Mast Cell Typing: Demonstration of a Distinct Hematopoietic Cell Type and Evidence for Immunophenotypic Relationship to Mononuclear Phagocytes

By P. Valent, L.K. Ashman, W. Hinterberger, F. Eckersberger, O. Majdic, K. Lechner, and P. Bettelheim

The immunologic surface marker profile of human mast cells (MCs) was established using a combined toluidine/imunofluorescence staining procedure [49 monoclonal antibodies (MoAbs)] tested. Ascites (n = 9) MCs as well as enzymatically dispersed cells from all organs tested (lung n = 11, skin n = 7, intestine n = 10) exhibited an identical marker profile. MCs were recognized by MoAbs clustered as CD9 (anti-gp24), CD33 (anti-gp67), and CD45 (anti-gp220) as well as by MoAbs directed against membrane-bound IgE. MoAB YB5B8 (anti-gp145) selectively recognized MCs. Most significantly, however, MCs were stained by MoAbs MAX1 (anti-gp65), MAX3 (anti-gp68), MAX11 (anti-gp65), and MAX24 (anti-gp65). These antibodies bind to surface membrane antigens associated with a late stage of monocyte/macrophage differentiation. Thus, our results provide definite evidence that MCs share surface membrane markers with mononuclear phagocytes. In contrast, MCs are stained neither by lymphatic markers (CD1-8, 10, 19-24) nor by myelomonocytic markers (CD11-17). MCs also lack the interleukin-2 (IL-2) receptor (CD25), the T10 antigen (CD38), and most of the myelocytic markers expressed on peripheral blood (PB) basophils. Thus, MCs displayed a unique phenotype within the hematopoietic system. This new approach enabled us to enrich human lung MCs to a purity >95% by means of negative selection with complement-mediated cell lysis. Purified MCs were subsequently stained with MoAbs and analyzed by flow cytometry, which confirmed the results obtained from the double-staining experiments. We next examined cultured metachromatic cells derived from bone marrow (BM) and peripheral blood colony-forming units (CFU). These metachromatic cells previously could not be classified by morphologic criteria alone and have therefore been termed basophil-like/MC-like cells. In this study, toluidine blue-positive cells obtained from either pooled multipotential colonies (day 14-CFU-GEM) or pooled myelocytic colonies (day 16/17-CFU-GM/G/M) were recognized by MoAbs MY7 (CD13), VIM12 (CD11b), and VIM2, as well as by an anti-IgE MoAb, after preincubation with IgE. In contrast, CFU-derived metachromatic cells were not stained by MoAb YB5B8. This marker profile corresponds to the immunologic phenotype of blood basophils and excluded a detectable formation of mature MCs in colonies derived from cultured hematopoietic stem cells.

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BOTH BASOPHILIC granulocytes and mast cells (MCs) are involved in the mechanisms of allergy and share cellular substrates necessary for allergic response. Most significantly, both cell types express high-affinity surface membrane receptors for IgE. Basophilic granulocytes usually circulate in the peripheral blood (PB), although they are capable of invading into tissue, probably on chemotactic stimulation. In rodents, mucosal mast cells (MMCs) differ from connective tissue-type mast cells (CTMCs) in their (bio)chemical and functional properties as well as in their dependence on T-cell-derived factors. More recently, a similar subclassification of human MCs was also established.

The origin of basophils and MCs from hematopoietic precursor cells is a widely accepted hypothesis recently confirmed by the observation that both cell types express the pan leukocyte antigen. In human colony assays, metachromatic cells may develop from bone marrow (BM) or PB stem cells. In particular, they have been detected in myeloid-restricted as well as in multipotential colonies. Metachromatic cells can also be cultured in suspension from hematopoietic precursors. By morphologic examination, however, an exact classification of cultured metachromatic cells (basophils or MCs) has not yet been achieved; therefore, these cells have often been termed basophil-like/MC-like cells.

Recently, we were able to establish the immunologic surface marker profile of human blood basophils. The present study was designed to (a) characterize the immunologic phenotype of human MCs derived from different organs, (b) characterize further the immunologic phenotype of human basophils and compare it with that of MCs, and (c) achieve a classification of so-called basophil-like/MC-like cells derived from cultured hematopoietic stem cells.

