

The nose, the lung and the bone marrow in allergic inflammation

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Introduction

In contemplating the relationship between the upper and lower airways in asthma, several important questions arise: is there a causal relationship between inflammation in either compartment, one way or the other? Or, is inflammation in these two compartments related to some other proximal cause? Alternatively, are the two compartments independent of each other? While substantial epidemiological, neurophysiological and clinical evidence exists for a relationship between rhinitis and asthma, and while it is well recognized that atopy itself is genetically and thus immunologically based throughout the organs, actual mechanisms for cell recruitment through a common pathway to both upper and lower airways in allergic inflammation have not yet been fully delineated. It is the intent of this article to describe and elaborate upon a potentially important systemic, contributing mechanism to cell recruitment in both rhinitis and asthma which serves to link these two diseases in a more definitive way. The specific role of the bone marrow in providing an ongoing source of differentiating inflammatory cells (eosinophils, basophils, and mast cells in particular) to the upper and lower airways will be the focus of this contribution.

Hemopoietic mechanisms in mucosal inflammatory disease

There is adequate precedent for the delivery of inflammatory cells to a mucosal site from a dynamic pool of progenitor cells derived from pluripotent stem cells in the bone marrow (Fig. 1); these restricted progenitors may move into the systemic circulation, and home to a tissue site.

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Experimentally, in nematode infestations involving the lung and gut such as is found in association with *Nippostrongylus brasiliensis*, several authors, including us, have shown that a readily accessible pool of circulating mast cell progenitors in the blood and thoracic duct ultimately home to the mesenteric lymph nodes and expand locally in the intestinal mucosa, differentiating into mature mast cells in the presence of locally derived growth factors such as interleukin (IL)-3 and stem cell factor (SCF) (1–3). Fluctuations in progenitors that are relevant to the intestinal mast cell hyperplasia are observed in peripheral blood in this model (3).

Along these lines, several clinical studies we have undertaken in patients with allergic rhinitis, with or without asthma, atopic eczema and/or nasal polyposis, have indicated repeatedly an upregulation of basophil/eosinophil (Eo-B) progenitors (Fig. 1) in the peripheral blood with fluctuations in relation to symptomatology, seasonal allergen exposure and extent of atopic disease (4–10). Together with overwhelming evidence by us and others over the last decade of the capacity of inflamed upper airways tissues from patients with allergic rhinitis and nasal polyposis to produce hemopoietic growth factors that drive the differentiation and maturation of Eo-B as well as mast cell progenitors *in vitro* and *ex vivo*, we have put forth the concept of an ongoing tissue-driven, “*in situ*” hemopoietic process in allergic inflammation, whether it involves the upper or lower airway (11–18). Thus, the mucosal tissues of the airways have the machinery to permit the differentiation of relevant inflammatory cell progenitors which are found in circulation and could theoretically home to tissues where they are proposed to differentiate and expand *in situ*.

In particular, the nasal polyp model has served to emphasize that a specific, seemingly isolated, tissue microenvironment, in which inflammatory cells such as eosinophils and mast cells are abundant, can be the repository of both hemopoietic growth factors and of inflammatory cell progenitors at the same time. Highlighting the latter is our recent observation that mononuclear cells bearing the marker for undifferentiated multipotential hemopoietic stem cells, CD34 (see Fig. 2A, B), reside within nasal polyp tissues in frequencies that are consistent with their distribution in other hemopoietic compartments such as blood. Such cells can be isolated from the nasal polyps and produce hemopoietic colonies containing varying types of inflammatory cells and, in particular, those of the Eo-B lineage (10, 13, 19).

