Brief Definitive Report

Cooperation between Interleukin-5 and the Chemokine Eotaxin to Induce Eosinophil Accumulation In Vivo

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Summary

Experiments were designed to study the effect of systemically administered IL-5 on local eosinophil accumulation induced by the intradermal injection of the chemokine eotaxin in the guinea pig. Intravenous interleukin-5 (IL-5) stimulated a rapid and dramatic increase in the numbers of accumulating eosinophils induced by i.d.-injected eotaxin and, for comparison, leukotriene B_4. The numbers of locally accumulating eosinophils correlated directly with a rapid increase in circulating eosinophils: circulating eosinophil numbers were 13-fold higher 1 h after intravenous IL-5 (18.3 pmol/kg). This increase in circulating cells corresponded with a reduction in the number of displaceable eosinophils recovered after flushing out the femur bone marrow cavity. Intradermal IL-5, at the doses tested, did not induce significant eosinophil accumulation. We propose that these experiments simulate important early features of the tissue response to local allergen exposure in a sensitized individual, with eosinophil chemoattractant chemokines having an important local role in eosinophil recruitment from blood microvessels, and IL-5 facilitating this process by acting remotely as a hormone to stimulate the release into the circulation of a rapidly mobilizable pool of bone marrow eosinophils. This action of IL-5 would be complementary to the other established activities of IL-5 that operate over a longer time course.

IL-5 was first described as a factor that induces differentiation and proliferation of bone marrow eosinophils (1–3). Subsequently, IL-5 has been shown to have several other properties, including the ability to activate or prime eosinophils (4–6) and to prolong their survival in vitro (7). IL-5 has also been implicated in local eosinophil recruitment into tissues during allergic inflammatory reactions, such as those induced by helminths (8). IL-5 mRNA expression is upregulated in a number of tissues during eosinophilic inflammatory reactions, including those in the airways (9–11), skin (12), bladder (13), intestinal mucosa (13), and heart (14). IL-5 message can be upregulated in eosinophils themselves, e.g., in the asthmatic lung (15). Furthermore, IL-5 protein has been detected in bronchoalveolar lavage (BAL) fluid from allergen-challenged/sensitized mice by ELISA (16), and several studies have shown that neutralizing antibodies to IL-5 suppress eosinophil accumulation and hyperreactivity in the lungs of experimental animals during allergic inflammation (17–20). Consistent with these observations, intratracheal administration of IL-5 has been shown to induce eosinophil accumulation in the guinea pig lung in vivo (21) and to induce chemotaxis of eosinophils in Boyden chambers in vitro (4, 6, 22, 23). Hence, IL-5 has been described as a selective eosinophil chemotactic factor (4, 23).

Against this background, we purified eosinophil chemoattractant activity from the BAL fluid of challenged/sensitized guinea pigs using three sequential HPLC steps and the accumulation of ^{111}In-eosinophils in guinea pig skin as the in vivo bioassay. No chemoattractant activity corresponding to IL-5 was detected in these experiments. A potent and selective eosinophil chemoattractant cationic protein of ~8 kD was isolated, however. This protein induced the rapid accumulation of ^{111}In-eosinophils in the skin (substantial accumulation seen within 30 min of intradermal injection [24]), but did not induce significant accumulation of ^{111}In-neutrophils. Microsequencing of the chemoattractant revealed a novel 73–amino acid C-C chemokine that we termed "eotaxin" (24, 25). Eotaxin has been cloned, and increased mRNA expression has been demonstrated in the lungs of sensitized guinea pigs after allergen challenge in vivo (26).

These results are apparently at odds with the notion that IL-5 is an important eosinophil chemoattractant in vivo. Paradoxically, however, other studies have clearly demonstrated that anti–IL-5 antibodies suppress eosinophil accumulation in the same model, as discussed above.

In this paper, we report results obtained from experiments on eosinophil accumulation in the skin, which show marked synergism between locally administered eotaxin

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and systemic IL-5 and thus provide a possible explanation for this paradox.

Materials and Methods

Animals. Male Dunkin Hartley guinea pigs (250–300 g) purchased from Harlan Olac Ltd. (Bicester, UK) were used in all skin assay experiments. Female Dunkin Hartley ex-breeder guinea pigs (600–800 g) from the same company were used as eosinophil donors for preparation of eosinophil peroxidase (EPO) standards.

Materials. Human rIL-5 was purchased from R & D Systems Ltd. (Oxford, UK). Leukotriene B_4 (LTB_4) was purchased from Cascade Biochem Ltd. (Reading, UK). Guinea pig eotaxin (native protein) was prepared from the BAL fluid of allergen-challenged animals as described previously (24). All other reagents and chemicals were purchased from Sigma Chemical Co. (Poole, UK).

