Relationship between Interleukin-5 and Eotaxin in Regulating Blood and Tissue Eosinophilia in Mice

Arne W. Mould, Klaus I. Matthaei, Ian G. Young, and Paul S. Foster
Division of Biochemistry and Molecular Biology, John Curtin School of Medical Research, Australian National University, Canberra, ACT, 0200, Australia

Abstract

The mechanism of cooperation between IL-5 and eotaxin for the selective accumulation of eosinophils at sites of allergic inflammation is unknown. In this investigation we have used IL-5 deficient mice to define the relationship between this cytokine and eotaxin in the regulation of blood eosinophilia and eosinophil homing and tissue accumulation. Both IL-5 and eotaxin could independently induce a rapid and pronounced blood eosinophilia in wild type mice when administered systemically. In contrast, only eotaxin induced a pronounced blood eosinophilia in IL-5 deficient mice. The eosinophilic response induced by intravenous eotaxin in wild type mice did not correlate with a significant reduction in the level of bone marrow eosinophils, whereas intravenous IL-5 resulted in depletion of this store. These results suggest the existence of two mechanisms by which eosinophils can be rapidly mobilized in response to intravenous eosinophil chemoattractants; first, mobilization of an IL-5 dependent bone marrow pool, and second, an eotaxin-induced sequestration of eosinophils from tissues into the blood. Subcutaneous injection of eotaxin induced a local tissue eosinophilia in wild type mice but not in IL-5 deficient mice. Furthermore, tissue eosinophilia in wild type mice, but not in IL-5 deficient mice, was enhanced by adoptive transfer of eosinophils or the administration of intravenous IL-5. However, pretreatment of IL-5 deficient mice with intraperitoneal IL-5 for 72 h restored eosinophil homing and tissue accumulation in response to subcutaneous eotaxin. We propose that eotaxin secreted from inflamed tissue may play an important role in initiating both blood and tissue eosinophilia in the early phases of allergic inflammation. Furthermore, IL-5 is not only essential for mobilizing eosinophils from the bone marrow during allergic inflammation, but also plays a critical role in regulating eosinophil homing and migration into tissues in response to eotaxin and possibly other specific chemotactic stimuli. (*J. Clin. Invest.* 1997. 99:1064–1071.) Key words: chemotaxis • eosinophils • eotaxin • homing • interleukin-5

Address correspondence to Paul S. Foster, John Curtin School of Medical Research, Australian National University, Canberra, ACT, 0200, Australia. Phone: +61-62-492-032; FAX: +61-62-490-415; E-mail: Paul.Foster@anu.edu.au

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Introduction

The proposed central role of eosinophils in asthma and allergic disease has initiated extensive investigations into the molecular and cellular mechanisms regulating eosinophil trafficking in response to inflammatory stimuli. The recruitment of eosinophils to sites of allergic inflammation is a complex process that potentially may be regulated by the inflammatory cytokines IL-1β, IL-3, IL-4, IL-5, granulocyte macrophage colony stimulating factor (GM-CSF) and tumor necrosis factor α (TNFα),[1] and the chemokines RANTES, monocyte chemoattractant protein-3 (MCP-3), macrophage inflammatory protein-1α (MIP-1α), and eotaxin. IL-3, IL-5 and GM-CSF have been shown to enhance eosinophil development, migration and effector function (1–7), while IL-1β, IL-4, IL-5, and TNFα may regulate eosinophil trafficking by activating adhesion systems in the vascular endothelium (8–13). In conjunction with chemokines (RANTES, MCP-3, MIP-1α, and eotaxin) and lipid mediators (platelet-activating factor and leukotriene B_4), IL-5 may also promote eosinophil chemotaxis within inflamed tissues (14–25). Despite this apparent complexity, it is clear from investigations with IL-5 deficient (IL-$5^{-/-}$) mice that the absence of IL-5 alone abolishes the tissue and blood eosinophilia normally generated by allergic responses and parasite infections (26, 27). Of the cytokines implicated in modulating eosinophilic inflammation, only IL-5 and eotaxin have been identified to selectively regulate eosinophil trafficking (16–18, 28). Currently, it is unknown which of these cytokines provides the essential signal for eosinophil homing and migration into tissues.

IL-5 has been identified as a central mediator in the regulation of eosinophilic inflammation and in the etiology of asthma and allergic disease (7, 26, 27, 29–31). IL-5 not only regulates the growth, differentiation, and activation of eosinophils (1–6), but also provides an essential signal for the induction of eosinophilia during allergic inflammation (7, 26, 27). Investigations with IL-$5^{-/-}$ mice have established that blood and airways eosinophilia, and the subsequent development of lung damage and airways hyperreactivity, which occurs in response to aeroallergens, is dependent on IL-5 (27). However, these investigations also indicate that other factors derived from the site of antigen presentation are required to amplify the IL-5 signal for eosinophil migration and are essential for widespread eosinophilic inflammation in tissues (27).

Eotaxin, a member of the C-C branch of chemokines, has been identified recently as a novel chemotactic agent for eosinophils (16, 17). The potency and rapid action of eotaxin in inducing selective pulmonary and intradermal eosinophil recruitment, suggests an integral role for this protein in the early

1. *Abbreviations used in this paper:* MOP, mini-osmotic pump, OVA, ovalbumin.

phases of the signaling mechanism for eosinophil homing and tissue recruitment (16–18). Eosinophils also express an eotaxin specific receptor which may regulate the selective recruitment of this leukocyte to sites of inflammation (32). Eotaxin is constitutively expressed in a number of tissues and may regulate basal tissue homing of eosinophils. Increased production of eotaxin in response to antigen stimulation, in association with the increased synthesis of cytokines and eosinophil chemoattractant chemokines, may regulate eosinophil tissue homing and accumulation to the site of inflammation (16–18). Investigations in guinea pigs suggest that eotaxin and IL-5 may act cooperatively to promote the recruitment of eosinophils into tissues (28). The number of eosinophils recruited to sites of intradermal injection of eotaxin correlated with an increase in the number of circulating eosinophils induced by systemic administration of IL-5. In mice, eotaxin-induced recruitment of eosinophils to the lung and skin was only consistently observed in IL-5 transgenic mice, which have elevated levels of IL-5 and a pronounced basal blood eosinophilia (33). Thus, during the inflammatory response, IL-5 may provide the signal for the release of a pool of eosinophils from the bone marrow, while eotaxin may elicit the signal for eosinophil localization to the site of inflammation (28, 33). Amplification of eotaxin-induced eosinophilia in the presence of increased levels of systemic IL-5 may be directly due to this cytokine or to the concomitant increase in the pool of circulating eosinophils. Furthermore, it is not known whether there is an essential requirement for IL-5 in the mechanism for eotaxin-induced recruitment of eosinophils to mucosal tissues.