Allergen challenge studies in asthma: human and animal models

Studies in lower airways inflammatory conditions associated with bronchial hyperresponsiveness, utilizing allergen inhalation as the provocative stimulus, have also highlighted the activation of hemopoietic mechanisms systemically. Gibson et al. first demonstrated that allergen inhalation associated with the development of the late-phase response and bronchial hyperresponsiveness in human atopic asthmatics cotemporaneously elicited a marked upregulation of Eo-B progenitors in circulation within 24 h of the challenge; this was specific for the Eo-B lineage and not the neutrophil-macrophage one (20, 21). Kinetics of this response in the blood were demonstrated further by Choudry in our group, indicating that this increase in progenitors abated as the degree of hyperresponsiveness returned to baseline over a 2-week period (22) (Fig. 3).

Based on studies we then undertook in canine models of *Ascaris suum*-induced bronchial hyperresponsiveness, it became clear that the bone marrow could be activated to upregulate myeloid progenitors within hours of an airway allergen challenge (23), and that this could be abrogated by pretreatment with inhaled corticosteroids (23). Furthermore, a serum hemopoietic activity was released, in the canine model described, following allergen provocation, acting on the bone marrow to elicit further upregulation of myeloid progenitors from naive, unchallenged progenitors *ex vivo*; this too was abrogated by pretreatment with inhaled corticosteroids *in vivo* (24–26). Accompanying this bone marrow response in the dog developing bronchial hyperresponsiveness was the appearance of actively dividing cells in both the blood and bronchoalveolar lavage fluid as marked by BrdU, within 24 h of the airway allergen challenge (27, 28). These results indicate an active communicative link between the lung and the bone marrow in allergic airways inflammation, and provide evidence for a beneficial systemic effect of inhaled corticosteroids on this process of cell recruitment to the airways.

Recent studies of the involvement of the bone marrow in human atopic asthmatic responses to inhaled allergen confirm what we have found in the canine model: CD34/45⁺ hemopoietic progenitors, increased in numbers in the blood and marrow of atopic individuals, can be upregulated specifically following airway allergen challenge eliciting bronchial hyperresponsiveness and the late-phase response in atopic asthmatics (29–30). An IL-5-responsive subset of progenitors, marking the Eo-B lineage specifically, is upregulated in the marrow within 24 h of allergen challenge