Bioassay of Eosinophil Accumulation in Guinea Pig Skin. Guinea pigs were sedated (Hypnorm: fentanyl citrate/fluanisone; Janssen Pharmaceutical Ltd., Oxford, UK), 0.8 ml/kg intramuscularly and, after collection of a heparinized (10 U/ml) blood sample (100 μl) from the ear vein, IL-5 (18.3 pmol/kg) or PBS vehicle (500 μl of 10 mM PBS, pH 7.4, containing 0.1% low, <0.1 ng/mg, endotoxin BSA) was administered intravenously. After 1 h, a second blood sample was obtained and test agents (eotaxin, LTB_4, or IL-5 in HBSS/0.1% BSA, pH 7.4) were injected intradermally into the shaved dorsal skin of the animals in duplicate sites (50 μl per site). 2 h after the intradermal injections, a final blood sample was collected and the animals were killed by anesthetic overdose. The dorsal skin was removed and individual skin sites (11 mm in diameter) were excised and frozen at −80°C. Total and differential circulating leukocyte numbers were determined in Kimura-stained preparations after red blood cell lysis using Immuno-Lyse reagent (Coulter Electronics Ltd., Luton, UK).

Bone Marrow Eosinophil Release Experiments. In separate experiments, the kinetics of onset of blood eosinophilia induced by intravenous IL-5 was studied. Furthermore, the potential role of the bone marrow as a source of these additional cells was investigated. Blood samples were collected before as well as 5, 15, 30, and 60 min after intravenous injection of IL-5 or PBS for total and differential leukocyte counts as described above. At the 1-h time point, the left femur was isolated, the femoral head and condyles were removed, and the displaceable cells were recovered by flushing the marrow cavity of the femur shaft with 25 ml HBSS containing 30 mM Hepes, 0.1% BSA, and 10 mM EDTA. After centrifugation (250 *g*, 10 min, 20°C), the cell pellet was resuspended in 1 ml of the HBSS buffer. Total nucleated cells and the number of eosinophils were determined in Kimura-stained preparations.

Preparation of Skin Homogenate for Measurement of EPO Activity. Skin samples were extracted for EPO using a modification of the method described by Pettipher et al. (27). Frozen skin sites were chopped during thawing, and after addition of 4 ml PBS containing 0.5% hexadecyltrimethylammonium bromide (HTAB), samples were homogenized (Ultra-turrax T25; Janke and KunKel GmbH and Co., Staufen, Germany, 3 × 15 s), sonicated (Soniprep 150; MSE Scientific Instruments, Crawley, UK, 10 s), and frozen at −80°C. On the day of EPO measurement, homogenate was thawed and centrifuged twice (2,800 *g*, 10 min, 20°C and 13,000 *g*, 20 min, 20°C). The recovered supernatant was then incubated at 60°C for 2 h before a final centrifugation step (13,000 *g*, 10 min, 20°C) to produce sample supernatant suitable for measurement of EPO.

Measurement of EPO in Skin Homogenate. EPO was measured using a modification of the method described by Strath et al. (28). Processed skin homogenate was placed in duplicate wells (100 μl per well) in a 96-well plate followed by addition of 100 μl substrate (8.6 mM *o*-phenylenediamine dihydrochloride and 2.9 mM hydrogen peroxide in 0.1 M Tris/HCl, pH 8.0). After 30 min at room temperature, the reaction was stopped by the addition of 50 μl 4 M sulphuric acid and the absorbance read at 492 nm.

Guinea pig EPO standard was prepared using eosinophils recovered from the peritoneal cavity of donor animals given repeated intraperitoneal injections of horse serum and purified (>98.5%) over Percoll gradients as described previously (29). After red blood cell lysis (resuspension in 0.2% NaCl for 20 s, then addition of an equal volume of 1.6% NaCl), aliquots of eosinophils (10^6 cells per ml PBS/0.5% HTAB) were frozen at −80°C. Thawed aliquots were sonicated (10 s), incubated at 60°C for 2 h, centrifuged (13,000 *g*, 20 min, 20°C), and the supernatant was used to construct an EPO calibration curve (80–10,000 cells per well). The cross-reactivity with guinea pig neutrophil myeloperoxidase was <1% using this assay. The equivalent number of eosinophils per skin site was calculated from the standard curve. EPO standards and skin homogenates were diluted in PBS/0.5% HTAB, pH 7.4.

