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Characterization of chemokine CCR3 agonist-mediated eosinophil recruitment in the Brown-Norway rat

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1 The ability of various C-C chemokines to elicit tissue eosinophil infiltration following intradermal injection or peripheral blood eosinophilia following intravenous injection were compared in the Brown-Norway rat.

2 Eotaxin ($0.1-3$ μg site^{-1}) of human and murine origin produced equivalent, dose-dependent increases in eosinophil peroxidase activity in rat dermis 4 h post-injection.

3 Human eotaxin-2 was equipotent with human eotaxin in terms of dermal eosinophil recruitment. Other human CCR3 agonists, such as MCP-3, RANTES and MCP-4 failed to increase dermal eosinophil peroxidase activity at doses up to 1 μg site^{-1} whereas the latter did produce a small effect at 3 μg site^{-1}.

4 Consistent with observations *in vivo*, human eotaxin displaced [^{125}I]-eotaxin from rat spleen membranes more potently ($IC_{50}=2$ nM) than did MCP-4 ($IC_{50}=500$ nM). RANTES did not compete with the radiolabelled chemokine at concentrations up to 1 μM.

5 Human eotaxin (5 μg) administered intravenously increased circulating eosinophils $\sim$3 fold whereas MCP-4 (5 μg, i.v.) increased circulating monocytes $\sim$3 fold without affecting eosinophil numbers.

6 Dexamethasone pretreatment inhibited eotaxin-induced dermal eosinophil influx only at a steroid dose (0.1 mg kg^{-1}, s.c.) which significantly reduced circulating eosinophil numbers. The steroid also reduced eosinophilia in peripheral blood resulting from systemic eotaxin administration (5 μg, i.v.).

7 These data suggest differences in rat CCR3 relative to other species as surmised from a distinctive rank order of chemokine potency. In addition to its chemotactic effects eotaxin, but not MCP-4, promotes eosinophil recruitment into the circulation. One of the mechanisms by which glucocorticoids, such as dexamethasone, acutely inhibits eotaxin-induced dermal eosinophil influx is to diminish the circulating numbers of these cells available for tissue recruitment.

Keywords: Brown-Norway rat; eosinophils; C-C chemokines; steroids

Abbreviations: B-N, Brown-Norway; BSA, bovine serum albumin; EPO, eosinophil peroxidase; PBS, phosphate buffered saline

Introduction

Asthma is currently defined as an inflammation of the airways in which the eosinophil is a major infiltrating cell (NIH/NHLBI, 1995). The eosinophil's contribution to the pathogenesis of the disease has been inferred from studies demonstrating a correlation between the clinical severity of asthma with airway eosinophils and their granular proteins, such as major basic protein and eosinophil cationic protein (Bousquet *et al*., 1990; Broide *et al*., 1991; Virchow *et al*., 1992). An increased understanding of the mechanisms by which eosinophils migrate out of the bone marrow and into the airways may offer new therapeutic opportunities for the treatment of allergic diseases. For example, the cytokine IL-5 has been shown to be a critical factor in eosinophil growth, differentiation, activation and recruitment from bone marrow (Sanderson, 1992; Palframan *et al*., 1998a). However, while IL-5 has been reported to be chemotactic for eosinophils, its activity is relatively weak (Wang *et al*., 1989) suggesting the importance of other factors in tissue specific recruitment of these cells.

Eotaxin is a C-C chemokine which was originally identified as an eosinophil specific chemotactic agent derived from the bronchoalveolar lavage fluid of sensitized guinea-pigs following antigen challenge (Jose *et al*., 1994). Murine eotaxin has also been identified and its lung tissue expression has been shown to parallel eosinophil accumulation during pulmonary inflammation (Gonzalo *et al*., 1996). The importance of this chemokine in human airway disease is supported by evidence of increased eotaxin expression in lung tissue which correlates with eosinophil numbers 4 h after allergen exposure (Brown *et al*., 1998). Eotaxin specifically binds to and activates the chemokine receptor CCR3 which has been cloned as a highly expressed receptor from human eosinophils (Combadiere *et al*., 1995; Ponath *et al*., 1996a). The human CCR3 receptor also binds other eosinophil chemoattractants including RANTES, MCP-2, 3 and 4 (Heath *et al*., 1997) and eotaxin-2 (White *et al*., 1997; Forssman *et al*., 1997). Like eotaxin, CCR3 mRNA has been shown to be upregulated in bronchial mucosal biopsies obtained from atopic relative to non-atopic patients which is consistent with the role C-C chemokines are proposed to play in the pathogenesis of asthma (Ying *et al*., 1997).

The Brown-Norway (B-N) rat has been used to model allergic airway diseases by a number of investigators since these antigen-sensitized animals exhibit early- and late-phase responses, marked lung eosinophilia and airway hyperresponsiveness following antigen challenge (Eidelman *et al*., 1988; Elwood *et al*., 1992; Haczku *et al*., 1995; Uyama *et al*., 1995). Although the rat CCR3 chemokine receptor has been cloned

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(Flanagan *et al*., 1998), little is known about its agonist specificity. In these studies, the B-N rat was used to evaluate the ability of C-C chemokines to recruit eosinophils from bone marrow into the blood upon systemic administration and into skin tissue following local injection. In the process, we have revealed species differences in rat responses to these chemokines relative to those which have been reported for guinea-pig and human. Finally, since glucocorticoids are currently standard anti-inflammatory therapy for the treatment of asthma, the effects of steroids on eotaxin-induced eosinophil effects were examined.

