

Anti-IL-5 (mepolizumab) therapy induces bone marrow eosinophil maturational arrest and decreases eosinophil progenitors in the bronchial mucosa of atopic asthmatics

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Background: Eosinophils develop from CD34⁺ progenitors under the influence of IL-5. Atopic asthmatic individuals have increased numbers of mature eosinophils and eosinophil progenitors within their bone marrow and bronchial mucosa. We have previously reported that anti-IL-5 monoclonal antibody treatment decreases total bone marrow and bronchial mucosal eosinophil numbers in asthma.

Objective: Using an anti-IL-5 monoclonal antibody, we examined the role of IL-5 in eosinophil development within the bone marrow and bronchial mucosa in asthma.

Methods: Blood, bone marrow, and airway mucosal biopsy specimens were examined before and after anti-IL-5 (mepolizumab) treatment of asthmatic individuals in a double-blind, placebo-controlled trial. Numbers of mature and immature eosinophils were measured by histologic stain (bone marrow myelocytes, metamyelocytes, and mature eosinophils), flow cytometry (bone marrow and blood CD34⁺/IL-5R α ⁺ cells), enumeration of bone marrow-derived eosinophil/basophil colony-forming units in methylcellulose culture, and sequential immunohistochemistry and in situ hybridization (bronchial mucosal CD34⁺/IL-5R α mRNA⁺ cells).

Results: Mepolizumab decreased mature eosinophil numbers in the bone marrow by 70% ($P = .017$) in comparison with placebo and decreased numbers of eosinophil myelocytes and metamyelocytes by 37% ($P = .006$) and 44% ($P = .003$), respectively. However, mepolizumab had no effect on numbers of blood or bone marrow CD34⁺, CD34⁺/IL-5R α ⁺ cells, or eosinophil/basophil colony-forming units. There was a significant decrease in bronchial mucosal CD34⁺/IL-5R α mRNA⁺ cell numbers in the anti-IL-5 treated group ($P = .04$).

Conclusion: These data suggest that anti-IL-5 therapy might induce partial maturational arrest of the eosinophil lineage in the bone marrow. The reduction in airway CD34⁺/IL-5 mRNA⁺ cell numbers suggests that IL-5 might also be required for local tissue eosinophilopoiesis. (*J Allergy Clin Immunol* 2003;111:714-9.)

Key words: Eosinophil progenitors, bone marrow, asthma, anti-IL-5

Selective eosinophil accumulation within the bronchial mucosa, with activation and release of basic proteins and membrane-derived lipid mediators, is characteristic of asthma.¹ There is strong evidence to suggest that the bone marrow plays an active role in promoting and perpetuating eosinophilic tissue inflammation.² Percentages of both mature and immature eosinophils are increased in the bone marrow of atopic asthmatic individuals in comparison with atopic, nonasthmatic, and normal control individuals.³ These findings, in conjunction with animal studies,⁴ suggest that in asthmatic individuals there is an increased bone marrow pool of eosinophils available for rapid mobilization.

In common with other leukocytes, eosinophils develop from a CD34⁺ progenitor.⁵ Eosinophil differentiation and maturation occurs largely in the bone marrow, and on release cells traffic rapidly to tissue.⁶ One of the earliest steps in development of the eosinophil lineage is upregulation of expression of the IL-5 receptor α chain (IL-5R α) on the surface of CD34⁺ cells.⁷ Sehmi et al⁸ have demonstrated an increase in the percentage of bone marrow CD34⁺/IL-5R α ⁺ cells 24 hours after inhaled allergen challenge in dual responder atopic asthmatic individuals. These findings suggest a direct signal between the bronchial mucosa and the bone marrow that is able to increase eosinophil production at times of increased allergic inflammation. Another method of examining early eosinophil progenitors involves measuring the number of peripheral blood- or bone marrow-derived eosinophil/basophil colony-forming units (Eo/B-CFUs) grown in methylcellulose culture.⁹ Eo/B-CFUs are increased in the blood of atopic individuals and fluctuate with seasonal allergen exposure, onset and maintenance of symptoms, and severity of atopic disease, and they increase in the bone marrow after inhaled allergen challenge.¹⁰