Statistical Analysis. Statistical analysis of circulating eosinophil numbers within animals and between control and treatment groups was determined on untransformed data using repeated measures analysis of variance followed by the Tukey-Kramer multiple comparisons test. Statistical analysis of skin responses and bone marrow eosinophil numbers was carried out on $\log_{10}$-transformed data using an unpaired two-tail Student's *t* test as indicated in the figure legends. $P < 0.05$ was considered to be statistically significant.

Results

Effect of Intravenous IL-5 on the Local Accumulation of Eosinophils Induced by Intradermal Eotaxin and LTB_4. Exper-

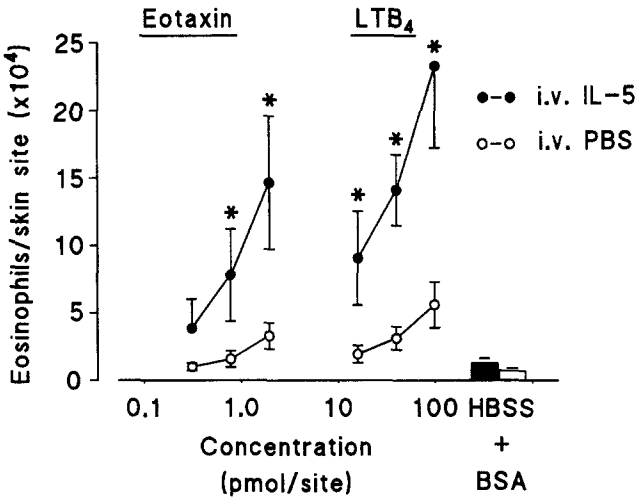

Figure 1. The effect of intravenous IL-5 on the local accumulation of eosinophils in response to intradermal eotaxin and LTB_4. Eosinophil accumulation over 2 h induced by intradermal eotaxin and LTB_4 (50 μl per site) 1 h after intravenous administration of IL-5 or vehicle (PBS/0.1% BSA). Results represent the number of eosinophils per skin site ± SEM ($n = 6$). A significant difference between test and control groups is indicated by *($P < 0.05$).

iments were aimed at investigating the effect of systemically administered IL-5 on local eosinophil accumulation induced by intradermally injected eotaxin and, for comparison, LTB_4. Preliminary experiments suggested that intravenous IL-5 had a remarkably rapid enhancing effect on eosinophil accumulation in the skin. The study was therefore designed to address specifically the acute effects of intravenous IL-5. IL-5 (18.3 pmol/kg) or vehicle was injected intravenously into six pairs of guinea pigs. 1 h later, eotaxin and LTB_4 were injected intradermally and eosinophil accumulation was measured after an additional 2 h.

Intradermal injection of either eotaxin (0.31–1.95 pmol per site) or LTB_4 (16–100 pmol per site) induced a small dose-related eosinophil accumulation in the control group of animals, which was significant ($P < 0.05$) at the highest dose of eotaxin and the two higher doses of LTB_4 (Fig. 1, *open symbols*). In animals injected intravenously with IL-5, the eosinophil accumulation in response to eotaxin and LTB_4 (Fig. 1, filled symbols) was dramatically increased at all the doses tested, except the lowest dose of eotaxin, when compared with the control group. Under these conditions, eotaxin was ~30 times more potent than LTB_4 as an eosinophil chemoattractant.

Effect of Intradermally Injected IL-5. IL-5 or vehicle was injected intravenously into another four pairs of guinea pigs. 1 h later, IL-5 (0.32–2.90 pmol/site) was injected intradermally and eosinophil accumulation was determined after an additional 2 h as before. No significant local eosinophil accumulation was induced by intradermal IL-5 in either set of animals at the doses used (Fig. 2). The positive control used in these experiments was LTB_4, which gave responses similar to those in Fig. 1.

