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Identification of Basophilic Cells that Express Mast Cell Granule Proteases in the Peripheral Blood of Asthma, Allergy, and Drug-Reactive Patients

Lixin Li,*‡ Yong Li,*‡ Stephen W. Reddel,† Maya Cherrian,‡ Daniel S. Friend,§ Richard L. Stevens,‖ and Steven A. Krilis*‡

Metachromatic cells in the peripheral blood of patients with asthma, allergy, or an allergic drug reaction were evaluated for their nuclear morphology, surface expression of the mast cell (MC) marker c-kit, surface expression of the basophil marker Bsp-1, and granule expression of MC proteases. Consistent with previous findings by others, Bsp-1+/metachromatic cells represented <1% of the cells in the peripheral blood of normal individuals. These cells generally contained segmented nuclei. Very little, if any, tryptase (Try), chymase (Chy), or carboxypeptidase A (CPA) was found in their granules, and very little, if any, c-kit was observed on their surfaces. The number of metachromatic cells increased in the peripheral blood of the three groups of patients. Like the basophils in normal individuals, most of these metachromatic cells contained segmented nuclei and expressed Bsp-1. However, in contrast to the basophils in normal individuals, many of the metachromatic cells in the three patient groups expressed c-kit, Try, Chy, and/or CPA. That the metachromatic cells in the blood of our patients have some features of MCs and some features of basophils suggests that human basophils and MCs are derived from a common progenitor. As assessed by the chloroacetate esterase cytochemical assay, the immunoreactive Chy in the peripheral blood of these patients is enzymatically active. Because MC proteases regulate numerous immunologic and other biologic systems, the expression of Try, Chy, and/or CPA in a peripheral blood-localized cell in an individual having asthma, allergy, or an allergic drug reaction has important clinical implications. The Journal of Immunology, 1998, 161: 5079–5086.

Asophils represent <1% of the leukocytes in the peripheral blood of normal individuals. Like tissue-localized mast cells (MCs), basophils are IgE-bearing cells that store histamine in their metachromatic granules. When their high-affinity receptors for IgE (FceRI) are cross-linked with Ag, both MCs and basophils release their preformed granule mediators and then generate and release various cytokines and arachidonic acid metabolites. Basophils and MCs isolated from normal individuals differ in their nuclear and granule ultrastructure (1) and in their expression of c-kit, the Bsp-1 epitope, and other surface proteins (2–5). In terms of granule constituents, human MCs express different combinations of carboxypeptidase A (CPA) (6–8), chymase (Chy) (9–12), and at least four homologous tryptases (Trys) (designated Try I, II/β, III, and α) (13–18). In contrast, basophils isolated from normal individuals have negligible amounts of Try and undetectable amounts of Chy (19) and CPA protein (20). Moreover, as assessed by quantitative RT-PCR technology, these basophils contain only a very small amount of Try α mRNA (21).

Mirza et al. (22) reported that recombinant human Try α is functionally active when transiently expressed in COS cells. Nevertheless, Sakai et al. (23) concluded, based on their inability to obtain functional enzyme in a baculovirus/insect cell expression system, that human Try α zymogen probably is not converted into functionally active protease in MCs. Because human Try α mRNA is the only Try transcript detected in the limited number of normal peripheral blood basophils that have been examined (21), it has been suggested that basophils would be unable to posttranslationally convert the expressed zymogen into active enzyme even if these cells contained abundant levels of the Try α transcript. Inasmuch as MC proteases regulate numerous immunologic and biochemical pathways, it is advantageous that these granule proteases be expressed only in the metachromatic, histamine-containing, IgE-bearing cells that reside in tissues.

Because basophils and MCs isolated from normal people differ from each other in their primary location in the body, their ultrastructure, and their expression of various cell surface and granule proteins, it has been assumed that the two populations of cells are developmentally unrelated. Nevertheless, the finding that MCs can reversibly alter their expression of granule proteases (24–28) and proteoglycans (29–32) in vivo and in vitro raised the possibility that in certain instances human peripheral blood basophils might express some of the granule proteases generally found in tissue MCs. We now demonstrate that many of the cells considered to be basophils in the peripheral blood of patients with asthma, allergy, or an allergic drug reaction contain substantial amounts of Chy, Try, and/or CPA in their secretory granules. These findings have...
broad implications for our understanding of the regulation and function of granule proteases in the circulating FcεRI-bearing, histamine-containing metachromatic cells found in these three patient groups.

