Investigation of the endogenous chemoattractants involved in ^{111}In -eosinophil accumulation in passive cutaneous anaphylactic reactions in the guinea-pig

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1 Eosinophil accumulation and plasma extravasation are features of type I allergic responses. In an attempt to characterize the mediators of these responses, we have examined the local accumulation of ^{111}In -eosinophils and leakage of ^{125}I -human serum albumin (^{125}I -HSA) during passive cutaneous anaphylaxis (PCA) reactions and in response to defined inflammatory mediators in the guinea-pig. Animals were passively sensitized by intradermal injection of anti-bovine gamma globulin antibody (50 μl , 1/50 dilution). After 20–24 h, animals were injected intravenously with ^{111}In -eosinophils and ^{125}I -HSA for the measurement of cell accumulation and plasma leakage, respectively.

2 When injected into sensitized sites, antigen caused a dose-related increase in the accumulation of ^{111}In -eosinophils and plasma leakage in guinea-pig skin. Time course experiments over 24 h revealed that the maximal rate of ^{111}In -eosinophil accumulation occurred over the first 90 min, with little accumulation at later time points. Plasma leakage was completed within the first 30 min after challenge. Responses to the mast cell degranulator, compound 48/80, exhibited very similar responses to the PCA reaction.

3 Co-injection of antigen with the PAF antagonist, WEB 2086 (10^{-7} mol/site) or the 5-lipoxygenase inhibitor, PF 5901 (10^{-7} mol/site) did not significantly alter the accumulation of ^{111}In -eosinophils or plasma leakage, whereas these drug doses abolished responses to exogenous PAF (10^{-9} mol/site) and arachidonic acid (AA, 3×10^{-8} mol/site), respectively. The H_1 receptor antagonist chlorpheniramine (2.5×10^{-8} mol/site) did not reduce antigen-induced ^{111}In -eosinophil accumulation. Drug combinations were also injected with antigen into sensitized sites, but were unable to reduce ^{111}In -eosinophil accumulation.

4 These results indicate that anaphylactic eosinophil accumulation in this model involves mediators other than histamine, PAF or lipoxygenase products. This is in contrast to plasma leakage in this reaction, which can be abolished by a combination of antagonists blocking these mediators.

Keywords: Passive cutaneous anaphylaxis (PCA); compound 48/80; eosinophils; PAF; leukotriene B_4 (LTB_4); histamine

Introduction

Type I hypersensitivity responses are conveniently studied in the form of the passive cutaneous anaphylaxis (PCA) reaction, as coined by Ovary in 1950 (Ovary, 1950). In the guinea-pig, both IgE and IgG₁ antibodies are capable of evoking anaphylactic reactions (Perini & Mota, 1972; Ovary *et al.*, 1976). Upon challenge with specific antigen the release of inflammatory mediators from sensitized mast cells leads to an increase in vascular permeability and consequently plasma extravasation into tissues. Infiltration of inflammatory cells such as neutrophils and eosinophils is also observed at PCA sites in different species (Kay, 1970; Nourshargh *et al.*, 1989; Wershil *et al.*, 1991). This type of response contributes to the pathology of several diseases such as extrinsic asthma, atopic eczema and allergic rhinitis (reviewed in Samter, 1978).

Eosinophil accumulation is a hallmark of the inflammation associated with infection with helminthic parasites and allergic disorders. Allergic patients have elevated levels of circulating eosinophils and these cells accumulate after antigenic challenge in the skin and airways (de Monchy *et al.*, 1985; Henocq & Vargaftig, 1988; Lozewicz *et al.*, 1991). There is now considerable evidence implicating the eosinophil in the pathophysiology of asthma. Following activation, eosinophils release a variety of preformed and newly synthesized

mediators (reviewed in Kay & Corrigan, 1992) which are thought to contribute to the development of this disease. Indeed, several studies show the presence of eosinophils and their products in bronchoalveolar fluid from asthmatics (de Monchy *et al.*, 1985; Kay *et al.*, 1986; Diaz *et al.*, 1989). In addition, it has been shown recently in an *Ascaris*-challenge primate model that an antibody to intercellular adhesion molecule 1 that inhibited lung eosinophil accumulation also reduced bronchial hyper-reactivity (Wegner *et al.*, 1990).

