Basophil and Mast Cell Lineages In Vitro and In Vivo

By Judah A. Denburg

Ever since Paul Ehrlich’s discovery that granule-laden cells ("mastzellen") in the frog mesentery, originally observed by von Recklinghausen, possessed metachromatic staining properties,12 cells in the basophil and mast cell lineages have been the subject of much scrutiny. The description by dermatologists at the turn of the century of the condition known as urticaria pigmentosa, involving mast cell infiltration in the skin and other organs,4 and subsequent findings that mast cells contain various preformed mediators of inflammation such as histamine,5 led gradually to a widespread fascination with the role of these cells in biologic processes. In parallel with these observations were descriptions by Ehrlich6 and later by hematologists of the presence of a blood-borne metachromatic, histamine-producing granulocyte, the basophil, significantly prominent in a number of myeloproliferative diseases, especially in chronic myeloid leukemia.8 Historically, because of their common metachromatic staining properties, the basophil and the mast cell have been thought to be intimately related in terms of hematopoietic lineage, and the literature is indeed replete with this assumption. The mast cell in the tissue and the basophil in the blood have, however, not been proven to be derived from a common stem cell in either animals or humans; moreover, there appears to be an inverse relationship between the presence of basophils or mast cells as the prominent representative of a metachromatic cell among various species.7 This review will focus on the ontogeny of basophils and mast cells, exploring knowledge in this field as it relates to cytokine biology, allergy and inflammation, and clinical hematology. The extensive literature on the biology of the mast cell in various processes, such as wound healing, repair, and fibrosis or on basophil and mast cell secretory function, will be touched upon only briefly within the scope of this review.

Murine Mast Cell Growth and Differentiation

The landmark observations of Ginsburg, providing evidence for the growth and differentiation in vitro of murine mast cells from lymphoid progenitors, paved the way for an understanding of conditions and growth factors for the differentiation of mast cells in vitro.8,9 Ginsburg observed that thymus, thoracic duct, or lymph node cells could be cultured, in the presence of serum, fibroblasts, and (at that time) unidentified growth factors, or after in vitro "immunization," to give rise to relatively large populations of almost pure mast cells.8,9 Following upon these observations, Ishizaka et al observed the growth and differentiation of rat mast cells from cultures of thymus in vitro,10 providing an explanation for the presence of mast cells within lymphoid organs and especially the thymus gland; indeed, Sir MacFarlane Burnet had already discovered and speculated upon the origin of mast cells in the thymus of autoimmune mice, relating them to the T-cell lineage.11

Ironically, the notion of a similarity, if not lineage relationship, between T cells and mast cells or T cells and basophils11 has come back to bear upon this field in a rather dramatic fashion with the discovery of mast cell or basophil cytokines, as well as differentiation markers for mast cells and basophils (see below).

Several different investigators, over the course of the last two decades, have provided in vitro culture systems for the growth and differentiation of large numbers of murine mast cells in vitro, in which the constant presence of a mitogen-stimulated conditioned medium was essential.14-18 The factor in these conditioned media responsible for mast cell growth and differentiation was discovered to be interleukin-3 (IL-3)19,20; indeed, to this date, murine mast cells grown in vitro from bone marrow, spleen, and/or lymphoid organs are routinely used to assay for cytokines with mast cell growth factor activity such as IL-3. In murine systems, the identification of IL-3 as the factor responsible for mast cell growth coalesced several different lines of hematopoietic investigation on growth factor activities that promoted the growth and differentiation of cells of such apparently disparate lineages as erythroid, megakaryocytic, and mast cell.19 Using models of graft-versus-host disease26 or nematicode infestation in vivo,22,23 it was shown that IL-3 was the active factor involved in vivo and in vitro in the promotion of the growth and differentiation of murine mast cells within mucosal tissues in these situations.24,25 For example, in the Nippostrongylus brasiliensis model of rat intestinal mastocytosis, in which Enerback first described a histochemically unique mucosa-associated mast cell,26 a systemic and local IL-3 response, which promotes mastocytosis, occurs in parallel with an IgE response, promoted by IL-4.27,28 In this system, Jarboe et al have dissected out two types of mast cell progenitors: one that is dependent, and another independent, of the IL-3 stimulus29,30; the latter responds to a fibroblast-derived factor.30 Others have observed that in vitro one can culture both large and small murine mast cell colonies,31 presumably from different progenitors; one of these is a self-replicating, murine mast cell, which can also be identified after degranulation with specific or nonspecific stimuli.32-34 These progenitors may give rise to different subtypes of the mast cell in vivo, "connective tissue-type" (serosal) or "mucosal-type" (mucosal) mast cells.34

Elegant adoptive transfer studies by Kitamura and his group, using the W/Wv mast cell-deficient mouse model have shown definitively that the origin of the murine mast
cell is in a bone marrow stem cell with self-renewal capacity (colony-forming unit-spleen [CFU-S]), which can give rise to the various subtypes (mucosal and serosal) of mast cells derived from their more immediate progenitors. Using a beige granule marker to follow mast cells or their progenitors adoptively transferred into mast cell-deficient recipients, these investigators also provided the first formal evidence of the common origin of both mucosal and serosal mast cells in the rodent; bidirectional "transdifferentiation" between these two types of mast cell, or at least differentiation of a (putative) relatively immature mucosal into a serosal mast cell can occur. In these processes, IL-4 can induce a phenotype switch of mouse mucosal to serosal mast cells, in the presence of IL-3. Fibroblast-derived factors, some requiring fibroblast-mast cell contact in vitro, have also been found to be important in rodent mast cell phenotype switching and functional activation.