MATERIALS AND METHODS

Patients and samples. BM and PB cells were obtained from normal donors (BM n = 11, PB n = 16) and from four chronic myelogenous leukemia (CML) patients (PB n = 4) after informed consent was given. BM was obtained from the iliac crest. Cells were collected in heparinized tubes, diluted with RPMI 1640 medium, and layered over Ficoll (1,077 density) to separate mononuclear cells (MCs). BM cells were obtained from ascitic fluid of nine patients suffering from liver cirrhosis, from digested lung tissue of 11 patients undergoing surgery for bronchiogenic neoplasms, from digested juvenile foreskin material after circumcision (n = 7, age 5 to 9 years), and from the intestinal mucosa of three patients undergoing small intestine resection for ulcer duodenai, of two patients suffering from gastric cancer, and of five patients suffering from colon neoplasm.
Monoclonal antibodies (MoAbs). MoAbs Leu1(CD5), Leu2a(CD8), Leu3a(CD4), Leu4(CD3), and Leu7 [natural killer (NK) cell-reactive MoAbs] were purchased from Becton Dickinson, Sunnyvale, CA; MoAbs MY7(CD13), MY9(CD33), B(A2CD20), B2(CD21), B4(CD19), and anti-interleukin-2 (IL2)R(CD25) were purchased from Coulter Immunology, Hialeah, FL; MoAb BA-2(CD9), were purchased from Hybritech, San Diego; MoAbs OKT9(anti-transferrin R), OKM1(CD11), and OKT10(CD38), were purchased from Ortho Pharmaceuticals, Raritan, NJ, MoAbs I0B2 (CD9) and E124-2-8 (anti-IgE MoAb) were purchased from Immunotech, Marseille, France; and MoAbs BMA 010 (CD45), BMA 022 (anti-HLA-DR), and BMA 011 (CD2) were purchased from Boehringer Mannheim, FRG. The following MoAbs were produced at the Institute of Immunology, University of Vienna, and kindly provided by the Professor W. Knapp: VIP-2b(CD38), VID-1 (anti-HLA-DR), VIL-1(CD10), VI M12(CD11b), VIM13(CD14), VIM-D5(CD15), VIM-2 (not yet clustered), VIB-C5(CD24), VIT-3b(CD3), VIT-4(CD4), VIT-6(CD1), ViFeR2(CD16), and VIE-G4 (anti-glycophorin A). MoAb WT-1(CD7) was kindly provided by Dr W.M.I. Tax, and anti-Tac MoAb(CD25) was provided by Dr T.A. Waldmann. MoAb YB5B8 (not yet clustered) was produced at the Institute of Immunology, University of Vienna. A number of MoAbs were selected from the second (Boston, 1984) and third (Oxford, 1986) “International Workshop on Human Leukocyte Differentiation Antigens,” including the anti-macrophage MoAbs MAX1, MAX3, MAX24; the myeloid markers TU3 (not yet clustered); L4F3 and L1B2 (both clustered as CD33); and MoAbs Ki-M1 (CD11c), T5A7(CD17), M MHM23 (CD18), MMH6 (CD23), CIKM5 (CD32), E11(CD35), 5FI(CD36), as well as the not-yet-clustered MoAb Ki-M3. MoAbs MAX2 and MAX11 were kindly provided by Dr Andreaesen. MoAb C1B-Ery3 was a gift of Dr Tetteroo. Antibodies known to recognize intracytoplasmic epitopes selectively were excluded from the study. The entire panel of MoAbs listed in Tables 1 through 3 was tested on each cell preparation (dispersed cells and cells derived from ascites) as well as on purified CML basophils obtained from four individual donors.

Table 1. Reactivity of Myelomonocytic Markers With MCs and Basophils

<table>
<thead>
<tr>
<th>MoAbs</th>
<th>CD</th>
<th>MCs</th>
<th>Basophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIM12</td>
<td>11b</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Km-1</td>
<td>11c</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>MY-7</td>
<td>13</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>VIM13</td>
<td>14</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>VIM-D5</td>
<td>15</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>VIFCR2</td>
<td>16</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>T5A7</td>
<td>17</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CIKM5</td>
<td>32</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MY-9</td>
<td>33</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MY-10</td>
<td>34</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>E11</td>
<td>35</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SFI</td>
<td>36</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>VIM-2</td>
<td>NK</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

CD, cluster of differentiation; NK, not known.
No reactivity of metachromatic cells observed (-); >95% of metachromatic cells reactive (+).

