

Molecular mechanisms in allergy and clinical immunology

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Eosinophil trafficking in allergy and asthma

Helene F. Rosenberg, MD,^a Simon Phipps, PhD,^b and Paul S. Foster, PhD^b Bethesda, Md, and Newcastle, Australia

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Blood eosinophilia and tissue eosinophilia are characteristic features of allergic inflammation and asthma, conditions associated with prominent production of T_H2 cytokines IL-4, IL-5, and IL-13. In this review, we will consider recent advances in our understanding of the molecular mechanisms that promote expansion and differentiation of eosinophil progenitors in bone marrow, eosinophil recruitment in response to chemokine receptor 3 agonists eosinophil transit mediated by specific ligand-receptor interactions, and prolonged survival of eosinophils in peripheral tissues. Novel rational therapies including antiselectin and antichemokine receptor modalities designed to block eosinophil development and trafficking are discussed, together with the implications of recent clinical studies that have evaluated the efficacy of humanized anti-IL-5 mAb therapy. (J Allergy Clin Immunol ■■■■;■■■;■■■-■■■.)

Key words: Inflammation, ILs, cytokines, hematopoiesis

Human eosinophils are distinguished phenotypically by their bilobed nuclei and large acidophilic cytoplasmic granules (Fig 1). They are granulocytic leukocytes and share many features with neutrophils, including growth and development in the bone marrow. However, in contrast with neutrophils, which exit the bone marrow and reside in the bloodstream, eosinophils are tissue leukocytes and are found primarily in the gastrointestinal tract under homeostatic conditions. During maturation, release into

Abbreviations used

IL-5R: IL-5 receptor
PSGL: P-selectin glycoprotein ligand
Siglec: Sialic acid-binding Ig-like lectin
VCAM: Vascular cell adhesion molecule
VLA: Very late antigen

the bloodstream, and recruitment to tissues, eosinophils respond to signals from cytokines, chemokines, and other proinflammatory mediators via cell-specific receptors. Perhaps most interesting, despite >100 years of research, is that the evolutionary role and biological function of the human eosinophil remain subjects of profound controversy. Although peripheral blood and tissue eosinophilia are hallmarks of parasitic helminth infection, the way in which this benefits the host remains unclear.¹⁻³ Likewise, although many studies have documented a role for eosinophils in the pathogenesis of acute and/or chronic asthma,⁴⁻⁷ it is important to recognize that in this setting, eosinophils are participating in an aberrant response—that is, one that does not benefit the host in any direct fashion. Recent explorations directed at modeling the hygiene hypothesis⁸⁻¹⁰ may ultimately elucidate mechanisms of inflammatory balance that have evolved to confront endemic parasitism. Ultimately, these studies may provide novel insights into the nature of the eosinophil and its role in host defense.

A CAVEAT ON HUMAN VERSUS MOUSE EOSINOPHILS

It is important to recognize that most of the findings to be discussed result from work performed in mouse models of disease. Although all investigations can suffer from overinterpretation of cross-species findings,^{11,12} the problems are particularly large when examining results related to the eosinophil. Although mouse eosinophils, like human eosinophils, can be identified by red-staining

From ^athe Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda; and ^bthe Center for Asthma and Respiratory Diseases, School of Biomedical Sciences, University of Newcastle and Hunter Medical Research Institute. Disclosure of potential conflict of interest: S. Phipps has received grant support from the Australian Lung Foundation. H. F. Rosenberg and P. S. Foster have declared that they have no conflict of interest.

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Reprint requests: Helene F. Rosenberg, MD, Laboratory of Allergic Diseases, Building 10, Room 11C215, National Institute of Allergy and Infectious Diseases, National Institutes of Health, 9000 Rockville Pike, Bethesda, MD 20892. E-mail: hrosenberg@niaid.nih.gov.