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Abbreviations used

APAAP:	Alkaline phosphatase–anti-alkaline phosphatase
Eo/B-CFU:	Eosinophil/basophil colony-forming unit
IL-5R α :	IL-5 receptor α chain
MNC:	Mononuclear cell
NAMNC:	Nonadherent mononuclear cell

Progenitors might also traffic from the bone marrow to the tissues, where they can differentiate into leukocytes. This phenomenon has been termed *in situ hemopoiesis*, and relevance to allergic disease is supported by the finding of increased numbers of CD34⁺/IL-5R α mRNA⁺ cells in the bronchial mucosa of atopic asthmatic individuals¹¹ and by the presence of CD45⁺/CD34⁺ cells with eosinophil/basophil colony-forming activity in nasal polyp tissue.¹² Further evidence comes from an explant model of nasal biopsy tissue taken from individuals with seasonal rhinitis, in which culture with IL-5 decreased the number of CD34⁺/IL-5R α mRNA⁺ cells and produced a concomitant increase in the number of major basic protein–positive eosinophils.¹³

Eosinophils are considered to be an important potential target for antiasthma therapies, and blockade of IL-5 has been extensively investigated as a potential treatment.¹⁴ IL-5 is a key cytokine in eosinophil differentiation and maturation in experimental models¹⁵; it is also key in the priming of eosinophils for enhanced chemotaxis¹⁶ and in delaying apoptosis.¹⁷

We have recently reported that a humanized monoclonal antibody against IL-5, mepolizumab, reduces total eosinophil numbers in the bone marrow and bronchial mucosa in asthma, though the extent of the change was less than what was seen in blood.¹⁸ Here we examine the effect of mepolizumab treatment on development of eosinophils in blood, bone marrow, and airway tissue in the same patients.

METHODS

Study design

The clinical study of anti-IL-5 (mepolizumab) in asthma has previously been described.¹⁸ In brief, 24 volunteers with mild asthma were recruited to a 2-center, double blind, placebo-controlled, parallel-group study. At baseline, venous blood sampling, bone marrow aspiration, and fiberoptic bronchoscopy with endobronchial biopsy were performed. Two days later, the study medication (750 mg mepolizumab) or an equal volume of placebo was given as an intravenous infusion in a double-blind fashion. The second and third infusions were given 4 and 8 weeks after the first. The blood sampling, bone marrow aspiration, and bronchoscopy were repeated 2 weeks after the final infusion. The study was approved by the local ethics committees; all volunteers gave informed consent.

Processing of bronchial biopsy specimens

Biopsy specimens were fixed immediately in 4% paraformaldehyde (BDH, Leicester, United Kingdom) before being mounted in OCT medium (BDH) and snap-frozen in isopentane (BDH) pre-cooled in liquid nitrogen.

Immunohistochemistry

Six- μ m sections were captured onto 0.1% poly-L-lysine–coated slides (BDH), and immunohistochemistry was performed through use of a modification of the alkaline phosphatase–anti-alkaline phosphatase (APAAP) technique, as described previously.⁸ In brief, a mouse monoclonal antibody against CD34 (BMA Biomedicals via Cedarlane Labs, Hornby, Ontario, Canada) was used as the primary antibody under appropriate conditions. After incubation with a rabbit antimouse secondary antibody (Dako, Carpinteria, Calif), alkaline phosphatase–mouse–anti-alkaline phosphatase (Dako) was added and the signal was enhanced by repeating the secondary antibody and APAAP steps before development with Fast Red solution (Sigma-Aldrich, Gillingham, United Kingdom). Appropriate isotype controls were included in staining runs.

In situ hybridization

The IL-5R α complementary DNA was used as a template for synthesis of a 92-base pair ³⁵S-labeled riboprobe specific for the membrane-associated isoform, as previously described.⁸ Negative controls used hybridization with the sense probe and pretreatment of slides with Rnase A (Promega, Southampton, United Kingdom) before hybridization with the antisense probe.

