Characterization of Human Mast Cells in Long-Term Culture

By M. A. Horton and H. A. W. O'Brien

Recent studies in rodents have demonstrated that mast cells derived from lymphoid tissues can be grown in long-term culture, provided that supportive growth factors or stromal fibroblasts are added; such findings have not been reported in man. Furthermore, although a hemopoietic origin for mast cells is supported by transplantation studies in mice, the exact origin of the human mast cell or its relationship to the circulating basophil and other hemopoietic cell lineages is unknown. We have investigated the requirements for in vitro growth of human mast cells derived from the infiltrated bone marrow of a patient with systemic mastocytosis, and have characterized both the mast cells proliferating in these cultures and those obtained from splenic infiltrates. Our data approached two questions: (1) Is there any evidence for the origin of mast cells from a bone-marrow-derived stem cell, and, if so, (2) what lineage relationship is there between mast cells and granulopoietic cells, including basophils? First, we have shown the expression of hemopoietic tissue-specific antigens by mast cells, strongly supporting a bone marrow origin for the mast cell in man (at least for those mast cells analyzed here). Second, the complete lack of granulocyte-monocyte markers contrasts with the phenotype of the basophil and suggests that mast cells diverge considerably from other granulopoietic cells during the acquisition of their differentiated specialized functions.

T HAS BEEN some 100 yr since the mast cell (MC) was described by Paul Ehrlich. Since then, the MC has become recognized as a fundamental component of the immediate hypersensitivity reaction and probably plays an important role in a variety of other pathologic processes. Since its first description, the question of the origin of the MC has given rise to a multitude of diverging hypotheses; cell types considered as possible precursors, from which the MC is derived, have included both hemopoietic and nonhemopoietic cells. In rodents, a certain amount of evidence has accrued for a T-lymphocyte derivation, or at least an involvement of T lymphocytes in the development of the MC; in particular, it is thought that the atypical gut-associated MC may originate from lymphocytes recirculating through intestinal lymphoid tissue and migrating to an intraepithelial location. More recently, a bone marrow origin, at least for some MCs in mice, has been conclusively demonstrated by transplantation experiments; in these studies, it has been shown that bone marrow from beige mutant mice was able to replenish the MC pools of hemopoietic stem-cell-deficient, W/Wv recipient mice. Furthermore, studies in a variety of other species demonstrate considerable phenotypic and functional heterogeneity among MCs, not only between species, but also between tissues in the same species.

At present, there is no evidence in man as to the nature of the MC progenitor or its relationship, if any, to the different hemopoietic cell lineages, in particular to the basophil. Although there are clear morphological and functional similarities between MCs and basophils, differences do exist between the two cell types and its not certain that they actually originate from a common, committed hemopoietic progenitor. In addition, there are no data to suggest whether, if derived from a common stem cell, these two cell types may be related at an early stage of development or if mature MCs and basophils can transform from one to another.

Among the possible approaches to the above problems is the use of culture systems, which have been successfully developed in rodents and can be used to generate permanently growing MCs for the study of the properties of their precursors and the phenotypic characteristics of their mature progeny. We have undertaken similar culture studies and have grown MCs from bone marrow infiltrates from a patient with systemic mastocytosis. Proliferating MCs have been cultured for several months in enriched growth media on bone-marrow-derived fibroblastic adherent layers and have been extensively characterized. In this article we report data on the long-term culture of human MCs and the phenotypic analysis of these cultured MCs and MCs from the spleen of a patient with systemic mast cell disease.

MATERIALS AND METHODS

Preparation of Mast Cells

Mast cells were obtained on four separate occasions from the bone marrow of a patient (S.W.) with systemic mastocytosis. Bone marrow aspirates were taken steriley into Hanks' basal salt solution (HBSS) containing 10 U/ml preservative-free heparin, spun at 1,200 rpm, and the buffy coat taken and disaggregated by repeated syringing through a 21-gauge needle. Bone marrow cells were then fractionated by centrifugation over Histopaque (Sigma, St. Louis, U.S.A.).
MO; density 1.077) and the high density cell pellet and mononuclear cell interface fractions removed and washed twice prior to culture.