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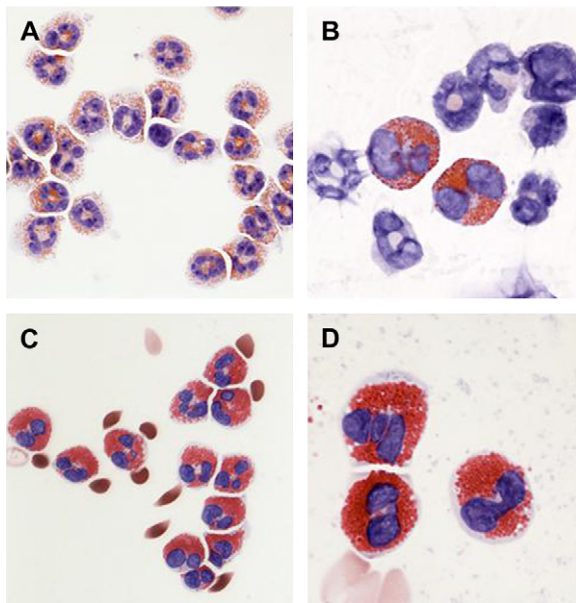


FIG 1. Microscopic anatomy of mouse and human eosinophils. The mouse eosinophils (**A** and **B**; original magnifications, $\times 40$, from bronchoalveolar lavage fluid, and $\times 100$, from bone marrow, respectively) display typical morphology, including red staining granules and bilobed nucleus. The human eosinophils (**C** and **D**; original magnifications $\times 20$ and $\times 63$, both from peripheral blood) likewise display the prominent and clearly defined, slightly larger cytoplasmic granules. Cells were stained with a modified Giemsa preparation (Dade Behring AG, Duding, Switzerland). **B-D**, Courtesy of Drs Kimberly D. Dyer and Jennifer M. Moser, Laboratory of Allergic Diseases, NIAID.

granules in blood, bone marrow, and tissue preparations stained either with hematoxylin and eosin or Giemsa stains, there are profound differences between these cell types (Fig 1). Structurally, mouse eosinophil granules are smaller and less refractile. Among the granule proteins are the mouse eosinophil-associated ribonucleases, which are remarkably divergent ($>50\%$) orthologs of human eosinophil-derived neurotoxin and eosinophil cationic protein,¹³ and Charcot-Leyden crystal protein, a major human eosinophil component, cannot even be identified in the mouse genome. Furthermore, human eosinophils express the high-affinity IgE receptor, Fc ϵ RI, whereas mouse eosinophils do not. Likewise, mouse eosinophils express Siglec F, a sialic acid-binding Ig-like lectin that is a functional correlate, but likewise a highly divergent ortholog of human Siglec 8.¹⁴⁻¹⁶ Finally, and highly relevant to asthma and allergy models, mouse eosinophils have distinct responses to chemotactic cytokines¹⁷ and have a very limited propensity to degranulate in our current models of disease, a feature that may be an inherent property of this cell type, or a limitation of the stimuli used to model specific disorders.¹⁸ Although these limitations do not preclude studies of eosinophil trafficking in mice, it is crucial to keep in mind that human versus mouse parallels may need to be explored quite carefully, given issues of species divergence.

EOSINOPHIL EXPANSION IN RESPONSE TO ALLERGIC PROVOCATION

An allergic response is initiated *in situ* as a CD4⁺ naive T lymphocyte responds to specific stimulation by developing into a T_H2 lymphocyte. T_H2 lymphocytes synthesize and secrete the cytokines IL-4, IL-13, and IL-5. IL-4 promotes eosinophilia indirectly via promoting autocrine development of T_H2 lymphocytes, and murine eosinophils themselves produce IL-4.^{19,20} Human eosinophils express IL-4 receptor α (IL-4R α).^{21,22} IL-4 also activates human vascular endothelial and respiratory epithelial cells to produce eosinophil chemoattractant cytokines²³ and directs IgE synthesis and mast cell growth and activation in both human and murine experimental systems.^{24,25} IL-13 also promotes eosinophilia indirectly in mouse models, as it induces eotaxin production and has been identified as the major factor activating the respiratory epithelium, promoting the subsequent development of bronchospasm.^{26,27}

