Basophil/Mast Cell Precursors in Human Peripheral Blood

By J. A. Denburg, M. Richardson, S. Telizyn, and J. Bienenstock

Semisolid (methylcellulose) hemopoietic cultures revealed the presence of histamine-containing granulocyte colonies derived from precursors (CFU-C) present in human peripheral blood. Light microscopy and histochemical studies of cells in individual histamine-containing colonies demonstrated homogeneous populations of metachromatic basophil/mast cells (BMC) at various stages of maturation. By inverted microscopy, pure BMC colonies were more often found to have the overall appearance of the previously described "eosinophil" (type II), rather than "neutrophil-macrophage" (type I), colony type. Histamine-positive colonies constituted 58% (50/86) of all (type I and type II) granulocyte colonies in repeated cultures from a patient with systemic mastocytosis (SM), and 19% (13/67) of colonies in cultures from 8 patients with chronic myeloid leukemia (CML); this was in contrast to 8% (12/153) of colonies in cultures from 4 patients with urticaria pigmentosa (UP) and 6 normal controls (p < 0.0001). Calculated frequency of BMC CFU-C was approximately 1 per 2 x 10^6 in normal and 1 per 2 x 10^6 nucleated cells in SM peripheral blood. Taking colony size into account, histamine content per cell in histamine-positive type II colonies in SM cultures was 1.1 ± 0.19 pg, compared to 0.29 ± 0.08 pg in CML and ±0.10 in normals and UP. Electron microscopy (EM) of individual colonies revealed electron-dense granules with ultrastructural features of BMC in histamine-positive, but not histamine-negative, colonies. Use of these methods may help to further clarify the nature of BMC precursors and the regulation of their proliferation in bone marrow disorders and allergic states.

THE GROWTH of colonies putatively derived from single cell precursors suspended in semisolid media is widely used to determine human hemopoietic cell lineages. 1  7 Erythroid or myeloid precursors that grow and differentiate, respectively, into colonies of mature erythrocytes,1  7 neutrophils-monocytes,2 eosinophils,3 megakaryocytes,4 or mixtures thereof5 have been found to exist in peripheral blood or bone marrow. However, there have been no descriptions of growth of human hemopoietic colonies consisting of either basophils or mast cells (BMC), apart from the rare occurrence of small clusters of mast cells in cultures of human bone marrow.6  7 BMC-like cell line have been described in long-term murine bone marrow cultures in suspension,5  but their counterpart in man has not yet been identified. Furthermore, although the exact relationship between human basophils and mast cells is not fully known, there is ultrastructural evidence for their derivation from a common precursor6  and evidence in ontogeny for a substitution of one type for the other across species.10 Mast cell precursors have recently been described by Zucker-Franklin in rat peripheral blood11; we report on the existence of BMC precursors in human peripheral blood, demonstrating increased numbers of these precursors in the blood of patients with systemic mastocytosis (SM) and chronic myeloid leukemia (CML).

MATERIALS AND METHODS

Cell Cultures

Ficoll-Hypaque density gradient (specific gravity 1.077) separated anticoagulated peripheral venous blood was cultured from 18 subjects (1 with SM, 4 with urticaria pigmentosa, 8 with CML, 6 normals) in 0.9% methylcellulose cultures, as previously described,1  using Iscove's modified Dulbecco's medium, 1% v/v penicillin-streptomycin (GIBCO, Long Island, N.Y.), 20% v/v fetal calf serum (GIBCO), 5 x 10^-6M 2-mercaptopoethanol (final concentration), and 5%-20% v/v conditioned medium (CM) containing colony-stimulating activity (CSA). Sources of CSA included human placental CM prepared by methods previously described,12 supernatants from a human T-lymphocyte leukemic cell line13 (kindly supplied by Dr. D. Golde), and CM from phytohemagglutinin-stimulated normal human peripheral blood lymphocytes. Granulocyte (CFU-C) colonies of 200–1000 cells were identified after 2 wk in vitro by their morphological appearances under an inverted microscope as being either "neutrophil-macrophage" (GM) like (type I) or "eosinophil" (Eo) like (type II) as previously described14 15 16 17 (see Fig. 1), picked from the methylcellulose by an elongated Pasteur pipette, and placed onto glass slides or into 100 µl phosphate-buffered saline (PBS) or 2% glutaraldehyde (see below). The ratio of type I to type II colonies was 1:1-1:2 in all cultures, a figure in keeping with what has been previously noted in analyses of human peripheral blood CFU-C.14