An increased understanding of the basic mechanisms underlying the recruitment of eosinophils *in vivo* may therefore have important therapeutic consequences. Nevertheless, despite much progress, the mechanisms that mediate and regulate the accumulation of this cell type in sites of allergic inflammation remain unclear. Several mediators such as platelet activating factor (PAF) (Wardlaw *et al.*, 1986), leukotriene B_4 (LTB_4) (Czarnetzki & Mertensmeier, 1985) and C5a (Ogawa *et al.*, 1981) have been described as chemotactic for eosinophils *in vitro*. However, results obtained with *in vitro* chemotaxis cannot be safely extrapolated in the *in vivo* situation since such test systems do not mimic all the requirements for cell adhesion and transmigration across the endothelial cell barrier. For this reason, it was important to establish an animal model that allowed detailed investigation of eosinophil accumulation in defined allergic inflammatory reactions. In a previous study, we developed a system for measuring the accumulation of ^{111}In -eosinophils *in vivo* and showed that guinea-pig C5a des Arg, human recombinant C5a, PAF and LTB_4 are able to induce eosinophil accumulation in guinea-pig skin (Faccioli *et al.*, 1991). Subsequently, we demonstrated that a monoclonal antibody to very late

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activation antigen-4 inhibits the accumulation of ^{111}In -eosinophils in response to inflammatory mediators and during a PCA reaction (Weg *et al.*, 1993). In the present study, in order to elucidate further the nature of the inflammatory mediators involved in eosinophil accumulation *in vivo*, we have used this method to examine eosinophil infiltration during PCA reactions in the guinea-pig. In addition, compound 48/80, was used in comparison to study non-immunological activation and degranulation of mast cells.

Methods

Animals

Ex-breeder Dunkin-Hartley cell donor guinea-pigs (800–900 g) and Dunkin-Hartley test guinea-pigs (300–500 g) of either sex were purchased from Harlan-Olac, Oxon.

Preparation of guinea-pig PCA serum

Guinea-pigs were immunised by subcutaneous injection of 0.2 mg BGG in 0.2 ml Freund's complete adjuvant, divided between the rear footpads and the nape of the neck. Animals were boosted by similar injections of antigen in incomplete adjuvant on day 21. Animals were exsanguinated and serum prepared on day 30. Aliquots were stored frozen at -20°C . The fixation time course and heat stability of this antibody are consistent with IgG₁-like activity (Weg *et al.*, 1991).

Separation and ^{111}In -labelling of peritoneal guinea-pig eosinophils

This technique has been described previously in detail by Faccioli *et al.* (1991). Briefly, ex-breeder guinea-pigs received 4 injections of horse serum at 2–3 days intervals. Peritoneal cells were collected by lavage 24 h after the last injection and purified over a discontinuous Percoll gradient. The cell population was used only when the eosinophil purity was above 90%. The predominant contaminating cell type was mononuclear and a major exclusion criterion was the presence of neutrophils. The cells were then incubated with $^{111}\text{InCl}_3$ (approximately 100 μCi in 10 μl) chelated with 2-mercaptopyridine-*N*-oxide (40 μg in 0.1 ml of 50 mM PBS, pH 7.4) for 15 min at room temperature. The labelled eosinophils were washed twice and resuspended in Hanks' solution (10^7 eosinophils ml^{-1}) pH 7.3 containing cell-free citrated guinea-pig plasma to a final concentration of 10%.

Induction and measurement of plasma leakage and eosinophil accumulation in guinea-pig skin

Eosinophil infiltration and plasma leakage in the guinea-pig skin were simultaneously measured using the local accumulation of i.v. injected ^{111}In -labelled eosinophils and ^{125}I -HSA (Faccioli *et al.*, 1991).