In order to provide important insights into the molecular mechanisms regulating eosinophil homing and selective eosinophil trafficking during complex tissue responses to inflammatory stimuli, it is essential to gain a better understanding of the interaction between IL-5 and eotaxin. In this investigation, we have used IL-$5^{-/-}$ mice to define the relationship between this cytokine and eotaxin in the regulation of blood eosinophilia and eosinophil homing and tissue accumulation. Our results suggest that eotaxin may have important roles in initiating both blood and tissue eosinophilia in the early phases of allergic inflammation. Furthermore, IL-5 was found to be essential for eotaxin-induced tissue eosinophilia suggesting that it may provide a key signal for eosinophil homing to tissues in response to specific chemotactic stimuli.

Methods

Animals. Male mice (C57BL/6, 6–8 wk of age) were used in all skin bioassay experiments. Donor eosinophils for homing experiments were obtained by sensitizing mice (C57BL/6, 8–10 wk of age) by intraperitoneal (i.p.) injection with 50 μg ovalbumin (OVA)/mg Alhydrogel (CSL Ltd., Parkville, Australia) in 0.9% sterile saline on days 0 and 12. On day 24 sensitized mice received injections of 50 μg OVA i.p., every second day for 8 d, and eosinophils were washed from the peritoneal cavity with 15 ml of RPMI-1640 medium. In some experiments mice were injected with supernatants (0.5 mg protein/100 μl) obtained from cultured SF9 insect cells, instead of OVA, as this was found to induce a stronger intraperitoneal eosinophilia. Eosinophils were purified by FACS using forward Vs side scatter and polarization of light. The purity of the enriched population of eosinophils was > 98% as determined by differential staining with Giemsa-May-Grunwald. Purified eosinophils were diluted as required into RPMI-1640 and 100 μl injected into the tail vein of recipient mice. Greater than 97% of injected cells were determined as viable by trypan blue exclusion. IL-$5^{-/-}$ mice and control littermates (IL-$5^{+/+}$) were derived as previously described (26).

Effect of IL-5 or eotaxin on levels of circulating and bone marrow eosinophils. Mice were injected i.v. with eotaxin (0.03–2.4 nmol/kg), IL-5 (10–500 pmol/kg), or control vehicle (100 μl of 10 mM PBS/0.1% BSA [< 0.1 ng/mg endotoxin], pH 7.4). Blood samples were taken before, at 30 min and hourly (as indicated in the text) after intravenous injection of eotaxin, IL-5 or control vehicle for differential quantification of leukocytes. At the peak of blood eosinophilia (30 min in IL-$5^{+/+}$ mice and 3 h in IL-$5^{-/-}$ mice with intravenous IL-5 and 30 min for all mice with intravenous eotaxin) mice were killed by cervical dislocation and the right femurs removed into ice cold HBSS. Epiphyses from both the proximal and distal ends of the femurs were removed and the bone marrow cavity lavaged with 2 ml of HBSS. The number of cells/ml of lavage fluid was determined using a hemocytometer and 2×10^5 cells were cytocentrifuged and stained with Giemsa-May-Grunwald for differential cell counting. Routinely, 300–400 cells were counted per slide.

Bioassay of eotaxin- or IL-5-induced eosinophil accumulation in skin. Mice were anesthetized with ether, the dorsal skin shaved, and injected i.v. with IL-5 (100 pmol/kg in IL-$5^{+/+}$ mice and 500 pmol/kg in IL-$5^{-/-}$ mice) or control vehicle (100 μl of 10 mM PBS/0.1% BSA [< 0.1 ng/mg endotoxin], pH 7.4). In some experiments, wild type mice were injected with eotaxin (1.2 nmol/kg i.v.) or in combination with IL-5 (100 pmol/kg). After 1 h mice were injected subcutaneously with 150 μl of air and the required concentration of eotaxin (1–80 pmol/site), IL-5 (0.1–10 pmol/site), combination of both cytokines (IL-5 [10 pmol/site] and eotaxin [5.0 pmol/site]) or control vehicle (100 μl HBSS/0.01% BSA [< 0.1 ng/mg endotoxin], pH 7.4). 2 h after subcutaneous injection of cytokine(s) or control vehicle, mice were killed by CO_2 asphyxiation, and the dorsal skin membrane (subcutaneous fascia [34], approximately 18 × 18 mm) below the air pouch excised directly onto a glass slide. Slides were allowed to air dry before being fixed with methanol and stained with Giemsa-May-Grunwald for differential cell counting. Eosinophils/mm^2 were determined by counting 10 fields of view (×250). Blood samples were taken before intravenous injection of IL-5, 30 min after administration, and hourly thereafter.

Role of IL-5 and eotaxin in eosinophil homing. IL-$5^{-/-}$ and IL-$5^{+/+}$ mice were injected i.v. with 1×10^6 or 4×10^6 donor eosinophils and 10 min later peripheral blood samples were taken. Mice were then injected subcutaneously with eotaxin (5.0 pmol/site), IL-5 (10 pmol/site), or control vehicle (100 μl HBSS/0.01% BSA, pH 7.4). After 2 h the mice were killed and the dorsal skin membrane was excised and prepared for differential cell counting (as above).

Restoration of eosinophil homing in IL-$5^{-/-}$ mice. IL-$5^{-/-}$ mice were anesthetized with ketamine (60 mg/kg i.p.) and rompun (8 mg/kg i.p.) by injection and the ventral skin was shaved and washed with hibitane. Mini-osmotic-pumps (MOP) containing IL-5 or control vehicle (10 mM PBS/0.1% BSA, pH 7.4, i.p.) were implanted under sterile conditions and the wound was sealed. MOP were calibrated to deliver control vehicle or 100 pmol/kg of IL-5 per h for 7 d. After 72 h, mice were injected i.v. with IL-5 (500 pmol/kg) or control vehicle (100 μl of 10 mM PBS/0.1% BSA, pH 7.4) at 0 and 30 min. Eotaxin (5.0 pmol/site), IL-5 (10 pmol/site) or control vehicle (HBSS/0.01% BSA, pH 7.4) was then injected subcutaneously and 2 h later the mice were killed and the dorsal skin membrane was excised for differential cell counts (see above). Blood samples were taken before intravenous injection of IL-5 and at intervals of 30 min thereafter.

