

Bone marrow events in animal models of allergic inflammation and hyperresponsiveness

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Allergic inflammation is associated with the marked infiltration of eosinophils in affected tissues. Eosinophilia, in turn, is a hallmark clinical feature associated with allergic rhinitis, atopic dermatitis, sinusitis, and asthma. There is considerable evidence in animal models and humans that the bone marrow plays an integral role in allergic inflammation. Evidence shows that, in response to allergen exposure in the airway, bone marrow (white blood cell) progenitors proliferate and differentiate, which leads to persistent increases in eosinophil numbers. These observations suggest that there is signaling between the lung and bone marrow after allergen exposure and provide further support for the proposition that allergy is a systemic disease. Although the nature of the signal-mediating activation of bone marrow after airway allergen exposure is unknown, several pathways have been implicated, including allergen-induced hemopoietic growth factors, cell trafficking, and stimulation of resident bone marrow cells. A common thread in all these pathways is the importance of IL-5. Evidence is reviewed in animal models for the role of bone marrow in allergic inflammation within the context of the systemic nature of allergic disease. (*J Allergy Clin Immunol* 2000;106:S235-41.)

Key words: Airway challenge, allergen, bone marrow, eosinophil, eotaxin, IL-5, progenitor

Evidence of an association between a systemic inflammatory cell hemopoietic response and allergen-induced inflammation was first obtained from patients with atopic airway disease. In these studies, it was observed that patients with symptoms of seasonal allergic rhinitis or acute asthma exacerbation had increased numbers of circulating eosinophil/basophil progenitors (Eo/B-CFU), when compared with healthy subjects.^{1,2} These observations stimulated an interest in the mechanisms by which these systemic hemopoietic responses occur and the degree to which these responses contribute to the severity of airway inflammation.

As reviewed by Denburg et al in this issue (p. S242), the role of bone marrow (BM) in allergic disease is under investigation in allergic patients and in human models of allergen exposure. In addition to studies in humans, there has been a growing interest in the exploration of systemic hemopoietic responses in animal models of allergic air-

Abbreviations used

BM: Bone marrow
GM-CFU: Granulocyte-macrophage colony-forming unit

way disease. Initially, evidence is presented indicating that stimulation of hemopoietic events is associated with allergic airway inflammation in animal models. Subsequently, evidence that implicates specific mediators and mechanisms that may be responsible for signaling between the airway and BM after allergen challenge is reviewed.

BM EFFECTS OF AIRWAY EXPOSURE TO ALLERGEN

The first studies of BM responses to airway allergen challenges were performed with a dog model of inhaled allergen-induced airway hyperresponsiveness and inflammation. This model is similar to human disease; that is, the inhaled allergen (*Ascaris suum*) is associated with immediate bronchoconstriction and delayed airway hyperresponsiveness. However, in contrast with humans with asthma, this model is not associated with a late bronchoconstrictor response, and only neutrophils have been observed to increase in the postallergen lavage.³ Woolley et al,³ using colony-forming unit assays of BM aspirate, observed that BM myeloid progenitor cells of the granulocyte/macrophage line (GM-CFU) were increased after allergen exposure in conjunction with airway neutrophilia and hyperresponsiveness. There was no response in BM progenitor cells to diluent challenge. We later showed that the increase in BM GM-CFU numbers was only observed in dogs that developed airway inflammation and hyperresponsiveness after airway allergen exposure.⁴ On the basis of these observations, we hypothesized that local allergen-induced inflammatory responses in the airway give rise to systemic signalling events that result in the increased production of relevant inflammatory cells by the BM and that this increase involved an expansion of the relevant progenitor population (Fig 1).

