Characterization of mast-cell tryptase–expressing peripheral blood cells as basophils

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Background: Mast-cell tryptase is a protease with proinflammatory activity, the expression of which by peripheral blood leukocytes (PBLs) has not been fully characterized.
Objective: We examined tryptase expression in human PBLs to further characterize this tryptase-expressing cell population for lineage and disease association.
Methods: PBLs were fixed, permeabilized, stained with antibodies to tryptase and a panel of mast cell– and basophil-specific markers, and analyzed by means of flow cytometry.
Results: Tryptase expression was restricted to a population of cells that stained positive for IgE and negative for the panel of lineage markers (IgE$^+$, lin$^-$). This IgE$^+$, lin$^-$ population did not stain for the mast-cell markers Kit or chymase but did stain for the basophil-specific granule proteins recognized by the 2D7 and BB1 mAbs. Per-cell tryptase expression demonstrated a greater than 100-fold range of expression among donors but did not correlate with disease status (asthma or mastocytosis), FEV_1, or serum tryptase concentration. Tryptase was released by purified basophils after anti-IgE activation.
Conclusions: The phenotype of tryptase-expressing PBLs and their lack of increase in patients with mastocytosis demonstrates that these cells are basophils. Per-cell basophil tryptase expression is highly variable between donors, with some donors expressing levels approaching those of mast cells. As such, anti-tryptase antibodies cannot be used to distinguish these 2 cell types from one another by means of flow cytometry. These results demonstrate that tryptase represents an additional mediator through which basophils may contribute to allergic inflammation. (J Allergy Clin Immunol 2002;109:287-93.)

Key words: *Basophil, mast cell, tryptase, human, asthma, mastocytosis, IgE, flow cytometry, FACS, Kit, 2D7, IgE, BB-1*

Basophils are peripheral blood leukocytes (PBLs) that express FcεRI, are activated by cross-linking of IgE, and release a spectrum of mediators that have been implicated in the pathogenesis of allergic diseases, including asthma. Basophils are potent producers of both IL-4 and IL-13 and are one of the major sources of these cytokines after allergen activation.[1,2] Additionally, basophils traffic to sites of late-phase allergic inflammation in the lung and skin.[3-5] In sum, these data suggest that basophils play a role in the pathogenesis of allergic disease and asthma.[6,7]

Abbreviations used
IgE$^+$, lin$^-$: Cells staining positive for IgE and negative for a panel of lineage markers
MEAPC: Molecules of equivalent allophycocyanin
PBL: Peripheral blood leukocyte
PBS-S: 0.1% Saponin in PBS
PE/Cy5: Phycoerythrin/cyananin 5

Mast-cell tryptases are trypsin-like serine proteases found in mast-cell granules and have also been reported to be expressed by peripheral blood basophils, albeit at levels less than 1% of those found in mast cells.[8] Several lines of investigation suggest that tryptase may play a role in allergic disease and asthma. Tryptase levels are increased in the bronchoalveolar lavage fluid from asthmatic subjects.[9,10] Human tryptase has been shown to cause inflammatory-cell trafficking[11] and to stimulate collagen synthesis and fibroblast proliferation.[12,13]

Tryptase expression in peripheral blood cells has been reported to be limited to the basophilic leukocyte population.[8] However, that understanding has become less clear with the description of a mast cell-basophil hybrid with characteristics of both mast cells (tryptase, chymase, carboxypeptidase, and Kit expression) and basophils (Bsp-1 expression and peripheral blood location).[14,15]

To identify the peripheral blood cell population expressing tryptase and to determine its lineage association, we used intracellular staining and flow cytometry. As shown within, tryptase expression was restricted to a subset of IgE$^+$, lineage-negative (IgE$^+$, lin$^-$; CD2, CD14, CD16, and CD19) cells that were determined to be basophils. Basophil tryptase expression was highly variable between donors, with some donors expressing levels of tryptase approaching that found in human mast cells. These data demonstrate that tryptase expression is another means whereby basophils may contribute to allergic inflammation.