Characterization of tissue and circulating leukocytes. Dorsal skin membrane preparations were stained with Giemsa-May-Grunwald solution and 10 fields/slide were counted for eosinophil infiltration. Blood samples were drawn from the tail vein into heparinized (10 U/ml) tubes. Blood eosinophil numbers were quantified in whole blood by staining with Discombe's solution (35). Total and differential circulating leukocyte numbers were also quantified on blood smears after staining with Giemsa-May-Grunwald solution. 200–300 leukocytes were counted on each slide. Cell types were identified by using morphological criteria.

Materials. Mouse recombinant eotaxin was purchased from PeproTech, Inc. (Rocky Hill, NJ). Mouse recombinant IL-5 was expressed and purified from the baculovirus expression system (36). IL-5 was purified to homogeneity by gel filtration (Superdex 75 Pharmacia LKB Biotechnology, Inc., Piscataway, NJ) and ion exchange (Mono Q Pharmacia LKB Biotechnology, Inc.) and protein levels estimated by optical density at 280 nm and Biorad protein assay using gamma globulin as the standard. MOP (model 2001) were purchased from Alza Corp. (Palo Alto, CA). All other reagents were purchased from standard commercial chemical houses.

Results and Discussion

Effect of intravenous IL-5 and eotaxin on circulating eosinophil levels. Intravenously administered IL-5 induced a rapid and sustained increase in circulating eosinophils in IL-$5^{+/+}$ mice (Fig. 1 *a*). The peak was at the first time point of 30 min and eosinophil numbers fell to basal levels over 5 h. This effect was concentration-dependent (10, 20, 50 pmol/kg, results not shown) and a dose of 100 pmol/kg induced an eosinophilia that was equivalent to that observed during allergic pulmonary inflammation in mice (27). The rapid action of intravenous IL-5 in guinea pigs has been associated with the mobilization of a bone marrow pool of eosinophils (28). This mechanism also appeared to be operating in the present experiments as the eosinophilia correlated with a fall in bone marrow eosinophil numbers (Fig. 2). In IL-$5^{-/-}$ mice, however, intravenous IL-5 at levels as high as 500 pmol/kg did not induce a pronounced blood eosinophilia, in comparison to wild type mice (Fig. 1 *a*), suggesting that the mechanism involved in the release and/or maintenance of this pool of eosinophils requires the presence of this cytokine. The effect of intravenous administration of eotaxin on circulating levels of eosinophils has not been previously reported. In this investigation systemic eotaxin potently induced a dose (0.03–2.4 nmol/kg) dependent blood eosinophilia (Fig. 1 *b*). The onset of eosinophilia was extremely rapid and blood levels were at least equivalent to that observed during eosinophilic inflammation in mice and in response to intravenous IL-5 (Fig 1 *a*). However, eotaxin was approximately threefold less potent than IL-5 in inducing an equivalent eosinophilia. Moreover, eotaxin induced a peripheral blood eosinophilia in IL-$5^{-/-}$ mice (Fig. 1 *c*). This response was diminished ($\sim$ 50%) in comparison to the blood eosinophilia observed in IL-$5^{+/+}$ mice. The increase in blood eosinophil numbers in IL-$5^{+/+}$ mice and IL-$5^{-/-}$ mice in response to intravenous eotaxin did not correlate with a significant reduction in the level of bone marrow eosinophils (Fig. 2). IL-$5^{-/-}$ mice have reduced basal blood levels of eosinophils which may affect the number of eosinophils migrating through tissues (26). Therefore, a reduction in the eosinophilic response to intravenous eotaxin may reflect the number of eosinophils available to be recruited to the blood, rather than a direct role for IL-5 in the recruitment mechanism. Thus, two mechanisms may exist whereby eosinophils can be rapidly mobilized in response to intravenous eosinophil-specific chemoattractant stimuli, mobilization of an IL-5-dependent bone marrow pool and eotaxin-induced recruitment of eosinophils residing in tissues into the circulation.

During allergic intradermal inflammation, two phases (peaks at 6 and after 24 h) of tissue eosinophilia were observed (37). The first phase of eosinophilia can occur independently of systemic IL-5, while the second phase is significantly reduced in the presence of IL-5-mAb. Expression of eotaxin oc-