Table 2. Reactivity of Lymphocytic and Not Lineage-Restricted MoAbs With MCs and Basophils

<table>
<thead>
<tr>
<th>MoAb</th>
<th>CD/Reactive Structure</th>
<th>MCs</th>
<th>Basophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-T</td>
<td>1-8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BA-2, IOB2</td>
<td>9</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>VIL-A1</td>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MHM23</td>
<td>18</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>B4</td>
<td>19</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B1</td>
<td>20</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B2</td>
<td>21</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MHM6</td>
<td>23</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>VIB-C5</td>
<td>24</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Anti-TAC, Anti-IL-2R</td>
<td>25</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>OKT10, VIP-2b</td>
<td>38</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>BMA010</td>
<td>45</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>VID-1, BMA022</td>
<td>HLA-DR antigen</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Anti-IgE</td>
<td>IgE-receptor</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>OKT9</td>
<td>Anti-transferrin R</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

No reactivity of metachromatic cells observed (-); >95% of metachromatic cells reactive (+).

Immunologic evaluation. The reactivity of cells with MoAbs was assessed as described previously. As second step reagent fluorescein-conjugated goat F(ab')2 anti-mouse IgG + IgM antibodies were used. Expression of cell-surface antigens on MCs was analyzed with a Leitz Ortholux microscope (Wetzlar, FRG). Pure populations of metachromatic cells (>95% purity), ie, highly enriched CML basophils, purified human lung MCs, and cells obtained from granulocyte-restricted colonies (one experiment) were analyzed with a FACS 440 (Becton Dickinson).

Enrichment of MCs. Ascitic fluid of liver cirrhosis patients was centrifuged and washed three times in PBS before being examined for the presence of MCs. Tissue MCs were enriched from various organs by enzymatic digestion by a modification of the technique described by Schultman et al. Samples were placed in TB immediately after resection. Tissue was cut into small pieces and extensively washed in the same buffer. Tissue fragments were then incubated (1 g tissue/mL) with collagenase (1.5 mg/mL) in RPMI 1640 medium supplemented with 3% fetal calf serum (FCS). After 60-minute incubation at 37°C, enzymatically freed cells were separated from tissue with Nytex cloth and washed three times in cold PBS. To exclude a collagenase-induced loss of antigenic structures, in two experiments, ascites cells as well as BM MNCs were enzymatically treated with collagenase under the conditions described above and subsequently phenotyped with all MoAbs used. No enzymatic effect on the expression of surface markers was observed.

Double-staining procedure. The presence of metachromatic cells was confirmed by Wright's staining after cytoxin preparation. The combined immunofluorescence/toluidine blue-staining proce-

Table 3. Reactivity of Macrophage-Associated Markers With Surface Antigens of Human Basophils and MCs

<table>
<thead>
<tr>
<th>MoAb</th>
<th>Reactive Structure</th>
<th>MCs</th>
<th>Basophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kim-3</td>
<td>26-27 kd</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>MAX-1</td>
<td>85 kd</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>MAX-24</td>
<td>85 kd</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>MAX-2</td>
<td>200 kd</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MAX-3</td>
<td>68 kd</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MAX-11</td>
<td>65 kd</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

No reactivity of metachromatic cells observed (-); >95% of metachromatic cells reactive (+).
dure was performed as described previously.11,32 Cells were incubated with MoAbs for 30 minutes at 4°C, washed twice in PBS, and conjugated with a second fluorescence-labeled antibody (30 minutes, 4°C). Cells were then fixed in 0.025% glutaraldehyde for one minute. After three washes, cells were stained with toluidine blue (0.0125%) for ten minutes at room temperature, again washed three times, and examined under a fluorescence microscope (Dialux Leitz, Austria).

**Purification of human lung MCs.** In four separate experiments, human lung MCs were purified from all contaminating cells present in dispersed lung cell suspensions by complement-mediated cell lysis according to the unique phenotype of MCs established by the double-staining procedure. To purify MCs from dispersed lung cells, the following MoAbs (and complement) were used: VIM-D5, VIM-2, VIM13, VIP-2b, VIB-C5, BMA-022, BMA-011, BI, OKM1, C1B-Ery3, Leu1, Leu7, and Leu9. Experimental procedures and conditions were otherwise identical to those described for purification of CML basophils (described below).