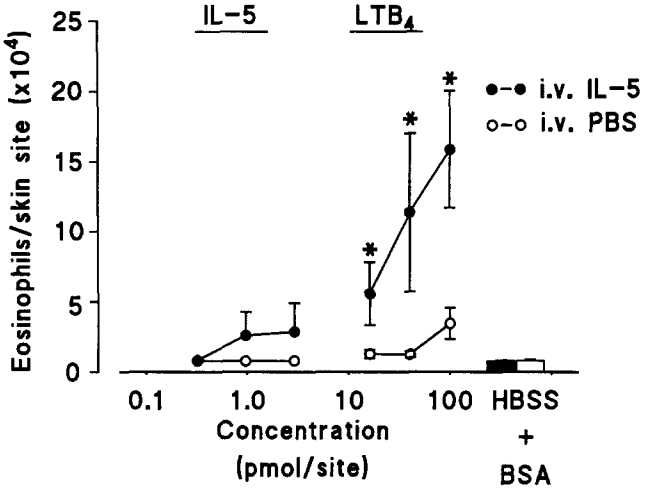

Figure 2. The effect of intravenous IL-5 on the local accumulation of eosinophils in response to intradermal IL-5 and LTB_4. Eosinophil accumulation over 2 h induced by intradermal IL-5 and LTB_4 (50 μl per site) 1 h after intravenous administration of IL-5 or vehicle (PBS/0.1% BSA). Results represent the number of eosinophils per skin site ± SEM ($n = 4$). A significant difference between test and control groups is indicated by *($P < 0.05$).

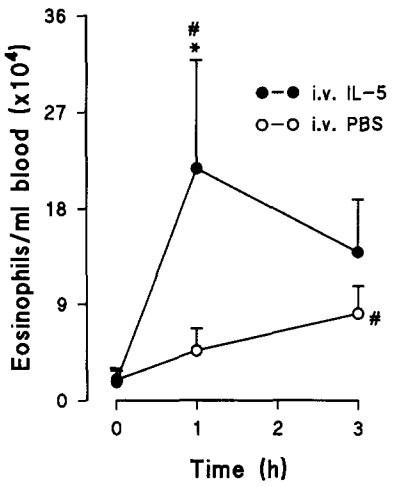

Figure 3. Circulating eosinophil numbers after intravenous IL-5 (results from the experiments shown in Fig. 1). Circulating eosinophil numbers over 3 h after intravenous administration of IL-5 or PBS/0.1% BSA. Results represent the number of eosinophils per milliliter of blood ± SEM ($n = 6$). A significant difference between circulating levels pre- and postintravenous treatment within a group is indicated by # ($P < 0.05$), and at a given time point between groups by *($P < 0.05$). Similar results were obtained in the group of animals used to investigate the eosinophil accumulation induced by i.d. IL-5 (Fig. 2), i.e., intravenous IL-5 ($n = 4$) induced a significant increase in circulating eosinophils after 1 h (17.3 ± 6.3 × 10^4 cells per ml; $P < 0.05$), but not at 3 h (9.9 ± 0.9 × 10^4 cells per ml) when compared with the preinjection level (0 h; 1.0 ± 0.6 × 10^4 cells per ml). In the vehicle-injected controls ($n = 4$), the number of circulating eosinophils did not change significantly during the 3-h period (i.e., 0-h level; 4.5 ± 2.7 × 10^4 cells per ml, 1-h level; 7.5 ± 3.6 × 10^4 cells per ml, 3-h level; 11.9 ± 2.4 × 10^4 cells per ml).

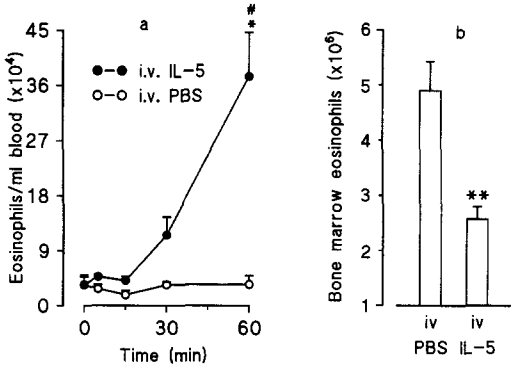

Figure 4. The kinetics of the onset of blood eosinophilia induced by intravenous IL-5 and the associated changes in the bone marrow pool of eosinophils. (*a*) Circulating eosinophil numbers over 1 h after intravenous IL-5 or PBS/0.1% BSA. Results represent the number of eosinophils per ml blood ± SEM ($n = 4$). A significant difference between circulating levels pre- and postintravenous treatment within a group is indicated by # ($P < 0.05$), and at a given time point between groups by *($P < 0.05$). (*b*) Femur bone marrow eosinophil numbers displaced by flushing 1 h after intravenous IL-5 or PBS/0.1% BSA. Results represent the number of bone marrow eosinophils ± SEM ($n = 6$). The total number of nucleated cells recovered from the femurs in the IL-5 and PBS groups was 6.4 ± 0.5 × 10^7 cells and 7.1 ± 0.8 × 10^7 cells, respectively. A significant difference is indicated by **($P < 0.01$). In these experiments, the circulating eosinophil numbers were: pre–IL-5, 8.1 ± 4.2 × 10^4 and 1 h post–IL-5, 28.8 ± 7.2 × 10^4; pre-PBS, 8.4 ± 3.3 × 10^4 and 1 h post-PBS, 3 ± 0.4 × 10^4.