Simultaneous in situ hybridization and immunohistochemistry

To examine expression of IL-5R α mRNA by CD34⁺ cells, sections were first stained with an anti-CD34 monoclonal antibody and developed with Fast Red, as described above; they were then processed for in situ hybridization through use of the ³⁵S-labeled riboprobe for IL-5R α membrane-associated isoform.

Quantification of immunohistochemistry and in situ hybridization

Slides were counted in duplicate; the counters were rendered blind to the volunteers' treatment by means of an eyepiece graticule. The results are expressed as numbers of cells per square millimeter of bronchial biopsy tissue.

Bone marrow aspiration

Bone marrow was aspirated from the posterior iliac crest and transferred to a sterile universal container with 1000 units of heparin. Before preparation of cytopins, red blood cells were removed by osmotic lysis with ice-cold distilled water before resuspension in McCoy's 5A medium (Sigma) at a concentration of 0.5 $\times$ 10⁶ cells per milliliter. One hundred μ L of the cell suspension was inserted into each Shandon 2 cytopsin device (Shandon, Inc., Pittsburgh, Pa), and the cells were captured onto 0.1% poly-L-lysine–coated slides. Cytopins were stained through use of a Hema-tek (Miles, Elkhart, Ind) in the Department of Haematology at the Royal Brompton Hospital and counted in a blinded fashion. Eosinophil numbers and level of maturity (myelocyte, metamyelocyte, or mature eosinophil) were determined as described previously³ and expressed as percentages of the total cells present. A minimum of 200 cells were counted per cytopsin.

Preparation of bone marrow and peripheral blood cells for quantification of CD34⁺/IL-5R α ⁺ cells

Low-density mononuclear cells (MNCs) were isolated by sedimentation on Histopaque (Sigma) density gradients (specific gravity, 1.077), as described previously.⁸ Monocytes were depleted from the MNC fraction by incubation in plastic flasks for 2 hours at 37°C. Samples of 1 $\times$ 10⁶ nonadherent MNCs (NAMNCs) were incubated with

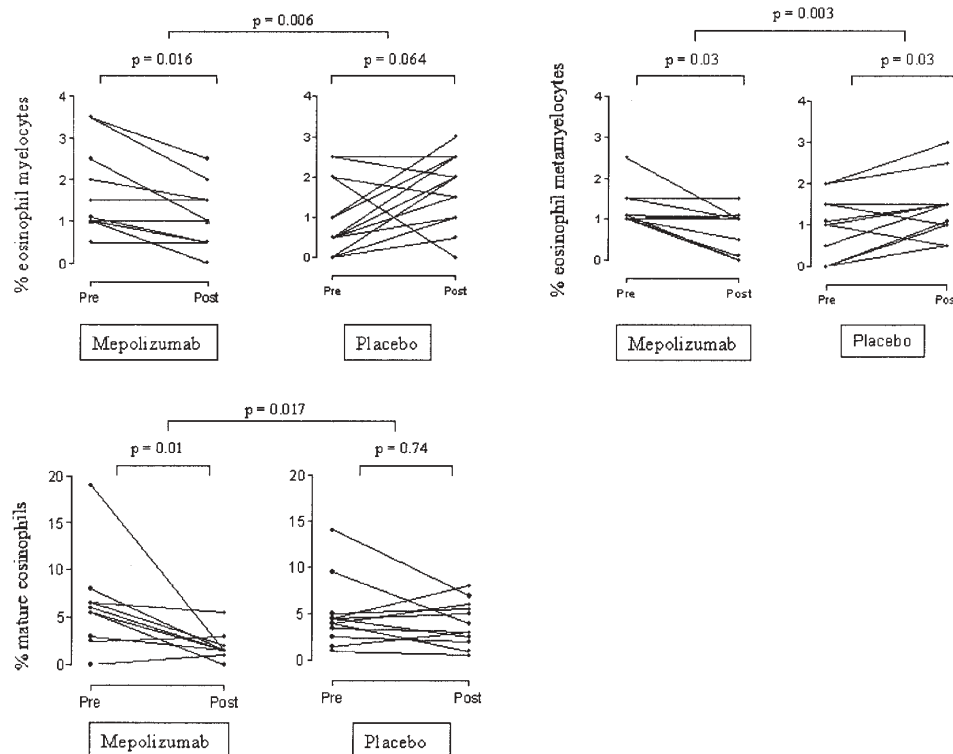


FIG 1. Effect of mepolizumab or placebo on eosinophil myelocytes, metamyelocytes, and mature eosinophils in the bone marrow (results are expressed as percentages of total cells counted; $n = 10$ and $n = 13$ in the mepolizumab and placebo groups, respectively).

