin? *Immunol. Today*, **13**, 487–490.

FACCIOLI, L.H., NOURSHARGH, S., MOQBEL, R., WILLIAMS, F.M., SEHMI, R., KAY, A.B. & WILLIAMS, T.J. (1991). The accumulation of ^{111}In-eosinophils induced by inflammatory mediators in vivo. *Immunology*, **73**, 222–227.

FAULDS, D., GOA, K.L. & BENFIELD, P. (1993). Cyclosporin. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in immunoregulatory disorders. *Drugs*, **45**, 953–1040.

FRANCISCHI, J.N., CONROY, D.M., MAGHNI, K. & SIROIS, P. (1993). Inhibition by rapamycin of leukocyte migration and bronchial hyperreactivity induced by injection of Sephadex beads to guinea-pigs. *Br. J. Pharmacol.*, **110**, 1381–1386.

FUKUDA, T., AKUTSU, I., MOTOJIMA, S. & MAKINO, S. (1991). Inhibition of antigen-induced late asthmatic response and bronchial hyper-responsiveness by cyclosporin and FK 506. *Int. Arch. Allergy Appl. Immunol.*, **94**, 259–261.

HASHIMOTO, M., SHINOZAKI, Y. & KATORI, M. (1993). Immeditae ihibitory effect of methylprednisolone suleptanate (U-67590A) on antigen-induced cutaneous and airway anaphylatic responses in guinea-pigs. *Br. J. Pharmacol.*, **108**, 604–612.

HOLBROOK, M., HUGHES, P., KINGABY, R., HUGHES, B. & HIGGS, G. (1994). Dexamethasone inhibits allergen-induced eosinophil infiltration and activation in the guinea-pig lung. *Br. J. Pharmacol.*, **112**, 590P.

KITA, H., ABU-GHAZALEH, R., SANDERSON, C.J. & GLEICH, G.J. (1991a). Effect of steroids on immunoglobulin-induced eosinophil degranulation. *J. Allergy Clin. Immunol.*, **87**, 70–77.

KITA, H., OHNISHI, T., OKUBO, Y., WEILER, D., ABRAMS, J.S. & GLEICH, G.J. (1991b). Granulocyte/macrophage colony-stimulating factor and interleukin 3 release from human peripheral blood eosinophils and neutrophils. *J. Exp. Med.*, **174**, 745–748.

LAGENTE, V., CARRE, C., KYRIACOPOULOS, F., BOICHOT, E., MENICA-HUERTA, J.M. & BRAQUET, P. (1994). Inhibitory effect of cyclosporin A on eosinophil infiltration in the guinea pig lung induced by antigen, platelet-activating factor and leukotriene B4. *Eur. Respir. J.*, **7**, 921–926.

LAMAS, A.M., LEON, O.G. & SCHLEIMER, R.P. (1991). Glucocorticoids inhibit eosinophil responses to granulocyte-macrophage colony-stimulating factor. *J. Immunol.*, **147**, 254–259.

MANCUSO, F., PERRETTI, M. & FLOWER, R.J. (1995). Leukocyte transmigration, but not rolling or adhesion, is selectively inhibited by dexamethasone in the Hamster post-capillary venule. Involvement of endogenous lipocortin 1. *J. Immunol.*, **155**, 377–386.

MANTHOUS, C.A., HALL, J.B. & SAMSEL, R.W. (1993). Endotoxin in human disease. *Chest*, **104**, 1572–1581.

MATSUMOTO, T., ASHIDA, Y. & TSUKUDA, R. (1994). Pharmacological modulation of immediate and late airway response and leukocyte infiltration in the guinea pig. *J. Pharmacol. Exp. Ther.*, **269**, 1236–1244.

MORLEY, J. (1992). Cyclosporin A in asthma therapy: a pharmacological rationale. *J. Autoimmunity*, **5**, 265–269.

PEERS, S.H. & FLOWER, R.J. (1991). Site of anti-inflammatory action of dexamethasone in rabbit skin. *Eur. J. Pharmacol.*, **196**, 37–41.

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

PETERSON, A.P., ALTMAN, L.C., HILL, J.S., GOSNEY, K. & KADIN, M.E. (1981). Glucocorticoid receptors in normal human eosinophils: comparison with neutrophils. *J. Allergy Clin. Immunol.*, **68**, 212–217.

RAMPART, M. & WILLIAMS, T.J. (1988). Evidence that neutrophil accumulation induced by interleukin-1 requires both local protein biosynthesis and neutrophil CD18 antigen expression *in vivo*. *Br. J. Pharmacol.*, **94**, 1143–1148.

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.

SANJAR, S., AOKI, S., KRISTERSSON, A., SMITH, D. & MORLEY, J. (1990). Antigen challenge induces pulmonary airway eosinophil accumulation and airway hyperreactivity in sensitized guinea-pigs: the effect of anti-asthma drugs. *Br. J. Pharmacol.*, **99**, 679–686.