The rat basophilic leukemia (RBL) cell line had been used for several decades as a focus for studies on IgE receptor biology and basophil/mast cell function. Recently, based on proteoglycan content, ultrastructure, and protease markers, this cell lineage had obviously been misnamed, because it represents a mucosal-type mast cell in vitro, and not a rodent basophil. Proteoglycan profiles can be shown to be unique for each species in IL-3-dependent murine mast cells, intestinal mucosal mast cells from helminth-infected rats, or retrovirally transformed murine mast cells. The switch from nonheparin to heparin-containing mast cells occurs in conjunction with predictable changes in gene expression for and production of combinations of (at least) six distinct murine mast cell serine proteases; the latter accompany phenotype switch from mucosal to serosal mast cells and represent another set of features associated with mast cell heterogeneity.

Such studies have culminated in the provision of more recent evidence for the existence of as yet another important hematopoietic and mast cell growth factor, stem cell factor (SCF), or c-kit ligand (KL), which is derived from fibroblasts. The discovery of this molecule provides an explanation for the reciprocal relationship between the two congenitally anemic mast cell-deficient mice, the aforementioned W/Wv and the Sl/St, in the latter, SCF, a microenvironmental determinant of mast cell differentiation, is lacking. The W/Wv mutant mouse is deficient in mast cell progenitor responses to SCF, a defect that has also been recently found in a new strain of rat, the Ws ("white spotting") mutant. Mutations at the c-kit (W) locus, a proto-oncogene that encodes a tyrosine kinase receptor for SCF, in both mice and rats appear to underlie mast cell deficiency, congenital anemia, melanocyte depletion, and sterility. Other factors, such as nerve growth factor (NGF) or extracellular matrices derived from fibroblasts, in conjunction with IL-3, may also be important in murine mast cell growth and differentiation, and could conceivably interact with c-kit in this process (see below) or with other mutations associated with osteopetrosis and mast cell deficiency in the m/m mouse. Fibroblast-derived cytokines (including SCF, NGF, and, speculatively, IL-11) may interact with IL-3, IL-4, IL-9, and IL-10 to determine changes in mast cell/basophil phenotype in vitro and in vivo (Table 1). This area of investigation has been a prime example of the use of mast cell lines to assay for novel hematopoietic cytokines, both in murine and human systems.

**MURINE BASOPHIL GROWTH AND DIFFERENTIATION**

By contrast with murine mast cells or human basophils (see below), murine basophil growth and differentiation have proved difficult to investigate. These differences reflect the relative abundance of basophils and mast cells, in humans and murine species, respectively. The case with which basophils or mast cells can be grown in vitro from various hematopoietic tissues generally reflects the prominence of the given metachromatic cell in vivo. Dvorak et al and others have provided ultrastructural criteria for the identification of murine basophils, and for interspecies differences among basophils and mast cell subtypes.

Many observations on the growth and differentiation of rodent basophils stem from a variety of observations on the prominence of both basophils and mast cells together with eosinophils in nematode-infested guinea pigs and rats. We demonstrated the bone marrow origin of guinea pig basophils, based on liquid suspension cultures of bone marrow in the presence of mitogen-stimulated or antigen-specific responses to SCF, a defect that has also been recently found in a new strain of rat, the Ws ("white spotting") mutant. Mutations at the c-kit (W) locus, a proto-oncogene that encodes a tyrosine kinase receptor for SCF, in both mice and rats appear to underlie mast cell deficiency, congenital anemia, melanocyte depletion, and sterility. Other factors, such as nerve growth factor (NGF) or extracellular matrices derived from fibroblasts, in conjunction with IL-3, may also be important in murine mast cell growth and differentiation, and could conceivably interact with c-kit in this process (see below) or with other mutations associated with osteopetrosis and mast cell deficiency in the m/m mouse. Fibroblast-derived cytokines (including SCF, NGF, and, speculatively, IL-11) may interact with IL-3, IL-4, IL-9, and IL-10 to determine changes in mast cell/basophil phenotype in vitro and in vivo (Table 1). This area of investigation has been a prime example of the use of mast cell lines to assay for novel hematopoietic cytokines, both in murine and human systems.