Materials and Methods

Patient groups and cell preparations

The clinical characteristics of the individuals studied are presented in Tables I and II. Of the 16 patients studied, 3 exhibited the clinical features of allergy, namely allergic rhinitis, urticaria, angioedema, and/or eczema; 5 had asthma; and 8 had experienced an allergic reaction to either allopurinol (All), isoniazid (Iso), amlopidine (Aml), penicillin (Pen), or ceftriaxone (Cef). Nine normal laboratory workers served as the control population. Peripheral blood was collected from all individuals. In the drug-reactive group (Table II), blood was obtained during the acute phase in four patients and the convalescent phase in four patients. All of the asthmatics were stable, and no blood was taken during an acute attack. The blood was collected in heparin-treated tubes to prevent coagulation, diluted 1:1 in Dulbecco’s PBS, and then layered on top of an equal volume of Histopaque-1077 (Sigma, St. Louis, MO). After a 30-min centrifugation at 400 × g, the fraction containing the metachromatic cells was washed and analyzed as described below.

Histochemistry, enzyme cytochemistry, and immunohistochemistry

Generally, replicate samples of the leukocytes obtained by the above density-gradient procedure were cytocentrifuged onto glass slides and air dried for histochemistry, enzyme cytochemistry, and immunohistochemistry. To identify the metachromatic cells in the preparations, the slides were incubated with 0.5% toluidine blue in 0.6 M HCl for 5 min. A modification (33) of the chloroacetate esterase procedure of Leder (34) was used, as described in an immunohistochemical evaluation of mouse MCs (26, 27), in some instances the Ab-treated slides were washed, placed in buffer containing biotin-labeled goat anti-mouse or anti-rabbit IgG, washed, placed in buffer containing Vectastain avidin biotin complex–alkaline phosphatase (AP) reagent (Vector Laboratories, Burlingame, CA), and finally placed in a buffer containing the AP substrate. In one instance, a pellet of cells derived from a patient with asthma was fixed in 4% paraformaldehyde for histochemical, enzyme cytochemical, and immunohistochemical analyses of serial sections.

For immunohistochemistry conducted on the same slide with two different Abs, slides were incubated with anti-Try IgG or anti-Chy IgG followed by either Bsp-1 IgM or anti-c-kit IgG. In these experiments, slides were exposed to the first Ab (0.5 μg/ml) for 30 min at room temperature, incubated for 30 min at room temperature in a solution containing a 1/50 dilution of rabbit anti-mouse IgG (Dakopatts, Glostrup, Denmark), exposed to AP anti-AP complex (1/50 dilution, Dakopatts), and then developed for 20 min in a 0.2 mg/ml solution of naphthol 3-hydroxy-2-naphthonic acid 2,4-dimethylamine solution (Pharmacia) containing 0.1 mg/ml Fast Red 4-chloro-4-dimethylaminobenzene diazonium (Sigma) in 100 mM Tris-HCl buffer (pH 8.2). Subsequently, cells were incubated either with mouse anti-Bsp-1 IgM followed by rabbit anti-mouse IgM conjugated with HRP (Silenus) or with anti-c-kit IgG followed by anti-rabbit IgG conjugated with biotin and streptavidin–HRP (Silenus). The slides were then treated with a freshly prepared solution of 10 mg of 3,3′-diaminobenzidine tetrahydrochloride in 10 ml of 100 mM Tris-HCl buffer (pH 7.6) containing 0.03% hydrogen peroxide, washed, and counterstained with hematoxylin (Sigma). In these double immunohistochemical reactions, cells that express Try or Chy are stained red, cells that express Bsp-1 or c-kit are stained yellow-brown, and cells that express a granule protease and one of the studied cell surface proteins are multicolored.