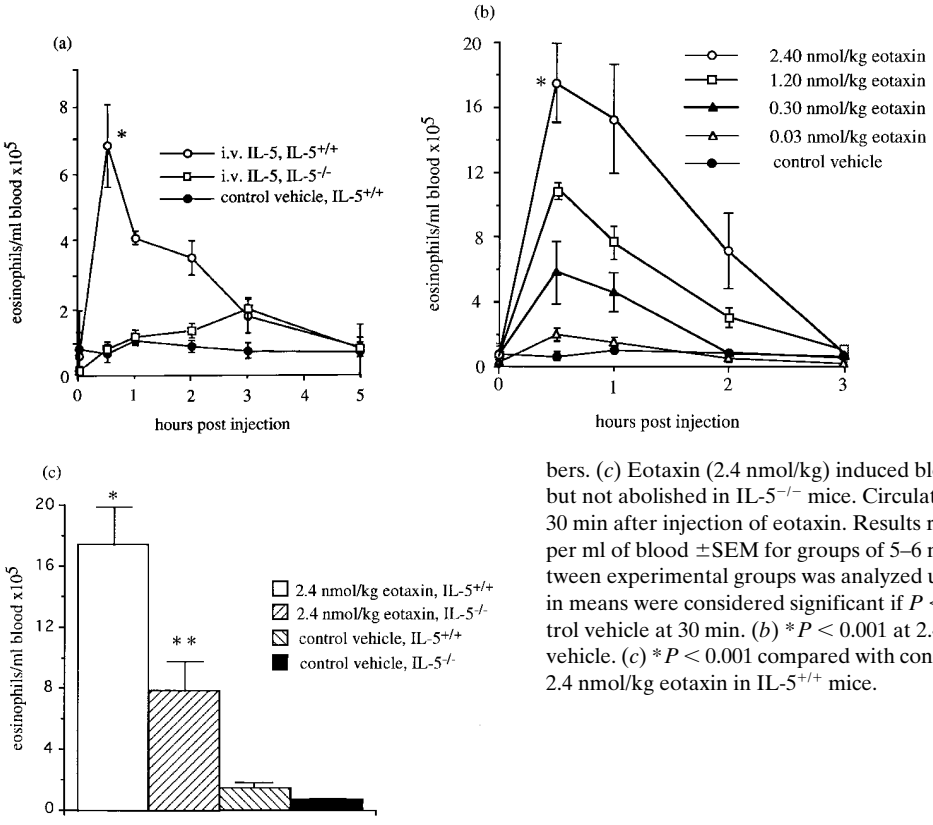

Figure 1. The effect of intravenous administration of IL-5 or eotaxin on circulating eosinophil numbers in IL-$5^{+/+}$ and IL-$5^{-/-}$ mice. Mice were injected with IL-5 (100 or 500 pmol/kg), eotaxin (0.03–2.4 nmol/kg) or control vehicle (100 μl of 10 mM PBS/0.1% BSA, pH 7.4). Blood samples were taken before, at 30 min, and hourly after intravenous injection of the cytokines or control vehicle for differential quantification of leukocytes. (*a*) Blood eosinophil levels induced by IL-5 (100 pmol/kg) in IL-$5^{+/+}$ mice and by IL-5 (500 pmol/kg) in IL-$5^{-/-}$ mice. (*b*) Eotaxin (0.03–2.4 nmol/kg) induced a dose dependent increase in circulating eosinophil numbers. (*c*) Eotaxin (2.4 nmol/kg) induced blood eosinophilia was significantly reduced but not abolished in IL-$5^{-/-}$ mice. Circulating eosinophil numbers were determined 30 min after injection of eotaxin. Results represent the mean number of eosinophils per ml of blood ±SEM for groups of 5–6 mice. The significance of differences between experimental groups was analyzed using Student's unpaired *t* test. Differences in means were considered significant if $P < 0.05$. (*a*) $*P < 0.001$ compared with control vehicle at 30 min. (*b*) $*P < 0.001$ at 2.4 nmol/kg eotaxin compared with control vehicle. (*c*) $*P < 0.001$ compared with control vehicle and $**P < 0.05$ compared with 2.4 nmol/kg eotaxin in IL-$5^{+/+}$ mice.

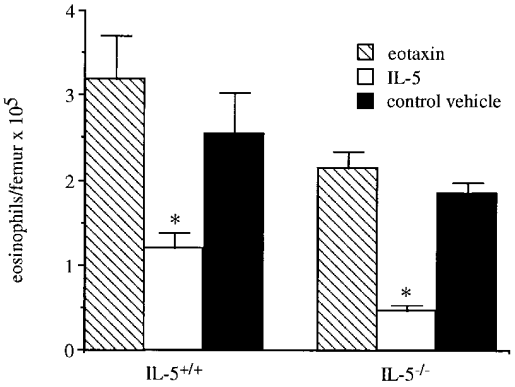

Figure 2. Eosinophil numbers in the bone marrow of IL-5$^{+/+}$ and IL-5$^{-/-}$ mice in the presence of intravenous IL-5 or eotaxin. IL-5 (100 pmol/kg and 500 pmol/kg, for IL-5$^{+/+}$ and IL-5$^{-/-}$ mice, respectively), eotaxin (2.4 nmol/kg) or control vehicle (100 μl of 10 mM PBS/0.1% BSA, pH 7.4) were injected intravenously into mice. Femurs were removed at the peak of the blood eosinophilic response (30 min in IL-5$^{+/+}$ mice and 3 h in IL-5$^{-/-}$ mice with intravenous IL-5 [Fig. 1 *a*] and 30 min for all mice with intravenous eotaxin [Fig. 1 *b*]) and the bone marrow cavity lavaged with 2 ml of HBSS. The number of cells/ml of lavage fluid was determined and 2×10^5 cells were cytocentrifuged and stained with Giemsa-May-Grunwald for differential cell counting. Results represent the mean number of bone marrow eosinophils ±SEM for groups of four mice. Differences in means were considered significant if $P < 0.05$. $*P < 0.05$ compared with control vehicle.

curs early in the inflammatory response and can be induced in cultured endothelial cells, alveolar epithelial cells, and T cell clones (Th1 and Th2 clones) (17, 18). Taken together, these results suggest that eotaxin secreted from inflammatory cells or tissue sites in the early phases of the inflammatory response may be able to initiate and supplement tissue eosinophilia by sequestering, into the circulation, eosinophils which are migrating through noninflamed tissues. This mechanism would utilize the eosinophil pool already participating in immune surveillance and provide a mechanism for an immediate response to allergic inflammation or parasite infection in mucosal tissues. Eosinophilic inflammation could then be amplified and sustained over the course of the inflammatory response by eosinophils mobilized by IL-5 from the bone marrow.

Effect of subcutaneously injected eotaxin and IL-5 on eosinophil accumulation in the skin. The role of IL-5 in the selective accumulation of eosinophils in tissue in response to eotaxin was analyzed in IL-5$^{+/+}$ and IL-5$^{-/-}$ mice. In IL-5$^{+/+}$ mice, as previously observed (18, 33), eotaxin was a highly potent chemotactic signal for the selective and rapid recruitment of eosinophils. Within 2 h, eotaxin (1.0–20 pmol/site) had induced a pronounced tissue eosinophilia which was highly dependent on concentration (Fig. 3 *a*). The effect of eotaxin was maximal at 5.0 pmol/site and subsequent increases in dose resulted in a concentration-dependent reduction of eosinophil accumulation. Similar results were also obtained in mice with dilutions of crude supernatants from COS cells transfected with eotaxin cDNA (17). These results, in part, may explain why some investigators see no or only poor chemotactic responses with eotaxin in the absence of systemic eosinophilia (33). The narrow range in which eotaxin stimulates eosinophilia suggests that chemotactic signals for the selective migration of a cell type are discretely regulated at the site of inflammation.

