Expression of α-tryptase and β-tryptase by human basophils

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Background: α and β-Tryptase levels in serum are clinical tools for the evaluation of systemic anaphylaxis and systemic mastocytosis. Basophils and mast cells are known to produce these proteins.

Objective: The current study examines the effect of the α,β-tryptase genotype on basophil tryptase levels and the type of tryptase stored in these cells.

Methods: Tryptase extracted from purified peripheral blood basophils from 20 subjects was examined by using ELISAs measuring mature and total tryptase and by using an enzymatic assay with tosyl-Gly-Pro-Lys-p-nitroanilide. Tryptase genotypes (4:0, 3:1, and 2:2/α/β ratios) were assessed by using a hot-stop PCR technique with α,β-tryptase-specific primers. Total α,β-tryptase mRNA was measured by means of competitive RT-PCR, and ratios of α to β-tryptase mRNA were measured by means of hot-stop RT-PCR.

Results: Tryptase in all but one of the basophil preparations was mature and enzymatically active. Tryptase quantities in basophils were less than 1% of those in tissue mast cells. Tryptase genotypes (β/α) among the 20 donors were 4:0 in 7, 3:1 in 7, and 2:2 in 6. Tryptase protein and mRNA levels per basophil were not affected by the tryptase genotype.

Conclusion: Basophils from healthy subjects contain modest amounts of mature and enzymatically active tryptase unaffected by the tryptase genotype. (J Allergy Clin Immunol 2004;113:1086-92.)

Key words: Basophil, tryptase

Tryptase is normally expressed by both human mast cells and basophils. In lung- and skin-derived mast cells, mean tryptase levels of 11 and 35 pg per mast cell account for a substantial portion of the cell protein.1 In contrast, mean levels in peripheral blood basophils (0.05 and 0.04 pg per basophil23) are typically less than 1% of those in mast cells, even though histamine levels per cell are similar. Tryptase expressed by human mast cells and basophils is derived principally from 2 genes that are arranged in tandem on chromosome 16p13.3, such that there is either one α-tryptase gene (TPS) and one β-tryptase gene (TPSB) or 2 β-tryptase genes per haploid chromosome.4,6 Approximately 25% of persons are α-tryptase deficient.7,9 Both α-tryptase and β-tryptase mRNAs are expressed in T mast cells and TC mast cells and in developing fetal liver–derived mast cells.10,11 In 2 samples of peripheral blood basophils10 and in blasts from certain subjects with acute myelocytic leukemia,12 levels of α-tryptase mRNA predominated. However, the basophil leukemia cell line KU812 expresses predominantly β-tryptase mRNA.13

Measurements of tryptase levels in serum have been used to assess mast cell activation in systemic anaphylaxis and mast cell load in systemic mastocytosis.8,14-16 β-Tryptase is processed from a pro to a pro' form by means of a heparin-dependent autocalytic step and from a pro' to a mature enzyme by means of dipeptidyl peptidase I.17 β-Tryptase is stored in secretory granules as an active tetramer and is released during degranulation.18,19 α-Tryptase appears to be processed only to the pro form by human mast cells.17 Precursor forms of both α-tryptase and β-tryptase are spontaneously secreted by mast cells, whereas mature β-tryptase is retained in secretory granules until its release by activated cells.8 Two immunoassays, one recognizing both precursor and mature forms of α-tryptase and β-tryptase (total tryptase) and another recognizing only mature tryptase, are used to assess these different forms of tryptase. Mature and total tryptase levels are increased in mast cell–dependent systemic anaphylaxis; total tryptase levels are increased in systemic mastocytosis. No significant difference in mean total tryptase levels is observed between subjects with and without this α-tryptase gene.8 Whether the tryptase genotype affects levels of tryptase mRNA or protein in peripheral blood basophils has not been determined. Basophils were isolated from 20 healthy human subjects and assessed at both the genomic and mRNA levels for α- and β-tryptases and at the protein level for the mature and immature forms of tryptase. The current study concludes that the tryptase genotype does not influence tryptase mRNA and protein levels and that essentially all of the tryptase retained in peripheral blood basophils, as in mast cells, is mature and enzymatically active.