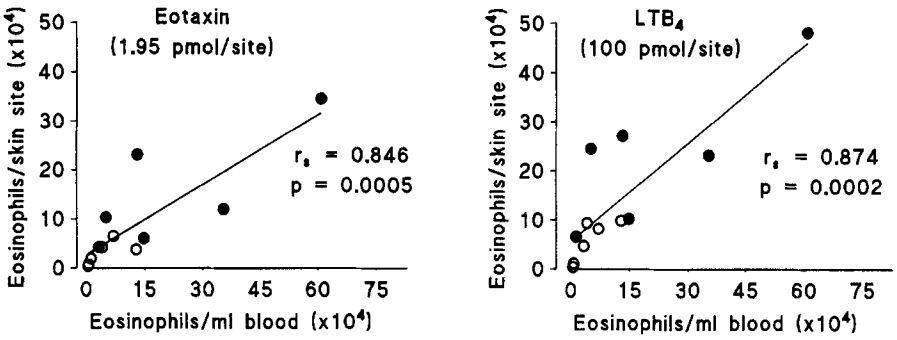

Figure 5. The relationship between circulating eosinophil numbers and the local accumulation of eosinophils induced by eotaxin and LTB_4. Regression analysis of circulating eosinophils at the time of intradermal injection of eotaxin (1.95 pmol per site) or LTB_4 (100 pmol per site) and the local accumulation of eosinophils induced by these agonists over 2 h (results from the experiments shown in Fig. 1). Each point represents the determination from a separate animal, those receiving intravenous IL-5 (n = 6) being indicated by the closed symbols and those receiving intravenous vehicle (n = 6) by the open symbols. The solid lines show linear regression, and the Spearman rank correlation coefficients (r_s) are also indicated. A linear regression with a significant r_s was also seen with the two lower doses of each agonist, i.e., eotaxin: 0.78 and 0.31 pmol per site giving r_s = 0.902 (P <0.0001) and r_s = 0.769 (P <0.004), respectively; LTB_4: 40 and 16 pmol per site giving r_s = 0.895 (P <0.0001) and r_s = 0.706 (P <0.02), respectively.

The Effect of Intravenous IL-5 on Circulating Eosinophil Numbers. In the experiments described above, blood samples were taken before intravenous injections, as well as 1 and 3 h thereafter, i.e., at the beginning and end of the measurement period of local cell accumulation. Blood eosinophil counts from the experiments using intradermal eotaxin (Fig. 1) are shown in Fig. 3. Intravenous IL-5 induced a remarkably rapid effect on circulating eosinophils, as reported in a preliminary study (30). Intravenous IL-5 (18.3 pmol/kg) induced a 13-fold increase in the numbers of circulating eosinophils at 1 h, when compared with preinjection levels (Fig. 3). Variability between animals was marked. Circulating eosinophil numbers also rose in vehicle-injected control animals, possibly because of handling or anesthesia; this increase was slow, with numbers significantly above preinjection levels at 3 h. The difference between circulating eosinophil numbers in animals injected intravenously with IL-5 or vehicle was significant at 1 h, but not at 3 h. A similar effect on circulating eosinophil numbers induced by intravenous IL-5 was observed in the second group of animals shown in Fig. 2. (see Fig. 3 legend).

A further set of experiments were designed to investigate the time of onset of blood eosinophilia induced by intravenous IL-5; these results are shown in Fig. 4 *a*. The significant increase in blood eosinophils observed 1 h after intravenous IL-5 was associated with a significant decrease in the number of displaceable eosinophils recovered from the shaft of the femur after a 25-ml flush of the marrow cavity (Fig. 4 *b*).

Analysis of the data on eosinophil accumulation in the skin revealed a significant direct correlation between eosinophil accumulation in response to a given dose of eotaxin or LTB_4 and the number of circulating eosinophils at 1 h after intravenous injection of IL-5 or vehicle, as shown in Fig. 5. This was significant for all three doses of the chemoattractants used (see Fig. 5 legend).

Discussion

This study on the accumulation of eosinophils in guinea pig skin demonstrates that IL-5, at the doses tested, has little chemoattractant activity in this in vivo system. A low dose of IL-5 administered intravenously, however, has a rapid and dramatic enhancing effect on eosinophil accumulation induced by intradermally injected eotaxin. The same effect was also observed with intradermal LTB_4, which at lower potency, also induces eosinophil accumulation in this species (29).