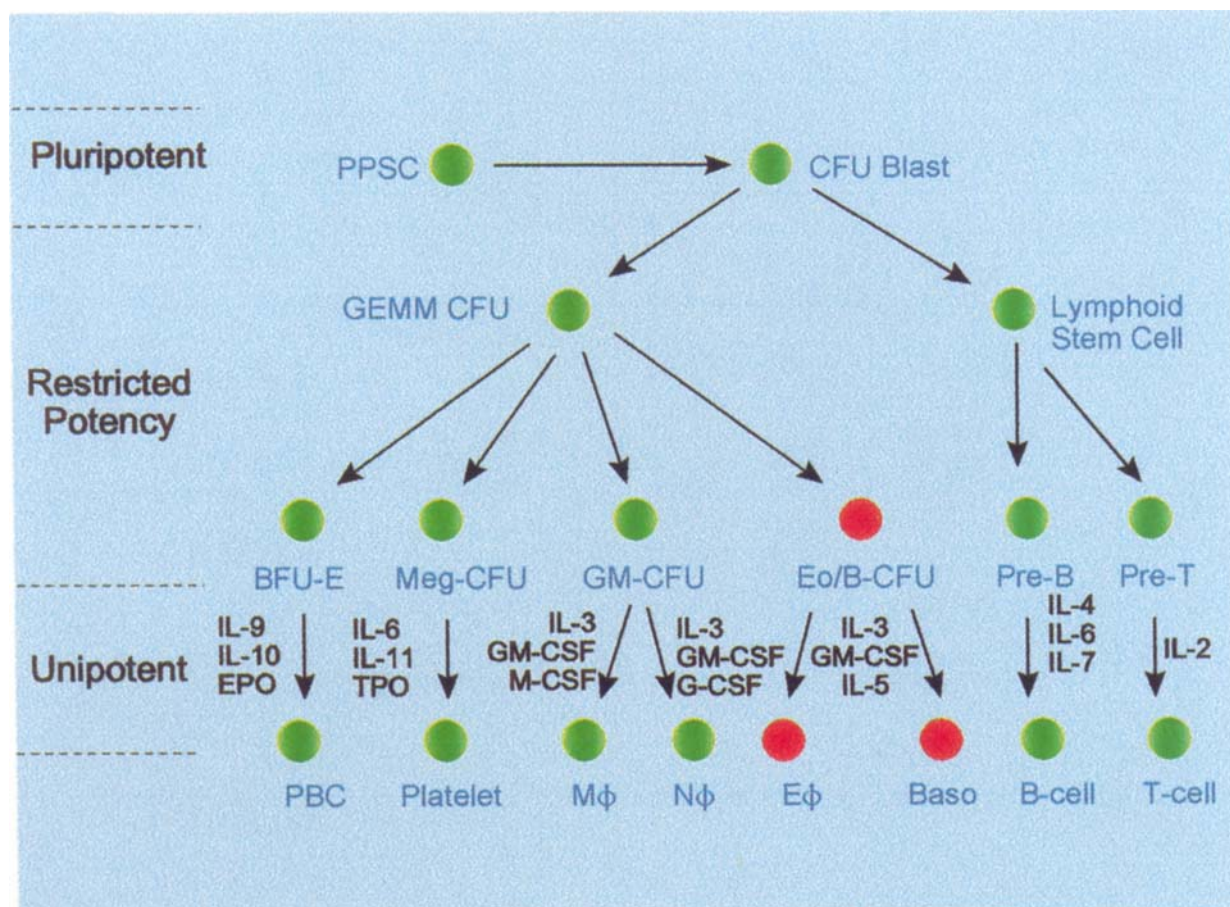


Figure 1. Hemopoietic differentiation depicted as a series of stochastic (random) steps in which bone marrow-derived pluripotent cells are committed and then restricted to specific pathways. The final differentiative phenotype is determined by the presence of specific hemopoietic growth factors. Highlighted is the common progenitor for eosinophils and basophils, which is specifically upregulated in allergic inflammation.

in dual responder asthmatics (27–30); using triple color flow cytometry, it can be shown that this subpopulation of progenitors in the marrow is one that bears high affinity receptors for IL-5 (IL-5R α) (Figs 4 and 5), existing as a subpopulation of early progenitors bearing CD34/45 (30). Thus, a readily mobilizable pool of Eo-B progenitors at a very early stage of lineage commitment is increased after inhalation of allergen only in those individuals who develop ongoing inflammatory responses. The nature of the signaling between the airway and the bone marrow, which upregulates IL-5R α on CD34⁺ progenitors in the bone marrow *in vivo*, is not yet known. Several studies in murine models of allergen-induced airways inflammation suggest that hemopoietic factors such as IL-5 and/or granulocyte-macrophage colony stimulating factor (GM-CSF) may be critical in signaling the bone marrow to produce more cells committed to the Eo-B lineage (31, 32).

The finding that in both humans and mice bone marrow transplantation can transfer the atopic diathesis, with its attendant eosinophil- and mast cell-rich inflammatory response, to previously nonatopic recipients supports the

view that critical elements in the bone marrow, possibly immunocompetent T cells or other, non-T-cell, populations, may be the source of this transplantable atopy (33–35). Indeed, we have observed recently that both a serum hemopoietic factor, as well as an innate tendency for bone marrow responsiveness, can combine to contribute to ongoing inflammatory responses in the canine airway (24). This extends further previous findings we have reported in patients with allergic rhinitis in which both the capacity of upper airways tissues to produce relevant hemopoietic growth factors, as well as increases in target circulating Eo-B progenitors, are critical in developing and maintaining an upregulation of hemopoietic responses in airways inflammation (8–13).