**BASOPHIL AND MAST CELL LINEAGES**

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Effect</th>
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<tbody>
<tr>
<td>A. Cytokines Involved in Rodent Mast Cell Growth and Differentiation</td>
<td></td>
</tr>
<tr>
<td>IL-3</td>
<td>Mast cell growth and differentiation; required for continued proliferation of mucosal mast cells.</td>
</tr>
<tr>
<td>IL-4</td>
<td>Mast cell growth and differentiation; enhances IL-3 effect and promotes mast cell phenotype switch from mucosal to serosal.</td>
</tr>
<tr>
<td>IL-9</td>
<td>Mast cell growth; enhances proliferative effects of IL-3; induces cytokine production by cultured mast cells.</td>
</tr>
<tr>
<td>IL-10</td>
<td>Mast cell growth; additive to IL-3 and/or IL-4.</td>
</tr>
<tr>
<td>NGF</td>
<td>Mast cell differentiation; synergizes with IL-3; promotes phenotype switch from mucosal to serosal.</td>
</tr>
<tr>
<td>SCF</td>
<td>Mast cell growth and differentiation; IL-3 independent, fibroblast-derived factor involved in prevention of mast cell deficiency and promoting phenotype switch from mucosal to serosal.</td>
</tr>
</tbody>
</table>

**B. Cytokines Involved in Human and Primate Basophil, Eosinophil, or Mast Cell Growth and Differentiation**

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM-CSF</td>
<td>Basophil and eosinophil growth and differentiation; promotes in vivo basophilia, eosinophilia and increase in circulating CFU-basoleo (primates); basophil and eosinophil activation/survival.</td>
</tr>
<tr>
<td>IL-3</td>
<td>Basophil and eosinophil growth and differentiation; basophil and eosinophil activation/survival; promotes in vivo basophilia and eosinophilia; some mast cell differentiating activity.</td>
</tr>
<tr>
<td>IL-4</td>
<td>Binds to human mast cells; down regulates c-kit gene expression.</td>
</tr>
<tr>
<td>IL-5</td>
<td>Basophil and eosinophil growth and differentiation; basophil and eosinophil activation/survival.</td>
</tr>
<tr>
<td>SCF</td>
<td>Basophil (and mast cell?) growth and differentiation; may synergize with IL-3 in cord blood cultures.</td>
</tr>
</tbody>
</table>
stimulated conditioned media from splenic T lymphocytes. However, the precise identity of the guinea pig growth factor was never ascertained; it was shown probably not to be any of the known guinea pig lymphokines, and not to possess either rat or murine IL-3-like activity. Observations on murine basophil growth and differentiation have even been harder to obtain. A basophilia accompanies certain parasitic infections in rodents, including the rat and mouse, and morphologic observations have indicated the presence of what appears to be a basophil in murine hematopoietic organs and blood. More recently, a non-B, non-T, IgE high-affinity receptor (Fɛ,R1)-bearing cell in the mouse has been shown to be a source of some cytokines, especially IL-4, heretofore ascribed to murine, bone marrow-derived mast cells.

The hematopoietic origins of murine and human basophils and mast cells, grown in vitro, are listed in Table 2.

**Table 2. Hematopoietic Origins of Basophils and Mast Cells In Vitro**

<table>
<thead>
<tr>
<th>Tissue or Cell Source</th>
<th>Basophils</th>
<th>Mast Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone marrow</td>
<td>Basophil- or mast cell-leukemic cell lines, human lung mast cells, and isolated leukemic or blood basophils have recently been used by a number of investigators to more</td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>Basophil- or mast cell-leukemic cell lines, human lung mast cells, and isolated leukemic or blood basophils have recently been used by a number of investigators to more</td>
<td></td>
</tr>
<tr>
<td>Cord blood</td>
<td>Basophil- or mast cell-leukemic cell lines, human lung mast cells, and isolated leukemic or blood basophils have recently been used by a number of investigators to more</td>
<td></td>
</tr>
<tr>
<td>Periphera blood</td>
<td>Basophil- or mast cell-leukemic cell lines, human lung mast cells, and isolated leukemic or blood basophils have recently been used by a number of investigators to more</td>
<td></td>
</tr>
</tbody>
</table>

HUMAN MAST CELL GROWTH AND DIFFERENTIATION

Despite the relative ease with which mast cell growth and differentiation and the cytokine biology associated with it in the mouse and rat have proceeded over the last few decades, transfer of this knowledge and application of these methods to human systems have been fraught with difficulty. First, unlike murine cultures, up to very recently it has been virtually impossible to grow and maintain cultures of human mast cells in vitro for prolonged periods of time. Horton and O'Brien provided evidence for the growth of mast cells from a patient with systemic mastocytosis under culture conditions that included the presence of steroids and various other supplemented media. However, this was not the norm for even mastocytosis cells, because numerous failed attempts at growing mast cell lines from these patients were shared by many investigators. In addition, neither murine IL-3 nor human IL-3, IL-4, or IL-5 could induce significant mast cell differentiation in human cord blood or fetal liver cultures, which were replete with basophils and eosinophils. Rare mast cells were observed in one study of human cord blood cultures stimulated by fractionated T-cell-conditioned media. We cultured a metachromatic cell with ultrastructural features intermediate between a basophil and a mast cell from the peripheral blood of patients with systemic mastocytosis, following up on observations by Zucker-Franklin on the presence of similar cells in rat blood and the common ultrastructural features between basophils and mast cells seen in some patients with chronic myeloid leukemia (CML). In addition, colony-forming cells (CFU-C) giving rise to metachromatic progeny lacking basophil (or eosinophil) granule-specific proteins were cultured from the peripheral blood of some, but not all, patients with mastocytosis. Some metachromatic progeny of CFU-C from allergic patient peripheral blood were found to stain histochemically like mast cells found in, for example, human nasal mucosal tissues, but these properties also characterize human blood basophils. Specific markers for the human mast cell lineage were not available at the time of such studies, and in retrospect the cells cultured in colony assays in methylcellulose from the peripheral blood of patients with either mastocytosis or CML were probably, for the most part, basophils (see below).

