

Review article

The role of the bone marrow in allergy and asthma

Denburg JA, Inman MD, Leber B, Sehmi R, O'Byrne PM. The role of the bone marrow in allergy and asthma.
Allergy 1996; 51: 141–148. © Munksgaard 1996.

**J. A. Denburg, M. D. Inman,
B. Leber, R. Sehmi, P. M. O'Byrne**

Department of Medicine, McMaster University,
Hamilton, Ontario, Canada

Key words: allergy; asthma; basophils; cytokines;
eosinophils; hemopoiesis; inflammation; progenitors.

Dr J. A. Denburg
Department of Medicine, HSC-3V46
McMaster University
Hamilton, Ontario, L8N 3Z5
Canada

Accepted for publication 28 November 1995

In allergic-type inflammation, basophil/eosinophil and mast cell infiltration into tissues is pathognomonic. The mechanisms whereby eosinophils, basophils, and mast cells reach tissue sites are classically believed to involve chemotaxis, cell adhesion, and infiltration of mature cells, as a result of immune activation and the consequent effects of proinflammatory and hemopoietic cytokines. Our laboratory has been examining another potential contributing mechanism to allergic inflammation; i.e., the differentiation and maturation of inflammatory effector cells and, thus, the involvement of the bone marrow in the delivery of inflammatory cells to the tissues. In this short review, the biologic and clinical relevance of the bone marrow, with its output of inflammatory cell progenitors, will be discussed. Studies we have done over the last decade specifically examining the identification, differentiation, and kinetics of eosinophil and basophil (Eo-Baso) progenitors in allergic inflammatory diseases of the lung will be highlighted.

A fundamental hypothesis is that the bone marrow is skewed in atopic states to differentiation of increased numbers or types of inflammatory cell (especially Eo-Baso) progenitors so as to give rise to the characteristic allergic inflammatory reaction in airways in asthma and related conditions.

General issues in hemopoiesis

Marker studies of progenitors

Until recently, progenitors for various hemopoietic lineages were identified and operationally defined by colony-forming assays in semisolid media such as agar or methylcellulose. Now, several hemopoietic progenitor cell-surface antigens which allow marker analysis and cell sorting of varying stages of progenitors in human and animal systems have been defined. The most relevant of these markers are CD34 (1–4), *c-kit* (the proto-oncogene cell product which binds SCF [5–7]), CD33, CD38, and CD45 (the common leukocyte antigen) (4). The earliest progenitors bear CD34 and the *flt3/flk-2* tyrosine kinase receptor on their surface (8) and later acquire CD33, *c-kit*, CD38, and/or CD45 (4, 9, 10). The CD34 molecule is a 120-kDa, *O*-sialated glycoprotein (9–11) expressed on the surface of progenitors, vascular endothelium (11, 12), and tissue structural cells (13).

Relationships of markers to differentiation

The relationships among hemopoietic progenitor cell antigens, cytokine receptors, and lineage commitment are not yet clearly delineated. The CD34 gene is induced at an intermediate stage of embryonic stem cell development, after induction of the

common β -subunit of the interleukin (IL)-3, IL-5, and granulocyte/macrophage colony-stimulating factor (GM-CSF) receptor (14, 15). CD34 may play an important role in progenitor cell adhesion and activation within the bone marrow stromal compartment, in a process involving carbohydrate interactions (16). From the practical point of view, marker studies combined with cell sorting have made large numbers of purified progenitor populations accessible for study *in vitro* and for replenishment of bone marrow *in vivo* (1–4, 10, 17, 18).

Cytokine receptors on progenitors

Cytokine receptors which appear to overlap in function or signaling may confer both "redundancy" and "subtlety" on lineage commitment (19); specificity may be achieved through mechanisms such as synergy, transactivation (20), or costimulation (19). Common receptor subunits such as gp-130 for IL-6, leukemic inhibition factor (LIF), tumor necrosis factor- α (TNF- α), and IL-11 (and partially, for IL-12 or granulocyte colony-stimulating factor [G-CSF]), or the shared β -subunit for IL-3, IL-5, and GM-CSF, may help to determine the direction and amount of differentiation, depending upon the availability of a given combination of cytokines at tissue sites.

Specific lineage commitment and expression for inflammatory effector cells

Basophil, eosinophil, and mast cell lineages

Basophil and eosinophil differentiation. *In vitro*, one can distinguish neutrophil and monocyte-macrophage ("GM" colonies) from eosinophil and basophil ("Eo" colonies) differentiation; each is derived from a committed progenitor (19, 21–27). Eo-Baso differentiation can be directed solely by IL-5 acting on a late-stage progenitor (28–30); nonetheless, IL-3 and GM-CSF also can be shown to play a role in commitment to this lineage (21, 22, 31). Binding studies reveal varying IL-3, IL-5, GM-CSF, and stem cell factor (SCF) receptor expression on mature basophils and mast cells (22, 31), but do not address the critical question of receptor expression on their respective progenitors. This is important, since the specific cytokine receptor-phenotype of each of these cells may help in understanding terminal differentiation events, as well as in developing potential therapeutic strategies for intervention in allergic diseases (see below).

Mast cell lineage. The human mast cell lineage is probably distinct from Eo-Baso (31, 32), since it can be stimulated uniquely by SCF both *in vitro* and *in vivo* (31, 33, 34–37). However, the precise

stage at which the mast cell pathway diverges from the Eo-Baso one is unknown. Progenitors in cultures of human fetal liver cells, cord blood, or bone marrow differentiate into mast cells and basophils by using combinations of SCF and IL-3 (31, 33–35, 38–42). The immunophenotype of mast cells is distinct from that of basophils and eosinophils (43). Thus, the *c-kit* mediates only mast cell differentiation (31, 32, 43, 44), with some exceptions of the hybrid basophil-mast cells in human bone marrow disorders (45–48). The uniqueness of the mast cell lineage is underscored by recent experiments done in the *W^s/W^s* rat suggesting a biologic dissociation from the basophil lineage (49, 50). Mutations in *c-kit* receptor domains at the *W* locus in mice and rats determine mast cell differentiation and function (51, 52).