These observations can provide an explanation for the results of previous studies, demonstrating that acutely administered neutralizing antibodies to IL-5 suppress eosinophil accumulation in allergen-challenged/sensitized animals in vivo. Our results, however, do not support the inference that IL-5 is an important eosinophil chemoattractant in vivo. It should be noted that previous studies showing a direct chemoattractant activity of IL-5 required high doses in vivo (21) and high concentrations in vitro (6). Higher intradermal doses of IL-5 were not used in our study to avoid the complications imposed by a proportion of the cytokine gaining access to the circulation.

The numbers of eosinophils accumulating in response to intradermal eotaxin correlated with an increase in circulating eosinophil numbers induced by intravenous IL-5. The blood eosinophilia induced by intravenous IL-5 was remarkably rapid in onset. This eosinophilia correlated with a significant decrease in the number of displaceable eosinophils recovered from the shaft of the femur, suggesting the presence of a rapidly mobilizable bone marrow pool of eosinophils. Further support for this is provided by the observation that the blood eosinophilia seen 1 h after IL-5 administered intravenously through the ear was substantially reduced by previous occlusion of the blood supply to the hind limbs (A. Das, S.M. Rankin, P.D. Collins, and T. J. Williams, unpublished observations). The cells released from the bone marrow by IL-5 may be at different stages of maturity; there are no available markers to distinguish these in the guinea pig. The cells appearing in the blood, however, are clearly able to migrate rapidly into tissues. The presence of a mobilizable pool of bone marrow eosinophils has been suggested previously (31, 32). In these studies, however, the release of the cells was demonstrated 1–2 d

after antigen challenge of sensitized guinea pigs. Our results indicate that this pool of eosinophils may in fact be available within a very short time (<1 h) after appropriate stimulation. In addition to releasing cells from the bone marrow, IL-5 may contribute to local eosinophil accumulation by further mechanisms, e.g., eosinophil priming and/or facilitating their migration through the microvascular endothelium, as suggested in other studies (4, 6, 33–35).

Taking all these observations together, we postulate the following sequence of events. Eotaxin expression is observed in naive guinea pig tissues (26) and may, therefore, be involved in a slow recruitment of eosinophils to maintain the tissue cell numbers under basal conditions. On exposure of a tissue to allergen in a sensitized animal, there is an upregulation of eotaxin mRNA (26) and an early appearance of protein (24). Eotaxin will be unable to induce recruitment to any great extent, however, if circulating cell numbers are low. IL-5 is also secreted locally in the tissue, but we propose that its initial role is hormonal, i.e., it circulates in the blood and stimulates the release of a rapidly mobilizable pool of bone marrow eosinophils. The synergism between remotely acting IL-5 and locally acting eotaxin will then induce a rapid recruitment of eosinophils in the allergen-stimulated tissue. In the longer term, the effect of IL-5 on eosinophil differentiation and proliferation would manifest itself in an increased pool of available eosinophils in the bone marrow and increased circulating cell numbers. Furthermore, together with other factors, IL-5 can enhance eosinophil survival (22) and responsiveness (20) once the cells have accumulated in the allergen-stimulated tissue.

We believe that these experiments, showing cooperation between a chemokine and IL-5, simulate important early events in the acute allergic reaction, which ensures a rapid accumulation of eosinophils in tissues exposed to allergen in the sensitized individual.

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References

1. Sanderson, C.J., D.G. Warren, and M. Strath. 1985. Identification of a lymphokine that stimulates eosinophil differentiation in vitro. Its relationship to interleukin 3, and functional properties of eosinophils produced in cultures. *J. Exp. Med.* 162:60–74.
2. Clutterbuck, E.J., and C.J. Sanderson. 1988. Human eosinophil hematopoiesis studied in vitro by means of murine eosinophil differentiation factor (IL-5): production of functionally active eosinophils from normal human bone marrow. *Blood.* 71:646–651.
3. Yamaguchi, Y., T. Suda, J. Suda, M. Eguchi, Y. Miura, N. Harada, A. Tominaga, and K. Takatsu. 1988. Purified interleukin 5 supports the terminal differentiation and proliferation of murine eosinophilic precursors. *J. Exp. Med.* 167:43–56.
4. Sehmi, R., A.J. Wardlaw, O. Cromwell, K. Kurihara, P. Waltmann, and A.B. Kay. 1992. Interleukin-5 selectively enhances the chemotactic response of eosinophils obtained from normal but not eosinophilic subjects. *Blood.* 79:2952–2959.
5. Warringa, R.A.J., H.J.J. Mengelers, P.H.M. Kuijper, J.A.M. Raaijmakers, P.L.B. Bruijnzeel, and L. Koenderman. 1992. In vivo priming of platelet-activating factor-induced eosinophil chemotaxis in allergic asthmatic individuals. *Blood.* 79: 1836–1841.
6. Coeffier, E., D. Joseph, and B.B. Vargaftig. 1991. Activation of guinea pig eosinophils by human recombinant IL-5. Selective priming to platelet-activating factor-acether and interference of its antagonists. *J. Immunol.* 147:2595–2602.
7. Tai, P.C., L. Sun, and C.J.F. Spry. 1991. Effects of IL-5, granulocyte/macrophage colony-stimulating factor (GM-CSF) and IL-3 on the survival of human blood eosinophils in vitro. *Clin. Exp. Immunol.* 85:312–316.
8. Coffman, R.L., B.W.P. Seymour, S. Hudak, J. Jackson, and D. Rennick. 1989. Antibody to interleukin-5 inhibits helminth-induced eosinophilia in mice. *Science (Wash. DC).* 245:308–310.
9. Sedgwick, J.B., W.J. Calhoun, G.J. Gleich, H. Kita, J.S. Abrams, L.B. Schwartz, B. Volovitz, M. Ben-Yaakov, and W.W. Busse. 1991. Immediate and late airway response of allergic rhinitis patients to segmental antigen challenge. *Am.*