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

SCHLEIMER, R.P., UNDEM, B.J., MEEKER, S., BOLLINGER, M.E., ADKINSON, N.F., LICHENSTEIN, L.M. & ADAMS, G.K. (1987). Dexamethasone inhibits the antigen-induced contractile activity and release of inflammatory mediators in isolated guinea pig lung tissue. *Ann. Rev. Respir. Dis.*, **135**, 562–566.

SCHREIBER, S.L. & CRABTREE, G.R. (1992). The mechanism of action of cyclosporin A and FK 506. *Immunol. Today*, **13**, 136–142.

STELLATO, C., PAULIS, A, CICCARELLI, A., CIRILLO, R., PATELLA, V., CASOLARO, V. & MARONE, G. (1992). Anti-inflammatory effect of cyclosporin A on human skin mast cells. *J. Invest. Dermatol*, **98**, 800–804.

TEIXERIA, M.M., DOENHOFF, M.J., MCNEICE, C., WILLIAMS, T.J. & HELLEWELL, P.G. (1993a). Mechanisms of the inflammatory response induced by extracts of *Schistosoma mansoni* larvae in guinea pig skin. *J. Immunol.*, **151**, 5525–5534.

TEIXEIRA, M.M. & HELLEWELL, P.G. (1994). Effect of a 5-lipoxygenase inhibitor, ZM 230487, on cutaneous allergic inflammation in the guinea-pig. *Br. J. Pharmacol.*, **111**, 1205–1211.

TEIXEIRA, M.M., REYNIA, S., ROBINSON, M., SHOCK, A., WILLIAMS, T.J., WILLIAMS, F.M., ROSSI, A.G. & HELLEWELL, P.G. (1994a). Role of CD18 in the accumulation of eosinophils and neutrophils and local oedema formation in inflammatory reactions in guinea-pig skin. *Br. J. Pharmacol.*, **111**, 811–818.

TEIXEIRA, M.M., ROSSI, A.G., WILLIAMS, T.J. & HELLEWELL, P.G. (1994b). 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. (1993b). E-type prostaglandins enhance local oedema formation and neutrophil accumulation but suppress eosinophil accumulation in guinea pig skin. *Br. J. Pharmacol.*, **110**, 416–422.

TEIXEIRA, M.M., WILLIAMS, T.J. & HELLEWELL, P.G. (1993c). Role of prostaglandins and nitric oxide in acute inflammatory reactions in guinea pig skin. *Br. J. Pharmacol.*, **110**, 1515–1521.

TEIXEIRA, M.M., WILLIAMS, T.J. & HELLEWELL, P.G. (1995). Anti-inflammatory effects of a short-acting and long-acting β_2-adrenoceptor agonist in guinea pig skin. *Eur. J. Pharmacol.*, **272**, 185–193.

WEG, V.B. (1993). Mechanisms of eosinophil accumulation and oedema formation in allergic inflammation in the guinea pig skin. *(PhD thesis). London: University of London.*

WEG, V.B., WALSH, D.T., FACCIOLI, L.H., WILLIAMS, T.J., FELDMAN, M. & NOURSHARGH, S. (1995). LPS-induced ^{111}In-eosinophil accumulation in guinea pig skin: evidence for a role of TNF-alpha. *Immunology*, **84**, 36–40.

WEG, V.B., WATSON, M.L., FACCIOLI, L.H. & WILLIAMS, T.J. (1994). Investigation of the endogenous chemoattractants invovled in ^{111}In-eosinophil accumulation in passive cutaneous anaphylatic reactions in the guinea pig. *Br. J. Pharmacol.*, **113**, 35–42.

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

WERSHIL, B.K., FURUTA, G.T., LAVIGNE, J.A. CHOUDHURY, A.R., WANG, Z-S. & GALLI, S.J. (1995). Dexamethasone or cyclosporin A suppress mast cell cell-leukocyte cytokine cascades. *J. Immunol.*, **154**, 1391–1398.

WHELAN, C.J., HUGHES, S.C. & WREN, G.P.A. (1995). Inhibition of some aspects of acute inflammation of guinea-pig lung by intraperitoneal dexamethasone and mifepristone: demonstration of agonist activity of mifepristone in the guinea-pig. *Inflamm. Res.*, **44,** 131–138.

WILLIAMS, T.J. & YARWOOD, H. (1990). Effect of glucocorticosteroids on microvascular permeability. *Am. Rev. Respir. Dis.*, **141,** S39–S43.

YARWOOD, H., NOURSHARGH, S., BRAIN, S.D. & WILLIAMS, T.J. (1993). Effect of dexamethasone on neutrophil accumulation and oedema formation in rabbit skin: an investigation of site of action. *Br. J. Pharmacol.*, **108,** 959–966.

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