THE PIVOTAL ROLE OF IL-5

The T_H2 cytokine IL-5 is a central factor mediating eosinophil expansion, priming, recruitment, and prolonged tissue survival in response to allergic stimuli. Originally identified as murine T-cell replacing factor, a B-cell growth factor and eosinophil differentiation factor, IL-5 is synthesized predominantly by T_H2 lymphocytes, but in smaller amounts by mast cells and eosinophils. IL-5 promotes differentiation of terminally committed human and murine eosinophil precursors in bone marrow.²⁸⁻³¹ The IL-5 receptor (IL-5R) includes a unique ligand-binding α -chain and β -chain shared with receptors for IL-3 and GM-CSF that mediates signaling via the Janus kinase (JAK)/signal transducer and activator of transcription pathway and via activation of phosphoinositide-3-kinase (PI-3Kinase).³² The IL-5R is expressed on human eosinophils and eosinophil progenitors. In mice, IL-5R can be detected on eosinophils and their progenitors and on CD5⁺ B1 lymphocytes,³³ which are prominent in both peritoneal and pleural cavities and produce IgM. Although IL-5 has a unique and specific impact on nearly all aspects of eosinophil biology, it works in synergy with IL-4, IL-13, and the family of chemotactic cytokines (or chemokines), termed *eotaxins*, to promote eosinophil-mediated inflammatory responses.

EOSINOPHIL DEVELOPMENT IN THE BONE MARROW

Committed eosinophil progenitors are derived from pluripotent CD34⁺ stem cells found in normal bone marrow. Of note, committed CD34⁺IL-5R⁺ eosinophil progenitor cells have also recently been identified in murine lung tissue.³⁴ Studies of *ex vivo* bone marrow cultures have shown that eosinophils develop from these progenitors in response to GM-CSF, IL-3, and IL-5. When they