Methods

Animals and antigen-sensitization

Male Brown-Norway rats (150–200 g) were purchased from Charles River Laboratories (Raleigh, NC, U.S.A.). In some experiments, animals were sensitized to chick egg ovalbumin by administration of a single 0.2 ml s.c. injection containing 1 mg ovalbumin and 4.5 mg alum (Imject®, Pierce, Rockford, IL, U.S.A.). Animals were used for experimentation 2 weeks following sensitization. All procedures were approved by the Pfizer Animal Care and Use Committee.

Dermal effects of chemokines

Animals were anaesthetized using methoxyflurane inhalation. Anaesthesia was determined by the failure of the animal to respond with flinching to a toe pinch or injection. The dorsal surface of the animal was shaved and intradermal injections of vehicle (0.1% bovine serum albumin (BSA)/phosphate buffered saline (PBS)) or inflammatory stimuli were made in a volume of 100 μl. Four (chemokine) or 24 (ovalbumin) h following injection, animals were euthanized by CO_2 asphyxiation. The dorsal surface was skinned and 13 mm dermal punch biopsies made. Biopsies were placed in 8 ml 0.5% hexadecyltrimethyl ammonium bromide (HTAB)/potassium phosphate buffer ($KHPO_4$), homogenized for 15 s at 15,000 r.p.m. using a Cyclone I.Q.[2] homogenizer (VirTis Co., Gardiner, NY, U.S.A.) and frozen at -20°C. Samples were thawed and refrozen twice then centrifuged at $2100 \times g$, 20°C for 30 min. The supernatant was assayed for eosinophil peroxidase (EPO) activity by the method of Strath *et al*. (1985). Samples (50 μl) diluted in 0.5% HTAB/KPO_4 buffer were incubated with 50 mM Tris HCl containing 0.1% Triton X-100 and 0.03% hydrogen peroxide at room temperature for 5 min. The reaction was terminated by the addition of 0.4 M sulphuric acid. Absorbance was read at 490 nm using a microtiter plate reader (Molecular Devices, Menlo Park CA, U.S.A.). Previous studies have demonstrated that EPO activity in dermal biopsies is predominantly (~90%) due to the presence of eosinophils (Pettipher *et al*., 1994).

For histological analysis, biopsies were placed in 10% PBS buffered formalin and fixed for at least 24 h. Skin was trimmed cross-sectionally and processed for standard light microscopy. Five μm paraffin-embedded sections were cut on a Reichert Jung rotary microtome. Tissue was stained with a Luna stain as modified for eosinophil detection. Cell counts were averaged from four different fields per section.

To examine effects of dexamethasone on chemokine-mediated dermal eosinophil influx, either vehicle (2% Tween 80 in 0.9% NaCl) or dexamethasone (0.001–0.1 mg kg^{-1}, s.c.) was administered 1 h prior to chemokine injection.

[^{125}I]-Human eotaxin binding assays

Since the rat spleen has been shown to contain numerous eosinophils (Tchernitchin *et al*., 1990), fresh tissues were homogenized in 50 mM Tris buffer pH 7.4 followed by centrifugation at $1300 \times g$ for 15 min, 4°C. The supernatant was then centrifuged at $43{,}000 \times g$ for 10 min, 4°C. The pellet was resuspended in 50 mM Tris buffer pH 7.4. Protein concentration was adjusted to approximately 2 mg ml^{-1} using the Bio-Rad assay (Bio-Rad Laboratories, Hercules, CA, U.S.A.). Membrane preparations were stored at -80°C until use. Receptor binding was initiated by addition of 100 μl membrane preparation (final protein concentra-

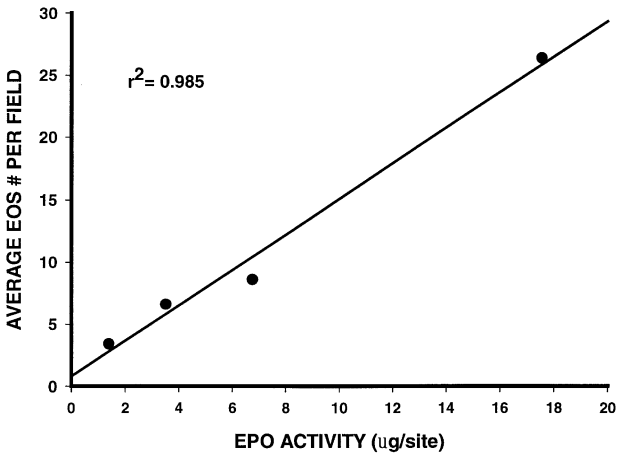

Figure 1 Correlation between eosinophil peroxidase activity and eosinophil number in dermal biopsies. Ovalbumin-sensitized rats received intradermal injections of the antigen at varying doses (0, 0.01, 0.1 and 1 mg) and skin biopsies were made 24 h later. Eosinophil peroxidase activity was measured in biopsies and expressed as average value ($n=8$) at each ovalbumin concentration. Cell counts represent the average value from four fields made on biopsies from $n=2$ rats. Using linear regression analysis, the correlation between EPO activity and eosinophil number was found to be 0.985.