Histamine Assays

Histamine assays on individual colonies picked into 100 µl PBS were performed by the enzymatic radiosotope technique previously described; this assay is sensitive to a lower limit of 100 pg. Since the volume assayed per colony was, practically, 80–90 µl, a cut-off of 120 pg was used to define a histamine-positive colony (100 pg in 80 µl = 120 pg in 100 µl). Histamine assayed in these cultures was >95% removed by 1 hr incubation at 37°C with diamine oxidase (histaminase, GIBCO), 0.5 U/ml.

Histochemical Stains

These were performed using the following procedures: May-Grunwald-Giemsa; Alcian blue after fixation in lead acetate at pH 9.5 18 19; Astra blue, purportedly specific for mast cells;20 Luxol fast

From the Host Resistance Program, Department of Medicine and Pathology, McMaster University, Hamilton, Canada.

Supported in part by a grant from the National Cancer Institute of Canada.

Submitted June 10, 1982; accepted November 8, 1982.

Address reprint requests to Dr. J. A. Denburg, Host Resistance Program, Department of Medicine and Pathology, McMaster University, Hamilton, Canada L8N 3Z5.

© 1983 by Grune & Stratton, Inc.

0006-4971/83/6104-0027$01.00/0

Blood. Vol. 61, No. 4 (April), 1983 775
Table 1. Histochemistry of Histamine-Containing Colonies

<table>
<thead>
<tr>
<th>Stain</th>
<th>Type I (pg/Colony)</th>
<th>Type II (pg/Colony)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcian blue</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Astra blue</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>Luxol fast blue</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Peroxidase</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Myelo</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Eosinophil</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

blue, purportedly specific for eosinophils,16; and myelo- or eosinophil peroxidase stains, by methods previously described.7,8,15,16 In some cases, both histamine content and morphology/histochemistry or electron microscopy were performed on an individual colony.

Electron Microscopy

Individual colonies were aspirated into 2% glutaraldehyde and 0.1M sodium cacodylate in a Beem (Ladd Research Industries, Burlington, Vt.) capsule. Fixation was continued at 4°C for 30 min-2 hr. After aspiration of the glutaraldehyde and wash in 0.2M sodium cacodylate, postfixation was performed with 1% osmium tetroxide in 0.1M sodium cacodylate for 15 min at 4°C. Samples were then stained en bloc for 15 min in saturated uranyl acetate, dehydrated, and embedded in Spurr resin, all within the Beem capsule.

RESULTS

Colony Morphology and Histochemistry: Relation to Histamine Content

Table 1 summarizes the histochemical findings according to colony type and histamine content. Histochemical staining of individual histamine-positive, type II colonies (Fig. 1A) by Alcian blue, Astra blue, or May-Grunwald-Giemsa techniques revealed the presence of prominent metachromatic granules homogeneously distributed in histamine-positive colony cells (Fig. 1B–E). A maturational sequence from red to blue staining corresponding to changing mucopolysaccharide content of granules in SM or CML type II histamine-positive colony cells by Alcian blue was demonstrated (Fig. 1C–E), resembling previously described BMC maturation.9,15,16 Cells in histamine-positive colonies were also partially eosinophil-peroxidase-positive, as demonstrated by light granular peroxidase staining (Fig. 1F). Type I, but not type II,
colonies in all cultures contained >90% myeloperoxidase-positive cells (not shown). Histamine-negative type II colonies were both Luxol-blue-positive and eosinophil-peroxidase-positive, but stained negatively with Alcian blue or Astra blue (Table 1).