Very recently, Furitsu et al made an important observation in long-term human cord blood cultures, following upon evidence for the growth and differentiation in this system of basophils, eosinophils, and other lineages by several investigators. After prolonged culture of cord blood in the presence of monolayers of murine 3T3 fibroblasts or soluble factors derived from these, but not human fibroblast lines, mast cells with characteristic protease granule markers, tryptase and chymase, could be cultured and maintained. Although as of this writing the identity of the human fibroblast-derived, mast cell growth factor has not been clarified, it is apparent that in the human system either IL-3 nor IL-4 nor any other known growth factor activity is primarily responsible for cord blood, bone marrow, or fetal liver-derived human mast cell growth and differentiation in vitro. (Ishizaka T, personal communication, October, 1991). SCF is produced by murine and presumably human fibroblasts, but it has not yet proven to be the factor responsible for mast cell growth in the human cord blood/3T3 fibroblast coculture system. Along these lines, several investigators have shown the growth and differentiation of both basophils and mast cells in CD34-positively selected fetal liver or bone marrow cell populations, a process that may involve SCF. Other investigators have shown that IL-3 is a human and primate basophil differentiation and activation factor, but does not bind to human mast cells. Indeed, as Valent et al have shown, human mast cells differ phenotypically from basophils, including differences in their respective expressions of cytokine receptors, especially for IL-3 and IL-4, as well as other immunophenotypic markers. The latter are summarized in Table 3.

Basophil- or mast cell-leukemic cell lines, human lung mast cells, and isolated leukemic or blood basophils have recently been used by a number of investigators to more
precisely identify differentiation-specific or mature immunophenotypic markers on human basophils and mast cells.\(^1\) Emerging is the concept that basophils express some T-cell activation markers, such as CD25 (IL-2R), leucocyte adhesion molecules such as CD11b/18, receptors for IgG (CDw32 or Fc,RII), and some unique markers (basophil-specific protein or BSP-1)\(^1\) on the other hand, mast cells express some monocyte-macrophage markers,\(^1\) ICAM-1 (CD54 or LFA-1 ligand), and some unique markers such as YSBB8,\(^1\) the latter recently shown to be identical to c-kit\(^1\) (Table 4). However, these studies have been performed in vitro on, primarily, leukemic cells; demonstration of in vivo or physiologic relevance of these findings is still lacking. In addition, HL-60 cell immunophenotyping during differentiation of basophilic differentiation,\(^1\) a process in which NGF synergizes with either granulocyte-macrophage colony-stimulating factor (GM-CSF) or IL-5,\(^1\) has led to a somewhat different understanding of the phenotypic profile of these cells, leaving open the question of lineage relationship between human basophils and mast cells, at least in terms of a possible immature myeloid stem cell giving rise to both these phenotypes in vitro.

Irani et al, using monoclonal antibodies developed against the human mast cell proteases, tryptase and chymase,\(^1\) have proposed a separate ontogeny of mast cell subpopulations.\(^1\) This model is at odds with the proposed in the mouse model, in which predictable speciation between human basophils and mast cells, at least in terms of a possible immature myeloid stem cell giving rise to both these phenotypes in vitro.

**Table 3. Cytokine Receptors on Human Basophils and Mast Cells**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Basophils</th>
<th>Mast Cells</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-2R (CD26)</td>
<td>+</td>
<td>-</td>
<td>Binding immunophenotyping</td>
</tr>
<tr>
<td>IL-3R</td>
<td>+</td>
<td>-</td>
<td>Binding</td>
</tr>
<tr>
<td>IL-4R</td>
<td>+</td>
<td>+</td>
<td>Binding</td>
</tr>
<tr>
<td>IL-5R</td>
<td>+</td>
<td>+</td>
<td>Binding</td>
</tr>
<tr>
<td>IL-8R</td>
<td>+</td>
<td>-</td>
<td>Binding</td>
</tr>
<tr>
<td>GM-CSFR</td>
<td>-</td>
<td>-</td>
<td>Binding</td>
</tr>
</tbody>
</table>

See Valent et al,\(^1\) Stain et al,\(^1\) Denburg et al,\(^1\) and Plaetinck et al,\(^1\)

\(*\) Using leukemic HL-60 cells.