PCA was prepared as previously described (Weg *et al.*, 1991). Guinea-pigs were sedated with Hypnorm and their dorsal skin shaved. Diluted antiserum (1/50) or purified IgG₁ (2 μg) were then injected i.d. in 50 μl volumes into the dorsal skin. After a fixation period of 20–24 h 1 ml of Hanks' solution containing 1×10^7 eosinophils mixed with ^{125}I -HSA (2.5 $\mu\text{Ci}/\text{animal}$) was injected i.v. via an ear vein. Five minutes later antigen (BGG or OA) was injected i.d. into serum-pretreated sites. This protocol was modified in time course experiments so that sites were challenged at specified time points prior to the injection of ^{111}In -eosinophils and ^{125}I -HSA.

In experiments performed to determine mediator involvement in the PCA response, antigen was injected locally in a mixture with antagonists. In each of these experiments exogenous agonists (histamine, PAF and AA) were injected into naive sites in the presence and absence of their respective

antagonist or in the case of AA, inhibitor of 5-lipoxygenase metabolism. Each test was performed in duplicate sites according to a balanced site injection plan with an injection volume of 100 $\mu\text{l}/\text{site}$. Except in certain time course experiments, a cardiac blood sample was collected after 2 h and animals were killed by an overdose of sodium pentobarbitone. The dorsal skin was then removed, skin sites were punched out with a 17 mm diameter punch and samples were counted together with plasma samples in an automatic gamma counter (Canberra, Berks.). The ^{111}In counts per eosinophil were determined and used to express eosinophil accumulation in each skin site in terms of number of labelled leukocytes. Leakage at each site was expressed as μl of plasma by dividing skin sample ^{125}I counts by ^{125}I counts in 1 μl of plasma.

Materials

Pentobarbitone sodium (Sagatal, 60 mg ml^{-1}) was purchased from May and Baker, Dagenham, Essex. Hypnorm (0.315 mg ml^{-1} fentanyl citrate and 10 mg ml^{-1} fluanisone) was purchased from Janssen Pharmaceutical Ltd, Grove, Oxford, $^{111}\text{Indium chloride}$ ($^{111}\text{InCl}_3$; 10 mCi ml^{-1} in pyrogen-free 0.04 N hydrochloric acid) and ^{125}I -human serum albumin (^{125}I -HSA; 20 mg albumin per ml of sterile isotonic saline, 50 $\mu\text{Ci ml}^{-1}$) were purchased from Amersham International, Amersham, Bucks. Histamine, bovine gamma globulin

Figure 1 Effect of antigen dose on ^{111}In -eosinophil accumulation (a) and plasma leakage (b) during passive cutaneous anaphylaxis responses in guinea-pig skin. Skin sites were sensitized by i.d. injection of a fixed dose of antiserum (1/50 dilution, 50 $\mu\text{l}/\text{site}$) and challenged with the indicated dose of BGG 24 h later. The open symbols represent responses detected at naive sites injected with the highest dose (25 μg) of BGG. Dashed lines indicate control responses to saline. Points indicate mean $\pm$ s.e. mean for $n = 6$ animals. Asterisks indicate a significant difference from the BGG responses: ** $P < 0.01$.