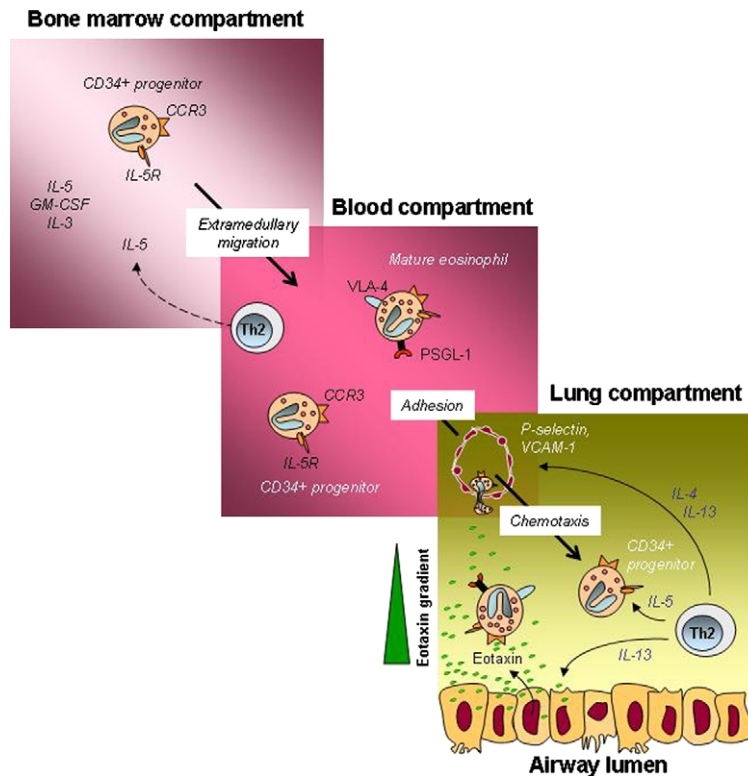


FIG 2. Pathways of eosinophil trafficking. Committed eosinophil progenitors ($CD34^+IL-5R^+$) differentiate and mature in response to cytokines IL-3, IL-5, and GM-CSF. Both forms exit the bone marrow and enter the bloodstream, where they respond to chemotactic signals from allergen-challenged respiratory epithelial cells. Eosinophils exit the bloodstream in response to interactions mediated by cell surface selectins and integrins and chemokine signaling (Fig 3) and enter the lung tissue and airways. The cytokine IL-13 results in augmented expression of P-selectin and integrin VLA-4 eosinophils as well as increased P-selectin/VLA-4 dependent adherence of eosinophils to IL-4/IL-13-treated endothelial cells.¹¹¹ Both phenotypically mature and immature eosinophil precursors can enter the lung, where the latter can differentiate into mature effector cells.³⁴ Eosinophil viability is maintained by cytokines and chemokines present in the allergic lung.

are phenotypically mature, eosinophils are released from the bone marrow into the circulation.

As noted, IL-5 is a strong and specific stimulus for eosinophil differentiation. Allergic stimulation results in elevated levels of serum IL-5, which leads to the expansion (10-fold to 20-fold or even greater) of $IL-5R^+$ eosinophil progenitors in the bone marrow and their differentiation into mature cells for release into the peripheral blood (Fig 2). Despite its pivotal role in allergic eosinophilia, IL-5 is not required for eosinophil growth and differentiation under homeostatic conditions. IL-5 gene-deleted mice have mature eosinophils in bone marrow and peripheral blood, albeit in reduced numbers.³⁵ Interestingly, no IL-5-dependent, eosinophil-specific transcription factors or transcriptional events have been elucidated, although the transcription factors PU.1, CAAT enhancer binding proteins α and ϵ , and GATA-1 have been implicated in eosinophil development.^{36,37} The recent finding that deletion of a specific enhancer in the proximal promoter of the gene encoding the hematopoietic transcription factor, GATA-1, results in selective eosinophil ablation³⁸ suggests that there are more subtle

GATA-mediated interactions directing eosinophil differentiation that remain to be elucidated.

EOSINOPHIL TRANSIT INTO TISSUES

Mature eosinophils leave the bone marrow and are attracted to sites of allergic inflammation by the actions of proinflammatory chemokines, which are produced in response to allergic stimulation by endothelial and epithelial cells activated by, among other mediators, IL-4 and IL-13. Eosinophils express receptors for the CC group of chemokines (those that contain adjacent cysteines near the amino terminus). Several CC chemokines have been characterized as eosinophil chemoattractants, including RANTES (chemoattractant cytokine ligand 5 [CCL5]) and macrophage inflammatory protein 1 α (CCL3), although neither of these is a specific eosinophil chemoattractant. The eotaxins, which interact with CCR3, 1 of the 7 transmembrane receptors common to all chemokines among other cell-signaling mediators, are the only known chemoattractants that interact uniquely with eosinophils. Eotaxin

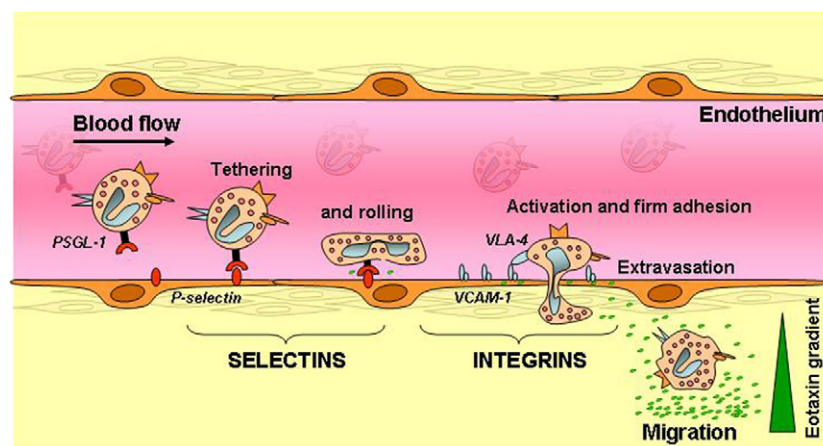


FIG 3. The role of selectins and integrins in promoting eosinophil transit from the bloodstream. Selectins and their ligands present on eosinophils and the vascular endothelium promote initial tethering and rolling of eosinophils. Integrins mediate eosinophil activation, adhesion, and transit through the endothelium in a direction determined via chemokine gradients. Eosinophil-specific interactions with the endothelium are mediated via the integrin VLA-4 and PSGL-1 binding to P-selectin.