Ohkawara et al⁵ have observed the time course of changes in airway, circulating, and BM eosinophil responses in a murine model of allergen sensitization and challenge. In this model, they observed increases in circulating eosinophils after the second of 2 intraperitoneal sensitizations to ovalbumin that, over a similar time course, was accompanied by an increase in BM eosinophils (Fig 2).⁵ When the mice were subsequently exposed to

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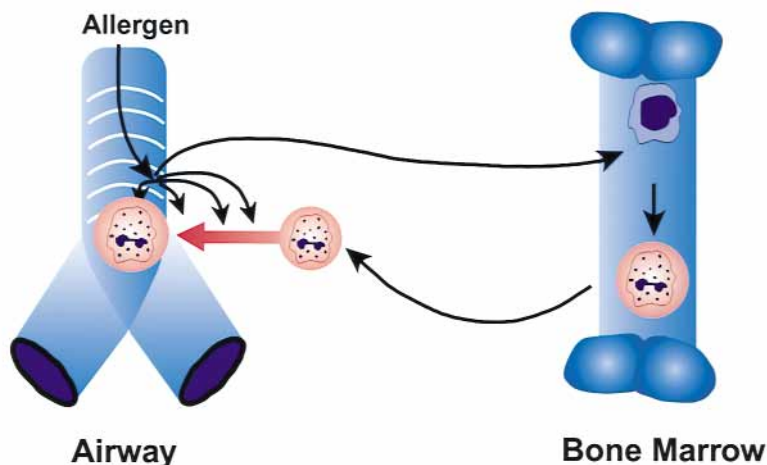


FIG 1. Schematic illustration of the events that take place in the airway and BM in response to airway exposure to allergen. In addition to releasing mediators responsible for recruiting inflammatory cells into the airway, signaling to the BM results in increased inflammatory cell production, mediated in part by progenitor expansion.

aerosolized ovalbumin, there was a period of airway eosinophilia that peaked 3 days after exposure and persisted for up to 10 to 15 days. This airway inflammatory response was accompanied by increases in both circulating and BM eosinophils that followed a similar time course. However, by Day 9, the BM response appeared to have partially resolved, whereas the airway and peripheral blood responses were near maximum. Increases in BM eosinophil numbers after airway allergen challenge of sensitized mice have also been observed by other investigators.⁶⁻⁸ Although eosinophil production rates cannot be determined from these data, it appears likely that local inflammatory events associated with allergen exposure in the peritoneum or airway are accompanied by an increased rate of eosinophil production. As a consequence, BM and peripheral blood eosinophil numbers are maintained at increased levels throughout the duration of the local response. Interestingly, in the study by Okhawara et al,⁵ there was a decrease in BM eosinophil numbers in the first 3 to 6 hours after airway allergen challenge, which suggests a brief period during which eosinophil recruitment from the BM exceeded production rates.

In a study by our group in a mouse model of ovalbumin sensitization and airway challenge, we observed increases in airway responsiveness and eosinophilia after allergen exposure, both of which resolved within 2 weeks.⁹ As illustrated in Fig 3,⁹ in the same mice we observed an increase in eosinophil/basophil progenitors that was limited to the 24- and 48-hour time points after challenge. This observation confirmed our hypothesis that increased production of eosinophils involves a period of progenitor cell expansion in the BM. However, the observation that the time course of the progenitor cell expansion was of shorter duration than airway inflammation suggested that progenitor expansion may occur only briefly at the onset of the inflammatory response.

Taken together, results from our laboratory⁹ and others⁵ have suggested that there are several distinct periods involved in the systemic BM response after airway exposure to allergen. Immediately after exposure is a period during which BM production of eosinophils is not sufficient to keep up with the demand. As a consequence, there is a transient decrease in BM eosinophils. In the next 48 hours, concurrent with progenitor cell expansion, BM eosinophils are restored and ultimately increased above normal levels through an increased production rate. The next period persists for the duration of the local airway inflammatory response and is characterized by elevated BM production of eosinophils, whereas progenitor levels have returned to normal. These results suggest that progenitor expansion may be required during periods of extreme demand but that increased eosinophil production can occur independently, presumably as a result of increased maturation rates of a normal population of progenitors. This concept is supported by observations in atopic patients; during the allergy season, the number of circulating basophils remains elevated despite the fact that the number of circulating basophil progenitors decreases.²