(BGG), bovine serum albumin (BSA), ovalbumin (OA), 2-mercaptopyridine-*N*-oxide, dimethyl sulphoxide (DMSO), arachidonic acid (AA), were purchased from Sigma Chemical Company, Dorset. Horse serum, sterile Hanks' Balanced Salt solution (Hanks' solution $10\times$) and HEPES (1 M) were purchased from Gibco Limited, Paisley, Renfrewshire. Percoll was purchased from Pharmacia Fine Chemicals, Uppsala, Sweden. Pyrogen- and preservative-free heparin sodium (5000 u ml^{-1}) was purchased from Pabyrn Laboratories, Greenford, Middlesex. Chlorpheniramine maleate (Piriton 10 mg ml^{-1}) was purchased from Allen and Hanburys Ltd., Middlesex. PAF (1-*O*-hexadecyl-2-acetyl-sn-glycero-3-phosphorylcholine) was purchased from Bachem, Bubendorf, Switzerland. Stock solutions of PAF ($3.6\times 10^{-3}\text{ M}$) and AA ($3.3\times 10^{-2}\text{ M}$) in ethanol were stored at -20°C and diluted in saline containing 0.1% bovine serum albumin (BSA) immediately before injection. The following substances were obtained as gifts: WEB 2086 (3-[4-(2-chlorophenyl)-9-methyl-6H-thieno [3,2-f] [1,2,4]-triazolo-[4,3,a] [1,4]-diazepin-2-yl]-1-(4-morpholinyl)-1-propanone) from Boeringher-Ingelheim AG, Ingelheim am Rhein, Germany; and PF 5901 (α -Pentyl-3-(2-quinolinylmethoxy) benzenemethanol) from Purdue Frederick, CT, U.S.A. WEB 2086 and chlorpheniramine were dissolved in saline. PF 5901 was dissolved in DMSO before dilution in saline. The final DMSO concentration injected was 3% v/v. Purified guinea-pig anti-ovalbumin IgG₁ was a

gift from Dr H.J. Showell, Pfizer Central Research, CT, U.S.A.

Statistical analysis

Results are expressed as the means $\pm$ s.e.mean for n animals where each datum unit is the average of responses in duplicate sites. Data were analyzed by two-way analysis of variance (ANOVA) of log transformed data and statistical significance determined with the Neuman-Keuls procedure for repeated comparisons (Snedecor & Cochran, 1967).

Results

Antigen-induced plasma leakage and eosinophil accumulation during PCA reactions

Injection of antigen into guinea-pig skin sites previously sensitized with 1/50 dilution of anti-BGG antiserum led to a marked and dose-dependent eosinophil accumulation measured over 2 h (Figure 1a). Even at the lowest antigen dose tested ($0.04\text{ }\mu\text{g/site}$) there was a 10 fold increase in ^{111}In -eosinophil accumulation compared with control sites. Oedema formation was also dose-related, although with a flatter dose-response curve (Figure 1b). In the same experiments the

Figure 2 Long time course of ^{111}In -eosinophil accumulation (a) and plasma leakage (b) during passive cutaneous anaphylaxis responses in guinea-pig skin. Sites were injected with antisera (1/50 dilution, $50\text{ }\mu\text{l/site}$) before injection of BGG ($1\text{ }\mu\text{g/site}$, solid columns) or saline (open columns). Responses obtained in sites pretreated with saline before injection of BGG are also shown (cross-hatched columns). BGG or saline was injected at intervals (as shown on the abscissa scale). ^{111}In -eosinophils and ^{125}I -HSA were injected i.v. and responses measured over a period of 2 h. Results are the mean $\pm$ s.e.mean for $n = 6-11$ animals. Asterisks indicate a significant difference from the BGG responses: ** $P < 0.01$.

Figure 3 Short time course of ^{111}In -eosinophil accumulation (a) and plasma leakage (b) during passive cutaneous anaphylaxis responses in guinea-pig skin. Sites were injected with antisera (1/50 dilution, $50\text{ }\mu\text{l/site}$) 24 h before injection of BGG ($1\text{ }\mu\text{g/site}$, solid columns). BGG was injected at intervals (as shown on the abscissa scale). Responses obtained in sites pretreated with saline before injection of BGG are also shown (open columns). Dashed lines indicate control responses to saline. ^{111}In -eosinophils and ^{125}I -HSA were injected i.v. and responses measured over a period of 30 min. Results are the mean $\pm$ s.e.mean for $n = 5$ animals. Asterisks indicate a significant difference from the BGG responses: * $P < 0.05$; ** $P < 0.01$.

highest dose of antigen (25 µg) did not cause exudation or cell accumulation at naive sites. The submaximal dose of 1 µg of BGG was used for subsequent experiments.

