

Basophil and eosinophil differentiation in allergic reactions

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Basophil and eosinophil participation in allergic reactions constitutes a hallmark of this type of inflammatory state. Mechanisms underlying the accumulation of these cells in tissues where allergen is present include the effect of cytokines and other inflammatory mediators on the egress from blood and migration of these cells into the tissue. Much progress has been made recently in the understanding of in vitro chemotactic and adherence mechanisms putatively involved in the accumulation of the mature basophil and eosinophil in IgE-dependent reactions in vivo.¹ A concept we have developed in our studies over the past decade is that the bone marrow, through the release of progenitors into the peripheral blood, contributes a pool of differentiating cells, which accumulate in response to signals from allergic inflamed tissues.²⁻⁸ In this review we summarize the basis for this concept, citing both basic and applied studies and expanding on the evidence supporting the progenitor hypothesis for accumulation of basophils and eosinophils by using recently acquired information in an animal model and in human beings.

BASIC STUDIES

Progenitors

The basophil-eosinophil progenitor is a common, committed hemopoietic cell present in bone marrow and peripheral blood, as well as in cord blood, which can differentiate into mature cells under the influence of specific cytokines.⁹⁻¹¹ Most of this information comes from studies of cells

Abbreviations used

GM-CSF: Granulocyte-macrophage colony-stimulating factor

IL: Interleukin

NGF: Nerve growth factor

SCF: Stem cell factor

separated from the blood or marrow by means of density gradients and placed in semisolid culture media, in which colonies form from single progenitors after 10 to 14 days in vitro. Using these standard hemopoietic approaches, we and others have demonstrated that a single cell is capable of giving rise to mixed colonies of basophils and eosinophils, pure basophil colonies, or pure eosinophil colonies¹¹; this indicates that a common progenitor exists for these cells but that the degree of differentiation toward one or the other lineage is stochastic.¹¹⁻¹³ The presence of interleukin (IL)-3 will stimulate basophil differentiation primarily,^{14, 15} whereas the presence of IL-5 will generally force differentiation of this progenitor along the eosinophil lineage.^{16, 17} In the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF), differentiation is of mixed colonies primarily; different amounts of histamine, marking the basophil, and granule proteins, primarily found in the eosinophil, can be measured in single colonies and may be related to the differentiation skewing of a given basophil-eosinophil progenitor.^{13, 18}

The presence and proportion of basophil-eosinophil progenitors depends on the compartment examined. Although in general the bone marrow is a source for a large number of progenitors, it is principally the neutrophil-macrophage progenitor that differentiates from the myeloid lineage-committed pool in the marrow; for reasons not yet clearly understood, the blood appears to be a repository of basophil-eosinophil progenitors,

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rather than neutrophil-macrophage progenitors, although overall there is a 10-fold reduction in the total number of progenitors in blood compared with marrow. The exception is cord blood, in which a surprisingly high number of basophil-eosinophil progenitors reside; these can be induced to differentiate *in vitro* with the cytokines mentioned above.^{17, 19, 20} Possible reasons for the presence of an increased proportion of basophil-eosinophil progenitors in the peripheral blood compartment are discussed in a separate section.

Cytokines involved in basophil-eosinophil differentiation

The cytokines involved in differentiation of basophils, mast cells, and eosinophils have been extensively reviewed.^{21, 22} There is clear evidence, in both murine and human systems, that IL-5 is a specific eosinophil lineage differentiation factor, stimulating a progenitor to form almost pure populations of eosinophils in both liquid and semisolid cultures.¹⁶ Transgenic IL-5 mice exhibit marked eosinophilia¹⁶ and anti-IL-5 antibodies block eosinophil differentiation.^{16, 18} There is also evidence that IL-5 stimulates basophil differentiation, as measured by histamine-positive colonies *in vitro*; this is not surprising, given the consideration that basophil and eosinophil progenitors are shared and that the process of differentiation is stochastic. Moreover, the commonality of the β -subunit of the receptor for IL-3, IL-5, and GM-CSF,²³ the biologic cross-reactivity of these three cytokines, and the presence of the gene encoding the receptors for all three cytokines on the same chromosome make it plausible that IL-5, although predominantly an eosinophil differentiating stimulus, can also stimulate basophil growth and differentiation. Although IL-3 is, in human beings, a basophil differentiating factor,¹⁵ IL-3, in rodent systems, is primarily a mast cell growth factor.²⁴ IL-3 is also, and primarily, a multi-hemopoietin, and it probably works in conjunction with other cytokines such as GM-CSF or stem cell factor (SCF) to promote myeloid differentiation along several distinct lineages.²⁵ *In vitro* IL-3 can thus probably aid in both eosinophil and basophil differentiation, and the types of colonies generated in the presence of IL-3 are usually mixed basophil-eosinophil colonies when peripheral blood is used as the source of progenitors.¹⁸