In situ hybridization

In situ hybridization was conducted using a 40-mer antisense oligonucleotide (5′-ACATTTCCCAAGGGGCAAAAAATGTTAGTGATGGGAA-3′) that corresponds to residues 406 to 445 in the human Chy transcript (10) and a 40-mer oligonucleotide (5′-GTTTTGGCATATGGGGACCTTCACCTGCTTCAGAGGAAAT-3′) that corresponds to residues 521 to 560 in the four known human Try transcripts (15–17). The more specific 20-mer oligonucleotide (5′-ATCGGCTCTAGCTGATGTA-3′) (15) was used to evaluate whether the metachromat-like cells in the peripheral blood of the three patients contained Try mRNA, whereas the 20-mer oligonucleotide (5′-GTCGCCCTGATCTGCGCGCG-3′) (16, 17) was used to determine whether or not these cells contained Try I, II, or III mRNA. The oligonucleotides were labeled with digoxigenin-AP using an oligonucleotide 3′ end labeling kit from Boehringer Mannheim (Indianapolis, IN). For control, each probe was transferred to a nitrocellulose membrane and incubated with an AP-conjugated polyclonal Ab raised against bovine pancreatic CPA (Boehringer Mannheim). The reaction product was visualized with nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate.

After centrifugation of the leukocytes onto slides, the cells were rehydrated in 50 mM Tris-HCl-buffered saline (pH 7.4), fixed in 4% paraformaldehyde for 20 min at room temperature, washed, and incubated for 45 min at 37°C with anti-Bsp-1 IgM. The slides were then rinsed, incubated for 45 min with HRP-labeled rabbit anti-mouse IgM, and treated with a freshly prepared solution of 50 mM Tris-HCl (pH 7.6) containing 1 mg/ml of 3,3′-diaminobenzidine tetrahydrochloride (Dakopatts). Bsp-1+ cells were identified by their developed brown color. After immunohistochemical staining, the cells on the slides were washed twice in PBS and peroxidized with proteinase K (1 mg/ml). Postfixation was performed in 4% paraformaldehyde/PBS for 10 min to destroy any residual proteinase K activity. After the slides had been washed with PBS twice and 0.5 mM of MgCl2/PBS once, they were prehybridized with a buffer consisting of 50% formamide, 4× SSC, Denhardt’s solution, salmon sperm DNA, and 10% dextran sulfate at 42°C for 30 min. The prehybridization solution was replaced by an amount of digoxigenin-labeled oligonucleotide in 50 ml of (2.5 μg/ml in PBS, pH 7.6) for 1 h at room temperature. The stained slides were washed, incubated with anti-rabbit Ig labeled with horseradish peroxidase (HRP) (Silenus, Hawthorn, Australia), and subsequently incubated with the diaminobenzidine substrate. The slides were viewed with a Leica microscope (Wetzlar, Germany). Bsp-1 IgM (2), anti-Try IgG (Chemicon, Temecula, CA), anti-Chy IgG (Chemicon), and anti-pancreatic CPA IgG (Sigma) were resuspended in PBS containing 1% BSA and used in immunohistochemical studies. As described in an immunohistochemical evaluation of mouse MCs (26, 27), in some instances the Ab-treated slides were washed, placed in buffer containing biotin-labeled goat anti-mouse or anti-rabbit IgG, washed, placed in buffer containing Vectastain avidin biotin complex–alkaline phosphatase (AP) reagent (Vector Laboratories, Burlingame, CA), and finally placed in a buffer containing the AP substrate. In one instance, a pellet of cells derived from a patient with asthma was fixed in 4% paraformaldehyde for histochemical, enzyme cytochemical, and immunohistochemical analyses of serial sections.

Table I. Clinical characteristics of the 25 individuals studied

<table>
<thead>
<tr>
<th>Number of Individuals</th>
<th>Age (yr) (mean ± SD)</th>
<th>Clinical Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>33 ± 3</td>
<td>Control group</td>
</tr>
<tr>
<td>3</td>
<td>37 ± 15</td>
<td>Allergy (allergic rhinitis (n = 2), urticaria (n = 1), eczema (n = 1), cutaneous angioedema (n = 2))</td>
</tr>
<tr>
<td>5</td>
<td>36 ± 21</td>
<td>Asthma (2 patients also had allergic rhinitis)</td>
</tr>
<tr>
<td>8</td>
<td>59 ± 22</td>
<td>Allergic drug reaction</td>
</tr>
</tbody>
</table>

* Many of the allergy and asthma patients exhibited heterogenous clinical manifestations.