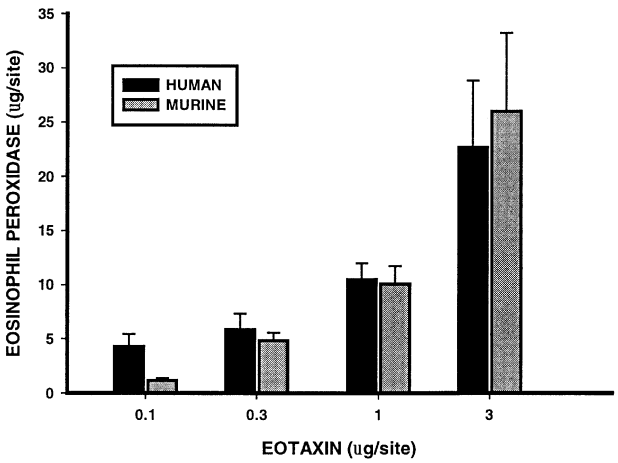

Figure 2 Dermal eosinophil peroxidase response to eotaxin from various species. Rats were injected intradermally with various eotaxin doses (0.1–3 μg) and biopsies were made 4 h later. Eosinophil peroxidase activity (EPO) was measured as described in Methods. Values are EPO activity expressed as average ± s.e.mean ($n=7$–8 animals per chemokine) above background (i.e. dermal biopsy from injection of 0.1% BSA/PBS; 3.73 ± 0.24 μg $site^{-1}$, $n=15$).

tion = 50 ng ml^{-1}) in duplicate to 0.5 nM of [^{125}]-human eotaxin (Amersham, Arlington Hts., IL, U.S.A., specific activity = 2000 Ci mmol^{-1}) in a final volume of 255 μl buffer containing 50 mM Tris, 10 mM $MgCl_2$ 0.5% heat inactivated foetal bovine serum (pH 7.4 at room temperature) and various doses of the chemokines. Incubations were performed at room temperature for 2.5 h. Binding was terminated by washing three times with 0.5 M NaCl, 10 mM HEPES buffer and filtration under vacuum through GF/B filters presoaked with 0.2% polyethyleneimine. Nonspecific binding was defined as binding in the presence of 0.2 μM unlabelled human eotaxin. Specific binding was calculated by subtracting nonspecific from total binding and was approximately 90%.

Systemic effects of chemokines

Animals were anaesthetized with pentobarbital (50 mg kg^{-1}, i.p.). Vehicle (0.1% BSA/PBS) or chemokine was injected in a total volume of 200 μl i.v. *via* tail vein. Thirty minutes following injection blood samples were taken by cardiac puncture and analysed for total and differential white blood cell counts using the Cell-Dyn 3500 automated cell counting system (Abbott, Chicago, IL, U.S.A.). To examine the effects of dexamethasone on eotaxin-mediated changes in circulating peripheral blood cells, animals were pretreated with vehicle (2% Tween 80 in 0.9% NaCl) or dexamethasone (0.1 mg kg^{-1}, s.c.) 1 h prior to chemokine injection.

Reagents

All chemokines, with the exception of guinea-pig eotaxin (which was synthesized by Glen Andrews, Pfizer Inc.) were obtained from PeproTech Inc (Rocky Hill, NJ, U.S.A.). Unless otherwise noted, other reagents were obtained from Sigma Chemical Company (St. Louis, MO, U.S.A.).

Statistics

All data is expressed as average ± s.e.mean. Statistical analyses of differences between two groups were made using Student's *t*-test and for multiple groups were made using one-way analysis of variance followed by multiple comparison procedures, such as Dunnett's Method (SigmaSTAT, Jandel Scientific, San Rafael, CA, U.S.A.). Significant difference was assumed at $P < 0.05$.

Results

Ovalbumin-induced dermal accumulation of eosinophils in sensitized B-N rats

Intradermal injection of ovalbumin into rats sensitized with the same caused a dose-dependent increase in dermal EPO activity at 24 h (Figure 1) but not 4 h (data not shown) post-injection. Light microscopic analysis confirmed a dose-dependent increase in eosinophils which correlated well with enzyme activity (Figure 1). Neutrophils were significantly and equivalently increased above background level at all ovalbumin concentrations (40 – 50 cells per field) whereas monocyte and lymphocyte numbers were not significantly different between the ovalbumin doses (data not shown). Hence dermal eosinophil peroxidase activity correlated with dermal eosinophil influx and was used as a measure of such in subsequent experiments.

Effects of eotaxin on eosinophil influx into B-N rat skin

Human eotaxin injected intradermally into sensitized rats dose-dependently (0.1 – 3 μg site^{-1}) increased EPO activity at 4 h post-administration (Figure 2) which was significantly different from vehicle injection at all doses. This was confirmed by light microscopic analysis of the dermis which revealed approximately 3 fold increase in the number of eosinophils (9.4 cells per field, $n = 3$) relative to vehicle treatment (2.8 cells per field, $n = 3$) following injection of 1 μg of eotaxin site^{-1}. A small increase (1.4 ×) in neutrophils was also observed following eotaxin but no significant differences in numbers of monocytes or lymphocytes. Dermal EPO activity measured was not significantly different between sensitized and non-sensitized rats following human eotaxin administration (data not shown). The potency of murine eotaxin was similar to that of human eotaxin (Figure 2). Guinea-pig eotaxin also increased dermal EPO activity to a similar degree at a dose of 1 μg site^{-1} (data not shown).

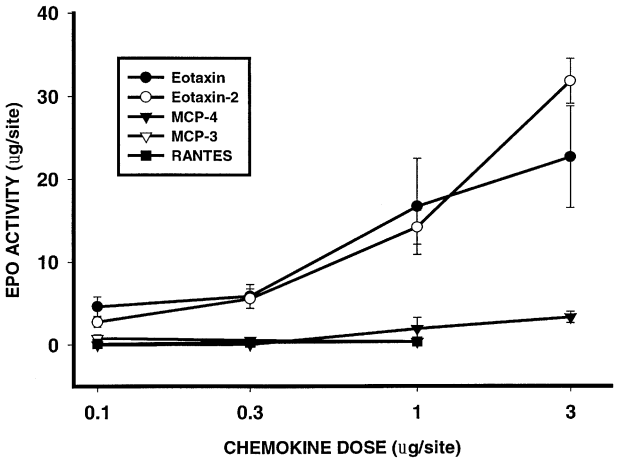

Figure 3 Effects of various human chemokines on dermal eosinophil peroxidase activity. Rats were injected intradermally with various chemokine doses (0.1 – 3 μg) and biopsies made 4 h later. Values are average ± s.e.mean ($n = 7 – 8$ per chemokine). Values are expressed as EPO activity above background (i.e. dermal biopsy from injection of 0.1% BSA/PBS; 4.23 ± 0.20 μg site^{-1}, $n = 25$).