GM-CSF is another multiply active hemopoietic growth factor, which can participate in directing differentiation of progenitors along the basophil-eosinophil pathway. The effects of GM-CSF, like those of IL-3, do not appear to be lineage-

specific; more likely, GM-CSF is permissive of the stochastic differentiation of mixed colonies of basophils and eosinophils, again when peripheral blood is used as the source of progenitors. However, all three of the cytokines, IL-3, IL-5, and GM-CSF, stimulate the differentiation of basophils and eosinophils from peripheral blood compartments, or even from the bone marrow, much more efficiently than granulocyte colony-stimulating factor, macrophage colony-stimulating factor, and SCF; each of the latter appears to aid in lineage commitment for several granulocytes including neutrophils, macrophages, and mast cells,²⁵ respectively.

Another factor that can cause mast cell hyperplasia in rodents and mast cell differentiation *in vitro* is nerve growth factor (NGF). This cytokine is active as a cofactor with IL-3 or IL-4 in rodent mast cell differentiation²⁶ and also stimulates human basophil-eosinophil progenitors,^{27, 28} especially when used in conjunction with GM-CSF or IL-5 *in vitro*.^{29, 30} The latter assays include both progenitors in peripheral blood semisolid cultures and cell lines such as HL-60, which are capable of basophil and eosinophil differentiation under alkaline conditions and in the presence of sodium butyrate, GM-CSF, or NGF.^{29, 30} This synergy between NGF and GM-CSF is particularly noteworthy in the HL-60 studies; the biologic role of NGF *in vivo* in human beings, and particularly in allergic reactions, remains to be determined.

There is some evidence that specific cytokines with hemopoietic inhibitory activity can also inhibit basophil and eosinophil progenitor growth and differentiation: these include transforming growth factor- β , which may aid in the switch from eosinophil to basophil commitment, suppressing the eosinophil pathway in particular³¹; IL-4, which can upregulate adhesion molecules and down-regulate *c-kit* expression on mast cells³²; tumor necrosis factor- α , which can inhibit myeloid colony growth *in vitro*³³; and, possibly IL-10, which has other more broadly based immunosuppressive effects.³⁴

The process of basophil-eosinophil differentiation

Much is yet to be determined about the stepwise process of basophil and eosinophil lineage commitment, with the use of the assays mentioned above, in either rodent or human systems. An orderly acquisition of cell surface markers for the basophil lineage has been examined in several studies.²¹ What is clear is that basophil and eo-

sinophil differentiating immunophenotypes are separate and distinct from mast cell-macrophage phenotypes and bear different profiles of cytokine receptors.^{21, 35-37} Characterizing basophil and eosinophil differentiating cell types are the presence of CD9, CD11b/18, CD25, CD35, and possibly CD40; conversely, mast cell progenitors have an immunophenotype that includes the presence of *c-kit*, the ligand for SCF,³⁸ and a number of macrophage-like determinants. During HL-60 cell differentiation to basophil and eosinophil lineage, CD15 is downregulated and CD35 is upregulated at a very early stage in vitro.³⁹ Studies on the intracellular processes of differentiation in basophils and eosinophils with molecular probes for differentiation-specific gene expression have revealed that a multistep process is involved; recent work in our laboratory indicates that retinoic acid, a differentiative stimulus for neutrophils, can suppress differentiation when present early in vitro during basophil-eosinophil lineage commitment.⁴⁰ Furthermore, the absence of a functional retinoic acid receptor on myeloid progenitors may favor basophil-eosinophil differentiation.⁴¹ Specific transcription factors such as GATA-1, -2, or -3 may be involved in commitment to the mast cell lineage and possibly to the basophil-eosinophil lineage.^{42, 43} A full discussion of the role of molecular transcription factors in these hemopoietic processes is beyond the scope of this article.