RELEVANCE OF BM RESPONSE

The importance of BM responses in the determination of the magnitude of airway inflammation was explored by Wang et al.¹⁰ We observed that, compared with wild-type mice, IL-5-deficient (IL-5 knockout) mice have fewer eosinophils in BM and peripheral blood. Furthermore, these mice displayed markedly reduced eosinophilic responses in BM, peripheral blood, and airway after ovalbumin sensitization and airway challenge. In an attempt to restore the airway eosinophilic response to allergen, we exposed mice to an adenoviral-based IL-5 transgene, delivered either intranasally to the airway or intramuscu-

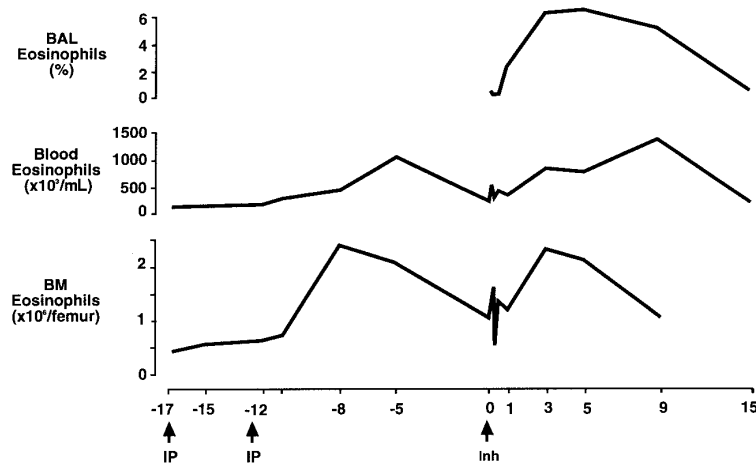


FIG 2. The time course of eosinophil responses in the airway, the peripheral blood, and the BM as a result of a protocol of intraperitoneal (IP) sensitization and inhalation (Inh) challenge of mice with ovalbumin. BAL, Bronchoalveolar lavage. (Adapted with permission from Ohkawara Y, Lei XF, Stampfli MR, Marshall JS, Xing Z, Jordana M. Cytokine and eosinophil responses in the lung, peripheral blood, and bone marrow compartments in a murine model of allergen-induced airways inflammation. *Am J Respir Cell Mol Biol* 1997;16:510-20. Official Journal of the American Thoracic Society. © American Lung Association.)