**Table 4. Immunophenotype of Mature Human Basophils and Mast Cells**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Basophils</th>
<th>Mast Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSP-1</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>YSBB8 (c-kit product)</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>CD11b/18</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>CD14</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CD25</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD54 (ICAM-1)</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>CD68</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Peripheral blood basophils or lung mast cells were used predominantly.\(^1\)

used as a source of human mast cells in vitro,\(^1\) again providing evidence for the lack of activity of human IL-3 in these experiments and the presence of factors from murine fibroblasts that appear essential for human mast cell growth in vitro.\(^1\) A newly discovered human mast cell line, HMC-1,\(^1\) has provided a focus of interest for the study of mast cells and their ontogeny in humans,\(^1\) but its derivation from a mast cell leukemic patient and its lack of high-affinity IgE receptors\(^1\) have cast some doubt on the applicability of findings based on these cells to the understanding of physiologic processes of mast cell differentiation in humans. Nonetheless, many of the observations on binding of cytokines, cell proliferation in the presence of cytokines, immunophenotyping, some functional studies, and microenvironmental influences on phenotype have shown analogies between mast cells derived from sources in vivo and the HMC-1 line.\(^1\)

**HUMAN BASOPHIL GROWTH AND DIFFERENTIATION**

A CFU-C in either methylcellulose or agar culture systems has been described by numerous investigators to give rise to pure or mixed colonies containing metachromatic, polymorphonuclear granulocytes,\(^1\) Using mediator content and release (primarily histamine), histochemistry, immunostaining, and/or ultrastructure, basophils or basophil-like cells have been shown to be the progeny of these progenitors (termed “CFU-baso” or “CFU-baso/co”) that can be grown in semi-solid or suspension cultures from human peripheral blood, bone marrow, or cord blood in the presence of various conditioned media or recombinant cytokines, from normal, atopic, or leukemic human specimens.\(^1\) Initially, in these systems, basophils were grown predominantly from patients with CML or related myeloproliferative disorders, acute myeloid leukemia (AML), and systemic mastocytosis,\(^1\) however, with observations of the presence of high numbers of histamine and metachromatic cell-containing colonies grown from normal and, especially, atopic individuals,\(^1\) it has been possible to routinely culture human basophils in vitro. A list of various cell sources for progenitors of human basophils in vitro is given in Table 2. Included in these are two cell lines derived from CML or AML patients, KU-812\(^1\) and HL-60, which can differentiate along the basophilic lineage, using appropriate inductive stimuli, cytokines and culture conditions.\(^1\)

As already noted, IL-3 is the principal recombinant cytokine in humans responsible for basophil growth and differentiation.\(^1\) However, other factors appear certainly to be important and include GM-CSF,\(^1\) IL-4,\(^1\) IL-5,\(^1\) which acts not only as an eosinophilopoietin,\(^1\) but also as a basophilopoietin, based on observations with HL-60 as well as peripheral and cord blood cells. It is not surprising that the human leukemic cell line, HL-60, can differentiate also along the basophilic lineage, because it was derived from a patient originally with an FAB-M2 type of myeloid leukemia that included the presence of atypical basophils and eosinophils
in the original bone marrow specimen. Conditions in vitro favoring basophilic differentiation of HL-60 include the presence of sodium butyrate, alkaline passage, cytokines such as GM-CSF, IL-3, IL-5, NGF, and a potentially novel basophil differentiation factor. In vivo parallels have been found: primates administered IL-3 together with GM-CSF develop prominent basophilia and eosinophilia, along with an egress from the marrow of granulocyte, and, specifically, basophil/eosinophil progenitors. Accompanying this is the development of a dermatitis that involves YB5B8-positive metachromatic cells as part of its histologic features. These observations provide support for the in vivo role of both IL-3 and GM-CSF in the promotion of basophilia and basophil growth and differentiation.

The use of CFU-baso or CFU-baso/ep assays to analyze various clinical conditions has proved interesting. In CML, mastocytosis and various allergic-type conditions of the airways, such as allergic rhinitis, nasal polyposis, and asthma, these CFU are increased in circulation. In these situations as well as under normal conditions, it can be shown that the basophil and eosinophil share a common committed stem cell, a fact confirmed by their parallel increase and decrease under both in vitro and in vivo conditions, as well as their sharing of numerous granular proteins, cell markers, and congenital anomalies. It should be noted that in bone marrow, cord blood, and peripheral blood cultures there is evidence that basophil and eosinophil differentiation is regulated reciprocally from this common progenitor; different cytokines, such as IL-3 and IL-5, as well as transforming growth factor-β (TGFβ) and perhaps other (novel?) factors can regulate basophil/eosinophil differentiation.

Recent observations on transient leukemia occurring in Down's syndrome (trisomy 21) as well as in megakaryoblastic leukemia, showing the presence of basophils or basophil-like cells in cultures derived from these leukemic cell populations as well as the increased numbers of mast cells in various hematologic malignancies underscore the relationship between the leukemic process and basophil or mast cell lineage commitment (see below). Whether cells in these various bone marrow disorders represent basophils, mast cells, or an atypical hybrid remains to be determined.