Basophil-eosinophil progenitors in circulation

Initial studies focused on the presence of an abundance of basophil-eosinophil progenitors in the blood of patients with myeloproliferative disorders, specifically chronic myeloid leukemia.^{9, 44} It became clear that atopic patients also have an increased number of these progenitors in circulation,⁴⁵ and in some individuals with severe allergy and asthma, the number is almost as high as in patients with chronic myeloid leukemia.⁴⁶ Studies in primates have indicated that parenteral administration of IL-3 and of GM-CSF can recreate this increased number of basophil-eosinophil progenitors in the peripheral blood: bone marrow release and subsequent circulation of high numbers of these progenitors can be elicited after successive daily administration of these two cytokines.^{14, 47} Whether or not in vivo IL-3, IL-5, and GM-CSF are released from tissue reactions involving allergen, IgE, T cells, and other accessory cells and then act on the marrow to increase release and circulation in the peripheral blood of basophil-

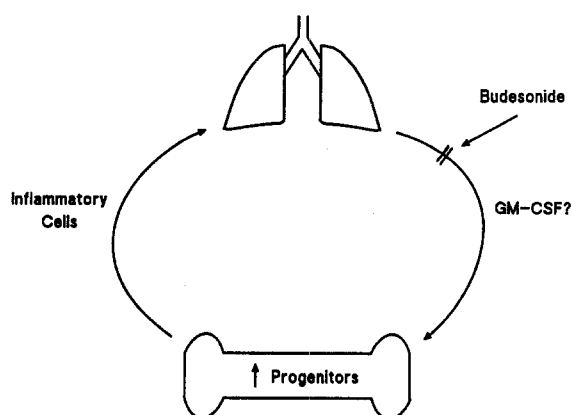


FIG. 1. Proposed communication between inflamed airway and bone marrow. Allergen challenge to the lungs in sensitized dogs leads to release of bone marrow colony-forming cells (i.e., inflammatory cell progenitors). This may be mediated by a systemic release of hemopoietic cytokines, such as GM-CSF, from the lung; the entire process can be blocked by inhaled corticosteroid (budesonide).

eosinophil progenitors has been the subject matter of studies we have performed in our laboratory over recent years. Use of animal models for an examination of the hypothesis termed *in situ hemopoiesis* or *microenvironmental differentiation*—support for which comes from studies of mast cell phenotype switch and differentiation in which various cytokines can act together or separately to induce specific genes for mast cell proteases that determine the ultimate outcome of the mast cell differentiative process—is now possible.⁴⁸⁻⁵⁰

APPLIED STUDIES Canine model

In human studies we had shown significant fluctuations in the number of circulating basophil-eosinophil progenitors during the course of allergic disease: an increase after allergen challenge or during an asthma exacerbation and a decrease after daily administration of inhaled corticosteroids to treat an asthma exacerbation,⁷ an inverse proportion of progenitors to symptoms in allergic rhinitis, and fluctuations during the course of natural allergen exposure in seasonal allergic rhinitis.⁶ In none of these studies was the bone marrow examined, and whether the circulating peripheral blood basophil-eosinophil progenitor pool was indeed related to and determined by bone marrow responses and release of progenitors into circulation during allergic responses remained conjecture. To examine this more directly,

TABLE I. CD34-positive cells in blood and marrow: Number of CD34⁺ cells ($\times 10^{-4}$) per 10^6 lymphocytes