Splenic (splenectomy performed for symptomatic relief) and bone marrow trephine biopsy tissue was processed, using standard histologic techniques, for morphological examination and cytochemistry.

Fresh spleen fragments were also snap-frozen in Tissue Tek II OCT embedding medium (Lab-Tek Products) and stored in liquid nitrogen for later processing for frozen section immunohistology.

**Morphology and Cytochemistry**

Cytospin preparations from MC cultures, bone marrow aspirate smears, and wax-embedded spleen and bone marrow cells were stained using routine hematologic and histopathologic methods. The different cytochemical stains used are given in Table 2. Metachromatic staining with Toluidine blue was performed after fixation of cytospin preparations with 50% acid ethanol or after dewaxing and rehydration of wax-embedded tissues; tissues were stained for 5 min with 0.1% Toluidine blue in 30% ethanol, previously adjusted to different pHs in the range of 2.5–6.5.

**Electron Microscopy**

Samples of washed and pelleted fresh bone marrow or cultured MCs were glutaraldehyde fixed, processed, and stained with uranyl acetate for transmission electron microscopy (TEM) using standard techniques. Scanning electron microscopy (SEM) was performed on glutaraldehyde-fixed, critical point dried, gold-shaded MCs from long-term cultures using standard techniques.

**Chromosomes**

Chromosomal analyses of bone marrow MCs, phytohemagglutinin (PHA) stimulated peripheral blood lymphocytes from patient S.W., cultured MCs and adherent layer (AL)-derived cell line cells (at various times, 6–24 hr, after passage) were carried out using standard techniques.

**Long-Term Culture of Mast Cells**

A quantity of 10^5/ml of high density or “Histopaque” interface cultures were cultured in 25 cm² flasks in either RPMI 1640 or α-minimal essential medium in the presence of various concentrations of serum and/or conditioned medium supplements, as detailed below. Cultures were routinely fed with fresh medium twice weekly, and cells were passaged either when cultures were established and were reaching high cell density or when required for experimental use.

**Conditioned Media and Culture Supplements**

Replicate cultures were set up in either RPMI or α-media and 10% fetal calf serum alone (3 different batches) or with one of the following supplements: 10% fetal calf serum, 10% horse serum, 10% autologous plasma, additional 20% conditioned media from placenta, primary or secondary mixed lymphocyte cultures, lectin-stimulated lymphocytes, murine factor producing cell lines (WEHI-3B, EL4), human T-cell (MOLT-4) or macrophage (U937) cell lines. In later experiments, the effect of culture supernatant from MC cultures and AL cell lines was also tested at a final concentration of 30%. The effects of 2-mercaptoethanol (5 x 10⁻⁴ M) and hydrocortisone (10⁻⁸ M) supplements were also tested. In some experiments, fibroblasts (foreskin or human embryonic lung) were used in place of syngeneic bone-marrow-derived AL cells.

**Self-Renewal Capacity of Cultured Mast Cells**

The ability of morphologically recognizable MCs from long-term cultures to self-replicate was tested in two systems. First, MCs were transferred from proliferating cultures into fresh growth media, with or without AL cells or fibroblasts, and with or without 30% conditioned media (from placenta, mixed lymphocyte cultures, MC, or AL cultures) in 2.5 ml well culture plates (Linbro). Second, both MCs or low density mononuclear cells were cultured in semisolid agar, with or without the above conditioned media, in order to assess whether either cell population contained cells with clonogenic potential.

**IgE Receptors**

The presence of Fc receptors for IgE was evaluated by assessing the binding of purified, FITC-conjugated IgE myeloma protein “P.S.” (a gift of Dr. T. A. E. Platts-Mills; chromatographically purified IgE, concentration 33 mg/mL IgE, less than 8 μg/ml other Ig class contaminants) to fresh or cultured MCs with a direct fluorescence assay. Twenty microliters (10^4) of MC in HBSS was added to 20 μl optimally diluted FITC-IgE for 30 min on ice, washed twice in HBSS, and examined for fluorescence; negative control cells consisted of freshly isolated peripheral blood neutrophil granulocytes, mononuclear cells, or cell lines (U937, K562).