Recently, the contribution of mast cell progenitors in the blood and lymph nodes to the development of mast cell hyperplasia in the intestinal or respiratory mucosa, after nematode infestation or viral bronchiolitis, respectively, has been confirmed (53, 54). These findings complement long-standing observations that we and others had made on mast cell progenitors in the rat *Nippostrongylus brasiliensis* model (55, 56) and on Eo-Baso progenitors in allergic rhinitis (57, 58). What appears to be the case for mast cell accumulation in response to a parasitic antigen is thus probably the same for Eo-Baso progenitors in human allergen-induced disease. The above studies have provided the conceptual and experimental framework for the bone marrow hypothesis we have pursued in human allergic reactions (below).

Cytokines and lineage specificity

Cytokine effects on mature cell phenotype. Lineage-specific cytokine effects on differentiated cells, which retain hemopoietic cytokine receptors on their surface, result in "activation" or "phenotype switching": IL-3, IL-5, and GM-CSF can activate and prevent apoptosis of basophils and eosinophils (42, 59); G-CSF and GM-CSF do the same for neutrophils (60). Autocrine stimulation by eosinophils, basophils, or mast cells of their own differentiation and/or activation is thus possible, since they produce a wide range of hemopoietic and proinflammatory cytokines, including IL-3, IL-4, IL-5, IL-6, and GM-CSF (33, 61–69).

Effects of single cytokines. Sanderson et al. have described IL-5 transgenic mice, in which massive eosinophilia in the peripheral blood and tissues is not accompanied by inflammatory disease unless concomitant nematode infection is present (30). This implies that eosinophil differentiation and

activation (presumably induced by the IL-5 transgene) are not sufficient to cause allergic-type inflammation; however, upregulated eosinophil differentiative processes may be necessary for this to occur.

In any case, anti-IL-5 antibody treatment *in vivo* by parenteral injection into guinea pigs, rats, or primates abrogates tissue eosinophilia (in the bronchoalveolar lavage) as well as bone marrow eosinophil increases after allergen challenge (70). In these experiments, a single dose of humanized anti-IL-5 abolished eosinophilia for 3 months, consistent with an effect on production of eosinophils by their progenitors.

Likewise, SCF, when administered exogenously to normal primates or rodents, causes a massive mastocytosis without disease, probably by preventing apoptosis of mature cells (33, 71, 72). Subcutaneous administration of SCF to human subjects causes a wheal-and-flare reaction and pigmentation, with increases in dermal mast cells locally and at distant sites (73). Whether anti-SCF treatment can abrogate mast cell-dependent inflammation in the same way that anti-TNF- α can (72) is still unknown.

Molecular events in eosinophil/basophil differentiation

HL-60 cells and pharmacologic modulation

Data from our laboratory suggest that several processes are involved in the generation of functional eosinophils and basophils from pluripotent progenitors. Several growth factors, including GM-CSF, IL-5, and nerve growth factor (NGF) – the latter as a synergistic cofactor (74, 75) – are potent stimulators of basophil differentiation. During myeloid differentiation, *bcl-2* expression is downregulated, rendering the mature cell susceptible to the process of programmed cell death, or apoptosis, without *preventing* induced differentiation. Retinoic acid (RA) and budesonide both inhibit Eo-Baso differentiation, as assessed by histamine production (76, 77). This suggests that the generation of Eo-Baso progeny from progenitors occurs in discrete steps that are inhibited by RA and corticosteroids. It will be of interest to examine which stages of Eo-Baso differentiation are inhibited by either of these two different agents (Fig. 1).

For example, RA inhibits early, but not later, stages of Eo-Baso commitment (78), and there appears to be a skewing toward Eo-Baso lineage in RA receptor-dominant mutant HL-60 cells (79). It is possible that this RA-induced inhibition of Eo-Baso lineage may be accompanied by modulation of surface expression of markers such as

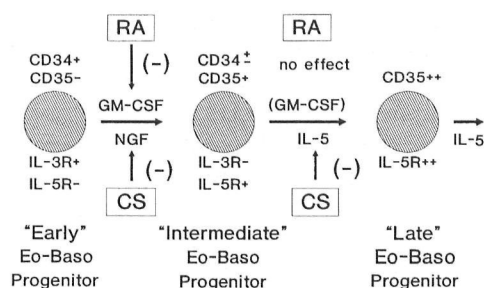


Fig. 1. Corticosteroids (CS) and retinoic acid (RA) can block development of Eo-Baso progenitors, but precise stage(s) at which this occurs is not known. Here we postulate that CS affect both "early" (CD34⁺) and "intermediate" (CD34[±]) Eo-Baso progenitors, while RA downregulates only former, according to preliminary data we have gathered.

IL-5R- α , CD34, CD45, or CD35. Since expression of the Eo-Baso granule protein genes for major basic protein (MBP), eosinophil cationic protein (ECP), and eosinophil-derived neurotoxin (EDN) is inhibited by RA (78), we can now examine whether expression of the earliest form of MBP, pro-MBP (80), is also blocked by RA (Fig. 1).