	Peripheral blood	
	Nonatopic	Atopic
Range	1.1-1.3	1.7-2.3
Mean	1.19	2.00

TABLE II. GM-CSF localization in human basophil-eosinophil colonies

Condition	Colony cells (% positive)	
	Nontopic (n = 2)	Atopic (n = 4)
Unstimulated	22	68 $\pm$ 17
GM-CSF (0.1 ng/ml)	14	89 $\pm$ 4.3
IL-3 (1 ng/ml)	6.0	82 $\pm$ 12
IL-5 (1 ng/ml)	24	89 $\pm$ 5.3

we have recently used a canine model of airway hyperresponsiveness to ozone or allergen. Ozone elicits primarily a neutrophilic response, which is associated with changes in airway responsiveness, whereas allergen elicits airway hyperresponsiveness especially in dogs that have pre-exposure eosinophilia in the bronchoalveolar lavage compartment (M. Woolley et al. Unpublished data). Using the latter group of dogs, we have recently demonstrated that a single allergen challenge to the airway can elicit a bone marrow granulocyte progenitor response and that this can be attenuated significantly by pretreatment with inhaled budesonide (M. Woolley et al. Unpublished data). Thus airway-specific challenges with allergen or corticosteroid can influence the bone marrow while effecting changes at a local tissue site. The mechanism for this "distal" effect on the bone marrow is hypothesized to be the stimulation or, in the case of corticosteroids, the inhibition of release of specific cytokines from the airway, which act on the bone marrow progenitor pool within minutes to hours of provocation (Fig. 1). The cytokines involved in this response *in vitro* include recombinant canine granulocyte colony-stimulating factor, SCF, and GM-CSF. We do not yet know which cytokines that are present *in vivo* may influence the bone marrow, but recent observations indicate that a serum-derived factor with hemopoietic activity is present within 24 hours of

allergen challenge (M. Inman et al. Unpublished data). The lack of availability of canine IL-3 or IL-5 has so far precluded efforts to examine specific lineages of basophils and eosinophils *in vitro* in this model. However, the principle that an allergic-type reaction in the airway can be accompanied by dynamic changes in bone marrow progenitor pools is highly instructive.

To examine the fate and traffic of inflammatory cell progenitors in this model, we have recently developed a retroviral marker for canine hemopoietic progenitors in long-term bone marrow cultures. Dogs reinfused with marked progenitor populations show repopulation of blood and marrow progenitor pools within days, which persists for weeks. These techniques will prove to be highly useful in monitoring progenitors for basophils and eosinophils from the marrow through the blood, and presumably, into tissues during the course of allergic reactions.

Allergic disease

We have extended the studies mentioned above, which had formed the basis of the *in situ* hemopoiesis and progenitor hypotheses in allergy, to further analysis of progenitor-progeny relationships in allergic reactions. Specifically, these fall into three categories: (1) new studies of circulating progenitors during natural allergen exposure, (2) quantitation of bone marrow progenitors in atopy with cell surface markers, and (3) cytokine expression of differentiating progenitors.

Natural allergen exposure. We have extended previous observations by studying multiple time points during the birch pollen allergy season in Sweden and by examining basophil-eosinophil progenitors (colony-forming cells) *ex vivo*. Previously, we had shown that at the height of the season there is a decrease in progenitors, but the mechanism for this decrease was unclear. We now have evidence that very early in the season there is an initial increase in progenitors, followed by a decrease late in the season. A very important new finding has been the GM-CSF unresponsiveness of progenitors studied *ex vivo* early in the season (M. Linden et al. Unpublished data). This is a point that was not examined in earlier studies and may indicate that the decreases we had originally observed were related to lack of GM-CSF binding to basophil-eosinophil progenitors because of receptor occupancy or competition. Results of these studies are consistent with the notion that GM-CSF receptor expression on progenitors for basophils and eosinophils may change dynamically