METHODS

Antibodies

Anti-CD2 FITC and phycoerythrin/cyananin-5 (PE/Cy5); anti-CD3 FITC; anti-CD19 FITC and PE/Cy5; anti-IL-3R/CD123 PE; mouse IgG1 isotype control unlabeled, biotin, PE, and streptavidin-

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allophycocyanin were obtained from PharMingen (San Diego, Calif). Anti-CD14 FITC (Immunotech, Marseille, France); anti-CD14 PE/Cy5; anti-CD16 FITC and PE/Cy5; goat anti-human IgE (Caltag, Burlingame, Calif); chimeric human IgE anti-nitrophenol (Serotec, Raleigh, NC); anti-IgE FITC and biotin (BioSource, Camarillo, Calif); anti-chymase; anti-tryptase (clone G3; Chemicon, Temecula, Calif); anti-CD117 (clone 104D2; Becton-Dickinson Biosciences, San Jose, Calif); polyclonal rabbit anti-Kit (Calbiochem, La Jolla, Calif); goat anti-mouse IgG PE; goat anti-mouse IgG1 PE/Cy5 (Caltag); goat-anti-rabbit PE (Southern Biotechnology Associates, Birmingham, Ala); and normal rabbit IgG (R&D Systems, Minneapolis, Minn) were also obtained. Anti-tryptase (clone G3) biotin[16] and the basophil granule–specific mAb 2D7[17] were derived in house. BB1 was a gift from Dr Andrew Walls (Southampton General Hospital, Southampton, United Kingdom).[18] Anti-IgE PE was a gift from PharMingen Corp.

Reagents

Normal mouse serum (Caltag), saponin (Fluka, Ronkonkoma, NY), paraformaldehyde, dimethyl sulfoxide, collagenase (Sigma, St. Louis, MO), ionomycin, deoxyribonuclease I (Calbiochem), and Sphero Rainbow Calibration Beads (PharMingen) were obtained commercially.

Cells and donors

The National Institute of Allergy and Infectious Diseases Institutional Review Board approved the clinical protocol and use of human tissues in this study. All subjects provided written informed consent. Healthy control subjects had no history of allergic disease or asthma and no more than one immediate-type skin test response of 2 mm induration or less (one healthy control subject had a single skin test response of 5 mm induration). Asthmatic subjects were defined by using American Thoracic Society criteria[19] and had 3 or more positive skin test responses (3-mm induration or larger). PBLs were isolated from EDTA-anticoagulated blood by means of density gradient separation with Histopaque-1083 (Sigma), fixed in 4% paraformaldehyde for 5 minutes at 37°C, washed, and cryopreserved according to previously published methods.[2]

Human lungs, spleens, and livers were obtained from the National Disease Research Interchange (Philadelphia, Pa). Tonsils were obtained as a byproduct of medically indicated tonsillectomies performed at the National Naval Medical Center (Bethesda, Md). The tissue was first cut into 1- to 2-mm^2 pieces and then forced through a wire mesh. Cells were treated with collagenase (50 U/mL) and deoxyribonuclease I (2.5×10^3 Dornase Units/mL) in a shaking water bath for 30 minutes at 37°C, filtered through a 100-μm mesh, fixed, washed, and cryopreserved.

Intracellular staining

Slightly different staining schemes, following previously described procedures,[2,20,21] were used in each of the 3- and 4-color flow cytometric experiments; however, the following example of 4-color staining for tryptase, 2D7, IgE, and lineage markers illustrates the methods used. Cryopreserved fixed cells were thawed, washed in PBS with 0.1% saponin (PBS-S), and then blocked in PBS-S/5% nonfat milk powder[21] for 1 hour on ice. Cells were incubated with 2D7 mAb or mouse IgG1 isotype control for 30 minutes, washed twice, incubated with goat anti-mouse IgG PE for 30 minutes, and again washed. Cells were then incubated with 2% normal mouse serum to block further goat anti-mouse binding. Cells were then incubated with lineage-specific antibodies (CD2, CD14, CD16, and CD19 PE/Cy5), anti-IgE FITC, and anti-tryptase biotin or mouse IgG1 biotin isotype control. The cells were then washed, incubated with streptavidin-allophycocyanin in PBS-S, and washed twice.

Extracellular tryptase staining

PBLs without fixation or permeabilization were incubated with the lineage-specific panel, anti-IgE FITC, and anti-tryptase biotin or mouse IgG1 isotype control for 30 minutes at 4°C. Cells were then washed in PBS-BSA, stained with streptavidin-allophycocyanin, washed, and analyzed.

Flow cytometry

Data were acquired with a 2-laser, 4-parameter FACSCalibur flow cytometer (Becton-Dickinson Biosciences) and analyzed on CellQuest software (Becton-Dickinson Biosciences). Typically, 300,000 to 600,000 total events were acquired to obtain adequate numbers of basophils (2000-5000).