**MAST CELL-BASOPHIL LINEAGE RELATIONSHIPS**

As can be derived from the above, the lineage relationship between basophils and mast cells either in rodents or humans remains unclear. While numerous in vivo situations can be pointed out in which basophils and mast cells, together with eosinophils, are stimulated to differentiate concurrently by antigen or other provocations, there is no proof, as yet, of a common mast cell-basophil progenitor. Both basophils and mast cells do arise in cultures of human cord blood, fetal liver or peripheral blood progenitors, ultrastructural evidence for hybrid basophils-mast cells in CML hints at their common origin, although a normal physiologic counterpart to these cells has not yet been described. The mast cell subpopulation in mucosal tissues, containing tryptase (MC₆₇-type), but not chymase, shares some histochemical features with basophils. The observation of mast cell heterogeneity in rodents and humans, wherein mast cells populating mucosal tissues (e.g., intestine, lung, and nose) differ in phenotype and function from mast cells in connective tissues such as skin, peritoneal cavity, and adventitia may also be related to the basophil-mast cell lineage question. For example, in studies of mast cells and basophils in the nasal mucosa of patients with allergic rhinitis, the phenotype of the mast cell in the stromal layers resembles that of skin (serosal) mast cells in that it contains both tryptase and chymase (MC₆₇-type), whereas the nasal epithelial mast cell resembles other mucosa-associated mast cells (MC₆₇-type) as well as (apart from tryptase) metachromatic cells (basophils) found in nasal secretions primarily.

Further circumstantial evidence for transitional forms among human or rodent mast cell subtypes and basophils includes the presence of both MC₆₇ and MC₆₇-types in 3T3-fibroblast-supported human cord blood cultures; the finding of large, tryptase-negative metachromatic cells (possibly basophils) in some cord blood cultures stimulated with SCF and IL-3 (Ishizaka T, personal communication, December 1990); an intermediate phenotype of murine neonatal peritoneal mast cells as well as the development of both serosal- and mucosal-type rat mast cells after in vivo treatment with SCF; and, in humans, the consistent inverse relationship between circulating basophil progenitors (CFU-baso) and nasal mucosal mast cells. Alternatively, definitive proof that human MC₆₇ can switch to MC₆₇ type in the presence of fibroblasts or factors derived from them, as has been established in the mouse W/W₅ and Sl/Sl² models as well as in vitro, is still lacking. Immunoelectron microscopy of human mast cells has provided evidence for characteristic ultrastructural appearances of MC₆₇ and MC₆₇-types; however, there are cells with hybrid appearances suggesting the possibility of phenotype switching (and thus lineage relationship) between the two human mast cell subtypes. The recent availability of mutant Ws and congenic control rats, a species in which both mucosal mast cell hyperplasia and peripheral blood basophilia are observed in response to nematode infection, may allow for definitive analysis of basophil-mast cell lineage relationships.

What is attractive about the hypothesis that there exist

| Table 5. Phenotypic Characterization of Basophils and Mast Cells in Human Nasal Mucosal Tissues |
|-------------------------------|-----------------|-----------------|
| **Basophils** | **Stromal** | **Epithelial** |
| Metachromasia* | Formaldehyde-sensitive* | - | - | + |
| | Formaldehyde-resistant* | - | + | - |
| | Safranin-positivity | - | + | - |
| | Tryptase | - | + | + |
| | Chymase | - | + | - |

*Using toluidine blue at acid pH.

Using alcian blue-safranin staining.
lineage relationships among mucosal and serosal mast cells and basophils in humans is that it provides a parsimonious explanation for the parallel presence of both cell types in relation to signs, symptoms, and treatment of nasal mucosal allergic reactions, their separation into compartments, and the observation that nasal polyp mononuclear cells contain a highly enriched basophil/mucosal mast cell-like progenitor.101-103,132,136 It is thus possible that the human blood basophil is more closely related in lineage to the mucosal type mast cell than to its serosal counterpart, and that microenvironmental differentiative stimuli are important in determining the final phenotype. Such observations, if they can be confirmed and extended, would be in accord with those by several groups showing evidence for transdifferentiation of mast cell subtypes in the mouse, and protease/proteoglycan expression dependent on the microenvironment with which the cells or their progenitors are in contact.150-154,155-157 Although marker studies showing distinct profiles of human mast cells and basophils promote the concept of different lineages for these two cell types,161,162 a precise phenotypic, functional, and hematopoietic characterization of human mucosal mast cell progenitors, compared with basophil progenitors, has not yet been undertaken.