(or eotaxin-1) was first described by Williams and colleagues³⁹ as a novel component in bronchoalveolar lavage fluid of allergen sensitized guinea pigs that promoted specific recruitment of eosinophils (see review⁴⁰). Eotaxin-1 is produced by epithelial cells in response to IL-4 and IL-13 via signal transducer and activator of transcription 6-dependent pathways⁴¹ and functions synergistically with IL-5 to promote eosinophil recruitment to the allergen-challenged lung.^{42,43} Several lines of evidence suggest that eotaxin-1 plays a central role in eosinophil recruitment to the lung tissue of patients with asthma.^{44,45} Eotaxin-1 regulates homeostatic levels of eosinophils in the gastrointestinal tract.⁴⁶ Eotaxin also promotes eosinophil hematopoiesis^{47,48} and is directly responsible for mobilizing eosinophil progenitors in the bone marrow for exit into the peripheral circulation.^{48,49}

Interestingly, the second member of the eotaxins group, eotaxin-2, discovered by Baggiolini and colleagues,⁵⁰ has little specific amino acid sequence homology (~30%) with eotaxin-1, but also serves as a specific eosinophil chemoattractant acting via CCR3. After allergen challenge in mouse models, eotaxin-1 expression is detected at earlier time points (6 hours), followed later by eotaxin-2 (24 hours).⁵¹ Eotaxin-2 also functions together with IL-5 to promote lung eosinophilia and production of IL-13.^{52,53} Cooperative interactions between eotaxin-2 and IL-13 promote eosinophil recruitment in response to allergen challenge⁵⁴ and specific synergy between eotaxins 1 and 2, with a dominant role for eotaxin-2 in the accumulation of eosinophils in the airway lumen.⁵⁵ Eotaxin-3, a third homolog, is also expressed at later time points in response to allergen challenge and most likely supports prolonged eosinophil recruitment, and has been implicated as a mediator of eosinophilia in postbypass pleural effusions.⁵⁶ Most recently, eotaxin-3 has been identified as a marker for the allergic disorder, eosinophilic esophagitis; a single nucleotide polymorphism

(+2496T>G) in the gene encoding eotaxin-3, with potential to disrupt an AU-rich RNA-stability regulatory element, has been identified in strong association with susceptibility to eosinophilic esophagitis.^{57,58} Specific polymorphisms in genes encoding eotaxins 2 and 3 have also been associated with elevated peripheral blood eosinophil counts in patients with asthma.^{59,60}

In addition to eosinophil-selective chemoattractants, there has been significant therapeutic focus on the role of eosinophil-specific adhesion molecules, particularly the $\beta 1$ integrin very late antigen (VLA)–4, a ligand for the integrin vascular cell adhesion molecule (VCAM)–1, and the P-selectin glycoprotein ligand (PSGL)–1 ligand for P-selectin, both with potential to mediate the specific transit of eosinophils across epithelial and endothelial barriers (Fig 3). Eotaxin-1 modulates expression of VLA-4 on eosinophils,^{61,62} and studies performed with anti-integrins and VLA-4 blockade on mice subjected to allergen sensitization and challenge suggest that this ligand is a crucial component of the eosinophil inflammatory response.^{63,64} Because the VLA-4/VCAM-1 interaction promotes specific adhesion of eosinophils (and not neutrophils), several small molecule inhibitors of the VLA-4/VCAM-1 interaction are under exploration as asthma therapeutics.^{65,66} Among these, IVL745, a specific VLA-4 antagonist, was studied in a placebo-controlled, double-blind, randomized, 2-way crossover study.⁶⁷ IVL745 had no statistically significant impact on symptoms but did yield a moderate reduction in sputum eosinophils. Likewise, the role of selectins in promoting eosinophil transit has been explored. PSGL-1 is expressed uniquely on eosinophils; thus, moieties that disrupt interactions between PSGL-1 and P-selectin have the potential to be eosinophil-specific. In contrast, L-selectins are common to all leukocytes, and inhibitory strategies are likely to have an effect on trafficking of both eosinophils and neutrophils. Interestingly, P-selectin gene-deleted mice display reduced pulmonary