Topical corticosteroid therapy to the airways: effects on hemopoietic processes

Therapy with topical corticosteroids in both upper and lower airway inflammation can be used as a tool to

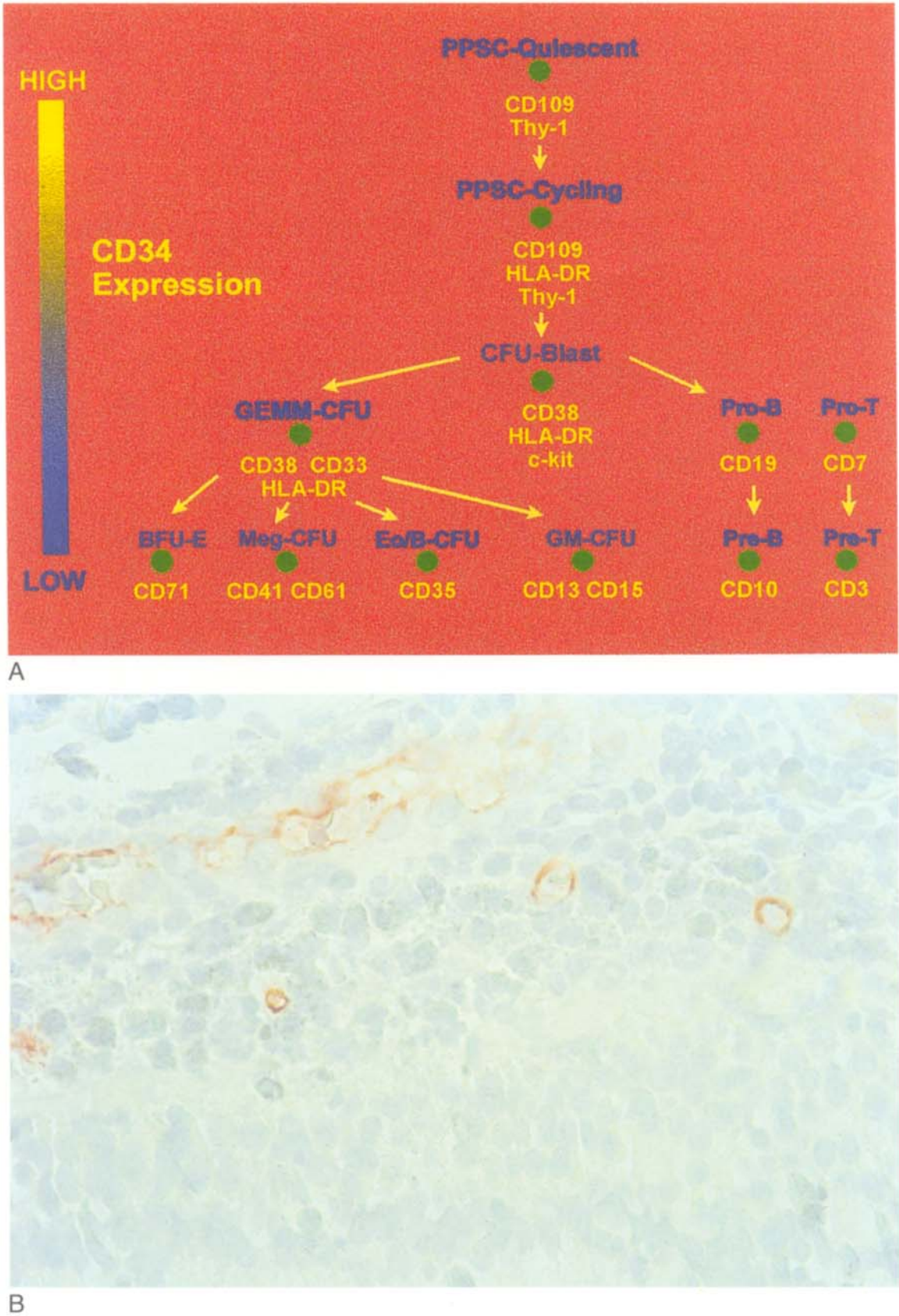


Figure 2. A) CD34 as a marker of progenitor cells. *In vitro* studies have shown that CD34 expression is restricted to primitive progenitor cells, and that purified CD34⁺ cells form colonies of various lineages *in vitro*. In addition, *in vivo* studies have shown that purified CD34⁺ cells can reconstitute hemopoiesis in lethally irradiated baboons and achieve engraftment in various malignancies in humans. B) Photomicrograph of CD34 immunostaining in nasal polyp tissue. Note both endothelial and mononuclear cell reactivity. The latter can be shown to represent myeloid progenitor cells.