**Purification of CML basophils.** Basophils were purified from the PB of four CML patients as described.13,32 CML MCs were first depleted of VIM-D5-positive cells by incubation of 1 x 10^7 cells with 20 μg MoAb VIM-D5 in RPMI at 4°C. After 45 minutes, cells were washed twice and resuspended in 5 mL rabbit complement (Behringwerke AG, Marburg, FRG). After 90-minute incubation at room temperature, cells were again washed and depleted of monocytes by scrubbed nylon wool. In a second step, a cocktail of MoAbs (VIT-3b, VIB-C5, Leu1, Leu7, BI, VIE-G4, anti-HLA-DR, VIM-D5, and CLB-Ery3, each MoAb 20 μg/10^8 cells) and rabbit complement 1 mL/10^8 cells was used in the same way as described above to eliminate remaining contaminating cells. After complement-mediated cell lysis, purified basophils were layered over Ficoll and washed three times to remove cell ghosts. Cell viability was assessed by trypan blue exclusion. CML basophils were enriched to a purity >95%.

**Neuraminidase treatment of MCs.** In two experiments, enriched human lung MCs were exposed to Vibrio cholerae neuraminidase (Behringwerke AG) as recently described for blood basophils.33 An amount of 4 x 10^6 cells were incubated with 0.2 U neuraminidase at 37°C for 30 minutes and then washed twice in RPMI 1640 medium and stained with CD15 MoAbs. MCs and highly enriched CML basophils were tested simultaneously.

**Preparation of PHA leukocyte-conditioned medium (PHA-LCM).** PHA-LCM was prepared as described previously.21 x 10^6 MNCs/mL were cultured at 37°C and 5% CO₂ in α-minimal essential medium (MEM) in the presence of 10% FCS and 1% phytomenadione (PHA, Difco Laboratories, Detroit). On day 7 of culture, supernatant was harvested and filtered through 0.45-μL sterile filters. Aliquots of conditioned media were stored frozen at −20°C until use. PHA-LCM from five different normal donors was tested at various concentrations for its capacity to generate metachromatic cells (data not shown). PHA-LCM that allowed optimal growth of metachromatic cells was used in stem cell assays.

**Stem cell assay for myeloid colonies.** BM MNCs of three normal donors and PB MNCs of five healthy volunteers were prepared for colony assay to generate myeloid colonies of various lineages by a modification of the technique described by Leary et al.22 Colony assay mixture contained 1 x 10^4 BM MNCs or 2 x 10^5 PB MNCs, Iscove's modified Dulbecco's medium (IMDM), 20% FCS, 1% bovine serum albumin (BSA), 5 x 10^-4 mol/L 2-mercaptoethanol, 8% PHA-LCM, and 0.8% methylcellulose (1,500 cp) as viscous support. One milliliter of this mixture was placed into 35-mm standard tissue culture dishes and incubated at 37°C in a humidified 5% CO₂ atmosphere. Colonies were examined on day 16 or 17 of culture.

** Colony assay for CFU-GEMM.** BM MNCs of eight normal donors and PB MNCs of three healthy volunteers were cultured for CFU-GEMM as described previously.46 x 10^3 BM MNCs or 2.5 x 10^5 PB MNCs were cultured in IMDM supplemented with 10% FCS, 1% BSA, 5% PHA-LCM, 0.8% methylcellulose, and 1 μg/mL purified erythropoietin (Toyobo, Osaka, Japan). Cultures were maintained at 37°C under 5% CO₂. Two to six plates were cultured per donor. Colonies were examined on day 14 of culture.