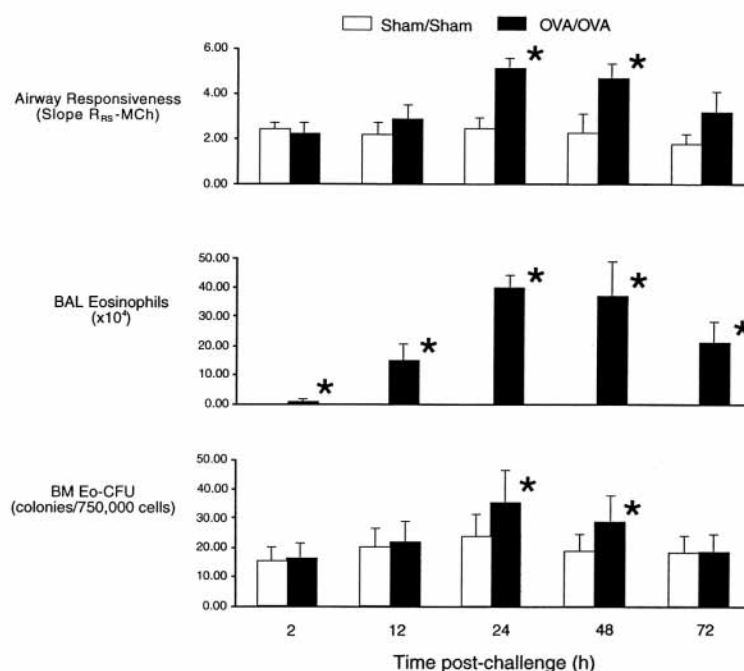


FIG 3. The time course of airway hyperresponsiveness, airway eosinophilia, and BM eosinophil progenitors (BM Eo-CFU) after the second of 2 daily intranasal exposures to ovalbumin in sensitized mice. Airway responsiveness was assayed as the slope of the curve of respiratory system resistance versus the log-transformed intravenous dose of methacholine. Airway eosinophils were assessed by bronchoalveolar lavage (BAL). BM eosinophil progenitors were assessed by semisolid cultures of BM perfusate in the presence of IL-5. OVA, Ovalbumin. (Adapted with permission from Inman MD, Ellis R, Wattie J, Denburg JA, O'Byrne PM. Allergen-induced increase in airway responsiveness, airway eosinophilia, and bone-marrow eosinophil progenitors in mice. *Am J Respir Cell Mol Biol* 1999;21:473-9. Official Journal of the American Thoracic Society. © American Lung Association.)

larly to the systemic circulation. These delivery routes produced selective increases in IL-5 in the airway and systemic circulation, respectively. Airway delivery of the IL-5 transgene, although sufficient to produce marked

eosinophilia in wild-type mice, failed to restore the ovalbumin-induced eosinophilia in the IL-5 knockout mice. However, when delivered intramuscularly, the IL-5 transgene restored ovalbumin-induced eosinophil responses in