The effect of eotaxin on eosinophil transmigration was significantly amplified (Fig. 3 *a*) by first inducing a blood eosinophilia by injection of IL-5 (100 pmol/kg i.v.) (Fig. 1 *a*) 1 h prior to eotaxin injection. Subcutaneously injected eotaxin or IL-5 did not induce blood eosinophilia (results not shown). These results support the concept that eotaxin and IL-5 act cooperatively to regulate the accumulation of eosinophils. However, subcutaneous injection of IL-5 (1–10 pmol/site) also induced a concentration dependent tissue eosinophilia which was also amplified by intravenous injection of IL-5 (Fig. 3 *b*). Tissue eosinophilia induced by IL-5 (10 pmol/site) was equivalent to that produced by eotaxin (5.0 pmol/site) (Fig. 3 *c*). Coinjection of eotaxin (5.0 pmol/site) and IL-5 (10 pmol/site) subcutaneously, significantly amplified eosinophil recruitment in comparison to eotaxin or IL-5 alone (Fig. 3 *c*). These results suggest that IL-5 secreted in tissue compartments can act as a chemoattractant for eosinophils and enhance local chemotactic signals for eosinophil accumulation (22, 24, 25).

In IL-5$^{-/-}$ mice, eotaxin (5.0 pmol/site), IL-5 (10 pmol/site), or a combination of both cytokines at the same site did not induce tissue eosinophilia (Fig. 3 *c*). However, IL-5$^{-/-}$ mice have reduced basal levels of circulating eosinophils. Intravenous administration of IL-5$^{-/-}$ mice with IL-5 (doses of 500 pmol/kg at 0 and 30 min before the subcutaneous injection of the cytokines at 1 h) was shown to restore circulating eosinophil numbers to the basal levels observed in IL-5$^{+/+}$ mice (see Fig. 5 *a*). However, this failed to promote eotaxin- or IL-5-induced tissue eosinophilia (see Fig. 5 *b*), suggesting a critical role for IL-5 in the migration of eosinophils into tissues in response to specific chemotactic stimuli.

The relationship between blood eosinophilia induced by eotaxin and/or IL-5, and the subsequent accumulation of eosinophils at subcutaneous sites of administration of these chemoattractants was also examined (Fig. 3 *d*). Interestingly, intravenous injection of eotaxin (1.2 nmol/kg) induced a blood eosinophilia in wild type mice (Fig. 1 *b*), but did not amplify chemotactic responses to subcutaneously administered eotaxin (5.0 pmol/skin site) or IL-5 (10 pmol/skin site) (Fig. 3 *d*). In contrast, injection of IL-5 (100 pmol/kg i.v.) significantly potentiated chemotactic responses to either cytokine, when administered subcutaneously (Fig. 3 *d*). Furthermore, intravenous injection of a combination of both IL-5 (100 pmol/kg) and eotaxin (1.2 nmol/kg) significantly amplified chemotactic responses to eotaxin (5.0 pmol/skin site) or IL-5 (10 pmol/skin site) when administered subcutaneously (Fig. 3 *d*). Eosinophil accumulation was significantly greater than responses obtained with intravenous IL-5 and subcutaneous eotaxin or with intravenous IL-5 and subcutaneous IL-5.

These results show that intravenous IL-5 and eotaxin can act cooperatively to potentiate local chemotactic responses. Interestingly, increased numbers of eosinophils in the blood in response to intravenous eotaxin did not significantly enhance responses to chemotactic stimuli in the skin, without intravenous IL-5. Intravenous IL-5 may enhance tissue accumulation by first priming circulating eosinophils. This data further shows the cooperative nature of the interaction between eotaxin and IL-5 for eosinophil homing.

Role of IL-5 and eotaxin in eosinophil homing. To determine whether the level of eotaxin-induced tissue eosinophilia