Quantitation of tryptase expression by means of flow cytometry

Tryptase expression was quantitated by using one of 2 methods: percentage of positive staining or expression of molecules of equivalent allophycocyanin (MEAPC) staining. For percentage of positive tryptase staining, statistical markers were placed on the basis of the corresponding isotype-matched control. The percentage of positive values were then corrected for nonspecific binding by subtracting out the isotype control value. Tryptase MEAPC value were calculated by generating a standard curve of the geometric mean versus MEAPC value for each experiment by using the calibration beads.[22,23]

Basophil purification

PBLs were isolated as described above, and basophils were purified with the MACS Basophil Isolation Kit (Miltenyi Biotec, Auburn, Calif) according to the manufacturer's instructions. These resulting basophil populations had a purity of 27% to 88% (mean, 67%).

Basophil stimulation

Purified basophils were suspended in RPMI supplemented with 10% FCS, 4 mmol/L glutamine, 100 U/mL penicillin, 100 U/mL streptomycin, 50 μmol/L 2-mercaptoethanol, 1 mmol/L sodium pyruvate, and nonessential amino acids (Biofluids, Rockville, Md) at a density of 1×10^6 cells/mL. Basophils were stimulated as noted with either 1 μmol/L ionomycin or goat anti-human IgE for 1 hour at 37°C. The supernatant was then collected, placed in an aliquot, and stored at –80°C.

Mediator release, tryptase expression, and serum tryptase levels

Histamine release was determined by using a competitive ELISA (Immunotech), according to the manufacturer's instructions. Tryptase levels in basophil pellets, cell-culture supernatant, and serum were determined by means of ELISA, as described previously.[24] Tryptase and histamine release were determined as the percentage relative to total tryptase obtained from lysed basophils.

Statistical analysis

The Mann-Whitney *U* test was performed to compare differences of tryptase expression between healthy donors and donors with asthma or mastocytosis. A *P* value of less than .05 was considered significant. The Spearman rank correlation test was used to evaluate correlative data.

RESULTS

Identification and characterization of tryptase-expressing PBLs

To determine whether we could detect tryptase-expressing cell populations in peripheral blood, we

examined the tryptase staining of PBLs by using flow cytometry and intracellular staining from 53 asthmatic and healthy control subjects. In this ungated population tryptase was exclusively expressed by IgE bright cells (Fig 1, *A*), suggesting that basophils or some other FcεRI-bearing cells were the source of tryptase.

We then used a previously validated approach to unambiguously identify basophils and allow their study by using flow cytometry.[2,25,26] Basophils were selected by gating on the cell population staining brightly for IgE (IgE^+) and staining dimly for a panel of lineage markers (lin^-: CD2, CD14, CD16, and CD19; Fig 1, *C*). Using this approach, we next examined basophil tryptase in a group of 17 allergic asthmatic and 7 nonatopic nonasthmatic control subjects. A wide range of basophil tryptase expression was found, ranging from 5.14% to 98.2% tryptase-positive cells (median, 42.1%; mean, 48.3%). Three representative subjects demonstrating low, intermediate, and high tryptase expression are shown (Fig 1, *D-F*). No tryptase-staining cells were noted outside the IgE^+, lin^- subset, further validating this approach.

To exclude the possibility of passively acquired surface-bound tryptase being responsible for the above results, we simultaneously examined basophil tryptase expression in permeabilized (Fig 1, *G*) and unpermeabilized (Fig 1, *H*) samples from 3 tryptase-positive subjects. These results clearly demonstrate that the tryptase detected by means of flow cytometry was intracellular. In sum, these results demonstrate that tryptase expression in peripheral blood is localized to an IgE^+, lin^- subset, presumably a basophil or basophil-like cell.

To further characterize the cell lineage of this tryptase-positive PBL, we next examined Kit and chymase expression. We could not detect IgE^+, lin^- cells that were positive for Kit with either a monoclonal anti-CD117 antibody (Fig 2, *A*; median, 0.019%; mean, 0.03%; range, 0%-0.22%; n = 27) or a polyclonal anti-Kit antibody (data not shown). Furthermore, in donors with intermediate to high levels of tryptase-positive PBLs, no greater numbers of Kit-positive cells were found within the IgE^+, lin^- or tryptase-positive populations (data not shown). No substantial chymase staining was found in the IgE^+, lin^- subpopulation (Fig 2, *E*; mean, 0.044%; median, 0.0002%; range, –0.067%-0.96%; n = 31). In contrast, IgE^+, lin^- tonsillar mast cells (n = 3) stained positive for both Kit (Fig 2, *B*; median, 48.3%; mean, 53.4%) and chymase (Fig 2, *F*; median, 28.6%; mean, 25.5%). These results demonstrate that tryptase-positive PBLs do not express the mast-cell markers Kit and chymase.