**Detection of Histamine and Monoamines**

For detection of intracellular histamine, the α-phthalaldehyde reaction was used. For detection of monoamines, cytospin preparations were treated with formaldehyde gas for 2 hr and then examined under ultraviolet light. Various hemopoietic cell lines (HL60, U937, and K562) were used as negative controls for these reactions.

**Degranulation Response**

MC degranulation was assessed morphologically, in Giemsa-stained cytospin preparations, after incubation of cultured MCs with purified IgE (10^5 cells in 20 μl with 30 μg IgE) for 30 min at 37°C, followed by crosslinking with either concanaavalin-A (Con-A) (10 μg) or optimally diluted monoclonal mouse anti-human IgE antibody (Fab specific) (BRL), again for 10 min at 37°C. Controls consisted of MCs incubated with IgE, Con-A, or anti-IgE alone.

**Antiseras**

Phenotypic analysis of cultured MCs, using various monoclonal antibodies (see Table 4), was performed by indirect immunofluorescence using standard techniques. HLA-specific antibodies (2A1, W6/32, CA2, DA2) were obtained from Drs. W. Bodmer or P. C. L. Beverley; T-cell-specific antibodies (Leu-1, S33, WT1, OKT1 9.6) were from Dr. F. Katz; antibody to glycoporphin-A (R10) was from Dr. P. A. W. Edwards. Antibodies detecting common leukocyte determinants (2D1, T29/33, A1/D8) were obtained from Drs. P. C. L. Beverley, I. Trowbridge, and B. F. Haynes. Antibodies reacting with granulocytes and/or monocytes formed part of the panel of antibodies studied in the First International Workshop on Human Leucocyte Differentiation Antigens; additionally OKM1 (Ortho), Mac-1 (Seralab), and MONO (BRL) antibodies were used. Surface immunoglobulin was assessed using a polyspecific FITC-conjugated goat anti-human Ig reagent (Nordic). Fc receptors were detected by standard rosetting techniques using rabbit IgG-coated sheep red blood cells.

**Immunohistologic Examination of Mast Cells in Infiltrated Spleen**

Five-micron frozen sections of infiltrated spleen, stored as described above, were cut, fixed in acetone for 10 min and frozen at −20°C until use. Sections were rehydrated for 10 min in HBSS containing 5% calf serum before use. The presence of various...
granulocyte-monocyte antigenic determinants (see above and Table 4) was determined by incubation of sections with 50 μl of optimally diluted monoclonal antibody at room temperature for 30 min, followed by washing in HBSS for 30 min, incubation with 50 μl prediluted polyclonal FITC-conjugated goat anti-mouse Ig (Coulter), and finally extensive washing in HBSS (60 min minimum) before examination by fluorescence microscopy.

Characterization of the Adherent Layer in Mast Cell Cultures

AL characterization, other than morphological, was performed on cell lines derived from MC-depleted long-term cultures. MC-depleted, AL cells were replated in 50-cm Petri dishes containing sterilized glass coverslips (19 mm diameter); wet preparations of preconfluent AL cells on coverslips were then mounted on glass slides without fixation and assessed by indirect immunofluorescence, as above, for the presence of fibronectin, factor-VIII-related antigen (Atlantic Antibodies), ABH blood groups (ulex lectin, monoclonal anti-blood-group A antibodies), DRw (1a) CA2, DA2), and common leukocyte and myeloid-monocyte determinants (see above). Morphology and cytochemistry of AL cells were determined as for MCs, as above. Granulocyte-monocyte colony-stimulating activity (GM-CSA) production by AL cells was determined by the use of 10× concentrated AL culture supernatant in agar cultures of normal bone marrow cells in a standard GM-CFC assay system.