Identification of human Eo-Baso progenitors

From data we have recently obtained, both in HL-60 and methylcellulose culture systems, it appears that the immunophenotype of an intermediate-stage Eo-Baso progenitor is a CD34-negative, CD35-positive cell (81). However, the precise immunophenotype of either eosinophil or basophil progenitor awaits further definition. Recent work on cultured eosinophils has detected a hybrid Eo-Baso cell (82) which we had identified in several earlier studies of colonies and cord blood (25, 26, 29, 77, 83). The terminal differentiation events separating these two cells are unclear. If the bone marrow is a key determinant of the accumulation of both these cell types in allergic tissues, one might predict a specific upregulation of precisely these progenitors in the marrow and blood of atopic individuals.

Cytokines produced by differentiating progenitors

Recent experiments by us have shown not only that peripheral blood Eo-Baso progenitors in atopic patients are increased in quantity (25), but also that their differentiating progeny (i.e., maturing eosinophils and basophils in colonies) produce increased GM-CSF compared with nonatopic progenitors (77, 84). Since this reflects an *ex vivo* expression of a presumably *in vivo* commitment, it suggests that differentiation might be skewed in the atopic individual, perhaps even at the bone marrow stem cell level or at intermediate stages of progenitor

differentiation. Indeed, studies of neonatal predictors of allergic diathesis suggest that, in addition to IgE levels, basophil and eosinophil production may be important (85). Taken together with our and others' previous demonstration of GM-CSF gene expression and production by eosinophils (65, 67), these findings suggest that eosinophils may exert autocrine control of their own differentiation in allergic states, amplifying their own numbers and maturation.

***In vivo* relevance and correlates of inflammatory cell progenitors**

Allergic inflammation

In vivo models: allergic rhinitis and asthma. We have extensive evidence of significant and relevant fluctuations in peripheral blood Eo-Baso progenitors in allergic airways disease (21–26, 57, 58, 86–88) (Table 1). In general, allergic rhinitis and atopy patients appear to have constitutive increases in these progenitors, observed mainly out-of-season (57, 58). Fluctuations in blood Eo-Baso progenitors occur during natural allergen exposure (57, 58, 89). Eo-Baso progenitors not only rise in number during seasonal allergen exposure, but also within 24 h of allergen provocation in a controlled setting (86, 88). Inhaled corticosteroid therapy reverses the rise in peripheral blood Eo-Baso progenitors observed during asthma exacerbation (87). These studies highlight the dynamic relationships that exist among allergen provocation, airways inflammation, corticosteroid therapy, and inflammatory cell progenitors.

An important question is whether the effect of allergen/topical corticosteroid on Eo-Baso progenitors in circulation in asthma is due to direct effects on the bone marrow, or is an indirect consequence of effects on airways tissue (and thus on production of cytokines which can act on the bone marrow) (Fig. 2). We have begun to address this key question in a canine model we have developed.

In vivo models: canine allergen-induced airway hyper-responsiveness. We have recently shown that airway allergen provocation in *Ascaris suum*-sensitized

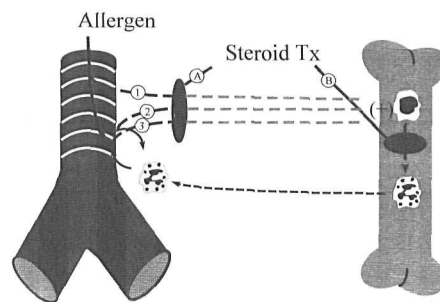


Fig. 2. Three potential mechanisms by which allergen exposure results in progenitor stimulation are illustrated: 1) direct effect of allergen on progenitors (unlikely), 2) hemopoietic factor(s) released from airway *directly* as result of allergen (possible, see text), 3) hemopoietic factor(s) released *indirectly*, either from inflammatory cells in airway or in circulation (to be studied). Two potential sites of steroid action are illustrated: A) blocking production/release of proinflammatory/prohemopoietic mediators (proven *in vitro*), B) blocking hemopoietic effect of these mediators on bone marrow (proven *in vitro*). Tx: treatment.

dogs, with bronchial hyperreactivity and eosinophilic infiltration, causes increases in bone marrow myeloid progenitors; prior inhalation of the potent corticosteroid, budesonide, abrogates this bone marrow response (90). A serum hemopoietic activity is released, probably from the lung, after allergen challenge, only in dogs that develop airway hyper-responsiveness, but not in nonresponders; an endocrine effect by this factor is likely (91) (Fig. 2). Together with the findings in human allergic rhinitis and asthma exacerbation (57, 58, 87, 89), such observations support the hypothesis that bone marrow progenitors respond to changes (allergen or steroids) in the airways during allergen-induced inflammatory responses. This would be in keeping with the knowledge we now have of cytokine production by inflamed airways tissues.

In vitro models: production of hemopoietic cytokines by allergic tissue structural cells. In the model of allergic inflammation we have proposed, cytokines released by inflamed airways tissue structural cells can determine the pattern of accumulation of inflammatory effector cells. The systemic release of tissue-derived cytokines may provide communication between the allergic response and the bone marrow–peripheral blood axis. We have evidence that IL-3, IL-6, IL-8, G-CSF, M-CSF, GM-CSF, TNF- α , TGF- β , and, recently, SCF are all expressed or produced in increased amounts by the epithelial cells and fibroblasts of chronically inflamed airways in patients with nasal polyposis, allergic rhinitis, asthma, and pulmonary fibrosis (76, 92–97). Since some of these are cytokines involved in Eo-Baso or mast cell differentiation, our hypothesis of “endocrine” effects by tissue cytokines on the bone marrow, potentially modulated by steroids, becomes