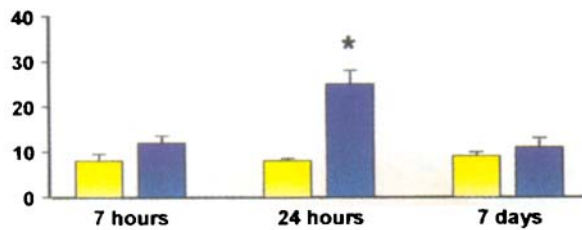
Eo/B-CFU (per 10⁶ cells plated)

Figure 3. Kinetics of eosinophil-basophil progenitor (Eo-B-CFU) changes after allergen (blue bars), compared to diluent (yellow bars) challenge in allergic asthmatics. The rise in progenitors is present at 24 h, at the time of maximal bronchial hyperresponsiveness, and abates by 7 days when bronchial responsiveness has returned to baseline (not shown).

examine the relevance of the contribution of hemopoietic processes to rhinitis and asthma. First, the elicitation of a mild exacerbation of asthma, by controlled withdrawal in stepwise fashion of topical inhaled corticosteroids, is accompanied by a rapid and sustained increase in peripheral blood Eo-B progenitors almost contemporaneous with

the development of symptoms and changes in peak flow rates (20, 21, 36). This suggests a degree of sensitivity of a systemic hemopoietic process to inhaled corticosteroids, further implying that the bone marrow is actively contributing cells that are being recruited as immature, not fully differentiated, inflammatory cells to the upper and lower airways. Further support for this comes from our recent observations of a block in differentiation, and thus increases in primitive CD34⁺ mononuclear cell populations, within the nasal polyp after corticosteroid therapy (19, 37). This striking elevation of CD34⁺ cells within nasal polyp tissues after corticosteroid therapy to the nose also implies the ongoing contribution of the bone marrow to the recruitment of cells in both upper and lower airways.

Finally, in recent studies we have just completed, inhaled corticosteroids deposited in the lung of atopic asthmatics developing late-phase responses to inhaled allergen are capable of downregulating the airway inflammatory response comprising eosinophils and mast cells (38, 39) while at the same time beginning to, but not completely