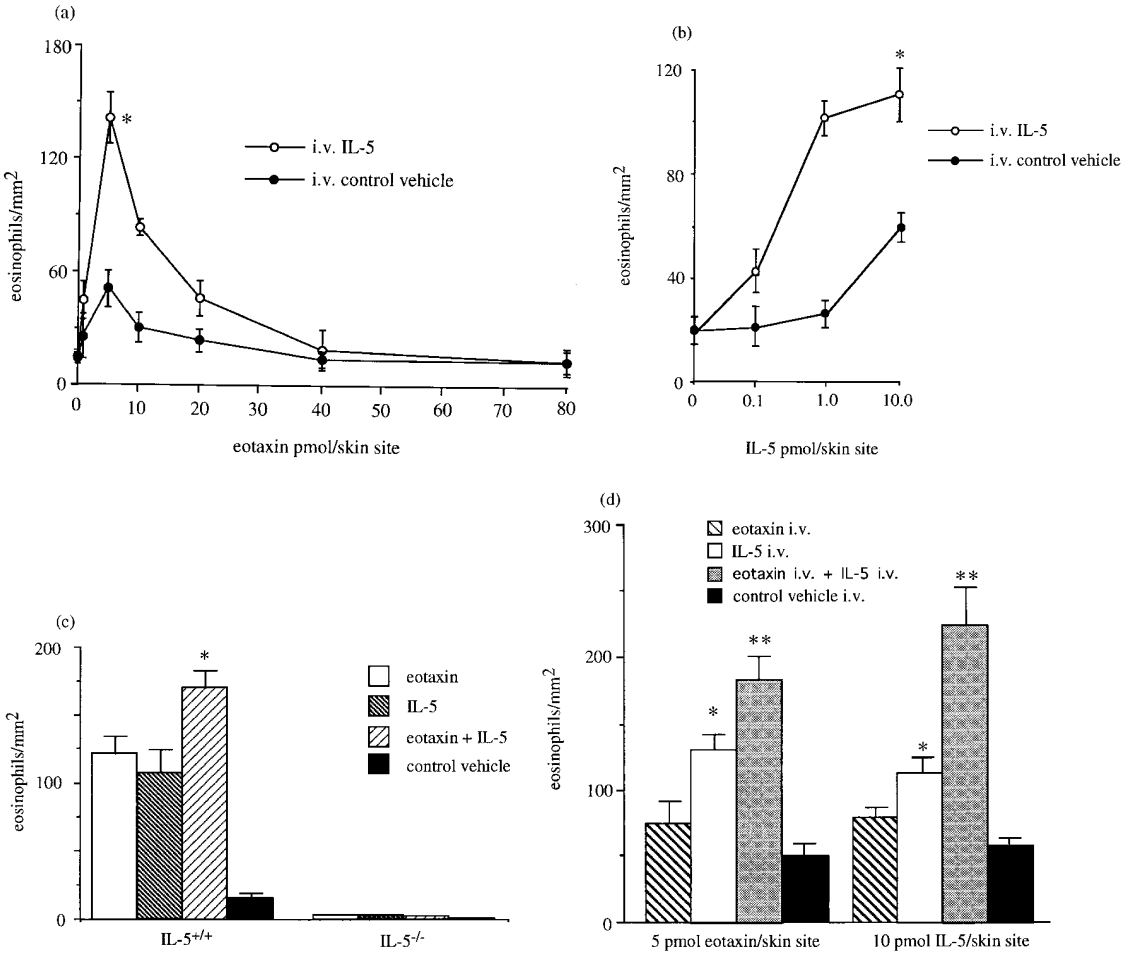

Figure 3. The effect of intravenous IL-5 on eotaxin- and IL-5-induced eosinophil recruitment into the skin in IL-$5^{+/+}$ and IL-$5^{-/-}$ mice. (*a*) Accumulation of eosinophils into dorsal skin membranes in response to subcutaneously injected eotaxin (1.0–80 pmol/site) or control vehicle (100 μl of HBSS/0.01% BSA, pH 7.4). Eotaxin-induced eosinophil recruitment was concentration dependent and was determined in the presence or absence of intravenous IL-5. Mice were injected intravenously with IL-5 (100 pmol/kg) or control vehicle (100 μl of 10 mM PBS/0.1% BSA, pH 7.4) and 1 h later eotaxin or control vehicle was injected subcutaneously. Eosinophil recruitment was maximal at 5.0 pmol/site eotaxin. (*b*) Accumulation of eosinophils into dorsal skin membranes was determined as described above, however, mice were subcutaneously injected with IL-5 (0.1 to 10.0 pmol/skin site) instead of eotaxin. IL-5-induced eosinophil recruitment was concentration dependent and was determined in the presence or absence of intravenous IL-5. Eosinophil recruitment was maximal at 10 pmol/site IL-5. (*c*) IL-$5^{+/+}$ mice were injected with IL-5 (100 pmol/kg i.v.) and then injected subcutaneously with either IL-5 (10 pmol/site) or eotaxin (5 pmol/site) or with a combination of both cytokines (IL-5 10 pmol/site and eotaxin 5.0 pmol/site). IL-$5^{-/-}$ mice were also injected subcutaneously with these cytokines, individually or in combination (at the above concentrations), after administration of IL-5 (500 pmol/kg i.v.). (*d*) Effect of i.v. injection of IL-5 (100 pmol/kg) or eotaxin (1.2 nmol/kg) or a combination of both cytokines on accumulation of eosinophils into dorsal skin membranes in response to subcutaneously administered eotaxin (5 pmol/site) or IL-5 (10 pmol/site) in IL-$5^{+/+}$ mice. Maximal accumulation of eosinophils occurred in the presence of a combination of intravenous eotaxin and IL-5. In the above experiments chemoattractant agents or control vehicle were injected into the skin 1 h after intravenous administration of IL-5, eotaxin or control vehicle. Eosinophil recruitment into dorsal skin membranes was determined 2 h after subcutaneous injection of eosinophil chemoattractants or control vehicle. Results represent the mean number of eosinophils/mm^2 ±SEM for groups of six mice. One dorsal membrane preparation was excised from each animal and prepared for differential cell counting. Ten fields per preparation were counted for eosinophil infiltration and the mean was obtained. Differences in means were considered significant if $P < 0.05$. (*a*) $*P < 0.001$ compared to intravenous control vehicle at the same subcutaneous concentration of eotaxin. (*b*) $*P < 0.001$ compared to intravenous control vehicle at the same subcutaneous concentration of IL-5. (*c*) $*P < 0.05$ compared to eotaxin or IL-5 alone in IL-$5^{+/+}$; and (*d*) $*P < 0.01$ compared to control vehicle intravenous, and $**P < 0.05$ compared to IL-5 intravenous. No significant difference was observed between control vehicle and eotaxin groups, both intravenous, in the presence of subcutaneous eotaxin or IL-5.

was solely dependent on IL-5 or on the available pool of circulating eosinophils and to ascertain the role of these cytokines in eosinophil homing, donor eosinophils were injected intravenously into IL-$5^{+/+}$ and IL-$5^{-/-}$ mice (Fig. 4).

Adoptive transfer of eosinophils (1×10^6 donor cells) to IL-$5^{+/+}$ mice significantly enhanced the accumulation of eosinophils in the skin in response to eotaxin (5.0 pmol/site) (Fig. 4 *a*) or IL-5 (10 pmol/site) (Fig. 4 *b*). These responses were significantly amplified by transferring 4×10^6 donor cells to IL-$5^{+/+}$ mice (result shown only for eotaxin, Fig. 4 *a*). In contrast, injection of up to 4×10^6 eosinophils in IL-$5^{-/-}$ mice did not promote eosinophil recruitment to sites of eotaxin (5.0

