

Hemopoietic progenitors and cytokines in allergic inflammation

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With the advent of more sophisticated cellular and molecular biologic techniques to clarify the regulation of IgE and IgE receptor (IgER), it has become apparent that a number of key inflammatory effector cells which characterize allergic inflammation bear high-affinity IgE receptors (IgERI). Included among these are basophils and eosinophils, which are abundant in both early- and late-phase responses to allergen in the airways and skin (1–3). Given that eosinophils and basophils share a common hemopoietic lineage, i.e., are derived from a common progenitor which circulates in the peripheral blood and is of bone-marrow origin (4–6), it is not surprising that both cell types can express IgERI. While this was known for quite some time in relation to the basophil, it has only recently been documented for the eosinophil (7, 8). This paper will highlight the contribution of the bone marrow to allergic inflammation by summarizing a body of work which has accumulated demonstrating the active upregulation, release, and recruitment of eosinophil-basophil progenitors (CFU-Eo/B) to tissue sites in response to allergen provocation or during exacerbations of allergic inflammatory diseases.

Hemopoietic mechanisms in mucosal inflammatory disease

There is precedent for the delivery of inflammatory cells to a mucosal site from a pool of hemopoietic progenitor cells which can home to tissue sites. In nematode infestations, a readily accessible pool of circulating mast-cell progenitors in the blood and

thoracic duct home to the mesenteric lymph nodes and differentiate locally within the intestinal mucosa into mature mast cells in the presence of growth factors such as interleukin (IL)-3 and stem-cell factor (SCF) (9–11). In addition, fluctuations in progenitors that are relevant to the intestinal mast-cell hyperplasia are observed in peripheral blood in this model (11).

Similarly, several clinical studies we have undertaken in patients with allergic rhinitis, with or without asthma, atopic eczema, and/or nasal polyposis, have demonstrated an upregulation of basophil/eosinophil progenitors (CFU-Eo/B) in the peripheral blood with fluctuations in relation to symptomatology, seasonal allergen exposure, and extent of atopic disease (12–18). There is also substantial evidence for the capacity of inflamed tissues from patients with allergic rhinitis and nasal polyposis to produce hemopoietic growth factors that can support the differentiation and maturation of CFU-Eo/B, as well as mast-cell progenitors, *in vitro* and *ex vivo*; the concept of an ongoing tissue-driven, “*in situ*” hemopoietic process in allergic inflammation, whether it involves the upper or lower airway, has been proposed (19–25). Thus, mucosal tissues have the capacity to permit the differentiation of circulating inflammatory cell progenitors as they home to tissues, where they are supposed to expand *in situ*.

The nasal polyp model has served to emphasize that a specific tissue microenvironment, in which IgER-positive inflammatory cells such as eosinophils and mast cells are abundant, can be the repository of both hemopoietic growth factors and

of inflammatory cell progenitors. Supporting this is our recent observation that mononuclear cells bearing the hemopoietic stem-cell marker for CD34 are present within nasal polyp tissues, can be isolated therefrom, and are an enriched source of CFU-Eo/B (18, 26).

Expression of IgERI on eosinophil-basophil progenitors

The presence of a CFU-Eo/B within tissues in which IgERI-positive inflammatory cells are abundant emphasizes that steps in lineage commitment of Eo/B progenitors involve the expression of IgERs in a potentially orderly fashion. In support of this, early observations made by us concerning the induction of differentiation of Eo/B in a leukemic cell line, HL-60, demonstrated both high- and low-affinity IgE binding to cells induced along this hemopoietic pathway (27). Using this cell line and its eosinophilic subclone 15, it has been shown that both IgER and IL-5R are expressed at stages of differentiation of the Eo/B lineage (28, 29).

In more physiologic studies of CD34+ cord-blood progenitors induced to differentiate to Eo/B lineage, Rottem et al. demonstrated the later appearance of IgERI on these cells by flow cytometry (30, 31). Although a formal study of the relationship between the acquisition of IgERI, IgERII, and other lineage markers such as IL-5R has never been performed, the results of these combined observations suggest an orderly sequence of the appearance of the lineage-specific markers, including IgERs, on nascent Eo/B progenitors (Fig. 1). This area may provide a basis for novel therapeutic approaches targeting the bone marrow and Eo/B progenitors in allergic inflammation.