The temporal development of the PCA response was then studied in two stages, using a long and a short time course (Figures 2 and 3, respectively). For the long time course sensitized sites were challenged at different time points (22, 5, 2 and 0 h) prior to i.v. administration of ^{111}In -eosinophils and ^{125}I -HSA and the reaction was then measured over a period of 2 h. Using this technique, eosinophil accumulation was detected only during the period after injection of radio-labelled cells, allowing the examination of rates of accumulation at different periods during the reaction. A pronounced eosinophil accumulation was observed at sites challenged at the time of ^{111}In -eosinophil injection ($t = 0-2$ h, Figure 2a). In contrast, no accumulation of ^{111}In -eosinophils was observed at sites challenged 2, 5 or 22 h before injection of radiolabel. Increased plasma leakage was also only apparent during the first measurement period ($t = 0-2$ h, Figure 2b). In short time course experiments we modified this protocol to examine ^{111}In -eosinophil and plasma leakage over 30 min time intervals during the first 2 h of the PCA reaction. Sensitized sites were challenged at 90, 60, 30 or 0 min prior to the i.v. injection of ^{111}In -eosinophils and ^{125}I -HSA. Figure 3 shows that while the leakage of plasma was almost complete during the first 30 min (Figure 3b), ^{111}In -eosinophil continued to accumulate throughout the 2 h period (Figure 3a).

Therefore, in subsequent experiments measurements were made over the first 2 h of the reaction.

Study of the mediators involved in the PCA reaction

The effects of chlorpheniramine (2.5×10^{-8} mol/site), an H_1 -receptor antagonist; WEB 2086 (10^{-7} mol/site), a PAF receptor antagonist; and PF 5901 (10^{-7} mol/site), a 5-lipoxygenase inhibitor; were tested against cutaneous anaphylactic responses elicited by 1 µg of BGG. It was observed that none of these drugs, when given alone, was capable of significantly reducing ^{111}In -eosinophil accumulation (Figure 4a) during the PCA reaction. Plasma leakage in response to this dose of antigen was partially inhibited by chlorpheniramine, but not by WEB 2086 or PF 5901 (Figure 4b). At control sites in the same experiments PAF (10^{-9} mol/site) and AA (3×10^{-8} mol/site) caused the accumulation of ^{111}In -eosinophils and these responses were abolished by WEB 2086 or PF 5901 respectively. The inhibition of AA by PF 5901 indicates that the effect of AA is probably mediated by metabolism to chemotactic 5-lipoxygenase products such as LTB_4 . Histamine (2.5×10^{-8} mol/site) was unable to induce ^{111}In -eosinophil accumulation (Figure 4c).

The effect of a combination of the three drugs (chlorpheniramine, WEB 2086 and PF 5901) was then tested against the PCA response. As shown in Figure 4b the combined treatment virtually abolished oedema formation, but

Figure 4 Effects of chlorpheniramine (Chlor, 2.5×10^{-8} mol/site); WEB 2086 (WEB, 10^{-7} mol/site) and PF 5901 (PF, 10^{-7} mol/site) on ^{111}In -eosinophil accumulation (a) and plasma leakage (b) during passive cutaneous anaphylaxis (PCA) responses in guinea-pig skin. BGG (1 µg/site) was injected without (solid columns) and with (open columns) drugs as indicated on the abscissa scale. Also shown are control responses (c and d) to histamine (H, 2.5×10^{-8} mol/site); PAF (PAF, 10^{-9} mol/site) and arachidonic acid (AA, 3×10^{-8} mol/site) in the presence (open columns) or absence (cross hatched columns) of their respective antagonists or inhibitor of metabolism. The dashed lines indicate control responses to saline. Results are the mean $\pm$ s.e. mean for $n = 4-10$ animals. * $P < 0.05$; ** $P < 0.01$.