**Immunologic examination of colonies.** Colonies were first classified by their typical appearance under an inverted microscope (Dialux). Cell aggregates >20 cells were estimated as colonies. In day 16/17 cultures, myeloid colonies could clearly be classified as either granulocytic or macrophage. However, irrespective of size or lineage, myeloid colonies of day 16/17 cultures were pooled for immunologic phenotyping unless otherwise noted. CFU-GEMM colonies were recognized by their content of RBCs and examined on day 14 of culture. Colonies were picked out from methylcellulose with an elongated Pasteur pipette. Twenty to 70 myeloid-restricted colonies from day 14 cultures of each individual donor and three to eight CFU-GEMM colonies from each individual donor (BM n = 3, PB n = 4) were pooled. Cells were freed from methylcellulose by washing in PBS. Suspended cells were split into four to six aliquots. One aliquot was first examined for the presence of metachromatic cells by Giemsa staining after cytopsin preparation. The percentage of metachromatic cells was calculated on the basis of 200 cells. The immunologic phenotype of metachromatic cells (one marker per aliquot) was evaluated using selected MoAbs and the combined toluidine/immunofluorescence staining procedure as described above. The reactivity of at least 20 metachromatic cells with MoAbs in each aliquot was examined. For anti-IgE MoAbs, suspended cells were preincubated with IgE at 37°C for three to ten hours as described previously. Human IgE was obtained from myeloma cell line U266 kindly provided by Professor K. Nilsson.

In one experiment, PB MNCs from one donor were cultured in 38 plates to generate myeloid-restricted colonies. On day 17 of culture 335 granulocytic (CFU-G) and 230 macrophage-containing (CFU-GM/M) colonies were separately pooled from all plates. Giemsa staining revealed 95% metachromatic cells in pooled granulocytic colonies and <10% metachromatic cells in pure and mixed macrophage colonies. The immunologic phenotype of cells derived from pooled granulocytic colonies was evaluated by FACs analysis. The percentage of metachromatic cells was evaluated in a blinded fashion by two independent observers. Evidence for specific metachromasia was provided by detection of histamine in cells derived from granulocytic colonies.

**Histamine measurement.** Histamine was quantified using a commercial radioimmunoassay (RIA, Immunotech) as described recently.47 In brief, histamine-containing standards (for standard curve setting), and samples of unknown histamine content were prepared by mixing with acetylation reagent (provided with the kit). Then 125I-radioabeled histamine was displaced by standards or samples to bind to antihistamine MoAbs coated on plastic tubes. After 18-hour incubation at 4°C, nonbound histamine was removed by aspiration and the surface-bound tracer was counted in a γ-counter. The histamine values were calculated by direct comparison with the standard curve values. Intracellular histamine was determined after cell lysis in aqua dest and subsequent freeze-thawing. The amount of histamine per metachromatic cell was calculated from total histamine and total cell count of pure populations of washed metachromatic cells.

Loss of intracellular histamine (ie, a significant subset of MCs during complement-mediated cell lysis) was excluded by comparing total histamine values of an equal volume of respective cell suspensions (1 x 10^7 cells/mL) obtained from dispersed lung; 1 x 10^6 cells...
Fig 1. Combined staining of an ascites MC with toluidine blue and the CD9 MoAb BA-2.

Fig 2. Purified human lung MCs obtained by complement-mediated cell lysis and stained with Giemsa.

Fig 3. FACS analysis of purified human lung MCs.

Fig 4. Metachromatic cells obtained on day 17 from pooled granulocytic colonies cultured from PB CFU.

RESULTS

Immunologic phenotype of human MCs and basophils. With a combined immunofluorescence/toluidine blue staining procedure, we recently established the surface-marker profile of human blood basophils. Using the same technique, we are now able to characterize the phenotype of human enzymatically dispersed MCs derived from different organs as well as of ascitic MCs (enzymatically untreated). Among dispersed cells of various organs, MCs were present at a concentration of 5% to 15% of total cell count, whereas in ascitic fluid MCs were present at a concentration of 0.3% to
1%. MCs obtained from various tissues or from ascites were easily recognized by their metachromatic granules after toluidine blue staining and could be distinguished from basophils by their typical round, centralized nucleus and dense packed granules (Fig 1).

MCs from all organs tested exhibited an identical marker profile for all MoAbs tested. Furthermore, each MoAb reactive with MCs showed a homogeneous staining pattern for all MCs derived from different organs. Therefore, in our study, no MC subtypes in humans could be recognized using surface-marker analysis. To compare the phenotype of MCs, and basophils, the immunologic characterization of basophils was extended in the present study. Similarly to MCs basophils showed a homogeneous staining pattern for all MoAbs reactive and a uniform pattern of reactivity for all MoAbs tested. The surface-marker profile of human MCs and human basophils is shown in Tables 1 through 3. MCs, but not basophils, are recognized by the macrophage-associated MoAbs MAX1, MAX11, MAX24, and KIM3. Moreover, MCs (as well as basophils) are recognized by MoAb MAX3, myelocytic markers CD33 and Tu3, and CD9 and CD45 MoAbs. In contrast, MCs lack almost all myeloid structures expressed on blood basophils as well as the activation-linked structure T10 and the IL-2 receptor. MCs were not stained by monocyte- or lymphocyte-associated MoAbs or by MoAbs directed against the HLA-DR antigen.