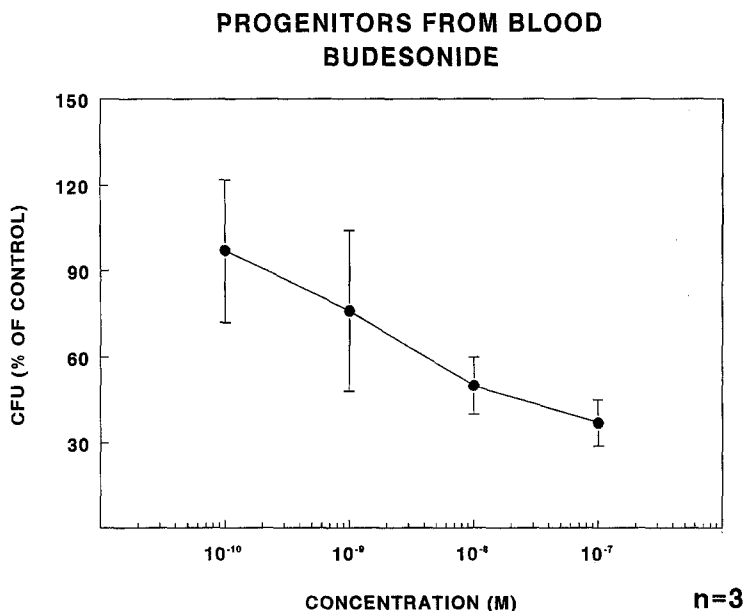


FIG. 2. Dose response of colony-forming units (CFU) in culture to the topical corticosteroid, budesonide.

during the course of allergen exposure in a seasonal model of allergic rhinitis. This idea has recently been advanced in a more general model of hemopoiesis.²⁵

In the same studies, it has also been shown that corticosteroids, in the form of budesonide *in vitro*, can suppress basophil-eosinophil progenitor differentiation at very low levels, i.e., 10^{-10} to 10^{-11} mmol/L (Fig. 2) (M. Linden et al. Unpublished data). This indicates that perhaps one mechanism which inhaled corticosteroids may affect circulating or bone marrow progenitor pools is through a system "leak" of topically active steroid.

Progenitor markers in atopy. We have recently used the cell surface marker CD34 to identify and quantitate progenitors by fluorescence-activated cell sorting using magnetic-activated cell separator (MACS) enrichment and flow cytometry in atopic and nonatopic subjects. As shown in Table I, CD34⁺ cells are twice as high in the blood of atopic subjects as in that of nonatopic subjects, supporting the results of earlier studies we had done with colony-forming cells in methylcellulose, in which atopic basophil-eosinophil progenitors were consistently twice as high as in nonatopic subjects. The precise cell surface immunophenotype of basophil-eosinophil progenitors and the way in which CD34 positivity is related to colony formation by these progenitors specifically remain to be determined.

Cytokine expression by progenitors. Likewise, we have found evidence for the spontaneous activation of differentiating basophil-eosinophil progenitors in atopic individuals by studying GM-CSF immunostaining of colony progeny derived from progenitors in the peripheral blood of atopic and nonatopic subjects. As shown in Table II, 80% to 90% of cells in colonies from atopic peripheral blood progenitors produce GM-CSF under all conditions of stimulation; the nonatopic colony-forming cells give rise to progeny which express GM-CSF at much lower levels or proportions.⁵¹ Studies of bone marrow are in progress, because it would be crucial to determine whether in the allergic state there is constitutive upregulation of cytokine gene expression or product during the course of differentiation of basophil-eosinophil progenitors to their mature counterparts, which have been shown to express various cytokines, including GM-CSF.⁵² Indeed, recent observations on hemopoietic stem cells reveal an orderly, sequential induction of cytokine and cytokine receptor genes during early lineage commitments; these include genes for the shared β -subunit of GM-CSF, IL-3 and IL-5 receptors, and CD34.⁵³

Bone marrow may clearly be one repository of the "allergic diathesis." Indeed, in bone marrow transplantation studies, there is evidence that both IgE levels and skin test reactivity, as well as

asthma, can be transferred.⁵⁴ To what extent bone marrow and basophil-eosinophil progenitors determine allergy and allergic reactions in individual patients, in the natural state, remains to be determined.

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New concepts about the mast cell

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