To confirm that these tryptase-positive PBLs were indeed basophils, we examined the expression of the basophil-specific markers 2D7[17] and BB1.[18] Almost all of the IgE^+, lin^- cells (median, 96.3%; mean, 90.6%; range, 40.3%-99.9%; n = 20 [allergic asthmatic, n = 13; healthy, n = 5; mastocytosis, n = 2]) and tryptase-expressing cells (median, 91.7%; mean, 84.7%; range, 31.1%-97.3%; n = 20) were also positive for 2D7 (Fig 2, *C*). Additionally, in a subset of 7 donors, the majority of IgE^+, lin^- cells stained positively for BB1 (median, 75.2%; mean, 73.0%; range, 51.8%-92.3%). In contrast, mast cells from human lung (Fig 2, *D*; n = 2), bronchus (n = 1), and liver (n = 1) tissue did not stain for 2D7 (median, 4.1%; mean, 5.3%; range, 0.16%-12.6%) or BB1 (median, 1.4%; mean, 1.0%). These data reconfirm the specificity of these antibodies and demonstrate that tryptase-expressing PBLs have a phenotype consistent with a basophil lineage.

Tryptase expression in asthma and mastocytosis

Because increased numbers of tryptase-positive PBLs have been reported in the blood of subjects with allergic asthma,[14] we examined whether we could correlate the level of basophil tryptase expression with the presence of allergic asthma. Additionally, because systemic mastocytosis is associated with hyperplasia of both mast cells and mast-cell progenitors,[27] we hypothesized that if tryptase-positive PBLs represent a mast cell-basophil hybrid, their numbers should be elevated in subjects with this disease. Accordingly, healthy nonatopic control subjects (n = 22), allergic asthmatic subjects (n = 18), and subjects with mastocytosis (n = 22) were evaluated. As can be seen from Fig 3, *A*, there was a large range of percentages of tryptase-positive cells in each subject group. However, tryptase expression was not significantly different in either the asthmatic subjects ($P = .62$) or the subjects with mastocytosis ($P = .58$) when compared with that of healthy control subjects.

We next determined the tryptase MEAPC value to further quantitate relative tryptase expression.[22,23] The MEAPC value obtained is directly proportional to the amount of antigen present per cell. Using the MEAPC assay, we again found no difference in tryptase expression between subject groups (asthma vs healthy donors, $P = .45$; subjects with mastocytosis vs healthy donors, $P = .63$). As expected, mast cells obtained from tissue specimens demonstrated a high level of tryptase expression (Fig 3, *B*, right column). Strikingly, the per-cell tryptase expression from some donors approached that of mast cells. In sum, these results demonstrate that tryptase expression by peripheral blood cells is not correlated to the presence of allergic asthma or mastocytosis.

In asthmatic subjects, tryptase expression did not correlate with the severity of asthma, as measured by FEV_1 (n = 13; $r = 0.02$, Spearman rank correlation; FEV_1 range, 54%-112% predicted). Additionally, tryptase expression did not correlate with sex, age, or mastocytosis category (data not shown).

We next verified that intrasubject tryptase expression determined by means of the MEAPC assay was reproducible by repeated determinations of tryptase MEAPC values over a 6-month period in 9 subjects. The tryptase MEAPC values in these subjects ranged from 414 to 48,900, with a geometric mean of 2115. The intersubject SD was 15,700, whereas the geometric mean intrasubject SD was 871. These data demonstrate that individual subject's tryptase MEAPC values were reproducible over a 6-month time span.