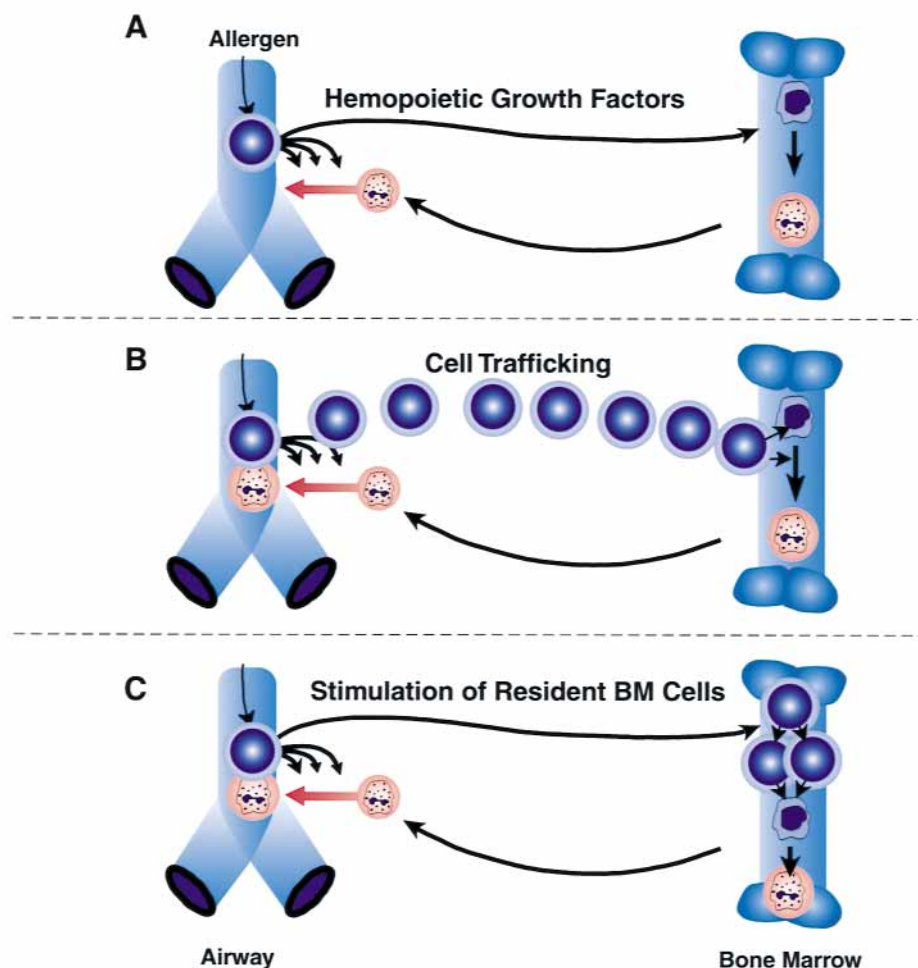


FIG 4. Schematic illustration of 3 possible mechanisms of communication between the lung and BM after airway exposure to allergen. **A**, Direct hemopoietic effect of mediators released into circulation; **B**, trafficking of T lymphocytes from the airway to the lung and subsequent release of hemopoietic mediators; and **C**, local release of hemopoietic mediators in response to blood-borne signals (possibly including allergen).

the BM, peripheral blood, and bronchoalveolar lavage of IL-5 knockout mice to levels comparable with those observed in ovalbumin-challenged wild-type mice. These results indicated that the absence of a normal BM and peripheral blood eosinophilic response in IL-5 knockout mice prevented the development of airway inflammation. These responses were prevented even when the local airway IL-5 level had been restored to that normally observed in association with allergen challenge. However, despite the fact that there was no IL-5 detected in the airway after intramuscular delivery of the IL-5 transgene, IL-5 knockout mice developed airway eosinophilia comparable with wild-type mice. These results clearly demonstrated that without the enhanced production of eosinophils after allergen challenge, the extent of eosinophilic airway inflammation was markedly reduced. These results also suggested that the mechanism by which allergic airway responses are prevented in anti-IL-5-treated¹¹⁻¹⁸ or IL-5 knockout animals^{12,19,20} is by preventing enhanced BM production of eosinophils.

SYSTEMIC SIGNALING BETWEEN THE LUNG AND BM

Allergen challenge models

The mechanism underlying airway exposure to allergen and increase in BM progenitors remains a question. The concept is summarized in Fig 1. The airway to the BM is a signaling mechanism whereby the BM response is activated as a result of local airway exposure to allergen. A simple interpretation of the study by Wang et al¹⁰ is that increased circulating IL-5 levels are required for the development of increased eosinophil production by the BM and maintenance of airway eosinophilia. However, the study only showed that IL-5 was required at some level of the signaling process and that increasing systemic IL-5 levels are capable of restoring BM eosinophil production in the IL-5 knockout mouse. Evidence that IL-5 was also required for the allergen-induced recruitment of eosinophils from BM was provided by Humbles et al.²¹ They observed that the transient decrease in BM eosinophils observed after allergen