saturating amounts of biotin-conjugated anti-IL-5R α (a kind gift of Dr Jan Tavernier, Ghent, Belgium) or IgG $_1$ isotype control antibody (BD Biosciences Pharmingen, Mississauga, Ontario, Canada) for 30 minutes at 4°C. Next, the cells were washed and stained with saturating concentrations of streptavidin-conjugated PerCP, anti-CD45 FITC, and anti-CD34 PE (all Becton Dickinson, Franklin Lakes, NJ) for 30 minutes at 4°C. Cells were then washed before fixing in PBS and 1% paraformaldehyde. Samples were analyzed on a BD FACSscan (Becton Dickinson) through use of a sequential gating strategy to enumerate CD45 $^{+}$ /CD34 $^{+}$ /IL-5R α $^{+}$ cells, as described previously.⁸

Enumeration of bone marrow Eo/B-CFUs

Semisolid methylcellulose cultures of NAMNCs were performed as described previously.⁹ In brief, NAMNCs were cultured in duplicate in supplemented Iscove's modified Dulbecco's medium (GIBCO, Grand Island, NY) with 1% penicillin/streptomycin and 5×10^{-5} mol/L 2-mercaptoethanol, 0.9% methylcellulose, and 20% FCS in the presence of 1 ng/mL of recombinant human IL-5 (BD Biosciences Pharmingen). Cultures were incubated for 14 days at 37°C in 5% CO $_2$, after which colonies were identified as Eo/B-CFUs according to previously described criteria⁹ and expressed as colony-forming units per 2.5×10^5 NAMNCs.

Statistical analysis

Nonparametric statistical methods were used for within-group paired comparisons (Wilcoxon signed rank test). For between-group comparisons, the delta values of the effect of mepolizumab and placebo were compared (Mann-Whitney test). P values of ≤ 0.05 were considered to be significant.

RESULTS

Eleven volunteers were randomized to receive mepolizumab, and 13 volunteers were randomized to receive placebo.

Eosinophil myelocytes, metamyelocytes, and mature eosinophils

Mepolizumab significantly reduced both the number of eosinophil myelocytes (mean suppression, 37%; $P = .006$ in comparison with placebo) and the number of metamyelocytes in the bone marrow (mean reduction, 44%; $P = .003$ in comparison with placebo; Fig 1 and Table I). Mepolizumab therapy also produced a 70% mean reduction in the number of mature eosinophils in the bone marrow ($P = .017$; Fig 1 and Table I). Not enough eosinophil myelocytes and metamyelocytes were detected in peripheral blood for quantification.

CD34 $^{+}$ and CD34 $^{+}$ /IL-5R α $^{+}$ cells

Despite the observed changes in immature eosinophils, there was no overall change in the total number of bone marrow or blood CD34 $^{+}$ cells after mepolizumab therapy and no difference in comparison with placebo (Table I). Mepolizumab therapy also had no effect on the percentage of CD34 $^{+}$ cells expressing the IL-5R α subunit in blood or bone marrow (Table I).

Bone marrow–derived Eo/B-CFUs

Mepolizumab therapy had no significant effect on the number of Eo/B-CFUs grown in methylcellulose culture with recombinant human IL-5 (Table I).

Bronchial mucosal CD34⁺/IL-5R α mRNA⁺ cells

Mepolizumab produced a significant decrease in the number of CD34⁺/IL-5R α mRNA⁺ cells within the bronchial mucosa ($P = .04$); however, there was no significant difference in comparison with placebo ($P = .55$) (Fig 2 and Table I).