RESULTS

Growth of Human Mast Cells in Long-Term Culture

Preliminary experiments with a variety of culture conditions, using different combinations of media, serum supplements, and conditioned media additions (detailed in Materials and Methods), failed to show any growth advantage for MCs; MCs did not outgrow other cell types in some combinations, for example, PHA-LCM with RPMI 1640 medium, where only lymphocytes and monocytes proliferated. Furthermore, no mast cell growth occurred in cultures of low density (<1.077) bone marrow mononuclear cells. Thus, simple growth conditions were selected for the later part of this study—namely, high density (>1.077) bone marrow cells were cultured in α-mimimal essential medium supplemented with glutamine, pyruvate, 2-mercaptoethanol, hydrocortisone, and 10% selected fetal calf serum (GIBCO, Grand Island, NY, batch L413705L).

During the first 2 wk of primary culture, no cell type predominated, but after this time, there was a selective growth advantage for MCs, which accounted for over 95% of the total suspension cell population by 3 wk. Cytologic examination of cultures older than this consisted entirely of MCs.

Due to the nature of the cultures, the majority of MCs grew in clumps in continuity with the AL—only a minority of cells were free or easily released into suspension. This made it impossible to make an accurate assessment of total MC numbers without destroying the integrity of an individual culture. However, we estimate from the numbers of MCs in suspension that doubling times were approximately 7 days during the first 4–6 wk and that this represented a true increase in MC numbers, rather than merely cell selection during culture.

Primary and passaged MC cultures showed a characteristic growth pattern (Fig. 1, A and B). At low MC density, the MCs grew as either single or small groups of round adherent and nonadherent cells, which were readily distinguishable morphologically from other cell types (Fig. 1A). In older cultures, the MCs were closely associated with one another in morulae of various sizes (Fig. 1B). In all cultures, MC growth always occurred in association with an AL with fibroblastic characteristics (see below); the cultures illustrated were at a relatively low density, but MCs also grew equally well on near-confluent ALs.

Although no systematic attempt was made to grow these MCs clonally, the question of the self-renewal capacity of the morphologically recognizable MC was approached in replating experiments. MCs from established cultures, transferred in the presence of AL cells, resulted in the reestablishment of proliferating MC populations. However, MCs replated without an adequate AL failed to proliferate and died within 48 hr. In preliminary experiments, we also found that fibroblast monolayers were unable to replace bone marrow AL cells in the maintenance of MC proliferation in these transfer experiments. The growth maintaining effect could not be substituted for by addition of conditioned media, including supernatants from established MC cultures or AL-derived cell lines.

Supporting these results was the inability to obtain growth of MC colonies in agar of bone marrow mononuclear cells in the absence or presence of placental, AL, or lectin-stimulated lymphocyte conditioned medium. The questions of whether MCs themselves or the stromal AL produce maintenance factors for normal MC proliferation or if the appropriate conditioned media were used is under study.

Characteristics of the Adherent Layer

The AL established in the MC cultures was considered to be composed predominantly of fibroblasts. Definitive evidence for this, obtainable by electron microscopy (to exclude the presence of endothelial-specific organelles, Weibel-Palade bodies) or biochemical analysis of the extracellular matrix, has not been sought. However, the morphology and growth pattern of the cells (Fig. 2), the presence of fibronectin,23 and the absence of detectable factor-VIII-related antigen,24 ABH blood groups,25 DRw antigens,23 alkaline phosphatase enzyme,26 or GM-CSA production,27 taken together with the lack of hemopoietic tissue-
Fig. 1. Mast cell culture photographed by bright field microscopy. (A) Small clumps, as well as individual mast cells, and (B) morulae of mast cells are shown growing in association with a nonconfluent adherent layer (×425).
specific common leukocyte or myeloid-monocyte determinants\textsuperscript{22} and the cytochemical profile (Table 1), strongly suggest that AL in the MC cultures is fibroblastic and does not contain a significant proportion of other stromal elements or other cell types.