eosinophilia,⁶⁸ and an L-selectin mAb was particularly effective in modulating disease in sheep.⁶⁹ Although selectin inhibitors have entered clinical trials, there is currently no successful therapeutic strategy based on this approach.⁷⁰⁻⁷² The most promising at this writing is Bimosiamose (TBC1269; Revotar Pharmaceuticals, Hemmingsdorf, Germany), a small molecule pan-selectin antagonist. In a recent randomized, double-blind, placebo-controlled clinical crossover trial, inhaled Bimosiamose effectively reduced late-phase asthmatic reactions in a series of 12 male subjects with mild allergic asthma, although the specific impact on eosinophil trafficking remains uncertain.⁷³

EOSINOPHIL SURVIVAL AND APOPTOSIS

A final aspect of eosinophil trafficking involves its survival and functioning within peripheral tissues. There are several excellent reviews that detail general findings relating to apoptosis signaling pathways described in eosinophils.⁷⁴⁻⁷⁶ Among the findings that relate uniquely to the biology of eosinophils, IL-5 clearly promotes eosinophil survival *in vitro*, and the absence of IL-5 results in spontaneous cellular apoptosis. Simon et al⁷⁶ and Simon⁷⁷ have shown that IL-5 is also associated with prolonged eosinophil survival in explants of nasal polyps. The cytokines IL-3 and GM-CSF also result in delayed eosinophil apoptosis in *in vitro* studies,^{78,79} as does the chemokine eotaxin,⁸⁰ and biochemical blockade of cysteinyl-leukotriene receptors (CysLT1) partially reversed the antiapoptotic effects promoted by GM-CSF.⁸¹ Interestingly, interactions with extracellular matrix proteins fibronectin and laminin also resulted in prolonged eosinophil survival, in large part as a result of autocrine production of GM-CSF.⁸²

In contrast with spontaneous apoptosis, resulting from withdrawal of cytokines necessary for prolonged cellular survival, cellular apoptosis can also be induced directly via extrinsic (receptor mediated) or intrinsic (direct mitochondrial perturbation) mechanisms.⁸³ Among the characterized proapoptotic or death receptors on the eosinophil cell surface, activation of CD95 (Fas) with a specific mAb has been shown to promote cellular apoptosis.^{84,85} In contrast, the cytokines TNF- α and TNF-related apoptosis-inducing ligand, ligands for the extrinsic pathway death receptors TNF receptor 1 and TNF-related apoptosis-inducing ligand receptor 1, appear to prolong eosinophil survival.^{86,87} Other eosinophil cell surface receptors for proapoptotic signals include CD137,⁸⁸ Siglec 8,⁸⁹ and CD30,⁹⁰ which induces the expression of the eosinophil-specific nuclear receptor, NOR1.⁹¹ Apoptotic signals are transmitted in eosinophils via members of inhibitor of apoptosis protein and Bcl-2 families; eosinophils express the proapoptotic pro-teins Bax at a particularly high level.⁹² The result is the activation of the proteolytic caspase cascade; caspases 3, 8, and 9 have been identified as mediating apoptotic responses in human eosinophils.⁹³

Although IL-5 appears to be the primary modulator of eosinophil survival *in vitro*, the results of the clinical