Figure 5 Effect of chlorpheniramine on ^{111}In -eosinophil accumulation (a) and plasma leakage (b) during 48/80-induced reactions. The indicated dose of 48/80 was injected without (●) or with (○) antagonist at 2.5×10^{-8} mol/site. Dashed lines indicate control responses to saline. Results are the mean $\pm$ s.e.mean for $n = 4$ animals. * $P < 0.05$; ** $P < 0.01$.

had no significant effect on cell accumulation (Figure 4a). In further experiments, purified anti-ovalbumin IgG₁ (2 $\mu\text{g}/\text{site}$) was used to sensitize the guinea-pig skin. Challenge of sites sensitized with IgG₁ elicited similar ^{111}In -eosinophil responses to those occurring at sites sensitized with unfractionated sera. Again a combination of chlorpheniramine, WEB 2086 and PF 5901 had no effect on the accumulation of ^{111}In -eosinophils, i.e. PCA = $21.45 \pm 6.27 \times 10^3$ ^{111}In -eosinophils/site and PCA + drug combination = $17.02 \pm 7.80 \times 10^3$ ^{111}In -eosinophils/site, for $n = 5$ animals.

^{111}In -eosinophil accumulation and oedema formation induced by compound 48/80 in the guinea-pig skin

Figure 5 shows that the local injection of compound 48/80 (1–100 $\mu\text{g}/\text{site}$) led to a dose-related increase in ^{111}In -eosinophil accumulation (Figure 5a) and oedema formation (Figure 5b) over 2 h in guinea-pig skin. The kinetics of this reaction are illustrated in Figure 6. As with the responses observed during the PCA reaction, the plasma leakage induced by 100 μg of compound 48/80 was almost complete during the first 30 min (Figure 6b) while ^{111}In -eosinophils continued to accumulate throughout the 2 h period (Figure 6a). Since compound 48/80 is a potent mast cell degranulating agent, one likely mediator to be involved in these reactions is histamine. The role of histamine was investigated by co-injecting different doses of compound 48/80 with the H₁ antagonist, chlorpheniramine. It was observed that chlorpheniramine significantly inhibited the oedema formation (Figure 5b) without significantly reducing cell accumulation in all 48/80 doses used (Figure 5a). In addition, as observed

Figure 6 Time course of ^{111}In -eosinophil accumulation (a) and plasma leakage (b) induced by 48/80 in guinea-pig skin. Compound 48/80 (100 $\mu\text{g}/\text{site}$) was injected at intervals (as shown on the abscissa scale). Dashed lines indicate control responses to saline. ^{111}In -eosinophils and ^{125}I -HSA were injected i.v. and responses measured over a period of 30 min. Results are the mean $\pm$ s.e.mean for $n = 4$ animals. Asterisks indicate a significant difference from the saline responses: $P < 0.01$.

with the PCA reaction, the combination of these drugs failed to inhibit significantly eosinophil accumulation induced by 100 μg of compound 48/80, i.e. 48/80 = $27 \pm 13.68 \times 10^3$ ^{111}In -eosinophils/site and 48/80 + drug combination = 16.85 ± 5.79 ^{111}In -eosinophils/site, for $n = 5$ animals.

Discussion

The present results show that antigen challenge at sensitized sites induced a dose-dependent ^{111}In -eosinophil accumulation and oedema formation in guinea-pig skin. Time course experiments over 24 h revealed that the maximal rate of ^{111}In -eosinophil accumulation occurred over the first 90 min while oedema formation was mainly completed within the first 30 min after antigen challenge. Thus, no evidence of a late response was found as far as these parameters were concerned. Although eosinophil accumulation is often considered a feature of the late phase response to antigen in both the skin and the airways, several investigators have also observed eosinophil infiltration within an hour of antigen challenge. Atkins *et al.* (1973) noted perivascular eosinophils in skin biopsies following ragweed challenge of sensitive patients which began at about 20 min and continued to increase over 4 h. Extracellular deposition of eosinophil granule contents such as eosinophil derived neurotoxin and major basic protein, can be detected within 15 min of challenge and are deposited throughout the dermis by 3 h (Leiferman *et al.*, 1990). An early phase influx of eosinophils (30 min after

challenge) was also observed in the nasal mucous membrane of patients with allergic rhinitis (Lozewicz *et al.*, 1991). It is also possible that in the present study the sensitization procedure employed accounts for the absence of a more prolonged ^{111}In -eosinophil accumulation. In models of actively, as opposed to passively sensitized guinea-pigs, two peaks of eosinophil recruitment were observed in the lungs and conjunctiva (Chan *et al.*, 1990; Makino *et al.*, 1991).