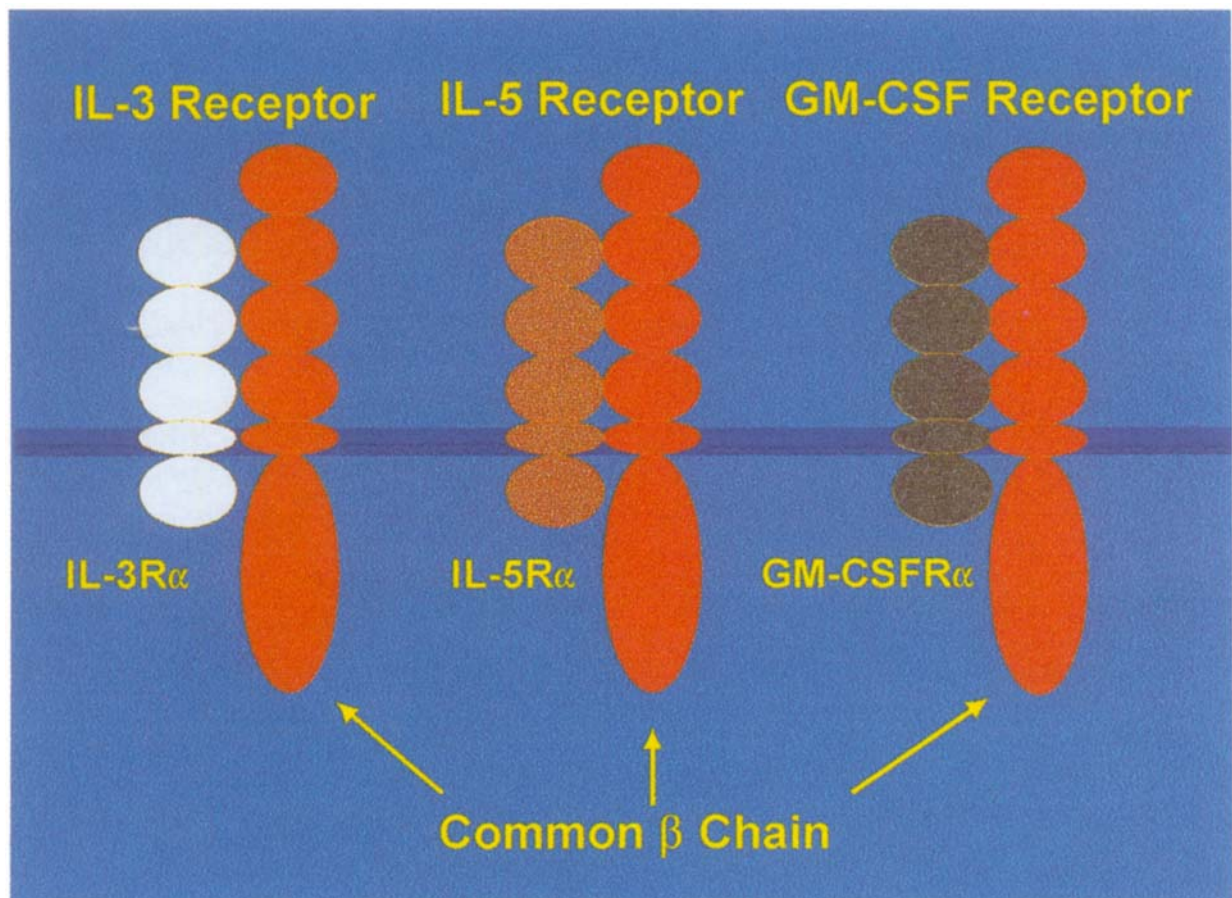


Figure 4. Cell surface receptors for the hemopoietic cytokines IL-3, IL-5 and GM-CSF. Specificity is conferred by high affinity binding to an α subunit; signaling occurs through association of the α subunit with a common β subunit shared by these three cytokines.