CYTOKINES WITH BASOPHIL OR MAST CELL GROWTH- AND DIFFERENTIATION-PROMOTING ACTIVITIES

Table 1A and B lists the cytokines involved in basophil or mast cell growth and differentiation in rodent or human systems.72,85,133 As noted, IL-3 is the prototypical mast cell growth factor in mouse and rat,14,39 and is a basophil differentiation factor in humans.101,111,147 IL-4, also possessing mast cell growth activity in murine systems, with the additional feature of promotion of rodent serosal mast cell differentiation,92 appears to have minor activity on human basophil growth and differentiation92,95,96 but not on human mast cell growth and differentiation. IL-4 receptors are present, however, on both basophils and mast cells in humans and induce ICAM-1 expression on mast cells.166,167 GM-CSF, on the other hand, present either in T-cell-conditioned media (eg, from the hairy cell leukemic line Mo164 or in recombinant form), is a prominent basophilopoietin and basophil activation factor both in vitro and in vivo in murine, primate, and human systems.85,90,94,97,100-102,112,113,120-122,127,128 The precise identity, however, of the fibroblast-derived hematopoietic malignancies. The prototypical hematopoietic malignancies providing experiments in nature in which basophil and mast cell lineages appear to be dysregulated are, respectively, CML and related myeloproliferative disorders on the one hand, and systemic mastocytosis on the other. The presence of basophilia or basophil crisis heralding terminal blast crisis in CML, has long been known.198-205 In addition, hyperhistaminemia is an important symptom in CML and myeloproliferative disorders, indicating increased numbers of turnover of basophils (and, possibly, mast cells).198,199,200,201,202,203 Several different studies or predictors have pointed out the poor prognostic implication of basophilia in CML.199,203 Related to this have been in vitro observations that indices of increased basophil growth and differentiation (ie, progenitor increases) are also poor prognostic indicators in CML and related myeloproliferative disorders.190,192,202,203 Such phenomena may relate, in turn, to fundamental aberrations of chromosomes that are associated clinicopathologically with atypical forms of leukemia in which there is prominent basophilia or eosinophilia.190,191,192,193,194 This has been described in an inversion of chromosome 16 (inv 16) associated with atypical eosinophils with basophilic granulation190,191; in a t(6;9) chromosomal translocation associated with basophilia and leukemia190, in trisomy 21175,176; and in various abnormalities of
other chromosomes associated with increased numbers of basophils in the marrow of patients with acute leukemia [e.g., t(15;17) with t(9;14)]. Basophils have been shown to be derived from the leukemic clones by analysis of chromosomal breakpoints (such as Ph) in relatively pure or separated populations present in the peripheral blood. To this date, the precise relationship between the chromosomal aberrations described and atypical or abnormal differentiation of basophils or their prominence in vivo has not been delineated. Moreover, the significance of increased numbers of mast cells in a variety of lymphoid malignancies and refractory anemias has also not yet been elucidated.

In systemic mastocytosis and the related cutaneous disorder, urticaria pigmentosa, proliferations of mast cells within tissues are common, while systemic elevations of mast cells appear only rarely as mast cell leukemia. Increased mast cell turnover, with or without overt mastocytosis, can be accompanied by increases in released mast cell mediators, leading to symptoms such as urticaria, anaphylaxis, asthma, and diarrhea. In addition, there is evidence that increased numbers of mast cell or basophil progenitors are present in circulation in these conditions, and there is inferential evidence for increased turnover of mast cell progenitors, based on studies in psoralen and ultraviolet A (PUVA) treatment of mouse skin mast cells. It is not yet fully clear in humans whether such progenitors represent basophil or mast cell lineages, or both; some cultures of peripheral blood in mastocytosis patients have shown the presence of metachromatic cells with ultrastructural features of mast cells or the absence of basophil/eosinophil granule proteins in colony cells, as is found in vivo. An increase in metachromatic cell CFU-C in the peripheral blood can be an indication of a switch from urticaria pigmentosa to the more systemic form of mastocytosis.

The cytokines involved in the induction of either the basophilia of myeloproliferative disorders or the mast cell hyperplasia of mastocytosis have not been fully defined in vivo. It is far from clear that abnormalities in the regulation of relevant hematopoietic cytokines represent a primary cause in these disorders, nor whether dysregulated cytokine genes related to leukemogenic breakpoints, such as is found for IL-3, IL-4, IL-5, and GM-CSF in 5q- leukemias, are responsible for basophilia or mastocytosis in hematopoietic malignancies. Recent observations of the expression of the GATA-1 transcription factor in mast cells, erythroblasts, and megakaryocytes may help explain the prominence of basophils (and, possibly, mast cells) in certain megakaryoblastic leukemias or in trisomy 21. Molecular probing for cytokine gene expression in these conditions, immunostaining for cytokines in tissues and in cells, and study of transgenic immunodeficient mice may help in understanding the pathogenesis of these conditions and their corresponding aberrations in hematopoietic cytokines or progenitors for basophils and mast cells.

**Allergy and inflammation.** More clearly defined over the last few years has been the prominence of CFU-baso or CFU-baso/eo in a variety of allergic inflammatory conditions such as rhinitis, polypsis, asthma, and atopic dermatitis. While mast cells are also prominent in inflammatory bowel disease and rheumatoid synovium, changes in progenitors or cytokines have not been examined carefully in these conditions. The elevated numbers of CFU-baso and CFU-baso/eo in allergic conditions, their fluctuation with signs and symptoms of disease, and increase in relation to the presence of cells such as mucosal mast cells in tissues or under conditions of natural or provoked allergen exposure all point out the clinical relevance and importance of increased availability of these CFU-C in allergic inflammation. Measurement of CFU-baso/eo represents a very sensitive, early marker of exacerbations of asthma. These findings appear to be related to those in the primate in which IL-3 and GM-CSF provoke CFU-baso/eo increases in the blood; one can speculate that allergic inflammation is attended by increases in these CFU-C because of in vivo allergen stimulation; production of such hematopoietins (basophilopoietins) as IL-3, IL-5, and GM-CSF from T cells, basophils, or mast cells.