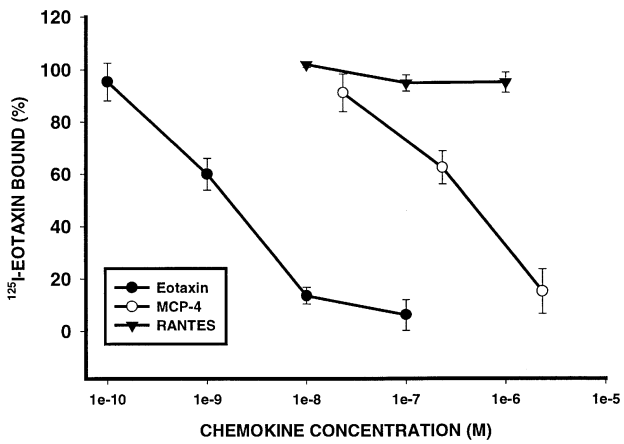

Figure 4 Displacement of [^{125}I]-human eotaxin binding to Brown-Norway rat spleen membranes by human chemokines. Values are average ± s.e.mean of data from three separate experiments.

Comparison of eotaxin-induced eosinophil influx with other CCR3 agonists

Eosinophil chemoattraction produced in response to other C-C chemokines known to be agonists for human CCR3 was compared to eotaxin in sensitized rats. Eotaxin-2 caused a dose-dependent (0.1 – 3 μg site^{-1}) increase in EPO activity 4 h post-injection which was similar in magnitude to that elicited by human eotaxin (Figure 3). MCP-3 (0.1 – 1 μg site^{-1}) elicited no measurable effects on eosinophil accumulation in the rat dermis. MCP-4 increased EPO acitivity at 1 μg which was significant at a dose of 3 μg site^{-1} (Figure 3) however this activity was approximately 6 fold less than that produced by eotaxin at the same dose. Neither human nor rat RANTES increased EPO activity at either 4 (Figure 3) or 24 h (data not shown) following dermal injection. Furthermore, EPO activity following co-injection of human eotaxin (1 μg) with human RANTES (0.1 – 1 μg site^{-1}) was not significantly different from that obtained from injection of eotaxin alone (data not shown).

Chemokine receptor binding affinities in rat spleen membranes

[^{125}I]-human eotaxin was displaced by non-labelled human eotaxin in rat spleen membrane preparations with $IC_{50} = 1.6 \pm 0.34$ nM (Figure 4). MCP-4 was less potent ($IC_{50} = 500 \pm 180$ nM) than eotaxin and RANTES failed to significantly displace [^{125}I]-eotaxin binding at concentrations up to 1 μM. In human eosinophils, [^{125}I]-eotaxin was displaced by eotaxin, MCP-4 and RANTES with IC_{50} s = 1.2, 0.9 and 29 nM, respectively (data not shown).

Effects of chemokines on circulating peripheral blood leukocytes

Systemic administration of human eotaxin (0.5 – 5 μg, i.v.) to rats caused a dose-dependent increase in peripheral blood eosinophils 30 min following chemokine injection (Figure 5). Unlike eotaxin, systemic administration of human MCP-4 (5 μg, i.v.) had no significant effects on circulating eosinophils (Figure 5) but did increase the circulating monocytes approximately 3 fold above control (Figure 6).

Effects of dexamethasone on eotaxin-induced dermal eosinophil influx and eosinophilia

Dexamethasone (0.001 – 0.1 mg kg^{-1}, s.c.) administered 1 h prior to intradermal eotaxin injection significantly reduced tissue EPO levels measured 4 h post-chemokine injection relative to vehicle-treated control only at the highest steroid dose (Figure 7). To understand the mechanisms by which dexamethasone may be affecting eotaxin-induced tissue eosinophilia, separate experiments were performed in which animals were dosed with dexamethasone (0.001 – 0.1 mg kg^{-1},

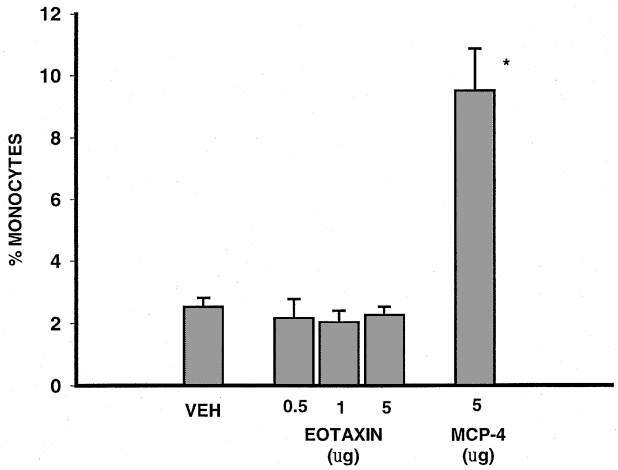

Figure 6 Effects of systemic chemokines on circulating monocytes. Rats received vehicle or chemokine by i.v. bolus. Thirty minutes later, blood samples were taken and differential white blood cell counts made. Values are average ± s.e.mean from 7 – 17 animals per group. *Indicates significant differences from vehicle control at $P < 0.05$.