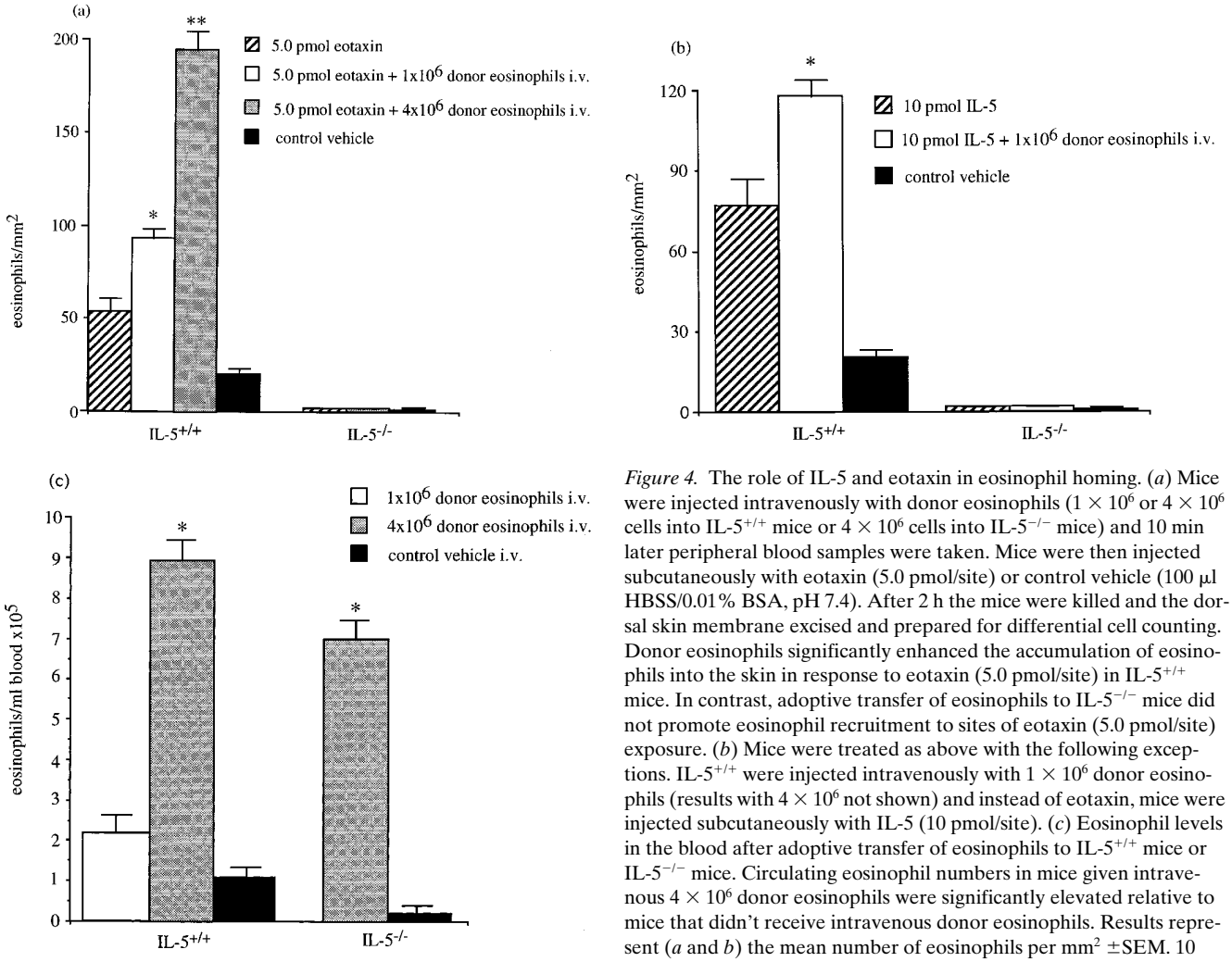

Figure 4. The role of IL-5 and eotaxin in eosinophil homing. (*a*) Mice were injected intravenously with donor eosinophils (1×10^6 or 4×10^6 cells into IL-$5^{+/+}$ mice or 4×10^6 cells into IL-$5^{-/-}$ mice) and 10 min later peripheral blood samples were taken. Mice were then injected subcutaneously with eotaxin (5.0 pmol/site) or control vehicle (100 μl HBSS/0.01% BSA, pH 7.4). After 2 h the mice were killed and the dorsal skin membrane excised and prepared for differential cell counting. Donor eosinophils significantly enhanced the accumulation of eosinophils into the skin in response to eotaxin (5.0 pmol/site) in IL-$5^{+/+}$ mice. In contrast, adoptive transfer of eosinophils to IL-$5^{-/-}$ mice did not promote eosinophil recruitment to sites of eotaxin (5.0 pmol/site) exposure. (*b*) Mice were treated as above with the following exceptions. IL-$5^{+/+}$ were injected intravenously with 1×10^6 donor eosinophils (results with 4×10^6 not shown) and instead of eotaxin, mice were injected subcutaneously with IL-5 (10 pmol/site). (*c*) Eosinophil levels in the blood after adoptive transfer of eosinophils to IL-$5^{+/+}$ mice or IL-$5^{-/-}$ mice. Circulating eosinophil numbers in mice given intravenous 4×10^6 donor eosinophils were significantly elevated relative to mice that didn't receive intravenous donor eosinophils. Results represent (*a* and *b*) the mean number of eosinophils per mm^2 ±SEM. 10 fields were counted from each membrane preparation and (*c*) the mean number of eosinophils per ml of blood ±SEM. Data was obtained from groups of 5–6 mice. Differences in means were considered significant if $P < 0.05$. (*a* and *b*) $*P < 0.01$ when compared with 5.0 pmol/site eotaxin or 10 pmol/site IL-5 in the absence of donor eosinophils and (*a*) $**P < 0.001$ when compared in the absence of donor eosinophils. (*c*) $*P < 0.01$ when compared in the absence of donor eosinophils.

pmol/site) (Fig. 4 *a*) or IL-5 (10 pmol/site) (Fig. 4 *b*) exposure. Donor eosinophils were administered to mice (Fig. 4 *c*) in numbers which established blood eosinophil levels equivalent to those which were induced by intravenous IL-5 in IL-$5^{+/+}$ mice (Fig. 1 *a*) and promoted enhanced tissue chemotactic responses to eotaxin (Fig. 3 *a*) and IL-5 (Fig. 3 *b*). Donor eosinophil levels in the blood were also similar to that observed during allergic responses in mice (27). These results in IL-$5^{-/-}$ mice indicate that increased levels of circulating eosinophils alone are not sufficient to promote the enhanced recruitment of eosinophils to sites of eotaxin or IL-5 exposure. Moreover, they indicate an essential role for IL-5 in eosinophil homing and migration into tissues in response to specific chemoattractant stimuli.