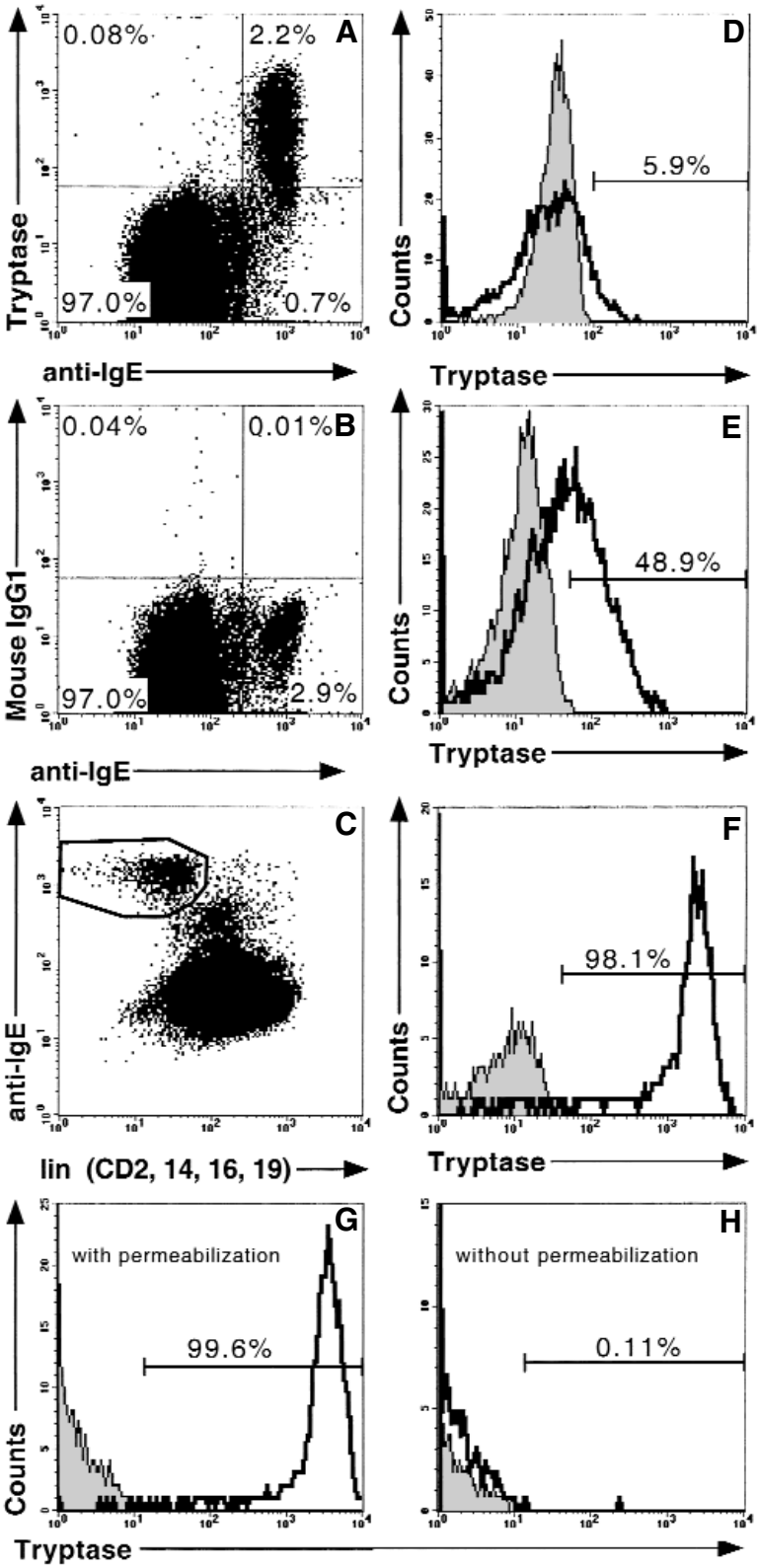

FIG 1. Tryptase expression by PBLs: a representative dot plot of ungated PBLs displaying anti-IgE staining versus tryptase staining **(A)** and the corresponding isotype control **(B)**. After gating on the IgE$^+$, lin$^-$ population **(C)**, histograms were examined for tryptase (*bold line*, **D-F**) versus the isotype-matched control (*shaded histogram*, **D-F**). Representative histograms from low **(D)**, intermediate **(E)**, and high **(F)** tryptase-expressing donors are shown. Tryptase staining of IgE$^+$, lin$^-$ PBLs with **(G)** or without **(H)** permeabilization is also shown.