Table 1. Changes in Eo-Baso progenitors in atopy and asthma

■	Increased number in atopic blood and bone marrow
■	Fluctuations during seasonal allergen exposure in allergic rhinitis
■	Increases in blood 24 h after allergen challenge in atopic asthma
■	Increases in blood during asthma exacerbation
■	Increase in canine and human bone marrow within 24 h of allergen challenge in airway hyperresponders
■	Inhibition of increases in blood and bone marrow by inhaled steroids in asthma exacerbation or canine airway hyperresponse

even more attractive. It is still possible that low systemic levels of corticosteroids achieved after topical therapy are sufficient to affect bone marrow function *directly*; this effect may depend on a process of active inflammation, since we did not observe such effects in patients with allergic rhinitis given inhaled (to the lung) steroids (88). Because several of the above cytokines, such as TGF- β and TNF- α , as well as some chemokines (98, 99), may also specifically *inhibit* hemopoiesis (63, 100–103), the concept has emerged that the airways tissue microenvironment provides a milieu which can regulate (up or down) differentiation of inflammatory cell progenitors in bone marrow and blood (92).

Evidence from bone marrow transplantation

There is only one study of the contribution of human bone marrow to the allergic diathesis: in nine of 12 patients receiving bone marrow from atopic donors, there was a change in skin test responses to standard allergens and/or airway hyperresponsiveness (109). An hypothesis derived from these observations is that hemopoietic stem cells are involved in the transfer of IgE and possibly other responses (basophilia, mastocytosis, eosinophilia) in allergic reactions. We must determine whether all this is due to the transfer of a TH2 cell (104), sets of progenitors for specific lineages, or other factors which confer increased capacity to produce the inflammatory effector cells of allergy. Further study is also needed to determine whether the bone marrow or the lung (105), or both, are both necessary and sufficient for the development of atopic asthma.

Application of progenitor markers and assays in allergic airways disease: recent in vivo findings

Atopic vs. nonatopic blood and marrow: validity of fluorescence-activated cell sorting (FACS) analysis of progenitors. In a recent study of patients undergoing thoracotomy (106), we have found that CD34/45-positive cells are increased in atopic blood and bone marrow. Both FACS analysis of CD34/45-positive cells and methylcellulose culturing have been performed on peripheral blood and bone marrow samples from atopic and nonatopic subjects. A significantly greater number of CD34/45-positive cells are present in the peripheral blood of atopic subjects than in normal nonatopic subjects; bone marrow findings indicate a similar trend (106). IL-5 responsiveness appears to be the most significant correlate of the increased progenitor population in these atopic samples (106).

Our studies also indicate that FACS and magnet-activated cell separation (MACS) estimation/purification of CD34/45-positive cells represents a valid alternative for enumeration of Eo-Baso progenitors. Clearly, further work is required to describe the composite phenotype of Eo-Baso progenitors, in order to quantify more accurately their presence in various biologic samples.

Allergen-induced bone marrow progenitor responses. As we have shown in the dog (above), we have recently observed that human bone marrow Eo-Baso progenitors increase 6–24 h after inhaled allergen in atopic asthmatics; this can be substantiated by both colony assays and by FACS analysis of CD34/45-positive cells (107, 108).

Summary

The above studies have begun to address the fundamental question of the mechanisms of bone marrow involvement and response to allergen challenge in allergic asthmatics. Further studies in this area should complement our investigations in human asthma – which suggest that a particular bias toward differentiation of Eo-Baso progenitors characterizes the atopic state – as well as our findings in the dog model of allergen-induced airway hyperresponsiveness, which indicate that the bone marrow responds to inhalation of allergen or corticosteroids. Taken in the context of previous indications that IgE and bronchial responsiveness may both be transferrable through bone marrow transplantation (109), these findings indicate a physiologic role for the bone marrow in allergic inflammation. Likewise, these concepts provide a basis for making certain predictions regarding management and novel therapeutic interventions in atopy and asthma.

References

1. Civin CI, Strauss LC, Brovall C, Fackler MJ, Schwartz JF, Shaper JH. Antigenic analysis of hematopoiesis. III. A hematopoietic progenitor cell surface antigen defined by a monoclonal antibody raised against KG-1a cells. *J Immunol* 1984;133:157–65.
2. Fritsch G, Buchinger P, Printz D, et al. Rapid discrimination of early CD34+ myeloid progenitors using CD45-RA analysis. *Blood* 1993;81:2301–9.
3. Kato K, Radbruch A. Isolation and characterization of CD34+ hematopoietic stem cells from human peripheral blood by high-gradient magnetic cell sorting. *Cytometry* 1993;14:384–92.
4. Lansdorp PM, Sutherland HJ, Eaves CJ. Selective expression of CD45 isoforms on functional subpopulations of CD34+ hemopoietic cells from human bone marrow. *J Exp Med* 1990;172:363–6.