Table II. Clinical characteristics of the eight individual patients having an allergic drug reaction

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Drug</th>
<th>Clinical Manifestation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>57</td>
<td>Allopurinol</td>
<td>Erythematous rash, lymphadenopathy, fever, eosinophilia, angioedema</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>69</td>
<td>Allopurinol</td>
<td>Exfoliative dermatitis, eosinophilia, fever</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>90</td>
<td>Isoniazid</td>
<td>Fever, erythematous rash (isoniazid prophylaxis while on steroids)</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>64</td>
<td>Amlopidine</td>
<td>Angioedema, erythema multiforme, fever</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>72</td>
<td>Amlopidine</td>
<td>Palpable purpura, eosinophilia, fever</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>66</td>
<td>Ceftriaxone</td>
<td>Fever, eosinophilia</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>23</td>
<td>Ceftriaxone</td>
<td>Fever, eosinophilia</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>30</td>
<td>Penicillin</td>
<td>Fever, rash, eosinophilia</td>
</tr>
</tbody>
</table>

* Blood was taken during the acute phase in patients 1 to 4 and during the convalescent phase in patients 5 to 8.

4 Although the overall amino acid sequences of the MC and pancreatic exopeptidases are only ~50% identical, many regions of five residues or greater in their sequences are 100% identical. Thus, rabbit polyclonal Abs raised against bovine pancreatic CPA will weakly recognize the MC homolog in immunohistochemical assays.
hybridization buffer. Slides were hybridized overnight at 42°C. The next morning, they were washed twice with 2× SSC and once with 1× SSC at room temperature, incubated in blocking solution (Boehringer Mannheim) for 30 min, and then incubated with anti-digoxigenin-AP Ab complex for 30 min at room temperature. The slides were washed twice in Tris-HCl-buffered saline and equilibration buffer (100 mM Tris-HCl (pH 9.5), 100 mM NaCl, and 50 mM MgCl2). After they were exposed to nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate, color development was performed according to the manufacturer’s instructions. The reactions were stopped by washing each slide with 10 mM Tris-HCl (pH 8.2). The resulting slides were mounted with a glycerol-gelatin solution (Sigma) and viewed with a Leica microscope. MCs derived in vitro from progenitors in umbilical cord blood (35, 36) and MC-containing sections of skin from normal individuals were used as positive controls for these Try and Chy mRNA analyses. Both peripheral blood leukocytes incubated with a non-labeled probe and human erythroleukemia cell line TIB-180 (American Type Culture Collection, Manassas, VA) incubated with the labeled probes were used as negative controls.

FIGURE 1. Identification of metachromatic cells that differ in their protease expression in the peripheral blood of a patient with asthma. A pellet of peripheral blood leukocytes was fixed, and serial sections were analyzed for metachromatic cells (a) that express Chy (b). Nearly every metachromatic cell (solid arrows) in the peripheral blood of this individual contains immunoreactive Chy. A second group of serial sections from the same cell pellet was analyzed for cells that expressed Try (c) and Chy (d). The solid arrows in c and d indicate cells that express both proteases; the open arrows in d indicate cells that express Chy but not Try.

FIGURE 2. Identification of cells having at least one MC protease in the peripheral blood of three patients who have experienced an allergic drug reaction. Representative cells in peripheral blood smears that contain immunoreactive Try (a and d), Chy (b and e), and/or CPA (d and e) are shown. The cell depicted in c contains large amounts of a functional Chy due to its ability to cleave the chloroacetate esterase substrate. The cells depicted in a, b, and c were obtained from the same patient. The cells depicted in d and e are from two different patients. A double immunohistochemical approach was used in d and e to show a cell in the peripheral blood of a patient with asthma that expresses both CPA and Try (d) and another cell that expresses both CPA and Chy (e). The brown color in this double immunohistochemical approach indicates the presence of immunoreactive CPA; the red color indicates the presence of immunoreactive Chy or Try.
the number of cells per 1000 leukocytes (mean groups generally were small in size (12–15 chromatic cells in the three groups of patients contained segment-Although some mononuclear cells were present, most of the metachromatic substrate (Fig. 2) indicated that the Chy was enzymatically active.