Chromosomal Analysis

MCs freshly isolated from infiltrated bone marrow or from long-term cultures were chromosomally normal, as were peripheral blood lymphocytes. AL cells from long-term cell lines showed an increased frequency (8\%) of metaphases with endoreduplication, but they otherwise had a normal karyotype; the significance of a high frequency of endoreduplication in AL cells is not clear.

Morphology of Mast Cells

The MCs present in both bone marrow and spleen showed the typical heavy cytoplasmic granulation described for MCs at other sites (Fig. 3), together with some spontaneous degranulation. On culture, there was a gradual decrease in granulation, such that cells analyzed by day 30 (Fig. 4) showed a wide range of granule density. This loss of granulation, associated with a gradual decrease in chloroacetate esterase enzyme activity (see below), has also been observed in murine long-term MC lines.\textsuperscript{18} The MCs all showed a centrally placed, round or indented nucleus, distinguishing them from the segmented nucleus of the circulating basophil.\textsuperscript{7}

Ultrastructural Features of Mast Cells

The morphology of MCs was further evaluated by TEM. Representative transmission electron micrographs of fresh bone marrow MCs and cells isolated from long-term cultures are shown in Figs. 5 and 6, respectively. In general, the characteristic ultrastructural features of MCs are seen. The MC illustrated in Fig. 5, which was directly isolated from bone marrow,
Fig. 3. Photograph of bone marrow smear showing infiltration by typical, heavily granulated, and partially degranulated mast cells in a case of systemic mastocytosis (stained by May-Grünwald-Giemsa) (×4,250).

Fig. 4. Detail of mast cells from a 30-day culture (stained by May-Grünwald-Giemsa) (×4,250).
shows prominent intracytoplasmic granules of uniform size and homogeneous electron density; these granules lacked any obvious intragranular structure and showed no evidence of active granule formation or degranulation. Another feature to note is the relatively smooth surface membrane; this is in contrast to the MC after in vitro culture. Cultured MCs have prominent membrane processes and folds, which are shown in Fig. 6 and the scanning electron micrograph (Fig. 7). In line with the reduction in granulation mentioned previously, cultured MCs showed fewer granules, which were more heterogeneous in size and electron density (Fig. 6). Furthermore, some of these showed intragranular crystalloid structures and rare lamellae (not shown); in this respect, the granule morphology of cultured MCs approached that of normal skin or gut MCs.

### Cytochemical Features of Cultured Mast Cells

The cytochemistry of MCs isolated from long-term MC cultures, in comparison to the pattern of reactivity of freshly isolated bone marrow MCs (or MCs analyzed in spleen or bone marrow trephine biopsy material), is summarized in Table 2. The cytochemical features of both fresh and cultured MCs are compara-

<table>
<thead>
<tr>
<th>Test</th>
<th>Fresh* Mast Cells</th>
<th>Cultured† Mast Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAS</td>
<td>+/+++/†</td>
<td>+/+++/†</td>
</tr>
<tr>
<td>Sudan Black</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Peroxidase</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>α-Naphthyl acetate esterase</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Chororacetate esterase</td>
<td>++</td>
<td>+/+++/§</td>
</tr>
<tr>
<td>Alcian blue, pH 2.5</td>
<td>−/−</td>
<td>−</td>
</tr>
<tr>
<td>Toluidine blue, pH 6.0</td>
<td>+/++</td>
<td>+/++</td>
</tr>
</tbody>
</table>

*Reactions similar for freshly isolated bone marrow mast cells or mast cells tested in paraffin-embedded spleen or bone marrow trephine sections.
†No change in reactivity between early (day 10) and late (day 100) cultures.
‡Variable diffuse positivity.
§Variable reactivity according to density of granulation.
||Occasional foci of positive cells seen in spleen.
ND, not done.
ble, except that, in line with the reduction in MC granulation on prolonged culture, the chloracetate esterase reactivity was less in cells grown in vitro. The metachromatic staining seen with Toluidine blue, typical of MCs, was observed in the pH range described for malignant, rather than normal, MCs. However, some acid-reactive cells were observed in the spleen sections, and these might represent a residual population of normal splenic MCs. One point to note is the low levels of acid or alkaline phosphatase activity of the MCs studied here; previous studies have reported high levels of phosphatases in MCs. The cytochemical profile of MCs is thus quite different from that described for the circulating basophil.