DISCUSSION

We have demonstrated that IL-5 blockade with mepolizumab significantly reduced terminal differentiation of eosinophils (myelocytes, metamyelocytes, and mature eosinophils) within the bone marrow but had no significant overall effect on numbers of early eosinophil progenitors (CD34⁺/IL-5R α ⁺ cells or Eo/B-CFUs). Anti-IL-5 also significantly decreased numbers of CD34⁺/IL-5R α mRNA⁺ cells in the bronchial mucosa. These findings are compatible with anti-IL-5–induced maturational arrest of bone marrow eosinophil differentiation, and they are in keeping with our earlier report that mepolizumab decreased, but did not deplete, total eosinophil numbers in the bone marrow and bronchial mucosa.¹⁸

There are several possible explanations for the apparent differential effect of mepolizumab on early (ie, CD34⁺ and CD34⁺/IL-5R α ⁺ cells) and late (from myelocyte to fully mature cells) eosinophil differentiation in the bone marrow. First, our findings are consistent with maturational arrest of the entire bone marrow eosinophil lineage. However, if both IL-5–induced upregulation of the IL-5R α subunit on CD34⁺ cells and the subsequent maturation of these cells toward the eosinophil lineage were blocked by anti-IL-5 treatment, the number of CD34⁺/IL-5R α ⁺ cells would be expected to remain constant or even increase, as was observed with CD34⁺ cells in nasal polyps from patients who had received topical corticosteroid treatment.¹² Thus, we favor the possibility that anti-IL-5 blocked all stages of eosinophil maturation from the CD34⁺ cell onward. However, it is possible that IL-5 blockade induced apoptosis of terminally differentiated eosinophils without effect on earlier progenitors. The later progenitors have yet to be examined for evidence of apoptosis after treatment, and this is an area of ongoing investigation.

A second explanation for the persistence of CD34⁺ and CD34⁺/IL-5R α ⁺ cells in the marrow is redundancy of cytokine function: although IL-5 is important in eosinophil development, other cytokines might sustain eosinophil development in its absence, as has been demonstrated in vivo in animal models.¹⁴ Obvious candidates are IL-3 and GM-CSF, both of which have been shown to be expressed at increased concentrations in asthmatic airways¹⁹ and are important in early eosinophil

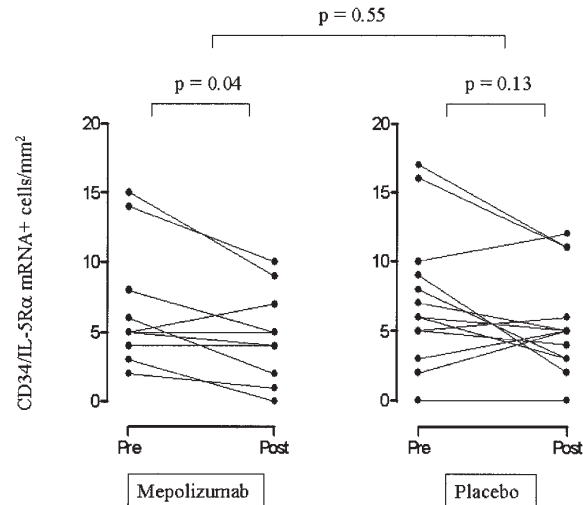


FIG 2. Effect of mepolizumab or placebo treatment on the number of CD34⁺/IL-5R α mRNA⁺ cells per square millimeter within the bronchial mucosa ($n = 10$ and $n = 13$ in the mepolizumab and placebo groups, respectively).

maturation, as developing eosinophils abundantly express IL-3R α and GM-CSFR α .²⁰

A third possibility is that the early eosinophil progenitors are able to overcome the IL-5 blockade by autocrine production of the cytokine. Indeed, we previously demonstrated that cord blood–derived CD34⁺ cells are capable of producing autologous IL-5 after stimulation with IL-3 or GM-CSF.⁷ Alternatively, paracrine effects of CD3⁺ cell–derived IL-5 within the bone marrow²¹ might prevent mepolizumab from depleting eosinophils.