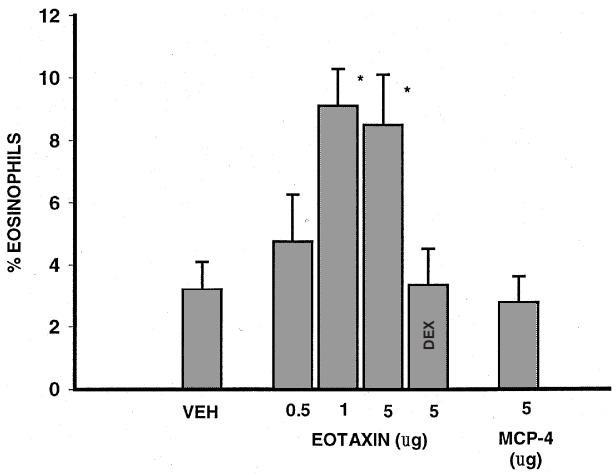

Figure 5 Effects of systemic chemokines on circulating eosinophils. Rats received vehicle (0.1% BSA/PBS) or chemokine by i.v. bolus. Thirty minutes later, blood samples were taken and differential white blood cell counts made. One group of rats received dexamethasone (0.1 mg kg^{-1}, s.c.) 1 h prior to eotaxin (5 μg) injection. Values are average ± s.e.mean from 7 – 17 animals per group. *Indicates significant differences from vehicle control at $P < 0.05$.

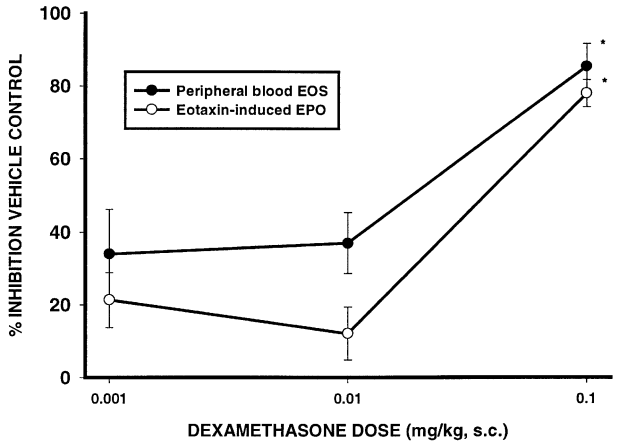

Figure 7 Dose-response for dexamethasone-induced inhibition of eotaxin (1 μg)-induced increase in dermal EPO activity vs reduction in peripheral blood eosinophils. Rats received dexamethasone (0.001 – 0.1 mg kg^{-1}) or an equivalent volume of vehicle (2% Tween 80 in 0.9% NaCl) subcutaneously 1 h prior to intradermal chemokine injection or, in separate experiments, 5 h prior to blood collection. Dermal biopsies were made 4 h following eotaxin injection. Data are expressed as average ± s.e.mean percentage of the vehicle control value. $n = 7 – 8$ animals per dose per treatment. *Indicates significant differences from vehicle control at $P < 0.05$.

s.c.) and blood samples taken 5 h following administration (Figure 7). Circulating eosinophil numbers were significantly reduced following dexamethasone 0.1 mg kg^{-1}, s.c. treatment. Dexamethasone (0.1 mg kg^{-1}, s.c.) administered 1 h prior to i.v. eotaxin (5 μg) also inhibited the chemokine-induced increase in circulating eosinophils (Figure 5).

These data demonstrate that the dose of dexamethasone which significantly inhibited eotaxin-induced dermal influx was equivalent to the dose which reduced both basal circulating eosinophil numbers and the increase which occurred in response to systemic eotaxin administration.

Discussion

CCR3 agonists, such as eotaxin, are known to be potent chemoattractants for human eosinophils (Heath *et al*., 1997; Stellato *et al*., 1997). The studies presented here demonstrate that eotaxin, derived from human, mouse and guinea-pig, are equivalent in their ability to elicit dermal eosinophil influx in B-N rats. It is not unexpected that murine eotaxin should be efficacious in the rat since it shares 97% amino acid sequence with the rat chemokine (Williams *et al*., 1998). However, overlap of biological activity is less expected in the case of human and guinea-pig chemokines which are only 62 and 64% similar to rat, respectively (Williams *et al*., 1998). Eotaxin-2, like eotaxin, has been shown to selectively recruit human eosinophils *via* CCR3 (Forssmann *et al*., 1997). In our experiments, the response to human eotaxin-2 was equivalent to eotaxin in spite of the fact that these two chemokines share only 39% identical amino acids (Forssmann *et al*., 1997). Dermal injection of the same dose of both chemokines also elicited a comparable degree of eosinophil influx in rhesus monkeys (Forssmann *et al*., 1997). These data suggest that common structural features responsible for the chemokine activity in the rat reside distally from the NH_2-terminal region since this is the area of greatest dissimilarity in eotaxin derived from various species as well as for eotaxin-2 (Ponath *et al*., 1996b).