**FACS analysis on purified human lung MCs.** According to their unique surface-marker profile, human lung MCs could be enriched to a purity >95% by negative selection using complement-mediated cell lysis (Fig 2). Subsequently, purified MCs were subjected to FACS for a nonsubjective quantification of their immunologic phenotype. As shown in Fig 3, FACS analyses of MCs confirmed the results obtained from the double-staining experiments.

In a separate experiment, loss of a significant subpopulation of MCs during complement-mediated cell lysis with a lytic cocktail of MoAbs (described above) could be excluded by demonstration of recovery of intracellular histamine in lung cell suspensions during complement lysis. Before lysis 1 × 10⁶ lung cells suspended in 1 mL medium contained 2,590 ng histamine. After complement lysis with the lytic cocktail, 1 mL purified MCs contained 2,210 ng histamine. After lysis with MoAb T5A7 or exposure to a nonlytic control antibody, a similar outcome was observed (T5A7: 2,245 ng and control antibody: 2,360 ng intracellular histamine in 1 mL medium). Nonspecific loss of histamine during complement lysis (~15% intracellular histamine) corresponded to the nonspecific loss of cells (15% to 20%).

**Effect of neuraminidase treatment on MCs.** Basophils bound CD15 MoAbs after neuraminidase treatment. However, under identical conditions, neuraminidase-treated human lung MCs failed to react with CD15 MoAbs.

**Incidence and morphology of metachromatic cells detected in stem cell assay colonies.** Giemsa staining of pooled myeloid-restricted day 16/17 colonies grown from PB MNCs of four different individuals revealed a percentage of metachromatic cells of 31%, 46%, 60%, and 62%, respectively. A less frequent outcome of metachromatic cells (9%, 15%, and 17%, respectively) was observed in myeloid colonies obtained from cultures of three different BM donors. In pooled multipotential (CFU-GEMM) colonies grown from either BM or PB precursor cells, metachromatic cells were detected at a concentration of 2% to 7%. In contrast to metachromatic cells obtained from tissue or from various biologic fluids, cultured metachromatic cells could not be classified as either basophils or MCs by morphologic means alone.

**Immunologic phenotype of metachromatic cells derived from pooled multipotential and myeloid colonies.** Metachromatic cells of pooled colonies were immunologically characterized with selected MoAbs by the combined immunofluorescence/toluidine blue staining method. Without exception, toluidine blue-positive cells obtained from either multipotential (day 14) or myeloid-restricted (day 16/17) colonies were detected by MoAbs MY7, VIM12, and VIM2 (Table 4). They were further detected by an anti-IgE MoAb after preincubation with IgE, which served as a positive control. In contrast, the MC-specific marker YB5B8 failed to detect metachromatic cells in pooled colonies. Identical results were obtained from colonies derived from BM and PB stem cells.

In one experiment, pure (98%) metachromatic cells (Fig 4) derived from cultured granulocyte-committed precursors of one PB donor were studied by FACS analysis. As shown in Fig 5, FACS staining of pure metachromatic cells confirmed the basophil phenotype (anti-IgE⁺, MY7⁺, VIM12⁺, YB5B8⁻) of previously termed basophil-like/MC-like cells. Metachromatic cells derived from pooled macrophage containing colonies expressed the same marker profile as the metachromatic cells derived from granulocytic colonies.

**Calculated histamine values for purified metachromatic cells.** The histamine value per purified metachromatic cell was calculated from total cell number and total histamine after cell washing. Purified CML basophils stored 0.7 to 1.8 pg histamine per cell, whereas purified human lung MCs contain ~2 to 5 pg histamine per cell. The histamine value of metachromatic cells derived from granulocyte-restricted colonies in this study (1.6 pg) corresponds to the histamine content of human blood basophils rather than to that of human MCs.