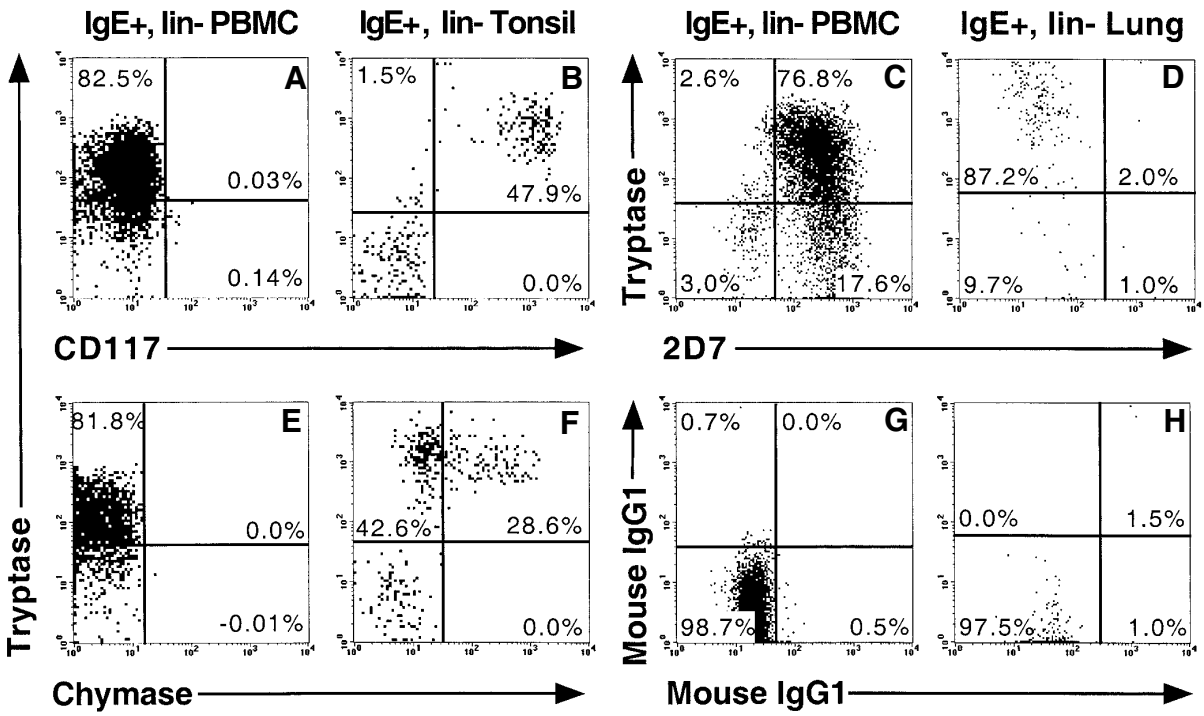

FIG 2. Expression of mast-cell and basophil markers by tryptase-positive PBLs. Dual tryptase and Kit (**A** and **B**) and dual tryptase and chymase staining (**E** and **F**) of IgE$^+$, lin$^-$ PBLs or tonsil cells, respectively, are shown. Quadrant markers were placed on the basis of corresponding isotype-matched controls. Dual 2D7 and tryptase staining of IgE$^+$, lin$^-$ PBLs **(C)**, as well as of human IgE$^+$, lin$^-$ lung mast cells **(D)**, along with the corresponding isotype-matched controls (**G** and **H**, respectively), are shown.

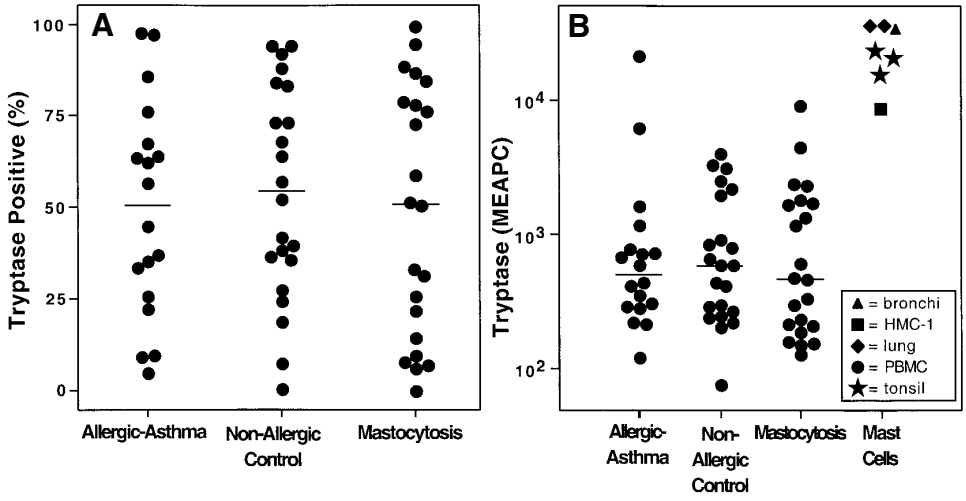

FIG 3. Basophil tryptase expression as a function of disease status. After gating on IgE$^+$, lin$^-$ basophils, tryptase expression was measured as follows: **A**, percentage of positive staining; **B**, tryptase MEAPC value. *Horizontal lines* denote median tryptase expression. Mast-cell tryptase MEAPC values are shown in **B**.