5. Huang E, Nocka K, Beier DR, et al. The hematopoietic growth factor KL is encoded by the *Sl* locus and is the ligand of the *c-kit* receptor, the gene product of the *W* locus. *Cell* 1990;63:225-33.
6. Williams DE, Eisenman J, Baird A, et al. Identification of a ligand for the *c-kit* proto-oncogene. *Cell* 1990;63:167-74.
7. Zsebo KM, Williams DA, Geissler EN, et al. Stem cell factor is encoded at the *Sl* locus of the mouse and is the ligand for the *c-kit* tyrosine kinase receptor. *Cell* 1990;63:213-24.
8. Rosnet O, Schiff C, Pebusque M, et al. Human *FLT3/FLK2* gene: cDNA cloning and expression in hematopoietic cells. *Blood* 1993;82:1110-19.
9. Eaves CJ. Peripheral blood stem cells reach new heights. *Blood* 1993;82:1957-9.
10. Lapidot T, Sirard C, Vormoor J, et al. A cell initiating human acute myeloid leukemia after transplantation into SCID mice. *Nature* 1994;367:645-8.
11. Fink L, Molgaard HV, Robertson D, et al. Expression of the *CD34* gene in vascular endothelial cells. *Blood* 1990;75:2417-26.
12. Nickoloff BJ. The human progenitor cell antigen (CD34) is localized on endothelial cells, dermal dendritic cells, and perifollicular cells in formalin-fixed normal skin, and on proliferating endothelial cells and stromal spindle-shaped cells in Kaposi's sarcoma. *Arch Dermatol* 1991;127:523-9.
13. Schlegelmann RD, Rietveld FJR, Waal RMW, et al. Leukocyte antigen CD34 is expressed by a subset of cultured endothelial cells and on endothelial abluminal microprocesses in the tumor stroma. *Lab Invest* 1990;62:690-6.
14. Miyajima A, Mui AL-F, Ogorochi T, Sakamaki K. Receptors for granulocyte-macrophage colony-stimulating factor, interleukin-3, and interleukin-5. *Blood* 1993;82:1960-74.
15. van Leeuwen BH, Martinson ME, Webb GC, Young IG. Molecular organization of the cytokine gene cluster, involving the human IL-3, IL-4, IL-5, and GM-CSF genes, on human chromosome 5. *Blood* 1989;73:1142-8.
16. Majdic O, Stockl J, Pickl WF, et al. Signaling and induction of enhanced cytoadhesiveness via the hematopoietic progenitor cell surface molecule CD34. *Blood* 1994;83:1226-34.
17. Berenson RJ, Andrews RG, Bensinger WI, et al. Antigen CD34-positive marrow cells engraft lethally irradiated baboons. *J Clin Invest* 1988;81:951-5.
18. Berenson RJ, Bensinger WI, Hill RS, et al. Engraftment after infusion of CD34+ marrow cells in patients with breast cancer or neuroblastoma. *Blood* 1991;77:1717-22.
19. Metcalfe D. Hematopoietic regulators: redundancy or subtlety? *Blood* 1993;82:3515-23.
20. Vadas MA, Lopez AF, Gamble JR, Elliot MJ. Role of colony-stimulating factors in leucocyte responses to inflammation and infection. *Curr Opin Immunol* 1991;3:97-104.
21. Denburg JA. Cytokine-induced human basophil/mast cell growth and differentiation *in vitro*. *Springer Semin Immunopathol* 1990;12:401-14.
22. Denburg JA. Basophil and mast cell lineages *in vitro* and *in vivo*. *Blood* 1992;79:846-60.
23. Denburg JA, Dolovich J, Harnish D. Basophil mast cell and eosinophil growth and differentiation factors in human allergic disease. *Clin Exp Allergy* 1989;19:249-54.
24. Denburg JA, Richardson M, Telizyn S, Bienenstock J. Basophil/mast cell precursors in human peripheral blood. *Blood* 1983;61:775-80.
25. Denburg JA, Telizyn S, Belda A, Dolovich J, Bienenstock J. Increased numbers of circulating basophil progenitors in atopic patients. *J Allergy Clin Immunol* 1985;76:466-72.
26. Denburg JA, Telizyn S, Messner H, et al. Heterogeneity of human peripheral blood eosinophil-type colonies: evidence for a common basophil-eosinophil progenitor. *Blood* 1985;66:312-18.
27. Ogawa M. Differentiation and proliferation of hematopoietic stem cells. *Blood* 1993;81:2844-53.
28. Clutterbuck EJ, Sanderson CJ. Human eosinophil hematopoiesis studied *in vitro* by means of murine eosinophil differentiation factor (IL-5): production of functionally active eosinophils from normal human bone marrow. *Blood* 1988;71:646-51.
29. Denburg JA, Silver JE, Abrams JS. Interleukin-5 is a human basophilopoietin: induction of histamine content and basophilic differentiation of HL-60 cells and of peripheral blood basophil-eosinophil progenitors. *Blood* 1991;77:1462-8.
30. Sanderson CJ, Warren DJ, Strath M. Identification of a lymphokine that stimulates eosinophil differentiation *in vitro*. Its relationship to interleukin 3, and functional properties of eosinophils produced in culture. *J Exp Med* 1985;162:60-74.
31. Valent P, Ashman LK, Hinterberger W, et al. Mast cell typing: demonstration of a distinct hematopoietic cell type and evidence for immunophenotypic relationship to mononuclear phagocytes. *Blood* 1989;73:1778-85.
32. Agis H, Willheim M, Sperr WR, et al. Identification of the circulating mast cell progenitor as a *c-kit*⁺, CD34⁺, CD14⁻, CD17⁻, Ly⁻, colony forming cell [Abstract]. *Blood* 1993;82 Suppl:102a.
33. Galli SJ. Biology of disease. New insights into "the riddle of the mast cells": microenvironmental regulation of mast cell development and phenotypic heterogeneity. *Lab Invest* 1990;62:5-33.
34. Irani AA, Craig SS, Nilsson G, Ishizaka T, Schwartz LB. Characterizations of human mast cells developed *in vitro* from fetal liver cells cocultured with murine 3T3 fibroblasts. *Immunology* 1992;77:136-43.
35. Irani AM, Nilsson G, Miettinen U, et al. Recombinant human stem cell factor stimulates differentiation of mast cells from dispersed human fetal liver cells. *Blood* 1992;80:3009-21.
36. Jarboe DL, Huff TF. The mast cell-committed progenitor. II. *W/W^v* mice do not make mast cell-committed progenitors and S1/S1d fibroblasts do not support development of normal mast cell-committed progenitors. *J Immunol* 1989;142:2418-23.
37. Jarboe DL, Marshall JS, Randolph TR, Kukolja A, Huff TF. The mast cell-committed progenitor. I. Description of a cell capable of IL-3-independent proliferation and differentiation without contact with fibroblasts. *J Immunol* 1989;142:2405-17.
38. Furitsu T, Saito H, Dvorak AM, et al. Development of human mast cells *in vitro*. *Proc Natl Acad Sci USA* 1989;86:10039-43.
39. Ginsburg H, Lagunoff D. The *in vitro* differentiation of mast cells. Cultures of cells from immunized mouse lymph nodes and thoracic duct lymph on fibroblast monolayers. *J Cell Biol* 1967;35:685-97.
40. Kirshenbaum AS, Goff JP, Dreskin SC, Irani A-M, Schwartz LB, Metcalfe DD. IL-3-dependent growth of basophil-like and mast-like cells from human bone marrow. *J Immunol* 1989;142:2424-9.
41. Kirshenbaum AS, Kessler SW, Goff JP, Metcalfe DD. Demonstration of the origin of human mast cells from CD34⁺ bone marrow progenitor cells. *J Immunol* 1991;146:1410-15.
42. Saito H, Hatake K, Dvorak AM, et al. Selective differentiation and proliferation of hematopoietic cells induced by