A final possibility is that IL-5 is a key cytokine for the terminal maturation of eosinophils, whereas early lineage differentiation is not directly influenced by IL-5.²²

Our findings differ somewhat from an IL-5 knockout mouse model of allergic rhinitis.²³ In this model, the IL-5–deficient mice had significantly lower numbers of Eo/B-CFUs in response to in vitro culture with IL-5 and significantly lower expression of IL-5R α on bone marrow CD45⁺/CD34⁺ cells than wild-type mice. The discrepancies between these findings and our results with mepolizumab can be explained in one of 2 ways. In the knockout model, there is a complete absence of IL-5 in the bone marrow during embryogenesis and thereafter, whereas this is not the case with mepolizumab treatment; in addition, in the latter situation anti-IL-5 might not penetrate the bone marrow sufficiently and/or IL-5 produced locally might provide a low-level signal that overrides the antibody effect. The alternative explanation is that murine eosinophils are more dependent on IL-5 than their human counterparts, though given the persistence of eosinophils in the absence of receptors for IL-3, IL-5, and GM-CSF in a double-knockout mouse, this seems less likely.²⁴

There are 2 possible reasons for the modest numeric fall (but not complete absence) of CD34⁺/IL-5R α

TABLE I. The effect of mepolizumab and placebo on early eosinophil progenitors in the bone marrow and blood

	Mepolizumab			Placebo		
	Pre	Post	<i>P</i> value of treatment	Pre	Post	Between-groups <i>P</i> value
Bone marrow						
CD34 ⁺ cells*	35,190 (23,620-62,200)	30,540 (11,230-40,750)	.43	34,140 (24,090-48,020)	28,820 (17,200-43,950)	.88
CD34 ⁺ /IL-5Rα ⁺ cells†	1.08 (0-1.9)	1.186 (0.67-2.21)	.84	1.118 (0.75-1.45)	1.258 (0.85-2.08)	.74
Eo/B-CFUs‡	16.19 (6.5-30)	16.06 (2.5-30)	.94	13.67 (1.5-32)	9.0 (3-19)	.33
Eosinophil myelocytes§	1.75 (0.5-3.5)	1.1 (0.0-2.5)	.016	1.0 (0.0-2.5)	1.692 (0.0-3.0)	.006
Eosinophil metamyelocytes§	1.25 (1.0-2.5)	0.7 (0.0-1.5)	.03	0.92 (0.0-2.0)	1.35 (0.5-3.0)	.003
Mature eosinophils§	6.25 (0.0-19.0)	1.9 (0.0-5.5)	.01	4.77 (1.0-14.0)	4.19 (0.5-8.0)	.017
Blood						
CD34 ⁺ cells*	1,443 (689-2,085)	1,601 (690-3,050)	.38	1,713 (740-3,004)	1,810 (946-2,758)	.92
CD34 ⁺ /IL-5Rα ⁺ cells†	3.661 (0.58-8.28)	2.475 (0-4.71)	.32	2.346 (0.46-8.18)	2.561 (0.89-4.32)	.32
Bronchial mucosa						
CD34 ⁺ /IL-5Rα mRNA ⁺ cells	6.7 (2.0-15)	4.7 (0.0-10.0)	.04	7.231 (0.0-17.0)	5.538 (0.0-12.0)	.55

Data are expressed as means (minimum-maximum values).

Eo/B-CFUs, Eosinophil/basophil colony-forming units.

*Per 10⁶ CD45⁺ cells.

†Percent of cells expressing IL-5Rα.

‡Per 2.5 × 10⁵ nonadherent mononuclear cells.

§Percent of cell type.

||Positive cells per square millimeter.

mRNA⁺ cells within the bronchial mucosal that is seen with mepolizumab. Blocking IL-5 might prevent progenitors from trafficking from the bone marrow to the bronchus. However, trafficking might still occur through the actions of eotaxin or other chemokines, inasmuch as CC chemokine receptor 3 expression by CD34⁺ progenitors has been demonstrated.²⁵ Alternatively, progenitors might be able to differentiate in situ despite the absence of IL-5, perhaps through the actions of GM-CSF or IL-3.