Allergen challenge studies in asthma: human and animal models

Studies in allergic asthmatics after allergen provocation have pointed out that activation of hemopoietic mechanisms systemically occurs during these responses. Gibson et al. demonstrated that allergen inhalation, associated with the development of late-phase responses, elicited a specific increase in CFU-Eo/B in circulation within 24 h (3, 32); this increase abated as the degree of hyperresponsiveness returned to baseline (33).

Studies in a canine model of allergen-induced bronchial hyperresponsiveness showed, similarly, an upregulation of myeloid progenitors within hours of an airway allergen challenge, which could be prevented by pretreatment with inhaled corticosteroids (34). In this model, a serum hemopoietic

activity is released after allergen provocation, which acts on the bone marrow to elicit further upregulation of myeloid progenitors; this also could be abrogated by pretreatment with inhaled corticosteroids *in vivo* (35–37). Accompanying this bone-marrow response is the appearance of actively dividing (presumably hemopoietic) cells in both the blood and bronchoalveolar lavage fluid within 24 h of the airway allergen challenge (38). These results indicate an active link between the airways and the bone marrow in allergic inflammation, providing evidence for a “beneficial” systemic effect of inhaled corticosteroids on hemopoietic cell delivery to the airways.

Recent studies of the bone marrow in atopic asthmatic patients after inhaled allergen confirm findings in the canine model: CD34/45+ hemopoietic progenitors and CFU-Eo/B, increased in numbers in the blood and marrow of atopic individuals in direct relation to serum IgE levels (39), can be further specifically upregulated after airway allergen challenge eliciting late-phase responses in atopic asthmatics (40, 41). An IL-5-responsive subset of CFU-Eo/B is upregulated in the marrow within 24 h of allergen challenge in dual responder asthmatics (39–41); using triple colour flow cytometry, it can be shown that this subpopulation of CD34/45+ progenitors in the marrow is one that bears high-affinity receptors for IL-5 (IL-5R α) (40). Thus, a readily mobilizable pool of Eo/B progenitors, at a relatively early stage of lineage commitment, is increased after inhalation of allergen in those individuals who develop more chronic inflammatory responses. Whether these progenitors bear IgERI or IgERII is currently being investigated.

The nature of the signalling between the airway and the bone marrow which induces IL-5R α on CD34+ progenitors in the bone marrow *in vivo* is not yet known. Several recent studies in murine models of allergen-induced airways inflammation suggest that IL-5 and/or granulocyte-macrophage colony stimulating factor (GM-CSF) may be critical in signalling the bone marrow to release and/or produce more cells committed to the Eo/B lineage (42, 43). We have recently found that GM-CSF *in vitro* can induce both IL-5R α and other markers of this lineage on clone 15 HL-60 cells *in vitro* (29, 44). Whether GM-CSF or other cytokines are essential for IgERI and IgERII expression on Eo/B progenitors needs to be further examined.

The finding that in man one can transfer, by marrow transplantation, aspects of atopy to non-atopic recipients supports the view that critical elements in the bone marrow – such as T cells or progenitors – may be the sources of this transplantable atopy (45, 46). Indeed, we have recently