Rev. Respir. Dis. 144:1274–1281.

10. Hamid, Q., M. Azzawi, S. Ying, R. Moqbel, A.J. Wardlaw, C.J. Corrigan, B. Bradley, S.R. Durham, J.V. Collins, P.K. Jeffery, D.J. Quint, and A.B. Kay. 1991. Interleukin-5 in the pathogenesis of asthma. *J. Clin. Invest.* 87:1541–1546.
11. Krishnaswamy, G., M.C. Liu, S.-N. Su, M. Kumai, H.-Q. Xiao, D.G. Marsh, and S.-K. Huang. 1993. Analysis of cytokine transcripts in the bronchoalveolar lavage cells of patients with asthma. *Am. J. Respir. Cell Mol. Biol.* 9:279–286.
12. Kay, A.B., S. Ying, V. Varney, M. Gaga, S.R. Durham, R. Moqbel, A.J. Wardlaw, and Q. Hamid. 1991. Messenger RNA expression of the cytokine gene cluster, interleukin 3 (IL-3), IL-4, IL-5, and granulocyte/macrophage colony-stimulating factor, in allergen-induced late-phase cutaneous reactions in atopic subjects. *J. Exp. Med.* 173:775–778.
13. Dubucquoi, S., P. Desreumaux, A. Janin, O. Klein, M. Goldman, J. Tavernier, A. Capron, and M. Capron. 1994. Interleukin 5 synthesis by eosinophils: association with granules and immunoglobulin-dependent secretion. *J. Exp. Med.* 179:703–708.
14. Desreumaux, P., A. Janin, S. Dubucquoi, M.-C. Copin, G. Torpier, A. Capron, M. Capron, and L. Prin. 1993. Synthesis of interleukin-5 by activated eosinophils in patients with eosinophilic heart diseases. *Blood.* 82:1553–1560.
15. Broide, D.H., M.M. Paine, and G.S. Firestein. 1992. Eosinophils express interleukin 5 and granulocyte macrophage-colony-simulating factor mRNA at sites of allergic inflammation in asthmatics. *J. Clin. Invest.* 90:1414–1424.
16. Yamaguchi, S., H. Nagai, H. Tanaka, M. Tsujimoto, and N. Tsuruoka. 1994. Time course study for antigen-induced airway hyperreactivity and the effect of soluble IL-5 receptor. *Life Sciences.* 54:471–475.
17. Chand, N., J.E. Harrison, S. Rooney, J. Pillar, R. Jakubicki, K. Nolan, W. Diamantis, and R.D. Sofia. 1992. Anti-IL-5 monoclonal antibody inhibits allergic late phase bronchial eosinophilia in guinea pigs: a therapeutic approach. *Eur. J. Pharmacol.* 211:121–123.
18. Van Oosterhout, A.J.M., R.S. Ladenius, H.F.J. Savelkoul, I. Van Ark, K.C. Delsman, and F.P. Nijkamp. 1993. Effect of anti-IL-5 and IL-5 on airway hyperreactivity and eosinophils in guinea pigs. *Am. Rev. Respir. Dis.* 147:548–552.
19. Mauser, P.J., A. Pitman, A. Witt, X. Fernandez, J. Zurcher, T. Kung, H. Jones, A.S. Watnick, R.W. Egan, W. Kreutner, and K.G. Adams. 1993. Inhibitory effect of the TRFK-5 anti-IL-5 antibody in a guinea pig model of asthma. *Am. Rev. Respir. Dis.* 148:1623–1627.
20. Coeffier, E., D. Joseph, and B.B. Vargaftig. 1994. Role of Interleukin-5 in enhanced migration of eosinophils from airways of immunized guinea-pigs. *Br. J. Pharmacol.* 113:749–756.
21. Iwama, T., H. Nagai, H. Suda, N. Tsuruoka, and A. Koda. 1992. Effect of murine recombinant interleukin-5 on the cell population in guinea-pig airways. *Br. J. Pharmacol.* 105:19–22.