- recombinant human interleukins. *Proc Natl Acad Sci U S A* 1988;85:2288-92.
43. Wang C, De Coteau J, Pinkerton PH, Reis MD. *C-kit* expression and response to *kit*-ligand by leukemic basophils from chronic myelogenous leukemia in basophilic transformation [Abstract]. *Blood* 1993;82 Suppl:230a.
44. Wong DA, Switzer J, Ohtoshi T, Delespesse G, Dolovich J, Denburg J. CD23 expression on HL-60 cells: evidence for both basophilic and monocytic differentiation [Abstract]. *J Allergy Clin Immunol* 1991;87:305.
45. Juhlin L, Michaelsson G. A new syndrome characterized by absence of eosinophils and basophils. *Lancet* 1977;1:1233-5.
46. Parkin JL, McKenna RW, Brunning RD. Philadelphia chromosome-positive blastic leukemia: ultrastructural and ultracytochemical evidence of basophil and mast cell differentiation. *Br J Haematol* 1982;52:663-77.
47. Soler J, O'Brien M, de Castro JT, et al. Blast crisis of chronic granulocytic leukemia with mast cell and basophil precursors. *Am J Clin Pathol* 1985;83:254-9.
48. Zucker-Franklin D. Ultrastructural evidence for the common origin of human mast cells and basophils. *Blood* 1980;56:534-40.
49. Ebi Y, Kasugai T, Seino Y, Onoue H, Kanemoto T, Kitamura Y. Mechanism of mast cell deficiency in mutant mice of *mi/mi* genotype: an analysis by co-culture of mast cells and fibroblasts. *Blood* 1990;75:1247-51.
50. Kasugai T, Okada M, Morimoto M, et al. Infection of *Nippostrongylus brasiliensis* induces normal increase of basophils in mast cell-deficient *W^s/W^s* rats with a small deletion at the kinase domain of *c-kit*. *Blood* 1993;81:2521-9.
51. Tei H, Kasugai T, Tsujimura T, et al. Characterization of cultured mast cells derived from *W^s/W^s* mast cell-deficient rats with a small deletion at tyrosine kinase domain of *c-kit*. *Blood* 1994;83:916-25.
52. Tsujimura T, Koshimizu U, Katoh H, et al. Mast cell number in the skin of heterozygotes reflects the molecular nature of *c-kit* mutation. *Blood* 1993;81:2530-8.
53. Kasugai T, Tei H, Okada M, et al. Infection with *Nippostrongylus brasiliensis* induces invasion of mast cell precursors from peripheral blood to small intestine. *Blood* 1995;85:1334-40.
54. Sorden SD, Castleman WL. Virus-induced increases in bronchiolar mast cells in brown Norway rats are associated with both local mast cell proliferation and increases in blood mast cell precursors. *Lab Invest* 1995; 73:197-204.
55. Denburg JA, Befus AD, Bienenstock J. Growth and differentiation *in vitro* of mast cells from mesenteric lymph nodes of *Nippostrongylus brasiliensis*-infected rats. *Immunology* 1980;41:195-202.
56. Guy-Grand D, Dy M, Luffau G, Vassalli P. Gut mucosal mast cells: origin, traffic and differentiation. *J Exp Med* 1984;160:12-28.
57. Otsuka H, Dolovich J, Befus AD, Bienenstock J, Denburg JA. Peripheral blood basophils, basophil progenitors, and nasal metachromatic cells in allergic rhinitis. *Am Rev Respir Dis* 1986;133:757-62.
58. Otsuka H, Dolovich J, Befus AD, Telizyn S, Bienenstock J, Denburg JA. Basophilic cell progenitors, nasal metachromatic cells, and peripheral blood basophils in ragweed-allergic patients. *J Allergy Clin Immunol* 1986;78:365-71.
59. Owen WF Jr, Rothenberg ME, Silberstein DS, et al. Regulation of human eosinophil viability, density and function by granulocyte/macrophage colony-stimulating factor in the presence of 3T3 fibroblasts. *J Exp Med* 1987;166:129-41.