Ultrastructure of Colonies: Relation to Histamine Content and Histochemistry

In order to examine ultrastructure of granules in SM colonies and relate this to histochemistry and histamine content, electron microscopy of cells from 12 individual colonies (6 type I, 6 type II) also assayed for histamine was undertaken. In all, an average of 200 cells was screened by transmission EM of fixed colonies in each of the colonies observed; EM photos were obtained of 30 separate cells, which were quite uniform in appearance according to colony type. In contrast to histamine-negative (type I) colony cells, histamine-positive (type II) colony cells contained more numerous, electron-dense granules, with features previously described as characteristic of either human basophils or mast cells (Fig. 1, F–H). Myelin forms, multivesicular bodies, and typical granular membranes and periodicity were all observed (Fig. 1, G–H). Most cells appeared polymorphonuclear (Fig. 1F), with mature nuclear chromatin and a Golgi zone and microtubules (Fig. 1G); the polymorphonuclearity suggests that these cells more likely fit the description of basophils rather than mast cells, although cells with combined basophil/mast cell features have been described.

Histamine Content of Individual Colonies

Both type I and type II histamine-positive colonies were found: 20/38 SM type I and 30/48 SM type II; 4/24 CML type I and 9/43 CML type II; and 3/45 type I and 9/108 type II colonies from UP or normal cultures contained ≥120 pg histamine per colony (Table 2 and Fig. 2). Taking colony size (approximate cell number) into account, the mean (± SE) calculated amount of histamine (pg/cell) was significantly higher in SM type I (0.29 ± 0.07; range 0–2) or type II
Histamine content of individual granulocyte colonies. GM (type I (C)) and Eo (type II (D)) histamine content is shown in nanograms per colony, according to patient group. Numbers in parentheses refer to total number of colonies in which histamine content was undetectable in a given condition. Mean histamine content (nanograms per colony) for type II colonies was significantly different from all other groups (p < 0.01); for type I SM colonies, it was different from normals or UP (p < 0.02). Histamine content of both CML type I and II colonies (nanograms per colony) was significantly different from normals or UP (p < 0.02, Student’s unpaired t test).

DISCUSSION

The presence in human peripheral blood of a precursor for histamine-positive colonies helps to delineate a simple quantitative assay for BMC precursors in semi-solid hemopoietic cultures. These findings supplement the suspension assay for BMC precursors we have described in CML peripheral blood cultures. Histamine content of individual colonies correlates well with accepted histochemical and ultrastructural features of BMC, with a calculated histamine content commensurate with that expected of mature BMC in most SM, some CML, and rarely in normal or UP colonies. Although histamine has been said to be present in small amounts in human eosinophils, such conclusions have been based on older fluorometric assays that underestimate the histamine content of basophils by tenfold when compared to current methodology. Conversely, no “pure” eosinophil colonies (by histochemical criteria) that we have picked contain detectable histamine (Tables 1 and 2). We thus have identified a relatively infrequent subset of eosinophil-like colonies that contains a histaminase-sensitive histamine not found in most type II colonies.

BMC colonies, which in SM almost invariably contain homogeneous cell populations, appear more frequently to be a subtype of type II (“eosinophil”) colonies overall; in SM or CML, higher proportions of all colonies (both type I and type II) contain BMC by morphological, histochemical, and biochemical criteria compared with normal or UP colonies (Table 2). These observations are consistent with the accepted notion of the common origin of neutrophils, eosinophils as well as basophils from a committed granulocyte progenitor; they also suggest that specific lineage commitment is probably made at a number of different stages of hematopoietic cell differentiation. Colonies with high histamine content (≥1 pg/cell) and tight

Table 2. Calculated Histamine Content of Human Peripheral Blood Granulocyte Colony Cells and Frequency of Histamine-Positive Colonies According to Colony Type