IgE Receptors, Histamine Content, and the Degranulation Response of Cultured Mast Cells

Both mast cells from infiltrated bone marrow and those in culture showed specific features characteristic of normal mast cells (Table 3). First, Fc receptors for IgE were readily detectable by fluorescence, using FITC-labeled purified IgE myeloma protein; the biochemical characteristics of the IgE–receptor interaction in these cells was not studied. Second, all cells showed strong fluorescence in both the o-phthalaldehyde reaction, demonstrating the presence of intragranular histamine, and after formaldehyde gas fixation, showing the presence of intracytoplasmic monoamines. In both cases, control reactions with peripheral blood mononuclear and polymorphonuclear leukocytes and hemopoietic cell lines were quite negative. The third specific feature exhibited by the cultured mast cells was degranulation in response to crosslinking of Fc-receptor-bound Ig, using either Con-A lectin or, to a lesser extent, by monoclonal anti-IgE antibody.

Surface Markers

The cell surface phenotype of the cultured MCs, as well as those in infiltrated splenic tissues, was analyzed and the results are summarized in Table 4. As expected, MCs express HLA and β₂-microglobulin, but in contrast to some reports of cultured murine MCs, DRw (1a) antigens and Fc receptors for IgG are not detectable. There was no evidence for the expression of T-lymphoid determinants, NK markers (Leu-7, OKMI), surface immunoglobulin, or erythrocyte-specific components.

The reactivity of MCs with a series of 46 monoclonal antibodies, which detected granulocytes and/or monocytes, was also determined; these included the panel of antibodies analyzed by the First International Workshop on Human Leucocyte Differentiation Antigens. We reasoned that an analysis of the cell surface profile of MCs would provide information on the cell lineage relationship among MCs, basophils, and other granuloepoietic cells; thus, a similar pattern of reactivity would support the view that two cell types may be closely related during development. None of the antibodies tested reacted with MCs (Table 4). Our results clearly show that the MCs studied here are phenotypically distinct from mature granulocytes and monocytes or their malignant counterparts. Furthermore, their phenotype differs greatly from that of the functionally and morphologically similar basophil, which expresses many granulocyte-monocyte markers and thus shows a greater affinity to other members of the granuloepoietic cell lineage than to MCs.

A Hemopoietic Origin for Human Mast Cells

One of the major questions of mast cell biology is whether MCs have an origin common to other bone-marrow-derived cell types. Although there is strong evidence in rodents for such a common ancestry, no
data appertaining to this problem exist for man. The expression by MCs, in infiltrated spleen and long-term culture, of three surface antigenic determinants (Hle-1, T29/33, and A3/D8) that are known to be only detectable on hemopoietic cells, strongly supports a bone marrow origin for the human MC, at least for the class of MCs analyzed here (Table 4).

DISCUSSION

It is clear that, compared with rodents, relatively little is known about the human MC; in particular, its in vitro growth, cell surface characteristics, origin, and relationship to hemopoietic cells are uncharacterized. The aim of this study was to investigate these questions.

The cells proliferating in long-term bone marrow culture in these experiments have many features characteristic of MCs; that is, they show typical metachromatic granule staining with Toluidine blue, an ultrastructural morphology compatible with the MC, intracellular histamine and monoamines, surface Fc receptors for IgE, and they degranulate on crosslinking of these receptors. Furthermore, they can be distinguished by morphology, cytochemistry, and surface phenotyping (see below) from basophils, and thus, we feel confident in concluding that these cells are indeed MCs.