In these studies the rank order of potency of human C-C chemokines for production of dermal eosinophil influx in the B-N rat was eotaxin = eotaxin-2 > > MCP-4 > > > RANTES, MCP-3. The rank order of potency *in vivo* was consistent with affinities observed for the chemokines in receptor binding studies; the IC_{50} for eotaxin displacement of eotaxin binding was 250 fold less than MCP-4 and RANTES was ineffective at concentrations up to 1 μM. The receptor binding affinity and chemotactic potency of these chemokines for human CCR3 are: MCP-4 ~ eotaxin > MCP-3 > RANTES (Stellato *et al*., 1997; Kudlacz *et al*., unpublished data). In cynomolgus monkeys, the rank order of potency for eliciting dermal eosinophil influx was MCP-4 > eotaxin > MCP-3 (Beck *et al*., 1998). Hence these data highlight significant species differences in response to the same CCR3 chemokines. Rat CCR3 has been identified and reported to possess a 'high' degree of similarity to mouse and human sequences, particularly in the transmembrane and carboxyterminal sequence (Flanagan *et al*., 1998). However, significant differences in the rank order of CCR3 agonist potencies suggest species differences in critical agonist binding regions of the receptor.

In our studies, human RANTES failed to elicit dermal eosinophil influx, nor did it antagonize eotaxin-induced eosinophil influx upon co-injection. The lack of RANTES effects *in vivo* were consistent with its inability to displace radiolabelled eotaxin from rat spleen and suggest that this chemokine does not bind to the rat eotaxin receptor. Similarly, RANTES does not elicit eosinophil recruitment in the guinea-pig (Campbell *et al*., 1997; Marleau *et al*., 1996). However, unlike the rat, RANTES has been shown to antagonize eotaxin-induced eosinophil accumulation in guinea-pigs and eotaxin has been shown to displace [^{125}I]-RANTES binding from guinea-pig eosinophils (Marleau *et al*., 1996) or CCR3 transfectants from this species (Sabroe *et al*., 1998). These data suggest species differences in RANTES binding to and activation of CCR3. Whereas neither rat nor guinea-pig eosinophils respond functionally to RANTES, the chemokine does promote monocyte recruitment into the site of administration in both species (Braciak *et al*., 1996; Marleau *et al*., 1996).

Systemic eotaxin administration increased circulating eosinophils in the rat 30 min post-injection by approximately 3 fold above basal levels. Eotaxin leakage into the peripheral blood following dermal injection may also increase circulating eosinophil numbers thereby enhancing the magnitude of its chemotactic effect. It has been previously demonstrated that eotaxin stimulates a rapid blood eosinophilia in guinea-pigs which corresponds to a selective, dose-dependent release of these cells from the bone marrow (Palframan *et al*., 1998b). However, not all chemokines which increased dermal EPO activity in the rat were capable of mobilizing eosinophils into the circulation. MCP-4, which also elicited a small, but significant increase in dermal eosinophil accumulation, did not increase circulating eosinophil numbers upon systemic administration. It is unlikely that the absence of this effect was due to insufficient circulating levels of the chemokine since monocyte numbers were increased relative to other cell populations presumably as a result of CCR2b activation (Garcia-Zepeda *et al*., 1996; Stellato *et al*., 1997).

The ability of glucocorticoids to reduce eosinophil influx by modulating adhesion molecule expression and chemokine levels may contribute to their overall efficacy in the treatment of allergic airway diseases (Van der Velden, 1998). In these studies, pretreatment of rats with dexamethasone at a dose $\geq$0.1 mg kg^{-1}, s.c. inhibited eotaxin-induced eosinophil influx. The same dose of dexamethasone also significantly reduced basal circulating eosinophil numbers and inhibited the rise in circulating eosinophils following systemic eotaxin administration. These data are consistent with reports which have demonstrated that cortisol treatment can selectively promote eosinophil migration from the blood into lymphoid organs, such as the spleen, lymph nodes and thymus, 6 h after administration (Sabag *et al*., 1978). The correlation between the dose of dexamethasone which inhibits eotaxin-mediated dermal eosinophil influx and which significantly reduces circulating eosinophils suggests that one of the mechanisms by which steroids might acutely inhibit inflammatory cell influx is by reducing the circulating pool of eosinophils available for tissue recruitment in response to a chemotactic stimuli.

In conclusion, eotaxin derived from various species promotes eosinophil recruitment in B-N rat dermis. However, the potency and intrinsic activity observed in response to other human C-C chemokines, such as MCP-4, MCP-3 and RANTES appears to vary significantly between species. The ability of eotaxin, but not MCP-4, to facilitate eosinophil mobilization into the circulation may serve to augment their tissue recruitment. Finally, down-regulation of circulating eosinophil numbers may contribute to the anti-inflammatory properties of steroids by reducing circulating cells available for recruitment into sites of chemokine injection.

References

BECK, L., BRUMMET, M., BICKEL, C., LEIFERMAN, K., ANDRESEN, C., GILMAN, S., GLADUE, R., MILCI, A.J. & SCHLEIMER, R. (1998). Cutaneous injections with C-C chemokines; a comparative study in cynomolgous macaques. *J. Allergy Clin. Immunol.,* **101,** S36.

BOUSQUET, J., CHANEZ, P., LACOSTE, J.Y., BARNEON, G., GHAVANIAN, N., ENANDER, I., VENGE, P., AHLSTEDT, S., SIMONY-LAFONTAINE, J., GODARD, P. & MICHEL, F.-B. (1990). Eosinophilic inflammation in asthma. *N. Engl. J. Med.,* **323,** 1033 – 1039.

BRACIAK, T.A., BACON, K., XING, Z., TORRY, D.J., GRAHAM, F.L., SCHALL, T.J., RICHARDS, C.D., CROITORU, K. & GAULDIE, J. (1996). Overexpression of RANTES using a recombinant adenovirus induces the tissue-directed recruitment of monocytes to the lung. *J. Immunol.,* **157,** 5076 – 5084.