Low levels of tryptase are detectable in normal human serum; however, in subjects with mastocytosis, tryptase serum levels are elevated as a result of the large mast-cell burden present in the disease.[24,28] To determine whether basophil tryptase expression was similarly reflected in serum levels, we compared the basophil tryptase MEAPC value with serum tryptase levels, to which there was no significant correlation (n = 11, $r = 0.01$).

To address a previous report demonstrating IgE$^+$, tryptase-positive PBLs expressing CD4,[15] we performed simultaneous tryptase and CD4 staining. CD4 staining of IgE$^+$, lin$^-$ basophils was low, with 96.8% of donors expressing a CD4 mean fluorescence intensity of 2-fold or lower than that of the isotype control (n = 62). There was no correlation between CD4 and tryptase staining within the IgE$^+$, lin$^-$ subset ($r = 0.0004$, $P = .998$, n = 24).

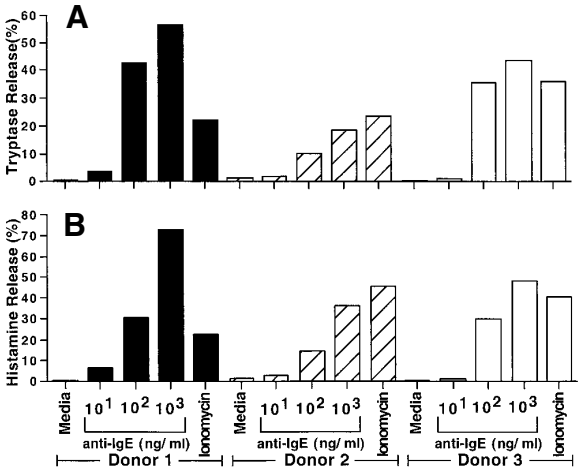

FIG 4. Basophil tryptase and histamine release. Tryptase **(A)** and total histamine **(B)** release from purified basophils by means of ELISA determined as the percentage of total amount of mediator is shown. Basophil purity for donor 1 is 72.4%, for donor 2 is 80.0%, and for donor 3 is 53.6%.

Correlation of basophil tryptase MEAPC to tryptase ELISA

We examined 12 subjects (8 asthmatic and 4 healthy control subjects) for tryptase expression in purified basophils by means of ELISA and MEAPC assay. Both assays demonstrated intersubject variations in per-cell tryptase expression of 100-fold. These 2 methods demonstrated a moderate correlation (P = .14 and r = 0.45, Spearman rank correlation). Basophils from the subject with the highest tryptase ELISA expressed 0.74 pg of tryptase per basophil. The median basophil tryptase expression by ELISA was 0.042 pg/cell, which is in agreement with the results of previous reports.[8]

Basophil tryptase release

We next determined whether this tryptase was released on basophil activation. Basophils from 3 donors released tryptase after activation with ionomycin and anti-IgE (Fig 4, *A*). The tryptase MEAPC value demonstrated a decrease parallel to the release noted by ELISA (data not shown). Histamine release demonstrated a parallel pattern of release to that of tryptase (Fig 4, *B*). Two additional donors failed to release either tryptase or histamine with anti-IgE (data not shown).

DISCUSSION

Our results verify and expand on earlier observations demonstrating tryptase-expressing peripheral blood cells.[8,14,15] We found that tryptase expression in PBLs was limited to a population of cells that stained brightly for IgE and was negative for a panel of lineage markers (CD2, CD14, CD16, and CD19; Fig 1), which is the basophil phenotype. Furthermore, these cells expressed the basophil-specific markers 2D7 and BB1 and did not express either of the mast-cell markers Kit/CD117 or chymase, despite our use of both mAbs and polyclonal antibodies to CD117. Lastly, there was no increase in tryptase-positive PBLs in patients with mastocytosis, a disease in which there is hyperplasia of both mast cells and their progenitors.[27] Taken together, these data provide convincing evidence that the tryptase-expressing cell lineage we have described is of a basophil, rather than mast-cell or hybrid, lineage.