Although some attempts have been made to determine the chemical mediators involved in tissue eosinophilia in cutaneous anaphylaxis (Kay, 1970; Hirashima *et al.*, 1983) this reaction has not yet been fully characterized. Histamine is a well-established mediator of mast cell-dependent responses and is partly responsible for the oedema formation observed in the PCA reactions in the guinea-pigs (Weg *et al.*, 1991). Since histamine was reported to be chemotactic for human eosinophils *in vitro* (Clark *et al.*, 1975) we investigated the potential role of this mediator in causing eosinophil accumulation in the PCA response. We observed that the intradermal administration of histamine while inducing plasma leakage caused little cell accumulation and that treatment of PCA sites with the H_1 antagonist, chlorpheniramine, failed to affect ^{111}In -eosinophil infiltration in this reaction. It is therefore unlikely that endogenous histamine can account for the ^{111}In -eosinophil accumulation in guinea-pig PCA reactions.

Other possible contributors to the accumulation of ^{111}In -eosinophils in the present model may include lipid mediators such as LTB_4 and PAF. LTB_4 , in addition to its potent effects on neutrophils (Ford-Hutchinson *et al.*, 1980), is also chemotactic for eosinophils *in vitro* (Czarnetzki & Mertensmeier, 1985). The intradermal injection of LTB_4 is also potent in inducing the accumulation of ^{111}In -eosinophils in the guinea-pig (Faccioli *et al.*, 1991). The eosinophil chemoattractant activity produced by challenged-sensitized guinea-pig lung fragments, formerly thought to reside in small peptides, was later identified as LTB_4 and 8,15-di-HETE (Sehmi *et al.*, 1991). Further, aerosolized LTB_4 administered by inhalation to guinea-pigs was shown to induce eosinophil accumulation (Silbaugh *et al.*, 1987) and a selective LTB_4 antagonist to inhibit eosinophil accumulation induced by antigen challenge (Richards *et al.*, 1989). PAF has been shown to be a potent mediator in stimulating human eosinophil chemotaxis *in vitro* (Wardlaw *et al.*, 1986) and induces the accumulation of eosinophils when administered to a skin window chamber in man (Henocq & Vargaftig, 1988). Furthermore, the ability of PAF to mimic several aspects of antigen challenge including bronchoconstriction, increased bronchial reactivity and tissue oedema formation (reviewed in Braquet *et al.*, 1987) suggests a potential role for PAF in the mediation of allergic responses.

In the present study, the local administration of a PAF antagonist, WEB 2086, had no significant effect on local eosinophil accumulation in the PCA suggesting that endogenous PAF is unlikely to be involved in attracting eosinophils. There was no evidence for the generation of chemotactic 5-lipoxygenase products since PF 5901 was without effect. However, PF 5901 is not a potent inhibitor and it is possible that other 5-lipoxygenase inhibitors may be active in this model. In addition, contrary to the effect on oedema formation, the combination of WEB 2086, PF 5901 and chlorpheniramine did not significantly affect the accumulation of radiolabelled eosinophils. Experiments were also performed with purified IgG_1 . Results from such experiments gave very similar results to those obtained with unfractionated sera, further indicating that IgG_1 is likely to be the predominant antibody type responsible for the anaphylactic reactions investigated in these experiments. The apparent lack of involvement of PAF and LTB_4 in the present system differs from other reports where these mediators appear to be implicated in the eosinophil accumulation observed in models of lung anaphylaxis in the guinea-pig (Silbaugh *et al.*, 1987; Lellough-Tubiana *et al.*, 1988; Richards *et al.*, 1989; Gulbenkian *et al.*, 1990). Several factors could account for differences

between cutaneous and lung anaphylaxis. Among those is the fact that subpopulations of mast cells show functional heterogeneity in their ability to respond to immunological (IgE/IgG_1 -dependent) or non-immunological (e.g. substance P, compound 48/80) stimuli (Church *et al.*, 1991).