22. Yamaguchi, Y., Y. Hayashi, Y. Sugama, Y. Miura, T. Kasahara, S. Kitamura, M. Torisu, S. Mita, A. Tominaga, K. Takatsu, and T. Suda. 1988. Highly purified murine interleukin 5 (IL-5) stimulates eosinophil function and prolongs in vitro survival. IL-5 as an eosinophil chemotactic factor. *J. Exp. Med.* 167:1737–1742.
23. Wang, J.M., A. Rambaldi, A. Biondi, Z.G. Chen, C.J. Sanderson, and A. Mantovani. 1989. Recombinant human interleukin 5 is a selective eosinophil chemoattractant. *Eur. J. Immunol.* 19:701–705.
24. Jose, P.J., D.A. Griffiths-Johnson, P.D. Collins, D.T. Walsh, R. Moqbel, N.F. Totty, O. Truong, J.J. Hsuan, and T.J. Williams. 1994. Eotaxin: a potent eosinophil chemoattractant cytokine detected in a guinea-pig model of allergic airways inflammation. *J. Exp. Med.* 179:881–887.
25. Griffiths-Johnson, D.A., P.D. Collins, A.G. Rossi, P.J. Jose, and T.J. Williams. 1993. The chemokine, Eotaxin, activates guinea-pig eosinophils in vitro, and causes their accumulation into the lung in vivo. *Biochem. Biophys. Res. Commun.* 197: 1167–1172.
26. Jose, P.J., I.M. Adcock, D.A. Griffiths-Johnson, N. Berkman, T.N.C. Wells, T.J. Williams, and C.A. Power. 1994. Eotaxin: cloning of an eosinophil chemoattractant cytokine and increased mRNA expression in allergen-challenged guinea-pig lungs. *Biochem. Biophys. Res. Commun.* 205:788–794.
27. Pettipher, E.R., E.D. Salter, and H.J. Showell. 1994. Effect of in vivo desensitization to leukotriene B_4 on eosinophil infiltration in response to C5a in guinea-pig skin. *Br. J. Pharmacol.* 113:117–120.
28. Strath, M., D.J. Warren, and C.J. Sanderson. 1985. Detection of eosinophils using an eosinophil peroxidase assay. Its use as an assay for eosinophil differentiation factors. *J. Immunol. Methods.* 83:209–215.
29. Faccioli, L.H., S. Nourshargh, R. Moqbel, F.M. Williams, R. Sehmi, A.B. Kay, and T.J. Williams. 1991. The accumulation of ^{111}In-eosinophils induced by inflammatory mediators in vivo. *Immunology.* 73:222–227.
30. Sanjar, S., P. McCabe, and L. Reynolds. 1992. Recombinant human IL-5 (rh-IL5) induces a rapid and selective blood eosinophilia in the guinea-pig. *Am. Rev. Respir. Dis. (Proc. Suppl.)* 143:A14P. (Abstr.)
31. Hudson, G. 1963. Changes in the bone marrow reserve of eosinophils following re-exposure to foreign protein. *Br. J. Haematol.* 9:446–455.
32. Hudson, G. 1964. The marrow reserve of eosinophils. Effect of cortical hormones on the foreign protein response. *Br. J. Haematol.* 10:122–130.
33. Schweizer, R.C., B.A.C. Welmers, J.A.M. Raaijmakers, P. Zanen, J.-W.J. Lammers, and L. Koenderman. 1994. RANTES- and interleukin-8-induced responses in normal human eosinophils: effects of priming with interleukin-5. *Blood.* 83:3697–3704.
34. Ebisawa, M., M.C. Liu, T. Yamada, M. Kato, L.M. Lichtenstein, B.S. Bochner, and R.P. Schleimer. 1994. Eosinophil transendothelial migration induced by cytokines II. Potentiation of eosinophil transendothelial migration by eosinophil-active cytokines. *J. Immunol.* 152:4590–4596.