<table>
<thead>
<tr>
<th>Clinical Group</th>
<th>Type I Colony Frequency</th>
<th>Histamine (pg/cell)</th>
<th>Histamine (pg/cell) Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>TA1, 10Normal</td>
<td>3/28</td>
<td>0.10 ± 0.04</td>
<td>0.17 ± 0.05</td>
</tr>
<tr>
<td>TA1, 10Urticaria pigmentosa</td>
<td>0/17</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td>TA1, 10Systemic mastocytosis</td>
<td>20/38</td>
<td>0.29 ± 0.07</td>
<td>1.06 ± 0.19</td>
</tr>
<tr>
<td>TA1, 10Chronic myeloid leukemia</td>
<td>4/24</td>
<td>0.16 ± 0.07</td>
<td>0.29 ± 0.08</td>
</tr>
</tbody>
</table>

*Number of histamine-positive colonies/number of colonies picked.
†p < 0.05, compared to normals or UP. Student’s unpaired t test.
‡p < 0.01.
§p < 0.0001.
compact cell aggregations (type II) in SM were predictably "pure" BMC, while in other instances (normal or UP or CML cultures, as well as some SM type I colonies) were "mixed," containing BMC as well as other cell types (Denburg JA, in preparation). This is reflected in the lower mean histamine content per cell in the latter groups in comparison to SM type II colonies (Table 2). Although we did find a relatively high frequency of histamine-positivity in SM type I or in CML type I or type II colonies, their lower histamine content and mixed cellular composition argue for the existence of circulating hematopoietic progenitors, especially in CML, which are capable of differentiation along neutrophil, eosinophil, and BMC pathways; findings supportive of this concept have been reported by Aglietta et al., using agar cultures in CML, and by Nakahata et al. in a recent elegant analysis of mouse mast cell colonies. Furthermore, we did observe serial increases in SM type I colony histamine content as our patient progressed to end-stage disease. This may mean that there is progressive restriction of lineage commitment to the BMC pathway as mast cell proliferation accelerates, a finding not unlike that which we have reported in blastic CML.

The numbers of circulating BMC precursors in UP are not different from normal, suggesting that in SM, a preleukemic state exists; this is consistent with the notion that a spectrum of mast cell proliferation exists from UP to SM and mast cell leukemia. Precursor frequency, based on a plating efficiency of 20 CFU-C/10^6 Ficoll-Hypaque separated peripheral blood cells, a white blood cell count of 5 × 10^7/ml, and a distribution of colony types as described in Table 2, can be calculated to be approximately 1 per 2 × 10^7 nucleated cells in SM and 1 per 2 × 10^6 in normal peripheral blood.

The specific morphological or histochemical criteria used commonly to distinguish basophils from other granulocytes require reevaluation, since we have found uniformly metachromatic as well as Luxol fast blue or eosinophil peroxidase-positive cells in histamine-positive colonies (Fig. 1); cells in these colonies have EM appearances characteristic of BMC (Fig. 1). Although considerable controversy exists as to whether or not basophils contain peroxidases, the consensus has been that mature basophils do not. Recently, however, Ackerman et al. have demonstrated Charcot-Leyden crystals in human basophils, suggesting that there may be more commonality between eosinophils and basophils than heretofore appreciated. This is in accord with numerous observations on the concurrence of presence or absence or abnormalities of both these cell types in pathologic states. The application of both eosinophil and BMC precursor assays may help to clarify the ontogeny of these cells in the normal state as well as in various bone marrow disorders. It may also shed light on the mechanisms of mobilization of eosinophils and BMC observed in allergic states.

ACKNOWLEDGMENT

Dr. David Golde kindly provided Mo conditioned medium. Janice Robertson typed the manuscript. We wish to thank Drs. R. Barr, J. Dolovich, M. M. Fisher, D. Hillyard, H. Messner, W. Nicholson, D. Rosenthal, J. Senn, and W. E. C. Wilson for their help in access to patients.

REFERENCES

18. Johnson GR, Metcalf D: Detection of a new type of mouse...
eosinophil colony by luxol fast blue staining. Exp Hematol 8:549, 1980


Basophil/mast cell precursors in human peripheral blood

JA Denburg, M Richardson, S Telizyn and J Bienenstock