Mast cells from guinea-pig lung and mesentery are almost totally unresponsive to non-cytotoxic concentrations of 48/80 (Ennis & Pearce, 1980; Barrett *et al.*, 1982). Similarly, dispersed human lung mast cells (Ennis, 1982), failed to release histamine on incubation with low doses of 48/80. In contrast, 48/80 injected intradermally in the guinea-pig liberates local histamine which is accompanied by mast cell degranulation (Kay, 1970). In the same way human skin mast cells are able to respond to non-immunological stimuli such as compound 48/80 (Atkins *et al.*, 1973; Dor *et al.*, 1983). Thus guinea-pig lung and cutaneous mast cells appear to respond similarly to the corresponding human cells when challenged with 48/80. Furthermore, the fact that guinea-pig skin, but not lung mast cells, respond to 48/80 indicates functional heterogeneity between mast cell populations within this species. These observations were further extended in this study. The local administration of 48/80 in guinea-pig skin led to a dose-related oedema formation which was accompanied by the accumulation of radiolabelled-eosinophils at doses ranging from 1–100 $\mu\text{g/skin}$ site of the secretagogue. Furthermore, the co-injection of 48/80 with the H_1 antagonist chlorpheniramine markedly inhibited the oedema formation induced by all doses of 48/80. In addition, like the results observed with the PCA responses, the PAF antagonist WEB 2086 and the 5-lipoxygenase inhibitor PF5901 alone or in combination failed to inhibit the local infiltration of ^{111}In -eosinophils.

Taken together these results suggest that mast cell products can be chemotactic for eosinophils and that these mediators can be released after immunological and non-immunological stimulation. From the results presented in this paper it seems unlikely that PAF, LTB_4 or histamine account for the eosinophil accumulation observed during guinea-pig PCA. Recently, it has been demonstrated that mast cells are able to produce a group of cytokines including interleukin-3 (IL-3), IL-4, IL-5 and GM-CSF (Plaut *et al.*, 1989; Wodnar-Filipowicz *et al.*, 1989), all of which have been implicated in the process of leukocyte accumulation (reviewed in Noursargh, 1993). Furthermore, normal mast cells contain substantial stores of preformed tumour necrosis factor- α (TNF α) that are available for immediate release upon appropriate stimulation of the cell (Gordon *et al.*, 1990). Some evidence suggests that TNF (Watson *et al.*, 1993) may play a role in eosinophil accumulation, perhaps because of its action in inducing adhesion molecule expression on the endothelium, or alternatively it may be an intermediate in triggering the release of eosinophil chemoattractants. Recently, two members of the C-C chemokine family, namely RANTES and MIP-1 α , have been shown to induce the migration and activation of eosinophils *in vitro* (Kameyoshi *et al.*, 1992; Rot *et al.*, 1992). Neither RANTES nor MIP-1 α exhibit neutrophil activating properties (Rot *et al.*, 1992; Kameyoshi *et al.*, 1992). Furthermore, intradermal injection of IL-8 in the guinea-pig caused significant accumulation of radiolabelled eosinophils *in vivo* (Collins *et al.*, 1993). It is possible that these and/or other unidentified factors are contributing for the eosinophil accumulation observed in the present study and the generation and activity of such mediators are currently under investigation.

In summary, the present study indicates that eosinophil accumulation in this model of allergic inflammation involves mediators other than histamine, PAF or 5-lipoxygenase products. This is in contrast with the plasma leakage in this reaction, which can be abolished by a combination of antagonists blocking the effects of these mediators (Weg *et al.*, 1991). The characterization of novel eosinophil attractants may allow the development of anti-inflammatory drugs

which inhibit eosinophil-mediated tissue damage in allergic disease.

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