in their granules that readily cleaved the chloroacetate esterase

Ab indicated that Try, Chy, and CPA preferentially reside in the

least one MC granule protease were readily found in the peripheral

drug reaction (Fig. 2). The staining pattern with the anti-protease

ever, metachromatic cells containing substantial amounts of at

metachromatic cells in one patient with asthma and two with an allergic

taining detectable amounts of Try, Chy, or CPA were not found in

When assessed immunohistochemically, metachromatic cells con-

Immunohistochemistry

Results

When assessed immunohistochemically, metachromatic cells con-

When either the double-staining approach or the serial section

FIGURE 3. Quantitation of cells in the peripheral blood of the individ-

FIGURE 4. Quantitation of cells containing immunoreactive Try or

2.3 ± 1.2% (mean ± SD, n = 8) of the total cells in this leukocyte fraction isolated from patients with allergy, asthma, or an allergic drug reaction, respectively. Try\(^+\) cells represented 0.5 ± 0.3% (mean ± SD, n = 3), 0.94 ± 0.48% (mean ± SD, n = 5), and 1.1 ± 0.8% (mean ± SD, n = 8) of the total cells in the peripheral blood of patients with allergy, asthma, and an allergic drug reaction, respectively (Fig. 3a). Bsp-1\(^+\) cells that lacked Try were found in the three patient groups, as were a few Try\(^+\) cells that lacked Bsp-1. However, many of the Try\(^+\) cells in the patients expressed the Bsp-1 epitope. The Chy\(^+\) cells in these three groups represented 0.07 ± 0.09%, 0.14 ± 0.30%, and 1.2 ± 1.7% of the total cells in the preparations, respectively (Fig. 3b). Although some of the Chy\(^+\) cells in the drug-reactive group were Bsp-1\(^-\), most of the Chy\(^+\) cells in the three patient groups expressed the Bsp-1 epitope.

Relative to the metachromatic cells in the peripheral blood of the patients with allergy or asthma, the metachromatic cells present in the drug-reactive patients were more heterogenous in terms of their Try and Chy expression (Fig. 4). Four of the patients who had an allergic reaction to Iso, Aml, or Cef contained 5- to 10-fold more Try\(^+\) cells in their peripheral blood than Chy\(^+\) cells. In contrast, four of the patients who had an allergic reaction to All, Pen, and Cef contained 1.3- to 2.2-fold more Chy\(^+\) cells than Try\(^+\) cells. Cells that expressed more than one protease were most often found in the patients with asthma (Fig. 3) or in the patients with an allergic drug reaction (Fig. 2).

Metachromatic cells in the peripheral blood of normal individuals contained negligible amounts of c-kit on their surfaces. However, 0.28 ± 0.43% (mean ± SD, n = 3), 0.60 ± 0.79% (mean ± SD, n = 5) and 1.1 ± 1.1% (mean ± SD, n = 8) of the cells in the peripheral blood of the patients with allergy, asthma, and an allergic drug reaction, respectively, expressed c-kit. A tremendous variation in c-kit expression (range: undetectable to 3%) was noted in those patients who had an allergic drug reaction. Thus, c-kit is expressed on more than one cell type in these patients. Metachromatic cells in one patient with asthma and two with an allergic drug reaction were examined with the double-staining approach for their expression of c-kit and either Try or Chy. All possible combinations of cells were found in the three patient groups (Fig. 5). Although some Try\(^+\) cells failed to express c-kit and some c-kit\(^+\) cells failed to express Try, many of the peripheral blood...
cells in the three patient groups expressed both. The same pattern held true for Chy and c-kit expression.

In situ hybridization

Metachromatic cells in the peripheral blood of normal individuals (n = 2) and of patients with asthma (n = 4), allergy (n = 2), or an allergic drug reaction (n = 3) were evaluated by in situ hybridization for their expression of Try mRNA (Fig. 6a) and Chy (Fig. 6b) mRNA to confirm and extend the immunohistochemistry studies. Quantitative analysis of three of the drug-reactive patients revealed that 25 ± 4% (mean ± SD) of their peripheral blood basophils that expressed Bsp-1 protein contained Chy mRNA, whereas 21 ± 20% (mean ± SD) of these cells contained Try mRNA. However, due to the problem of mRNA stability during sample preparation, it is likely that a higher percentage of the Bsp-1+ cells in the blood of these patients contain Try mRNA and/or Chy mRNA. To determine whether one Try was selectively expressed in these cells, more restrictive primers were used in subsequent experiments. At least two Try transcripts were found (Fig. 6, c and d). In control experiments, no Try transcript could be detected in HL-60 cells or in any of the basophils isolated from two normal patients (data not shown). In a third normal individual, only 3 of 1000 leukocytes contained Try α mRNA and only 1 of 1000 leukocytes contained Try I, II/β, and/or III mRNA. In contrast, in vitro-developed MCs and the MCs that reside in the skin of normal individuals contained Try and Chy mRNA (data not shown).