60. Gasson JC. Molecular physiology of granulocyte-macrophage colony-stimulating factor. *Blood* 1991;77: 1131-45.
61. Bradding P, Feather IH, Howarth PH, et al. Interleukin 4 is localized to and released by human mast cells. *J Exp Med* 1992;176:1381-6.
62. Desreumaux P, Janin A, Colombel JF, et al. Interleukin 5 messenger RNA expression by eosinophils in the intestinal mucosa of patients with coeliac disease. *J Exp Med* 1992;175:293-6.
63. Finotto S, Ohno I, Marshall JS, et al. TNF-alpha production by eosinophils in upper airways inflammation (nasal polyposis). *J Immunol* 1994;153:2278-89.
64. Kita H, Ohnishi T, Okubo Y, Weiler D, Abrams JS, Gleich GJ. Granulocyte/macrophage colony-stimulating factor and interleukin 3 release from human peripheral blood eosinophils and neutrophils. *J Exp Med* 1991;174: 745-8.
65. Moqbel R, Hamid Q, Ying S, et al. Expression of mRNA and immunoreactivity for the granulocyte/macrophage colony-stimulating factor in activated human eosinophils. *J Exp Med* 1991;174:749-52.
66. Nonaka M, Nonaka R, Woolley K, et al. Distinct immunohistochemical localization of IL-4 in human inflamed airway tissues. IL-4 is localized to eosinophils *in vivo* and is released by peripheral blood eosinophils. *J Immunol* 1995; 155:3234-44.
67. Ohno I, Lea R, Finotto S, et al. Granulocyte/macrophage colony-stimulating factor (GM-CSF) gene expression by eosinophils in nasal polyposis. *Am J Respir Cell Mol Biol* 1991;5:505-10.
68. Ohno I, Lea RG, Flanders KC, et al. Eosinophils in chronically inflamed human upper airway tissues express transforming growth factor β 1 gene (TGF β 1). *J Clin Invest* 1992;89:1662-8.
69. Wong DTW, Weller PF, Galli SJ, et al. Human eosinophils express transforming growth factor α . *J Exp Med* 1990;172:673-81.
70. Kung TT, Stelts D, Zurcher JA, et al. Mechanisms of allergic pulmonary eosinophilia in the mouse. *J Allergy Clin Immunol* 1994;94:1217-24.
71. Iemura A, Tsai M, Ando A, Wershil BK, Galli SJ. The *c-kit* ligand, stem cell factor, promotes mast cell survival by suppressing apoptosis. *Am J Pathol* 1994;144:321-8.
72. Wershil BK, Wang ZS, Gordon JR, Galli SJ. Recruitment of neutrophils during IgE-dependent cutaneous late phase reactions in the mouse is mast cell-dependent. Partial inhibition of the reaction with antiserum against tumor necrosis factor-alpha. *J Clin Invest* 1991;87:446-53.
73. Costa JJ, Demetri GD, Harrist TJ, et al. Recombinant human stem cell factor (rhSCF) induces cutaneous mast cell activation and hyperplasia, and hyperpigmentation in humans *in vivo* [Abstract]. *J Allergy Clin Immunol* 1994;93:225.
74. Matsuda H, Coughlin MD, Bienenstock J, Denburg JA. Nerve growth factor promotes human hemopoietic colony growth and differentiation. *Proc Natl Acad Sci U S A* 1988;85:6508-12.
75. Tsuda T, Wong D, Dolovich J, Bienenstock J, Marshall J, Denburg JA. Synergistic effects of nerve growth factor and granulocyte-macrophage colony-stimulating factor on human basophilic cell differentiation. *Blood* 1991;77:971-9.
76. Denburg JA, Dolovich J, Marshall J, et al. Eosinophil differentiation and cytokine networks in allergic inflammation. In: Gleich GJ, Kay AB, editors. *Eosinophils in allergy and inflammation*. New York: Marcel Dekker, 1994:211-23.
77. Denburg JA, Woolley M, Leber B, Linden M, O'Byrne P.