BROIDE, D.H., GLEICH, G.J., CUOMO, A.J, COBURN, D.A., FEDERMAN, E.C., SCHWARTZ, L.B. & WASSERMAN, S.I. (1991). Evidence of ongoing mast cell and eosinophil degranulation in symptomic asthma airway. *J. Allergy Clin. Immunol.,* **88,** 637 – 648.

BROWN, J.R., KLEIMBERG, J., MARINI, M., SUN, G., BELLINI, A. & MATTOLI, S. (1998). Kinetics of eotaxin expression and its relationship to eosinophil accumulation and activation in bronchial biopsies and bronchoalveolar lavage (BAL) of asthmatic patients after allergen inhalation. *Clin. Exp. Immunol.,* **114,** 137 – 146.

CAMPBELL, E.M., PROUDFOOT, A.E., YOSHIMURA, T., ALLET, B., WELLS, T.N., WHITE, A.-M., WESTWICK, J. & WATSON, M.L. (1997). Recombinant guinea pig and human RANTES activate macrophages but not eosinophils in the guinea pig. *J. Immunol.,* **159,** 1482 – 1489.

COMBADIERE, C., AHUJA, S.K. & MURPHY, P.M. (1995). Cloning and functional expression of a human eosinophil CC chemokine receptor. *J. Biol. Chem.,* **270,** 16491 – 16494.

EIDELMAN, D.H., BELLOFIORE, S. & MARTIN, J.G. (1988). Late airway responses to antigen challenge in sensitized inbred rats. *Am. Rev. Respir. Dis.,* **137,** 1033 – 1037.

ELWOOD, W., BARNES, P.J. & CHUNG, K.F. (1992). Airway hyperresponsiveness is associated with inflammatory cell infiltration in allergic Brown-Norway rats. *Int. Arch. Allergy Immunol.,* **99,** 91 – 97.

FLANAGAN, B.F., HARRINGTON, P.M., NEWTON, D.J. & COLEMAN, J.W. (1998). Conserved sequence and tissue expression of the rat CCR3 chemokine receptor. *J. Allergy Clin. Immunol.,* **101,** S42.

FORSSMANN, U., UGUCCIONI, M., LOETSCHER, P., DAHINDEN, C.A., LANGEN, H., THELEN, M. & BAGGIOLINI, M. (1997). Eotaxin-2, a novel CC chemokine that is selective for the chemokine receptor CCR3, and acts like eotaxin on human eosinophil and basophil leukocytes. *J. Exp. Med.,* **185,** 2171 – 2176.

GARCIA-ZEPEDA, E.A., COMBADIERE, C., ROTHENBERG, M.E., SARAFI, M.N., LAVIGNE, F., HAMID, Q., MURPHY, P.M. & LUSTER, A.D. (1996). Human monocyte chemoattractant protein (MCP)-4 is a novel CC chemokine with activities on monocytes, eosinophils, and basophils induced in allergic and nonallergic inflammation that signals through the CC chemokine receptors (CCR)-2 and -3. *J. Immunol.,* **157,** 5613 – 5626.

GONZALO, J.-A., JIA, G.-Q., AGUIRRE, V., FRIEND, D., COYLE, A.J., JENKINS, N.A., LIN, G.-S., KATZ, H., LICHTMAN, A., COPELAND, N., KOPF, M. & GUTIERREZ-RAMOS, J.-C. (1996). Mouse eotaxin expression parallels eosinophil accumulation during lung allergic inflammation but it is not restricted to a Th2-type response. *Immunity,* **4,** 1 – 14.

HACZKU, A., CHUNG, K.F., SUN, J., BARNES, P.-J., KAY, A.B. & MOQBEL, R. (1995). Airway hyperresponsiveness, elevation of serum-specific IgE and activation of T cells following allergen exposure in sensitized Brown-Norway rats. *Immunology,* **85,** 598 – 603.

HEATH, H., QIN, S., RAO, P., WU, L., LAROSA, G., KASSAM, N., PONATH, P.D. & MACKAY, C.R. (1997). Chemokine receptor usage by human eosinophils. *J. Clin. Invest.,* **99,** 178 – 184.

JOSE, P.J., GRIFFITHS-JOHNSON, D.A., COLLINS, P.D., WALSH, D.T., MOQBEL, R., TOTTY, N.F., TRUONG, O., HSUAN, J.J. & WILLIAMS, T.J. (1994). Eotaxin: a potent eosinophil chemoattractant cytokine detected in a guinea pig model of allergic airways inflammation. *J. Exp. Med.,* **179,** 881 – 887.

MARLEAU, S., GRIFFITHS-JOHNSON, D.A., COLLINS, P.D., BAKHLE, Y.S., WILLIAMS, T.J. & JOSE, P.J. (1996). Human RANTES acts as a receptor antagonist for guinea pig eotaxin in vitro and in vivo. *J. Immunol.,* **157,** 4141 – 4146.

NATIONAL INSTITUTES OF HEALTH, NATIONAL HEART, LUNG AND BLOOD INSTITUTE. (1995). Global initiative for asthma. Global strategy for asthma management and prevention. NHLBI/WHO Workshop Report.

PALFRAMAN, R.T., COLLINS, P.D., SEVERS, N.J., ROTHERY, S., WILLIAMS, T.J. & RANKIN, S.M. (1998a). Mechanisms of acute eosinophil mobilization from the bone marrow stimulated by interleukin 5: the role of specific adhesion molecules and phosphatidylinositol 3-kinase. *J. Exp. Med.,* **188,** 1621 – 1632.