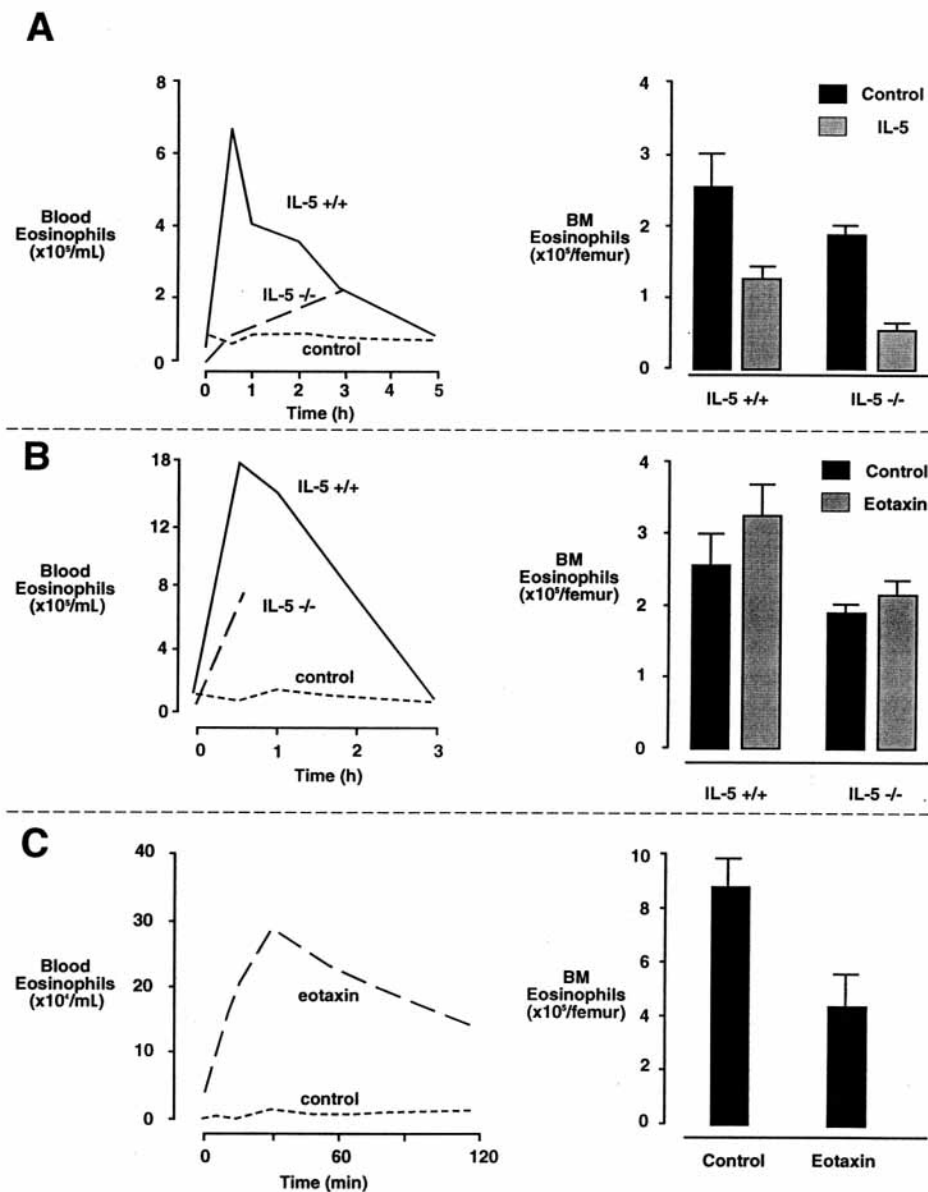


FIG 5. The response of blood eosinophils (left) and BM eosinophils (right) after intravenous administration of IL-5 and eotaxin to mice and guinea pigs. **A**, The effect of IL-5 or control in wild-type (IL-5 +/+) and IL-5 knockout (IL-5 -/-) mice; **B**, the effect of eotaxin or control in wild-type (IL-5 +/+) and IL-5 knockout (IL-5 -/-) mice; and **C**, the effect of eotaxin or control in guinea pig isolated perfused hindlimb. (Adapted from Mould AW, Matthaëi KI, Young IG, Foster PS. Relationship between interleukin-5 and eotaxin in regulating blood and tissue eosinophilia in mice. *J Clin Invest* 1997;99:1064-71. With permission.)

challenge of the guinea pig airway could be prevented by treatment with an anti-IL-5 blocking antibody. Although the studies by Wang et al¹⁰ and Humbles et al²¹ both illustrate the central importance of IL-5 in activating BM responses, neither study implies that increases in systemic IL-5 levels are a required signaling component in animal models or in allergic asthmatic responses. Although the specific nature of signaling between the lung and BM (in the context of allergic inflammation) is unknown, several studies have addressed this question and provided some insight.