Restoration of eotaxin- and IL-5-induced tissue eosinophilia and eosinophil homing in IL-$5^{-/-}$ mice. The central role of IL-5 in eotaxin-induced eosinophil recruitment was confirmed in IL-$5^{-/-}$ mice pretreated intraperitonealy for 72 h with IL-5 using MOP. The continuous administration of intraperitoneal IL-5 in the pretreatment did not induce blood eosinophilia. However, subsequent intravenous administration of IL-5 induced a rapid blood eosinophilia (Fig. 5 *a*) equivalent to that induced by IL-5 in IL-$5^{+/+}$ mice (Fig. 1 *a*) and which promoted enhanced tissue chemotactic responses to eotaxin (Fig. 3 *a*) and IL-5 (Fig. 3 *b*). The ability of eotaxin (5.0 pmol/site) and IL-5 (10 pmol/site) to induce tissue recruitment of eosinophils was also restored (Fig. 5 *b*). These results establish the essential role of IL-5 in the chemoattractant signal elicited by subcutaneous eotaxin and IL-5 and suggest that basal levels of IL-5 may play a critical role in regulating eosinophil homing, perhaps by activating adhesion systems in the vascular endothelium (9, 13). Moreover, results indicate an essential role for IL-5 in the homing of eosinophils to sites of specific chemotactic stimuli. Thus, IL-5 may have multiple functions in the regulation of eosinophil trafficking, mobilizing eosinophils from bone marrow stores, eliciting chemotactic responses for eosinophils in tissues and by operating systems at a tissue level which regulate eosinophil homing and migration into tissues in response to specific chemoattractant stimuli.

In summary, the selectivity, rapid action, and kinetics of tissue expression of eotaxin suggest a central role for this chemokine in the early phases of the signaling mechanism for

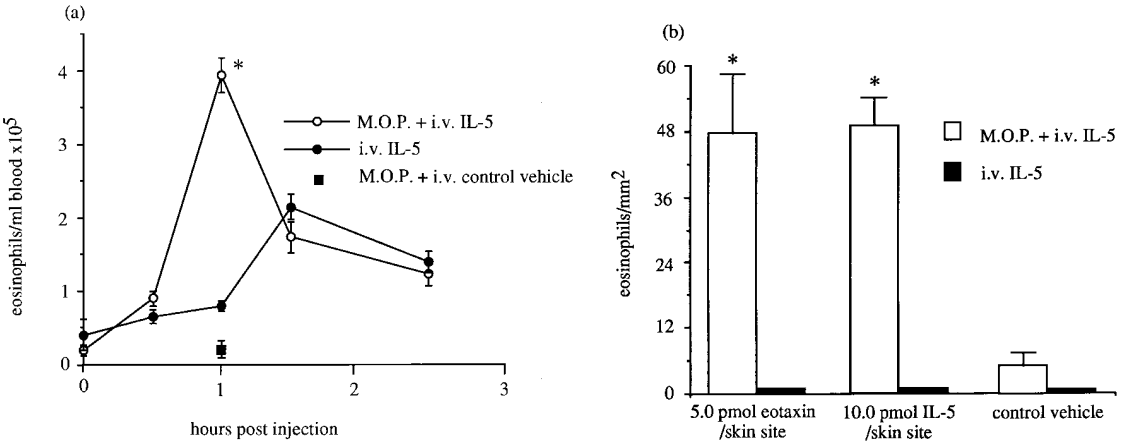

Figure 5. Restoration of eotaxin- and IL-5- induced tissue eosinophilia and eosinophil homing in IL-$5^{-/-}$ mice. (*A*) IL-$5^{-/-}$ mice were pretreated for 72 h with intraperitoneal IL-5 using MOP. Peripheral eosinophilia was induced in these mice by two injections of IL-5 (500 pmol/kg i.v.) at 0 and 30 min. Intravenous injection of IL-5 in mice which were not pretreated for 72 h with intraperitoneal IL-5 failed to induce a pronounced blood eosinophilia. Blood eosinophilia was not induced by intravenous injection of control vehicle (100 μl of 10 mM PBS/0.1% BSA, pH 7.4) in IL-5 pretreated mice. Blood samples were taken before intravenous injection of IL-5 and at intervals of 30 min thereafter. The resulting eosinophilia at 30 min after the second intravenous injection of IL-5 in IL-5 pretreated mice was equivalent to that induced by this cytokine (Fig. 1 *a*) at the time of subcutaneous administration of eotaxin (Fig. 3 *b*) or IL-5 (Fig. 3 *b*) in IL-$5^{+/+}$ mice. Blood eosinophilia was not induced in mice solely by pretreatment with intraperitoneal IL-5 for 72 h. (*b*) Eotaxin (5.0 pmol/site), IL-5 (10 pmol/site) or control vehicle (HBSS/0.01% BSA, pH 7.4) were injected subcutaneously 30 min after the second intravenous injection of IL-5 or control vehicle and 2 h later the mice were killed and the dorsal skin membrane excised for differential cell counts. Eotaxin- and IL-5-induced recruitment of eosinophils to sites of exposure was only restored in IL-$5^{-/-}$ mice which were pretreated with intraperitoneal IL-5 for 72 h. Results represent (*a*) the mean number of eosinophils per ml of blood ±SEM, and (*b*) the mean number of eosinophils per mm^2 ±SEM. 10 fields were counted from each membrane preparation. Data was obtained from groups of four mice. Differences in means were considered significant if $P < 0.05$. (*a*) $*P < 0.001$ when compared with intravenous IL-5 in the absence of IL-5 pretreatment for 72 h and (*b*) $*P < 0.001$ when compared with intravenous IL-5 in the absence of IL-5 pretreatment for 72 h.

eosinophil homing during allergic inflammation (16–18, 28). Under basal conditions, eotaxin and IL-5 may act cooperatively to regulate eosinophil homing and tissue accumulation (28, 33). In response to inflammatory stimuli IL-5 provides an essential signal for the mobilization of a bone marrow pool of eosinophils and to maintain peripheral eosinophilia (7, 26, 27). This study indicates a second critical role for IL-5 in regulating eosinophil homing and tissue migration in response to chemoattractant stimuli. It will be of interest to determine if IL-5 regulates homing by activating eosinophil-specific adhesion systems at the vascular endothelium (9, 13). Eotaxin secreted from inflammatory cells and tissue may augment the IL-5 signal by supplementing blood eosinophilia in the early phases of the inflammatory response as well as eliciting a selective chemoattractant signal for eosinophil polarization and migration (16, 33).

We conclude that IL-5 is not only essential for the rapid mobilization of eosinophils from the bone marrow but also has other critical biological functions in regulating eosinophil homing and migration into tissues in response to specific chemotactic stimuli. Furthermore, eotaxin in the early phases of the inflammatory mechanism may promote blood and tissue eosinophilia. This investigation further supports the concept that IL-5 and eotaxin are key therapeutic targets for the improved treatment of acute and chronic allergic disease.

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