PALFRAMAN, R.T., COLLINS, P.D., WILLIAMS, T.J. & RANKIN, S.M. (1998b). Eotaxin induces a rapid release of eosinophils and their progenitors from the bone marrow. *Blood,* **91,** 2240 – 2248.

PETTIPHER, E.R., SALTER, E.D. & SHOWELL, H.J. (1994). Effect of in vivo desensitization to leukotriene B4 on eosinophil infiltration in response to C5a in guinea-pig skin. *Br. J. Pharmacol.,* **113,** 117 – 120.

PONATH, P.D., QIN, S., POST, T.W., WANG, J., WU, L., GERARD, G.P., NEWMAN, W., GERARD, C. & MACKAY, C. R. (1996a). Molecular cloning and characterization of a human eotaxin receptor selectively expressed on eosinophils. *J. Exp. Med.,* **183,** 2437 – 2448.

PONATH, P.D., QIN, S., RINGLER, D.J., CLARK-LEWIS, I., WANG, J., KASSAM, N., SMITH, H., SHI, X., GONZALO, J.-A., NEWMAN, W., GUTIERREZ-RAMOS, J.-C. & MACKAY, C.R. (1996b). Cloning of the human eosinophil chemoattractant, eotaxin. Expression, receptor binding, and functional properties suggest a mechanism for the selective recruitment of eosinophils. *J. Clin. Invest.,* **97,** 604 – 612.

SABAG, N., CASTRILLON, M.A. & TCHERNITCHIN, A. (1978). Cortisol-induced migration of eosinophil leukocytes to lymphoid organs. *Experentia,* **34,** 666 – 667.

SABROE, I., CONROY, D.M., GERARD, N.P., LI, Y., COLLINS, P.D., POST, T.W., JOSE, P.J., WILLIAMS, T.J., GERARD, C.J. & PONATH, P.D. (1998). Cloning and characterization of the guinea pig eosinophil eotaxin receptor, C-C chemokine receptor-3: blockade using a monoclonal antibody in vivo. *J. Immunol.,* **161,** 6139 – 6147.

SANDERSON, C.J. (1992). Interleukin-5, eosinophils, and disease. *Blood,* **79,** 3101 – 3109.

STELLATO, C., COLLINS, P., PONATH, P.D., SOLER, D., NEWMAN, W., LA ROSA, G., LI, H., WHITE, J., SCHWIEBERT, L.M., BICKEL, C., LIU, M., BOCHNER, B.S., WILLIAMS, T. & SCHLEIMER, R.P. (1997). Production of the novel C-C chemokine MCP-4 by airway cells and comparison of its biological activity to other C-C chemokines. *J. Clin. Invest.,* **99,** 926 – 936.

STRATH, M., WARREN, D.J. & SANDERSON, C.J. (1985). Detection of eosinophils using an eosinophil peroxidase assay. Its use as an assay for eosinophil differentiation factors. *J. Immunol. Meth.,* **83,** 209 – 215.

TCHERNITCHIN, A.N., CARTER, W., SOTO, J. & BAUMANN, P. (1990). Effect of eosinophil-degranulating estrogens on spleen eosinophils and white pulp/red pulp ratio. *Agents and Actions,* **31,** 249 – 256.

UYAMA, O., IHAKU, D., KITADA, O., MIYASAKA, M. & SUGITA, M. (1995). Role of eosinophils and cell adhesion molecules in the allergen-induced asthmatic response of rats. *Res. Commun. Mol. Pathol. Pharmacol.,* **90,** 3 – 15.

VAN DER VELDEN, O. (1998). Glucocorticoids: mechanisms of action and anti-inflammatory potential in asthma. *Med. Inflamm.,* **7,** 229 – 237.

VIRCHOW, J.C., HOLSCHER, U. & VIRCHOW, C. (1992). Sputum ECP levels correlate with parameters of airflow obstruction. *Am. Rev. Respir. Dis.,* **146,** 604 – 606.

WANG, J.M., RIMBALDI, A., BIONDI, A., CHEN, Z.G., SANDERSON, C.J. & MANTOVANI, A. (1989). Recombinant human interleukin-5 is a selective eosinophil chemoattractant. *Eur. J. Immunol.,* **19,** 701 – 705.

WHITE, J.R., IMBURGIA, C., DUL, E., APPELBAUM, E., O'DONNELL, K., O'SHANNESSY, D.J., BRAWNER, M., FORNWALD, J., ADAMOU, J., ELSHOURBAGY, N.A., KAISER, K., FOLEY, J.J., SCHMIDT, D.B., JOHANSON, K., MACPHEE, C., MOORES, K., MCNULTY, D., SCOTT, G.F., SCHLEIMER, R.P. & SARAU, H.M. (1997). Cloning and functional characterization of a novel CC chemokine that binds to the CCR3 receptor and activates human eosinophils. *J. Leukoc. Biol.,* **62,** 667–675.

WILLIAMS, C.M., NEWTON, D.J., WILSON, S.A., WILLIAMS, T.J., COLEMAN, J.W. & FLANAGAN, B.F. (1998). Conserved structure and tissue expression of rat eotaxin. *Immunogenetics,* **47,** 178–180.

YING, S., ROBINSON, D.S., MENG, Q., ROTTMAN, J., KENNEDY, R., RINGLER, D.J., MACKAY, C.R., DAUGHERTY, B.L., SPRINGER, M.S., DURHAM, S.R., WILLIAMS, T.J. & KAY, A.B. (1997). Enhanced expression of eotaxin and CCR3 mRNA and protein in atopic asthma. Association with airway hyperresponsiveness and predominant co-localization of eotaxin mRNA to bronchial epithelial and endothelial cells. *Eur. J. Immunol.,* **27,** 3507–3516.

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