Using the dog model of *Ascaris* challenge, we have demonstrated the presence of a serum-borne hemopoietic signal, present at the time of allergen-induced airway inflammation.⁴ In this study, naive BM aspirate was obtained from dogs before allergen challenge and incubated with serum obtained from the same dogs 20 minutes, 2 hours, and 24 hours after allergen challenge. Although we observed no hemopoietic effect from the 20-minute or 2-hour serum, the 24-hour serum showed an increase in GM-CFU that was similar to the increase

observed in BM obtained from the dogs after allergen challenge. The results of these studies implicated the presence of hemopoietic factors after allergen exposure. These factors, in turn, activated progenitor expansion. Actually, it was unlikely that progenitor numbers were expanded in our *ex vivo* culture assays. Rather, it is probable that hemopoietic mediators in the serum after allergen challenge acted to prevent apoptosis of progenitor cells in a manner similar to lineage regulation in the BM (ie, through local cytokine levels stimulating survival of specific stem-cell populations).^{22,23}

Because of the lack of canine-specific mediators and blocking antibodies, we have not been able to characterize the nature of the serum-signaling mechanism observed in dogs. Additionally, we have not been successful in our attempts to reproduce the canine serum-signaling results in mice. However, Gaspar Elsas et al⁶ have demonstrated that plasma taken from mice after allergen challenge was capable of increasing BM eosinophil (mature) numbers when injected into naive recipient mice, which supports the concept that mediators in systemic circulation may be involved in stimulating BM responses after exposure to allergen.

The nature of the signaling mechanism has recently come under investigation by Minshall et al.⁷ They reported an increase in IL-5 immunostaining and expression of IL-5 mRNA in cells resident in mouse BM after airway allergen challenge, which suggests that a component of the BM eosinophilic response may be mediated through local production of hemopoietic cytokines at the level of the BM. In this same study, there was an almost 5-fold increase in the number of CD3⁺ T lymphocytes in the BM after allergen challenge, and one-half of these cells were positive for IL-5 mRNA. These results suggested that T-cell production of IL-5 may contribute significantly to BM responses. Whether the increase in T cells was due to trafficking from the site of allergen presentation or attributed to a local T-cell expansion (perhaps because of systemic levels of antigen) could not be determined from these studies. In another study, Murali et al⁸ observed that the supernatant from spleen cell cultures of allergen-challenged mice had a greater eosinopoietic effect on BM cultures than did supernatant from allergen-naïve mice. These results are in agreement with those of Minshall et al⁷ that hemopoietic mediators may be released from cells in compartments other than the airway. However, these findings were unable to distinguish whether cell trafficking or systemic T-cell activation/expansion was taking place.

Although the airway-BM signaling mechanism remains unknown, a number of factors have been implicated and are summarized in Fig 4. Although it is likely that several mechanisms coexist, the results from the study by Wang et al¹⁰ suggest that IL-5 is critically involved in the process.

Exposure to exogenous mediators

Although Wang et al¹⁰ have demonstrated the crucial role of IL-5 in stimulating BM eosinopoiesis in mouse models of airway allergen challenge, evidence also exists