**DISCUSSION**

Immunologic surface-marker analyses using MoAbs have been performed for years to evaluate distribution of surface membrane structures on hematopoietic cells. According to
findings from these studies, an immunologic classification of almost all leukocyte subsets has been achieved. In this study, extensive surface-marker analysis was performed to establish the immunologic phenotype of human MCs. In a first step, MCs derived from different organs were analyzed by a combined immunofluorescence/toluidine blue staining technique. In a second step, according to their unique phenotype, lung MCs were enriched to a purity >95% by negative selection with complement-mediated cell lysis. Subsequently, purified MCs were stained with MoAbs and analyzed by FACS for a nonsubjective quantification. As expected, almost identical results were obtained from the combined immunofluorescence/toluidine blue staining experiments and from FACS analyses.

MCs from all organs tested displayed an identical immunologic phenotype and a homogenous staining pattern for each reactive MoAb. Thus, in our study, no subtypes of human MCs could be detected in terms of their immunologic surface-marker profile. These results appear to contrast a suggested model of MC heterogeneity,\(^1\) which has been discussed to imply discrete stages of MC differentiation.\(^4\)\(^8\) However, the concept of MC heterogeneity has been established on biochemical and functional features rather than on surface-membrane analyses. Furthermore, murine MMCs recently were shown to change their intracellular phenotype to become CTMCs and vice versa, depending on the micro-environment to which they are exposed.\(^5\)\(^6\)\(^1\) This apparent capacity of transdifferentiation indicates a closer relationship between different types of MCs. Our results demonstrate that mature human MCs have almost identical surface antigen features independent of the “differentiation pathway” (CTMCs or MMCs) they have entered. However, certain stages (i.e., more immature stages) of MC differentiation may not have been detected by the staining procedures used in our study.

The exact origin of MCs and their relationship to various types of leukocytes remain to be clarified. A possible relationship between mast cells and blood basophils has been considered.\(^5\) Our results demonstrate that mature basophils and MCs can clearly be distinguished by immunologic phenotyping. In particular, MCs do not express the majority of myelocytic and activation-linked surface membrane structures detectable on blood basophils.

Perhaps our most striking finding was that MCs (but not basophils) are stained by a number of MoAbs raised against macrophages and known to indicate a late stage of macrophage differentiation.\(^5\) To our knowledge, this is the first description of a significant immunophenotypic relationship between MCs and macrophages in humans.

To achieve an immunologic characterization of cultured metachromatic cells which have not yet been clearly classified as either basophils or MCs by morphologic means alone, we extended our studies on stem cell assay colonies. Toluidine blue-positive cells obtained from either pooled multipotential (CFU-GEMM, day 14) colonies or from pooled myeloid (day 16/17) colonies were recognized by MoAbs MY7, VIM12, and VIM2. Furthermore, these cells were stained by an anti-IgE MoAb after preincubation with IgE. This marker profile strongly suggests the presence of basophils. In contrast, MoAb YB5B8, which is an excellent and rather specific marker for MCs,\(^5\)\(^4\)\(^5\) did not stain metachromatic cells obtained from CFU colonies. Thus, almost all metachromatic cells derived from hematopoietic stem cells in vitro resemble PB basophils and not MCs as defined by their immunologic surface-marker profile. Our findings do not exclude the origin of MCs from unipotent and/or multipotent human hematopoietic precursor cells. One explanation for the failure to detect cultured MCs in our studies may be that mature MCs are end-stage products of a multistep differentiation pathway (probably with the mononuclear phagocyte as an intermediate stage as suggested by Czarnezki et al.,\(^7\) excluding their detectable formation in a simpler short-term culture system. Recently, it was found that in prolonged stem cell cultures, persisting cells forming colonies probably share features with MCs.\(^5\)\(^7\)\(^8\) Another explanation is that MC development requires distinct growth factors not present in the conditioned media used. A third explanation is that the technique applied in this study was less sensitive to detect an extremely rare cell type among colony-derived cells. However, the latter possibility is unlikely because the combined toluidine blue/immunofluorescence staining procedure detected cell subsets <1% in a given cell population.\(^3\)\(^1\)\(^2\)

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MAST CELL TYPING


Mast cell typing: demonstration of a distinct hematopoietic cell type and evidence for immunophenotypic relationship to mononuclear phagocytes

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