Topical corticosteroids given to asthmatics will prevent or suppress many inflammatory cell functions as well as the increase in CFU-baso/eo, suggesting that local production by the tissue microenvironment in the airways of cytokines that promote systemic increases in progenitors may be the mechanism responsible for this phenomenon. Indeed, recent observations on tissue structural cell (epithelial cell, fibroblast, and endothelial cell) production of GM-CSF and other proinflammatory cytokines, as well as T-cell production and in situ expression of the IL-3, IL-4, IL-5, and GM-CSF gene cluster in atopic dermatitis and asthma, provide evidence for the elaboration within and by inflamed allergic tissues, after induction with proinflammatory cytokines such as IL-1, of hematopoietic cytokines capable of inducing the growth and differentiation of basophils, eosinophils, and, possibly, mast cells that are so prominent in these reactions (Table 6). What is still not clear is whether human basophils and/or mast cells can feed back positively on this process by elaborating their own cytokines, such as in the mouse in vitro and in vivo systems described.

### Table 6. Cytokines and Basophil/Eosinophil Progenitors in Allergic-Type Diseases: Facts and Predictions

<table>
<thead>
<tr>
<th>Clinical Paradigm</th>
<th>Hematopoietic Cytokine(s) Expressed</th>
<th>Basophil/Eosinophil Progenitors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Allergic rhinitis, in season</strong></td>
<td>No changes</td>
<td>↑ IL-3/IL-5</td>
</tr>
<tr>
<td></td>
<td>↑ IL-6/IL-8</td>
<td>↑ GM-CSF</td>
</tr>
<tr>
<td>Allergic rhinitis, out of season</td>
<td>No changes</td>
<td>↑ IL-3/IL-5</td>
</tr>
<tr>
<td></td>
<td>↑ GM-CSF</td>
<td>↑ TGF-β</td>
</tr>
<tr>
<td>Allergic asthma or nasal polyposis</td>
<td>↑ IL-3/IL-5</td>
<td>↑ IL-3/IL-5</td>
</tr>
<tr>
<td></td>
<td>↑ GM-CSF</td>
<td>↑ IL-6/IL-8</td>
</tr>
<tr>
<td></td>
<td>↑ IL-6/IL-8</td>
<td>↑</td>
</tr>
</tbody>
</table>

*Already established.*

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The use of basophil or mast cell markers to monitor allergic diseases and certain hematopoietic malignancies is also now possible. Immunooassay of mast cell tryptase in blood during anaphylaxis is already in use; immunophenotypic analysis of human mast cell subpopulations, as well as basophils, using protease (eg, tryptase and chymase) markers, granule proteins (eg, major basic protein and Charcot-Leyden crystal protein) and BSP-1, surface marker analysis using monoclonal antibodies to c-kit, YB5B8, as well as other more specific markers of either basophils or mast cells based on immunophenotype of cells or cell lines analyzed during differentiation to basophils or mast cells can also be now used both in tissues and on cells in circulation. In situ hybridization and immunostaining for cytokine gene expression and production by mature granulocytes such as human basophils and mast cells (as has been done in murine mast cells) would provide new vistas in understanding inflammation as it affects various organs, as well as useful knowledge of basophil and mast cell biology for the purposes of clinical investigation and management. Indeed, recent evidence has been provided that eosinophils in humans, either from blood or in nasal tissues express and produce TGFα and TGFβ as well as GM-CSF. Whether or not human mast cells and/or basophils possess such cytokine profiles in vitro or in vivo remains to be determined.

FUTURE DIRECTIONS

Both cellular and molecular approaches will be required to fully understand basophil and mast cell lineage commitment and phenotype switching. Knowledge of the biology of these specialized granulocytes will likely prove relevant to a better understanding of chronic inflammation, allergic diseases, fibrosis, neoplasia, and wound healing and repair. A pertinent example of the application of molecular techniques to the understanding of inflammatory cell dysregulation in relation to cytokine gene activation due to chromosomal translocation has been recently provided in a study of cosinophils and IL-3 in acute lymphocytic leukemia. Similar studies need to be performed in nonmalignant states.

USEFUL

Useful for the last three decades as targets of cytokines, mast cells and basophils, along with eosinophils, also represent potential sources of cytokines that may impact on inflammatory or neoplastic processes. Understanding the growth and differentiation processes of these cells may thus lead to a better general understanding of cytokine biology and its curious interweaving of autocrine, paracrine, and endocrine patterns of cell-to-cell interaction. Finally, in hematopoiesis, mapping of cytokine genes and the relationship of cytokine gene expression to chromosomal aberrations in various leukemic and dysmyelopoietic disorders may provide important biologic and diagnostic information into the relationships among basophils, mast cells, and disease expression.

ACKNOWLEDGMENT

I am indebted to my numerous colleagues, students, and collaborators for their ideas and openness. Special thanks to Lynne Larocque for her painstaking review of the manuscript.

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Basophil and mast cell lineages in vitro and in vivo [published erratum appears in Blood 1992 Aug 1;80(3):855]

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