that suggests that eotaxin contributes to the systemic mobilization of eosinophils. Mould et al²⁴ have observed that intravenous administration of both IL-5 and eotaxin can produce increases in circulating eosinophils in wild-type mice. In this study, IL-5 100 pmol/kg produced a rapid increase in circulating eosinophils, which was accompanied by a decrease in BM eosinophils (Fig 5A).²⁴ In IL-5 knockout mice that received 500 pmol/kg IL-5, there was a slower and a 3-fold smaller increase in circulating eosinophils, which was again accompanied by a decrease in BM eosinophils (Fig 5A). Interestingly, the basal (IL-5 preadministration) number of eosinophils in the BM was similar in the wild-type and IL-5 knockout mice. Thus, these results demonstrated that increased circulating levels of IL-5 were capable of recruiting eosinophils from the BM. The diminished ability of IL-5 to increase circulating eosinophil levels in the IL-5 knockout mice, despite near-normal basal BM eosinophils, could be attributed to several factors. Although circulating IL-5 may be capable of directly stimulating eosinophil recruitment from the BM to a small extent (Fig 5A), the level of recruitment observed in the wild-type mice may have required amplification of the IL-5 signal at the level of the BM. It is known that eosinophil activation occurs in response to IL-5²⁵ and that, once activated, eosinophils synthesize IL-5.²⁶ It was possible that amplification of the eosinopoietic signal occurred only in the wild-type mice. Alternatively, IL-5 may upregulate expression of the IL-5 receptor on the eosinophil. Thus, it is possible that the IL-5 knockout mice had lower levels of IL-5 receptor, accounting for the smaller effect of IL-5 on increasing circulating eosinophil numbers in these mice. Neither of these explanations accounts for the observation by Mould et al²⁴ that IL-5 induced similar decreases in BM eosinophils in IL-5 knockout and wild-type mice (Fig 5A). One explanation is that, although IL-5 knockout mice were able to mobilize eosinophils from the BM, these cells rapidly disappeared from the circulation.

Mould et al²⁴ also observed that systemically administered eotaxin resulted in increases in circulating eosinophil numbers in wild-type mice. In that study, eotaxin produced a rapid increase in circulating eosinophils that was not associated with a decrease in BM eosinophils (Fig 5B). When the same dose of eotaxin was given to IL-5 knockout mice, there was also a rapid, but 50% smaller, increase in circulating eosinophils compared with wild-type mice. Additionally, eotaxin was not associated with a change in BM eosinophils in IL-5 knockout mice. These observations suggested that eotaxin could mobilize eosinophils from sites other than the BM, using an IL-5-independent mechanism. Interestingly, different results were observed by Palframan et al²⁷ in guinea pigs that were given intravenous eotaxin. In that study, eotaxin produced a rapid increase in peripheral blood eosinophils that was associated with a decrease in BM eosinophils (Fig 5C), which suggested some recruitment from this compartment. Additionally, in response to eotaxin administration in isolated perfused guinea pig

hindlimb, there was increased release of eosinophils and their progenitors into the circulation.²⁷

Although there are discrepancies among different animal models, it appears that both IL-5 and eotaxin can contribute independently to the release of eosinophils and progenitors from BM. The observation that allergen-induced increases in eotaxin follow a relatively short time course, with levels returning to near basal values within 12 to 24 hours,²¹ suggests that eotaxin may be more important in early eosinophil and progenitor recruitment. In contrast, because IL-5 levels remain elevated for up to 5 days after allergen challenge,⁵ the ongoing maintenance of BM activation is likely mediated through IL-5. Although the specific mechanisms by which IL-5 promotes release of eosinophils from the BM are unknown, it has been demonstrated that IL-5-induced release of eosinophils from the isolated perfused guinea pig hindlimb is associated with an increased expression of β_2 -integrin and is prevented by a blocking antibody to this adhesion molecule ligand.

SUMMARY

As a result of airway exposure to allergen of sensitized animals, there is a rapid efflux of eosinophils from the BM. After this, there is an increased production of eosinophils by the BM, mediated through progenitor expansion and increased maturation rates. The signaling mechanisms responsible for these effects may include distal effects of mediators released at the airway, or possibly trafficking of cells from the airway to the BM. Although both eotaxin and IL-5 are capable of producing some of the effects observed in the BM after allergen challenge, it appears that IL-5 is critically important in mediating this response. The potential role of airway-BM signaling in allergen-induced eosinophilic responses associated with asthma and other allergic conditions remains an important issue for further investigation.

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