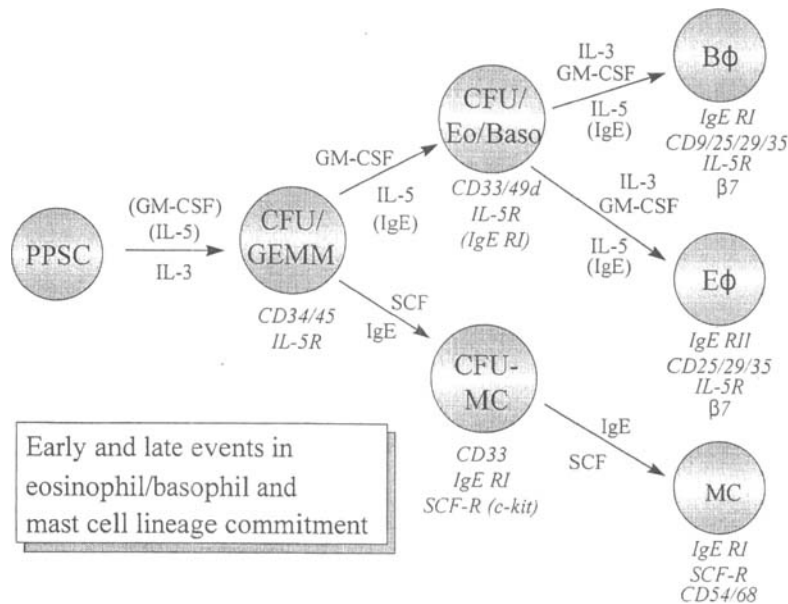


Fig. 1. Although a formal study of the relationship between the acquisition of IgERI, IgERII, and other lineage markers such as IL-5R has never been performed, the results of these combined observations suggest an orderly sequence of appearance of the lineage-specific markers, including IgERs, on nascent Eo/B progenitors.

observed that in addition to the aforementioned serum hemopoietic factor, an innate tendency to bone-marrow responsiveness can contribute to ongoing inflammatory responses (47). This further extends previous findings we have reported in patients with allergic rhinitis in which both locally produced hemopoietic growth factors and increased responsiveness of circulating CFU-Eo/B are important in developing and maintaining hemopoietic responses in allergic airways inflammation (21).

Topical corticosteroid therapy to the airways: effects on hemopoietic processes

Topical corticosteroids in airway inflammation can be used as a tool to examine the relevance of the contribution of hemopoietic processes to allergic responses. First, the elicitation of a mild exacerbation of asthma, by controlled withdrawal of inhaled corticosteroids, is accompanied by a rapid increase in peripheral blood CFU-Eo/B, together with the development of symptoms and changes in peak flow rates (32, 48). This demonstrates the sensitivity of a systemic hemopoietic process to inhaled corticosteroids, implying that the bone marrow is actively contributing immature, inflammatory cells that are being recruited to the upper and lower airways. Further support for this comes from our recent observations of increases in CD34+ mononuclear cell populations within the nasal polyp after corticosteroid therapy (26). This elevation of CD34+ cells within nasal polyp tissues after inhaled

or systemic corticosteroid therapy, implying a block in differentiation, is consistent with an ongoing contribution of the bone marrow to the process of inflammatory cell recruitment.

Finally, in studies recently completed, inhaled corticosteroids given to atopic asthmatics developing late-phase responses to inhaled allergen prevent both the airway inflammatory response – comprising mature eosinophils and mast cells (49) – and the upregulation of IL-5R α on CD34+ cells in the bone marrow (unpublished data) (40). Whether or not, in addition to changes in IL-5R α on CD34+ cell populations in the marrow, there is also modulation of IgER on these cells, after 1-week pretreatment with inhaled budesonide, remains to be determined. It should be noted that as low a concentration as 10^{-9} M of budesonide is sufficient *in vitro* to inhibit CFU-Eo/B differentiation (50, 51); this is a concentration readily achieved systemically with current regimens of inhaled corticosteroid.

The bone marrow in allergic inflammation: a target for anti-inflammatory therapy

We have proposed that the bone marrow is an ongoing source of chronic inflammation that seeds atopic tissues with cells capable of maturing into IgER-positive effector cells of allergy (14). Therapies directed at the bone marrow, whether via an effect of inhaled corticosteroids or, more specifically, through antagonism of hemopoietic cytokine

receptors for factors such as IL-5, or anti-IgE therapy which could theoretically interfere with maturation of Eo/B progenitors, need to be considered in the treatment of chronic allergic inflammation. Recent findings on the specific Eo/B-lineage inhibitory effects of retinoic acid may provide new insights in this regard (44, 52). A corollary of these observations is that allergic inflammation is a systemic process in which the bone marrow actively contributes to maintain and sustain disease and symptoms. Both Eo/B progenitors and mature cells can acquire an altered, autostimulatory, and chronic inflammatory phenotype, expressing IgERs and producing their own growth factors such as IL-5 and GM-CSF, thus perpetuating the inflammatory process (53). The chief goal of therapy in these conditions may well be to give sufficient anti-inflammatory and hemopoietic-modulating therapy so as to achieve blunting of the bone-marrow contribution to the development of allergic inflammation.

Conclusion

The bone marrow actively participates in the production of IgER-positive inflammatory cells (eosinophils, basophils, mast cells), which are typically recruited to tissues in atopic individuals. Understanding the signalling between the tissue and the bone marrow at the molecular level may well open up new avenues of therapy for allergic inflammation.

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