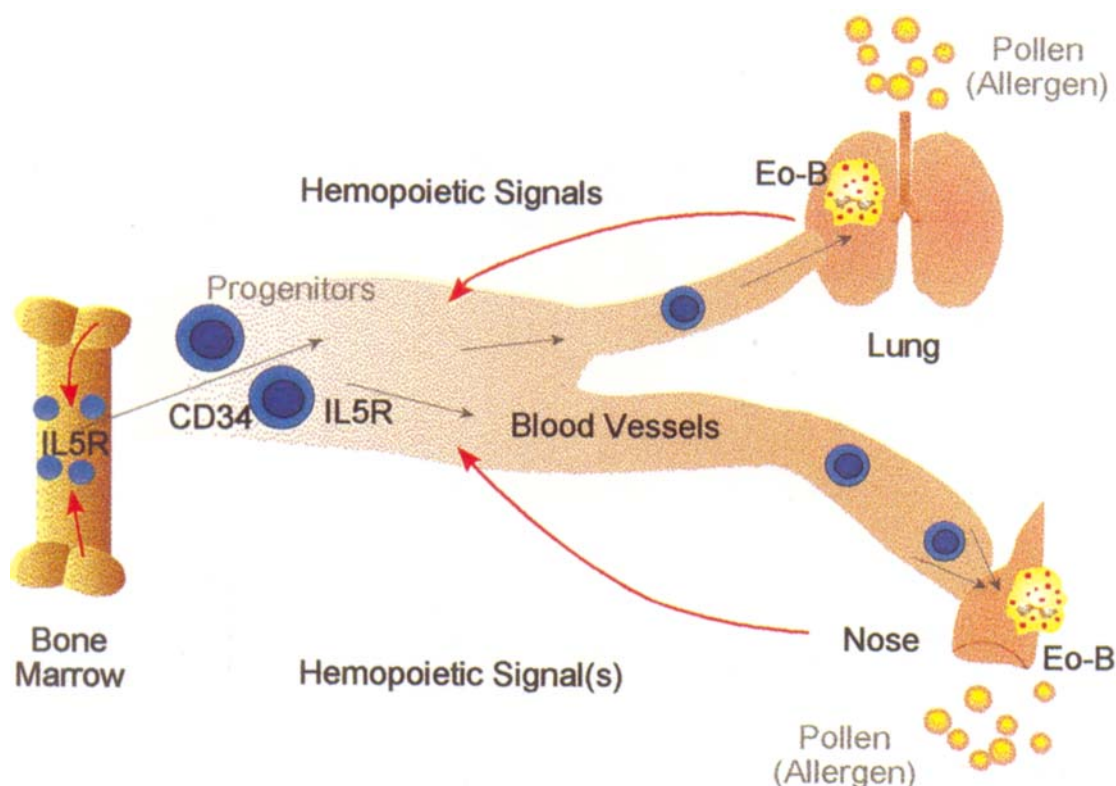


Figure 5. Communication among the nose, the lung and the bone marrow in allergic inflammation. Allergen provocation of the airways can, through hemopoietic signaling (cytokines), elicit a rapid upregulation of Eo-B lineage commitment in the marrow, as evidenced by increased expression of IL-5R α on CD34/45⁺ progenitors [30]. Such cells, or their differentiating progeny, are postulated to migrate through the circulation and home to the site of allergen exposure, where they can amplify the inflammatory response.

abolishing, the upregulation of Eo-B progenitors in the bone marrow in these same individuals (27–30). This suggests that the response in the marrow requires more sustained treatment with inhaled corticosteroids in order to abrogate chronic inflammation in the upper and lower airways. Further studies are in progress to examine this critical pathway and its kinetics. It should be noted that as low a concentration as 10^{-9} M of budesonide is sufficient *in vitro* to inhibit Eo-B growth and differentiation [40, 41]; this is a concentration readily achieved systemically with current dosing regimens of inhaled corticosteroid.

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

Based on the above accumulating evidence, we propose that the bone marrow is an ongoing source of chronic inflammation that seeds both upper and lower airways with cells capable of maturing into characteristic inflammatory effector cells of allergy. Therapies directed at the bone

marrow, whether through beneficial systemic effects of inhaled corticosteroids or more specific targeting of the upregulated cytokine receptors for eosinophilopoietic factors such as IL-5, need to be considered in the treatment of chronic inflammation seen in both rhinitis and asthma in allergic individuals.

A corollary of these observations is that both asthma and rhinitis are part of the same systemic process in which the bone marrow actively contributes, interacting with tissue-derived hemopoietic and chemotactic signals to maintain and sustain chronic allergic-type inflammation in the airways (Fig. 5). Effector cells such as eosinophils, and the progenitors of these cells, such as Eo-B progenitors, both acquire an autostimulatory and chronic inflammatory phenotype, attended by the production of their own growth factors such as IL-5 and GM-CSF [38], thus perpetuating the inflammatory process in asthma and rhinitis. Thus, the chief goal of therapy in these conditions is to give sufficient anti-inflammatory and hemopoietic-modulating therapy so as to achieve blunting of the bone marrow contribution to the development of both asthma and rhinitis.

Conclusions

Bone marrow participates actively in the production of inflammatory cells (eosinophils, basophils, mast cells) which are recruited typically to the nose and lung in

atopic individuals with rhinitis and/or asthma. Understanding the signaling between the airways and the bone marrow at the molecular level may well open up new avenues of therapy for both upper and lower airways inflammation.

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