Work in rodents has partially characterized the clonogenic cell for the bone-marrow-derived MC. Also, the growth factor(s) required for the permanent proliferation of MCs in the mouse have been purified to homogeneity from a variety of sources. In this study we have not investigated fully the characteristics of the MC stem cell, which is proliferating in long-term culture, and have only provided preliminary, negative data on possible sources of growth factors needed for the production of permanent human MC lines. However, the inability to maintain proliferation of morphologically recognizable MCs after transfer from high density primary bone marrow cultures, in the absence of an established stromal adherent cell layer, suggests the following possibilities.

First, the progenitor for morphologically recognizable MCs might not be present within the transferred MC population, and thus, the mature MC population has only limited self-renewal capacity. The implication for this would be that the true stem cell lay in the adherent MC subpopulation or as a morphologically unrecognizable cell type within the bone marrow AL. Second, a soluble growth factor may be required for prolonged proliferation of MCs in the absence of a bone marrow-derived adherent layer. Either the MC itself, or adherent layer cells, might conceivably produce such a factor; in the former instance, factor levels sufficient for maintenance of continued cell proliferation are not achieved. Another interpretation would be that MCs require direct cell-to-cell contact with stromal cells (or the influence of short range factors) to provide the correct cellular niche, allowing continued cell proliferation. Such an interaction between MCs and fibroblasts in vitro has been reported by Ginsburg in the mouse.

Why is the cell surface phenotype of the MC of particular interest? The cell lineage origin of MCs, especially in man, has been in dispute; even if a bone marrow origin for the MC is accepted, then its relationship to other hemopoietic cell types is unknown. Data on the cell surface characteristics of the MC would then allow the question of heterogeneity among MC populations from different sites (for example, those in connective tissue versus gut-associated MCs) to be approached. Also, one could explore further any possible affinity between MCs and the functionally and morphologically similar circulating basophil.

In this article we have shown that MCs express three “panhemopoietic” cell surface determinants (Table 4); this finding strongly supports a bone marrow origin for the human MC, at least for the class of MCs analyzed here. However, an approach utilizing genetic markers, in combination with appropriate culture systems, would be required to be conclusive.

Our results also show that MCs (Table 4) derived from bone marrow in culture or infiltrated spleen from patients with systemic mast cell disease are phenotypically distinct from mature granulocytes or monocytes or their malignant counterparts. Whether their surface antigenic profile accurately reflects the characteristics of normal MCs, or if there are differences between MCs from different tissues, remains to be determined and is presently under study. Furthermore, preliminary studies have also shown that the cell surface phenotype and cell membrane glycoprotein profile of the MC differs greatly from that of the basophil, which expresses markers and a glycoprotein pattern similar to granulocytes and monocytes (data not shown); the basophil thus appears to show a greater affinity to other members of the granulopoietic cell lineage rather than to the MC. Despite these data, it is not, as yet, possible to assess whether MCs at any stage of their development show similarities to early myeloid cells, altering later during terminal maturation and diverging from them, on acquiring their characteristic morphological features and functional repertoire. Likewise, the question of whether MCs and basophils share a common stem cell will remain unanswered until clonogenic culture assays are devised for each cell type.

In conclusion, the cell surface phenotype of MCs from spleen and those grown in long-term bone marrow culture has been analyzed. A hemopoietic origin
for the human MC is supported by the expression three "panhemopoietic" cell surface markers. The MCs studied here fail to express any granulocyte- or monocyte-specific markers, demonstrating a wide developmental separation between these cell lineages and from basophils. However, as these MCs were derived from a patient with systemic mastocytosis, this interpretation should be viewed with some caution, as they might show some phenotypic abnormalities.

ACKNOWLEDGMENT

We would like to thank J. Pettitt for performing electron microscopy and B. Kirk for carrying out the chromosomal analysis (Departments of Histopathology and Haematology, St. Bartholomew's Hospital, respectively). We also thank S. W. for permitting repeated bone marrow biopsies.

REFERENCES

17. Schrader JW: The in vitro production and cloning of the P cell, a bone marrow derived null cell that expresses H-2 and Ia antigens, has mast cell like granules and is regulated by a factor released by activated T cells. J Immunol 126:452, 1981
Characterization of human mast cells in long-term culture

MA Horton and HA O'Brien