Although mepolizumab might have caused partial maturational arrest of the eosinophil lineage, additional factors might act in eosinophilopoiesis, and this might explain the failure of mepolizumab to fully deplete bone marrow or bronchial mucosal eosinophils.¹⁸ These include GM-CSF and IL-3 and cysteinyl leukotrienes, which have been shown to synergize with GM-CSF in eosinophil development in vitro.²⁶ In addition, work with animal models suggests that both IL-5 and the eotaxin-CC chemokine receptor 3 axis need to be blocked to effectively deplete eosinophils from the tissue.¹⁴

The inability of mepolizumab to fully deplete tissue eosinophils might explain its lack of clinical efficacy in the studies published to date. Mepolizumab had no effect on the magnitude of the late asthmatic reaction or airway hyperresponsiveness after inhaled allergen challenge²⁷ or on the FEV₁ and histamine PC₂₀ of mild atopic asthmatic

individuals.¹⁸ However, both of the studies involved small numbers of volunteers, and the value of mepolizumab as an asthma therapy cannot be determined until the results of larger clinical trials are published.

Our data with selective blockade using mepolizumab has confirmed that IL-5 plays an important role in eosinophil differentiation, but other pathways are also likely to be involved in eosinophil development. Future strategies targeting the eosinophil will have to be more effective at disrupting both eosinophil development and survival if we are to understand the true role of the eosinophil in asthma and other allergic diseases.

REFERENCES

- Bosquet J, Chané P, Lacoste JY, Bameon G, Ghavanian N, Enander I, et al. Eosinophilic inflammation in asthma. *New Engl J Med* 1990;323:1033-9.
- Denburg JA, Sehmi R, Saito H, Pil-Seob J, Inman M, O'Byrne PM. Systemic aspects of allergic disease: bone marrow responses. *J Allergy Clin Immunol* 2000;106:S242-6.
- Zeibecoglou K, Ying S, Yamada T, North J, Burman J, Bunge J, et al. Increased mature and immature CCR3 messenger RNA⁺ eosinophils in bone marrow from patients with atopic asthma compared with atopic and nonatopic control subjects. *J Allergy Clin Immunol* 1999;103:99-106.
- Palframan RT, Collins PD, Severs NJ, Rothery S, Williams TJ, Rankin SM. 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* 1998;188:1621-32.