Discussion

We now report that many of the metachromatic cells in the peripheral blood of three allergic patient groups exhibit features of both normal MCs and basophils. The Bsp-1 Ab selectively recognizes basophils in normal individuals (2, 3), and the percentage of Bsp-1+ basophil-like cells in the leukocyte preparations from our control group is in the range reported previously by Bodger and coworkers. Increased numbers of basophils have been identified in the blood and sputum of some patients during acute asthma attacks (37). We also noted a modest increase in the number of metachromatic/Bsp-1+ cells in the peripheral blood of patients with allergic disease (Fig. 1); more importantly, approximately half of the Bsp-1+ cells expressed at least one granule protease previously thought to be selectively expressed by mature MCs (Figs. 1–6). Unlike the basophils in the peripheral blood of normal individuals, all populations of human MCs examined to date contain substantial numbers of c-kit on their surfaces. The observation that many of the Try+ and/or Chy+ cells in the peripheral blood of our patients also express c-kit (Fig. 3) confirms the unusual characteristics of these cells. The drug-reactive patients appear to be very different from the asthma and allergy patients, in that the former group tends to have acute disorders, whereas the latter groups tend to have chronic disorders. Nevertheless, because the number and phenotype of the metachromatic cells in the peripheral blood were not evaluated in a single patient at different time points over a 1-yr period, it is not known how long the unusual metachromatic cells reside in the blood of any patient.
Because peripheral blood basophils and tissue-localized MCs in normal humans are different morphologically, functionally, and biochemically, they have been considered to be developmentally unrelated. When human hemopoietic progenitors are cultured in the presence of IL-3 or T cell-conditioned medium, metachromatic cells are obtained that more closely resemble peripheral blood basophils than tissue MCs (38–42). Cells with eosinophil- and basophil-like granules have been identified in culture (43, 44) and in the bone marrow of patients with chronic myeloid leukemia (45). Thus, it has been concluded that human basophils are derived from the same population of hemopoietic progenitors that give rise to eosinophils. Trysa mRNA is present in the transformed Mono Mac 6 human monocytic cell line, Try1 mRNA is present in the transformed U-937 human monocytic line after exposure to PMA (46, 47), and CPA mRNA is present in the two transformed human myelomonocytic cell lines KG-1 and HL-60 (8). Based on these and other data (4, 5), it has been concluded that MCs are derived from CD34+ progenitors (48) that also can give rise to monocytes and macrophages.

Despite the above findings, cells with ultrastructural features of both normal peripheral blood basophils and tissue MCs have been found in the bone marrow and peripheral blood of patients with chronic myelogenous leukemia (49). Seldin et al. (50) noted that metachromatic cells possessing granules with structural features of both basophils and MCs can be obtained when human fetal liver progenitors are cultured for 3 wk in the presence of conditioned medium derived from a lectin-activated mouse T cell line. Although nuclear morphology has been a major criterion for assessing the lineage of metachromatic cells of hemopoietic origin, polynuclear cells that express c-kit and numerous MC granule proteases have been identified in the mouse (51). Moreover, Li and coworkers (35, 36) observed that a metachromatic/FceRI+/Try+/Chy+ population of cells resembling basophils in terms of nuclear morphology developed when human bone marrow cells from normal donors were cultured in the presence of c-kit ligand/stem cell factor and conditioned medium derived from the HBM-M cell line. Thus, the nuclear profile of a mouse or human metachromat cell does not, by itself, give insight about the type of progenitor cell from which it has developed.