TABLE I. Key points

1. Eosinophils are bone marrow–derived granulocytes that are effector cells in asthma and allergic responses.
2. The T_H2 cytokine IL-5 is a central modulator of eosinophil differentiation and survival in response to allergen provocation.
3. The eotaxins are chemoattractant cytokines that promote eosinophil recruitment to tissues in response to allergic provocation via CCR3 receptors expressed predominantly on eosinophils.
4. Eosinophil transit to the tissues is mediated via cell surface selectins and integrins and their ligands, which permit specific interactions with the vascular endothelium.
5. Clinical trials with humanized anti-IL-5 mAbs in patients with allergic asthma resulted in diminished peripheral eosinophil counts but persistent eosinophilia in lung tissue and disappointing clinical results. However, anti-IL-5 therapy appears to be more successful in the effective treatment other eosinophil-mediated disorders, including idiopathic hypereosinophilic syndrome and eosinophilic esophagitis, although not atopic dermatitis.
6. Anti-CCR3, anti-integrin, and antiselectin strategies are currently in development as antieosinophil strategies for treatment of asthma and other allergic conditions.

studies designed to evaluate the therapeutic potential of humanized mAbs demonstrate that eosinophils are clearly capable of persisting in the tissue despite effective blockade of endogenous IL-5. This result implies that there may be other, as yet to be characterized signals that promote eosinophil survival *in vivo* in response to allergen challenge, a point that is clearly worthy of experimental exploration.

CLINICAL IMPLICATIONS

Given the pivotal role of IL-5, it seemed reasonable to assume that blockade of IL-5 would result in the elimination of eosinophilia and thus the reduction in symptomatology associated with allergic asthma. This hypothesis was tested in clinical trials using 2 humanized monoclonal anti-IL-5 antibodies, SCH55700 (Schering-Plough Research Institute, Kenilworth, NJ) and mepolizumab (GlaxoSmithKline, Middlesex, United Kingdom). In a randomized, double-blind study of mepolizumab, clinical symptoms of patients with asthma were unaffected despite a dramatic decline in peripheral blood eosinophils. Most interesting, despite repeated administration of anti-IL-5 therapy, eosinophils persisted in the lung tissue and in the airway. An independent trial performed with SCH55700 likewise resulted in a depletion of peripheral blood eosinophils without improvement in clinical symptoms.⁹⁴⁻⁹⁶

Although disappointing, the existence of residual eosinophils in tissues suggests that IL-5 may not act alone in promoting eosinophil survival in lung tissue. There is evidence suggesting that IL-3, GM-CSF, and/or eotaxin may also contribute to eosinophil viability.^{78,79} Likewise, eosinophil trafficking in other tissues might be more readily altered by these monoclonal reagents.⁹⁷⁻⁹⁹ Similarly, a study by Liu et al¹⁰⁰ indicated that airway eosinophils are

relatively unresponsive to IL-5, and that bronchoalveolar lavage fluid from atopic individuals contained elevated concentrations of the soluble IL-5 receptor.

It is important to recognize that the disappointing results may be also be related to complexities of specific disease states. Although anti-IL-5 therapy has likewise been of little benefit in the treatment of atopic dermatitis despite clear reduction in peripheral blood eosinophil counts,^{101,102} several groups have reported success using anti-IL-5 therapy for hypereosinophilic syndromes^{94,103,104} and eosinophilic esophagitis.¹⁰⁵

CONCLUSION AND FUTURE DIRECTIONS

Despite some controversial findings, the balance of data suggests that eosinophils promote 1 or more aspects of respiratory dysfunction characteristic of allergic asthma. As such, the possibility that drugs directed at inhibition of eosinophil migration or activation or even outright eosinophil ablation might prove to be effective therapeutic strategies certainly remains worthy of further exploration. Among the avenues that might be considered is the possibility that coordinate inhibition of IL-5 and CCR3 might result in more effective eosinophil depletion in lung tissue and airways. Several small molecule antagonists of CCR3 are under experimental review,¹⁰⁶⁻¹⁰⁹ and an exploration of the therapeutic potential of the newly characterized eosinophil inhibitory receptors certainly warrants further consideration.¹¹⁰ Furthermore, a directed exploration of the factors permitting prolonged survival of eosinophils in tissue even in the presence of effective IL-5 blockade might uncover additional eosinophil depletion strategies (Table I).

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