- Basophil and eosinophil differentiation in allergic reactions. *J Allergy Clin Immunol* 1994;94:1135-41.
78. Leber B, Switzer J, Denburg JA. Retinoic acid modulation of induced basophil differentiation. 1996 (submitted).
79. Tsai S, Collins SJ. A dominant negative retinoic acid receptor blocks neutrophil differentiation at the promyelocyte stage. *Proc Natl Acad Sci U S A* 1993;90:7153-7.
80. Popken-Harris P, Thomas L, Oxvig C, et al. Biochemical properties, activities, and presence in biologic fluids of eosinophil granule major basic protein. *J Allergy Clin Immunol* 1994;94:1282-9.
81. Wong DA, Valent P, Bettelheim P, Switzer J, Denburg JA. Immunophenotypic analysis of HL-60 cells during basophilic differentiation. *Int Arch Allergy Immunol* 1996 (in press).
82. Boyce JA, Friend D, Matsumoto R, Austen KF, Owen WF. Differentiation *in vitro* of hybrid eosinophil/basophil granulocytes: autocrine function of an eosinophil developmental intermediate. *J Exp Med* 1995;182:49-57.
83. Tanno Y, Bienenstock J, Richardson M, Lee TDG, Befus AD, Denburg JA. Reciprocal regulation of human basophil and eosinophil differentiation by separate T-cell-derived factors. *Exp Hematol* 1987;15:24-33.
84. Denburg JA, Howie K, Girgis-Gabardo A. Expression of granulocyte macrophage-colony stimulating factor (GM-CSF) during maturation of eosinophils and basophils [Abstract]. *J Allergy Clin Immunol* 1994;93:194.
85. Björkstén B, Gamrelidze A, Vanto T, Kjellman M. Seasonal variation of IgE synthesis *in vitro* by human peripheral blood mononuclear cells. *Allergy* 1990;45:572-6.
86. Choudry NB, Watson R, Denburg J, O'Byrne PM. Time course of circulating inflammatory cells and their progenitors after allergen inhalation in asthmatic subjects [Abstract]. *Am Rev Respir Dis* 1992;145:A35.
87. Gibson PG, Dolovich J, Girgis-Gabardo A, et al. The inflammatory response in asthma exacerbation: changes in circulating eosinophils, basophils and their progenitors. *Clin Exp Allergy* 1990;20:661-8.
88. Gibson PG, Manning PJ, O'Byrne PM, et al. Allergen-induced asthmatic responses: relationship between increases in airway responsiveness and increases in circulating eosinophils, basophils, and their progenitors. *Am Rev Respir Dis* 1991;143:331-5.
89. Linden M, Svensson C, Andersson M, et al. Increased numbers of circulating leucocyte progenitors in patients with allergic rhinitis during natural allergen exposure [Abstract]. *Am J Respir Crit Care Med* 1994;149:A602.
90. Woolley MJ, Denburg JA, Ellis R, Dahlback M, O'Byrne PM. Allergen-induced changes in bone marrow progenitors and airway responsiveness in dogs and the effect of inhaled budesonide on these parameters. *Am J Respir Cell Mol Biol* 1994;11:600-6.
91. Inman MD, Ellis R, Wattie J, et al. Stimulation of canine bone marrow granulocyte progenitor growth by a serum factor present 24 hours after allergen inhalation [Abstract]. *J Allergy Clin Immunol* 1995;95:376.
92. Denburg JA, Dolovich J, Ohtoshi T, Cox G, Gaudie J, Jordana M. The microenvironmental differentiation hypothesis of airway inflammation. *Am J Rhinol* 1990;4:29-32.
93. Jordana M, Vancheri C, Ohtoshi T, et al. Hemopoietic function of the microenvironment in chronic airway inflammation. *Agents Actions Suppl* 1989;28:85-95.
94. Kim YK, Nakagawa N, Sulakvelidze I, Dolovich J, Denburg JA. Immunolocalization of stem cell factor in inflamed human nasal tissues [Abstract]. *J Allergy Clin Immunol* 1996;97:282.
95. Nakagawa N, Howie K, Switzer J, Marshall JS, Denburg JA. Human nasal polyp fibroblasts produce stem cell factor (SCF) [Abstract]. *J Allergy Clin Immunol* 1995;95:292.
96. Ohnishi M, Ruhno J, Bienenstock J, Dolovich J, Denburg JA. Hematopoietic growth factor production by cultured cells of human nasal polyp epithelial scrapings: kinetics, cell source, and relationship to clinical status. *J Allergy Clin Immunol* 1989;83:1091-1100.
97. Ohtoshi T, Tsuda T, Vancheri C, et al. Human upper airway epithelial cell-derived granulocyte-macrophage colony-stimulating factor induces histamine-containing cell differentiation of human progenitor cells. *Int Arch Allergy Appl Immunol* 1991;95:376-84.
98. Gautam SC, Pindolia KR, Noth CJ, Janakiraman N, Xu YX, Chapman RA. Chemokine gene expression in bone marrow stromal cells: downregulation with sodium salicylate. *Blood* 1995;86:2541-50.
99. Gewirtz AM, Zhang J, Ratajczak J, et al. Chemokine regulation of human megakaryocytopoiesis. *Blood* 1995;86:2559-67.
100. Costa JJ, Matossian K, Resnick MB, et al. Human eosinophils can express the cytokines tumor necrosis factor-alpha and macrophage inflammatory protein-1 alpha. *J Clin Invest* 1993;91:2673-84.
101. Roodman GD. TNF and hematopoiesis. In: Beutler B, editor. *Tumor necrosis factors: the molecules and their emerging role in medicine*. New York: Raven Press, 1992:117-29.
102. Sillaber C, Geissler K, Scherrer R, et al. Type beta transforming growth factors promote interleukin-3 (IL-3)-dependent differentiation of human basophils but inhibit IL-3-dependent differentiation of human eosinophils. *Blood* 1992;80:634-41.
103. Wahl SM. Transforming growth factor beta (TGF-beta) in inflammation: a cause and a cure. *J Clin Immunol* 1992;12:61-74.
104. Corrigan CJ, Kay AB. T cells and eosinophils in the pathogenesis of asthma. *Immunol Today* 1992;13:501-7.
105. Corris PA, Dark JH. Aetiology of asthma: lessons from lung transplantation. *Lancet* 1993;341:1369-71.
106. Sehmi R, Howie K, Schragge W, O'Byrne PM, Denburg JA. Increased levels of CD34⁺ hemopoietic progenitor cells in atopic subjects. *Am J Respir Cell Mol Biol* 1996 (in press).
107. Sehmi R, Wood L, Watson R, Inman MD, O'Byrne PM, Denburg JA. Increases in circulating CD34⁺ hemopoietic progenitors in allergen-induced late asthmatic responses [Abstract]. *J Allergy Clin Immunol* 1996;97:277.
108. Sehmi R, Wood LJ, Inman MD, et al. Increases in bone marrow derived CD34⁺ hemopoietic progenitor cells expressing the alpha-subunit of IL-3 receptors following allergen challenge in mild asthmatics [Abstract]. *Am Rev Respir Dis* 1996 (in press).
109. Agosti JM, Sprenger JD, Lum LG, et al. Transfer of allergen-specific IgE-mediated hypersensitivity with allogeneic bone marrow transplantation. *N Engl J Med* 1988;319:1623-8.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.