The presence of metachromatic cells with features of both basophils (e.g., blood location, segmented nuclei, and Bsp-1 expression) and MCs (e.g., expression of c-kit, Try, Chy, and/or CPA) in the peripheral blood of all of our patient groups suggests that human MCs and basophils arise from the same progenitor. In the case of the patients who had an allergic drug reaction, it has been reported that the drugs induce complex side effects that are, in part, T cell-mediated (52, 53). Drug-specific CD4+ and CD8+ T cells, which also have the activation marker CD25, have been identified in the peripheral blood of these patients, and analysis of T cell clones from these individuals have revealed that the drugs do not induce the production of identical panels of cytokines in each patient. For example, T cell clones isolated from some patients with an allergic drug reaction preferentially express large amounts of IL-4 and IL-5, whereas those isolated from other patients preferentially express large amounts of IL-2, IFN-γ, and TNF-α (53). Thus, if the phenotype of a basophil is not fixed but rather depends upon the differentiation- and maturation-enhancing factors with which it comes in contact in its microenvironment, the failure of most peripheral blood basophils in normal individuals to express high steady-state levels of c-kit, Try, Chy, and/or CPA mRNA and protein simply may be a consequence of that cell failing to localize in a tissue site containing the appropriate combination of regulatory factors. In the case of our patients, there could be an aberrant production of certain cytokines in the bone marrow and/or peripheral blood that induce the circulating basophil to increase its surface expression of c-kit and its expression of those proteases normally found in the granules of mature MCs. Thus, the individual variations in Try and Chy expression in the metachromatic cells of our patients may reflect different exposures to cytokines originating from activated T cells.

The metachromatic cells in the blood of our patients appear to be immature relative to tissue-localized MCs in that they do not have much cytoplasm and do not contain large granules. Although most MC-committed progenitors in the adult mouse originate from the bone marrow (54), certain tissue sites in this species constitutively have large numbers of poorly granulated MC-committed progenitors (55). Thus, we presently cannot rule out the possibility that the metachromatic cells found in the blood of our patients are predominately immature MC-committed progenitors that left the bone marrow, skin, or another connective tissue site. It is unlikely that these cells are derived from mature mononuclear MCs, because they would have had to degranulate and/or metabolize most of their granules, undergo nuclear segmentation, remove most of their cytoplasm, and up-regulate their expression of the Bsp-1 epitope during their transient movement from a tissue site into the circulation.

Although the identification of metachromatic cells in the peripheral blood of patients that contain immunoreactive Try, Chy, and CPA is relevant to the understanding of the development and fate of MCs and basophils in humans, the findings are even more relevant clinically. The numbers of circulating basophils are increased in patients with asthma, and the level of immunoreactive Try has been used to assess the degree of MC activation (56–58). Increased amounts of immunoreactive Try have been detected in the blood of patients undergoing allergic reactions, some of which are drug-mediated (59). Nevertheless, normal basophils have very little, if any, Try, Chy, and CPA in their granules (19–21). Thus, it was concluded that the immunoreactive Try in the blood of these patients probably originated from tissue-localized MCs that had degranulated. Because it was assumed that the circulating Try was not stored in a protected state in the granules of a metachromatic cell in the circulation, the functional significance of the previous observations was not apparent. We now report that levels of Try, Chy, and CPA are all increased in the blood of allergy, asthma, and drug-reactive patients. However, the more clinically important observation is that these neutral proteases are enzymatically active because they are sequestered in the granules of the circulating metachromatic cells.

Based on in vitro studies, human MC Trys can degrade and/or activate receptors on the surfaces of cells (60) and can cleave a large number of circulating proteins and/or biologically active peptides (14, 61–67). It is now apparent that human MCs express a large number of homologous Trys (15-18). The observation that the two mouse Trys cleave very different peptide sequences (68, 69) suggests that the specific substrates cleaved by a human MC or basophil probably depend upon the combination of the individual Trys that are in the analyzed preparation. In situ hybridization studies conducted with different primer sets indicate that the metachromatic cells in the peripheral blood of our patients express more than one Try. This finding raises the possibility that the exocyted Trys from these cells can exert multiple and diverse effects on the body.

Many of the metachromatic cells in the blood of our patients contain immunoreactive Chy. Because these metachromatic cells readily cleave a Chy-susceptible substrate, the immunoreactive Chy is functionally active. Although a large number of substrates have been reported to be cleaved in vitro by MC Chy purified from...
different species, it appears that one of the major functions of human MC Chy is the proteolytic conversion of the inactive, 10-residue peptide angiostatin I to the 8-residue hypertensive factor angiostatin II (11). Thus, the release of this Chy from the circulating metachromatic cells could indirectly affect various cell types via the G protein-coupled receptors AT1, AT1b, and/or AT2. The presence in the circulation of cells that contain substantial amounts of biologically active MC proteases in their granules consequently has important biologic implications in the regulation of homeostasis. In addition, the aberrant expression of Try, Chy, and CPA by these cells also may be a factor in some of the clinical manifestations seen in these patients.

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