In contrast to our results, Li et al,[14,15] using immunocytochemistry, have identified a tryptase-expressing PBL and concluded that it represents a mast cell-basophil hybrid, primarily on the basis of the cell's expression of both chymase and CD117. The frequency of tryptase-staining cells identified by these investigators (0.5%-2%) is similar to the frequency of tryptase-positive basophils we have identified. Furthermore, we were unable to detect tryptase in any PBL subpopulation other than IgE^+, lin^- cells, suggesting that the cell subset we have characterized in this study is identical to the tryptase-positive PBLs identified in the previous study.

We observed a great amount of variation in tryptase expression by peripheral blood basophils, with some donors expressing 100 times greater per-cell tryptase than others. This wide range of basophil tryptase expression is a novel finding and was apparent by both ELISA and flow cytometry (Fig 3). Despite this large intersubject variation, individual subjects demonstrated reproducible basophil tryptase expression over a 6-month period. These results suggest the possibility that basophil tryptase may play a role in the pathogenesis of asthma and may explain some of the heterogeneity seen in this disease in certain high-expressing donors.

Previous studies have shown that basophils express less than 1% of the tryptase of mast cells.[8] Our results generally agree with these, with tissue mast-cell tryptase MEAPC value approximately 100 times greater than the median basophil tryptase values. However, the tryptase MEAPC values from some donors expressed per-cell quantities of tryptase approaching that of mast cells (Fig 3). Although we did not directly compare per-cell basophil and mast-cell tryptase by means of ELISA, our basophil tryptase expression, as determined by means of ELISA, was substantially lower than the 12 to 35 pg/cell noted for mast cells in previously published reports.[8] These results suggest that in specific donors activated basophils may be an important contributor to tryptase production. Using basophil samples, the tryptase MEAPC assay and ELISA demonstrated a correlation; however, we did not validate the MEAPC assay for mast-cell tryptase staining. Thus it is possible that the relatively high level of basophil tryptase MEAPC staining we noted is due to greater accessibility of basophil tryptase to antibody staining relative to that in mast cells.

We believe this pattern of heterogeneity of tryptase expression between donors is the result of some undetermined environmental, pathologic, or genetic variable that we have not yet determined. One attractive possibility is that in specific subjects or diseases there is a cytokine milieu that favors basophil tryptase expression. However, we were unable to influence basophil tryptase by culturing PBLs with IL-2, IL-3, IL-4, IL-5, IL-13, eotaxin,

RANTES, GM-CSF, C5a, N-formyl-methionyl-leucyl-phenylalanine, or anti-IgE (data not shown).

Tryptase has been widely used as a specific marker for identifying human mast cells by means of immunohistochemistry. Although basophils have been reported to stain for tryptase with indirect immunohistochemistry, they did not stain with a directly labeled mAb,[16] presumably reflecting the lower amounts of tryptase in basophils. In contrast, in this study of flow cytometry, we have definitively shown that peripheral blood basophils readily stain with anti-tryptase antibodies. These results demonstrate that tryptase is not an exclusive marker of mast cells. Anti-tryptase antibodies cannot be used to distinguish these 2 cell types from one another by using flow cytometry, and therefore basophils should be considered when tryptase-expressing cells are detected.

Tryptase release from purified basophils paralleled histamine release consistent with localization to the basophil granules. These findings suggest that basophils have the capacity to release tryptase in vivo.

We found no statistical difference in the level of tryptase expression between the allergic asthmatic and nonatopic healthy control subjects (Fig 3) and were unable to correlate tryptase expression to asthma severity. Despite this, there did appear to be a subset of asthmatic subjects with high per-basophil tryptase. We were thus unable to confirm the results of previous investigators correlating tryptase-positive PBLs with the presence of allergic asthma.[14,15] However, it should be noted that Li et al[14,15] examined additional allergic populations (ie, drug allergic), which may express mast cell-basophil hybrid cells.

In summary, we have phenotypically identified the tryptase-expressing cells in the peripheral blood of both normal and allergic asthmatic subjects as basophils. The range of basophil tryptase expression varied greatly between donors and, in some donors, approached the level seen in mast cells. However, basophil tryptase expression did not correlate to the presence of allergic asthma or mastocytosis. These results suggest that tryptase may be an additional mediator through which basophils act to produce allergic inflammation.

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