5. Sehmi R, Howie K, Sutherland DR, Scragge W, O'Byrne PM, Denburg J. Increased levels of CD34+ hemopoietic progenitor cells in atopic subjects. *Am J Respir Cell Mol Biol* 1996;15:645-55.
6. Steinbach KH, Shick P, Trepel F, Raffler H, Dohrmann J, Heilgest G, et al. Estimation of kinetic parameters of neutrophilic, eosinophilic and basophilic granulocytes in human blood. *Blut* 1979;39:27-38.
7. Tavernier J, Van der Heyden J, Verhee A, Brusselle G, Van Ostade X, Vanderkerckhove J, et al. Interleukin-5 regulates the isoform expression of its own receptor α -subunit. *Blood* 2000;95:1600-7.
8. Sehmi R, Wardlaw AJ, Cromwell O, Kurihara K, Waltmann P, Kay AB. Interleukin-5 selectively enhances the chemotactic response of eosinophils obtained from normal but not eosinophilic subjects. *Blood* 1992;79:2952-59.
9. Wood LJ, Inman MD, Watson RM, Foley R, Denburg JA, O'Byrne PM. Changes in bone marrow inflammatory cell progenitors after inhaled allergen in asthmatic subjects. *Am J Respir Crit Care Med* 1998;157:99-105.
10. Cyr M, Denburg J. Systemic aspects of allergic disease: the role of the bone marrow. *Curr Opin Immunol* 2001;13:727-32.
11. Robinson DS, Damia R, Zeibecoglou K, Molet S, North J, Yamada T, et al. CD34+/interleukin-5R α messenger RNA+ cells in the bronchial mucosa in asthma: potential airway eosinophil progenitors. *Am J Respir Cell Mol Biol* 1999;20:9-13.
12. Kim YK, Uno M, Hamilos D, Beck L, Bochner B, Schleimer R, et al. Immunolocalization of CD34 in nasal polyposis. *Am J Respir Cell Mol Biol* 1999;20:388-97.
13. Cameron L, Christodoulou P, Lavigne F, Nakamura Y, Eidelman D, McEuen A, et al. Evidence for local eosinophil differentiation within allergic nasal mucosa: inhibition with soluble IL-5 receptor. *J Immunol* 2000;164:1538-45.
14. Foster PS, Hogan SP, Yang M, Young IG, Matthaei KI, Kumar RK, et al. Interleukin-5 and eosinophils as therapeutic targets for asthma. *Trends Mol Med* 2002;8:162-7.
15. Yamaguchi Y, Duda T, Suda J, Eguchi M, Miura Y, Harada N, et al. Purified IL-5 supports the terminal differentiation and proliferation of murine eosinophil precursors. *J Exp Med* 1988;167:43-56.
16. Sehmi R, Wardlaw AJ, Cromwell O, Kurihara K, Waltmann P, Kay AB. Interleukin-5 selectively enhances the chemotactic response of eosinophils obtained from normal, but not eosinophilic subjects. *Blood* 1992;79:2952-9.
17. Rothenberg ME, Petersen J, Stevens RL, Silberstein DS, McKenzie DT, Austen KF, et al. IL-5 dependent conversion of normodense human eosinophils to the hypodense phenotype uses 3T3 fibroblasts for enhanced viability, accelerated hypodensity and sustained antibody-dependent cytotoxicity. *J Immunol* 1989;143:2311-6.
18. Flood-Page PT, Menzies-Gow AN, Kay AB, Robinson DS. Eosinophil's role remains uncertain as anti-interleukin-5 only partially depletes numbers in asthmatic airway. *Am J Respir Crit Care Med* 2003;167:199-204.
19. Robinson DS, Hamid Q, Ying S, Tsicopoulos A, Barkans J, Bentley AM, et al. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* 1992;326:298-304.
20. Hashida R, Ogawa K, Miyagawa M, Sugita Y, Matsumoto K, Akasawa A, et al. Gene expression accompanied by differentiation of cord blood-derived CD34+ cells to eosinophils. *Int Arch Allergy Immunol* 2001;125 Suppl 1:2-6.
21. Wood LJ, Sehmi R, Dorman S, Hamid Q, Tulic MK, Watson RM, et al. Allergen-induced increases in bone marrow T lymphocytes and interleukin-5 expression in subjects with asthma. *Am J Respir Crit Care Med* 2002;166:883-9.
22. Ogawa M. Differentiation and proliferation of hematopoietic stem cells. *Blood* 1993;81:2844-53.
23. Saito H, Matsumoto K, Denburg AE, Crawford L, Ellis R, Inman MD, et al. Pathogenesis of murine experimental allergic rhinitis: a study of local and systemic consequences of IL-5 deficiency. *J Immunol* 2002;168:3017-23.
24. Nishinakamura R, Miyajima A, Mee PJ, Tybulewicz VL, Murray R. Hematopoiesis in mice lacking the entire granulocyte-macrophage colony-stimulating factor/interleukin-3/interleukin-5 functions. *Blood* 1996;88:2458-64.
25. Sehmi R, O'Byrne PM, Dorman S, Wood L, Baatjes A, Watson R, et al. Upregulation of eotaxin receptor, CCR3, on bone marrow CD34+ progenitor cells following allergen-inhalation challenge in asthmatic subjects: a mechanism of progenitor cell egress from the bone marrow during allergic inflammation. *J Allergy Clin Immunol* 2001;107:S373.
26. Braccioni F, Dorman SC, O'Byrne PM, Inman MD, Denburg JA, Parameswaran K, et al. The effect of cysteinyl leukotrienes on growth of eosinophil progenitors from peripheral blood and bone marrow of atopic subjects. *J Allergy Clin Immunol* 2002;110:96-101.
27. Leckie MJ, ten Brinke A, Khan J, Diamant Z, O'Connor BJ, Walls CM, et al. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 2000;356:2144-8.