Abbreviations used
G3PDH: Glyceraldehyde-3-phosphate dehydrogenase
Mac-6: Mono Mac-6
MES: 2-[N-Morpholino]ethanesulfonic acid

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METHODS
PBS, 2-[N-Morpholino]ethanesulfonic acid (MES), HEPES, Tris, sodium azide, EDTA, Percoll, controlled processed serum replacement medium 3, porcine heart glycosaminoglycan (1200-20,000 d), Trypan blue, Giemsa, BSA, p-nitrophenyl phosphate, tosyl-Gly-Pro-lys-p-nitroanilide, agarose, Isocoves medium, t-glutamine, penicillin, streptomycin, amphotericin D, FCS, α-thioglycerol, RPMI 1640 medium, MgCl₂, and gelatin (Sigma Chemical Co, St Louis, Mo); HBSS (Invitrogen, Carlsbad, Calif); streptavidin-AP conjugate (Roche Diagnostics GmbH, Mannheim, Germany); and basophil isolation kit, glycophorin A, and LS columns (Miltenyi Biotec, Bergisch Gladbach, Germany) were obtained as indicated. mAbs G4, G5, and B12 were produced and purified as previously described. The pHGAP plasmid containing human glyceraldehyde-3-phosphate dehydrogenase (G3PDH) cDNA was purchased from American Type Culture Collection (Rockville, Md). Moloney murine leukemia virus reverse transcriptase (Life Technologies, Grand Island, NY); dNTP mix (Boehringer Mannheim, Indianapolis, Ind); genomic DNA purification kit, RNase-free DNase, BstEII, EcoRV, Oligo(dT)15 primer, 100-bp ladder, and T4 polynucleotide kinase (Promega, Madison, Wis); RNA isolation kit (Quigen, Valencia, Calif); ProbeQuant G-50 Micro-column (Amersham, Piscataway, NJ); AmpliTaq (Perkin-Elmer Corp, Foster City, Calif); polyacrylamide gels (Invitrogen); and SYBR Green I (molecular probe Inc, Eugene, Ore) were obtained as indicated. The HMC-1 mast cell leukemia cell line was provided by Dr Gerald Gleich and Dr Joseph Butterfield (Mayo Clinic, Rochester, Minn). The Mono-mac-6 (Mac-6) myelomonocytic leukemia cell line was provided by Dr H. W. Ziegler-Heitbrock (University of Munich, Munich, Germany). Primers were prepared and sequencing was performed by the Nucleic Acid Core facility at Virginia Commonwealth University.

Purification of human basophils
Percoll density centrifugation. Freshly prepared buffy coats were obtained from the Virginia Blood Services (Richmond, Va). The contents of the buffy coats (34–40 mL) were further anticoagulated with 0.01 mol/L EDTA, pH 7.6, and diluted 1:1 with 1× HBSS, pH 7.4. Cells (approximately 20 mL) were layered over 20-mL cushions of 65% (1.090 g/mL) Percoll in 50-mL tubes. After centrifugation at 700 g for 15 minutes at room temperature, leukocytes were collected from the Percoll/cell medium interface, and erythrocytes were found at the bottom of the tube. Leukocytes were retrieved, diluted 1:1 with 1× HBSS, and layered (10-12 mL) over 12 mL of 45% (density, 1.070 g/mL), 12 mL of 57.5% (density, 1.080 g/mL), and 12 mL of 65% (density, 1.090 g/mL) Percoll, as previously described. Densities were confirmed with a Leica refractometer (Buffalo, NY). After centrifugation at 700 g for 15 minutes at room temperature, the basophils were collected predominantly from the 45%/57.5% interface at purities ranging from 3% to 16%. Percoll-enriched cells were washed 3 times (10 minutes at 500 g and 4°C) with cold PBS containing 1% controlled processed serum replacement medium 3 and 0.01 mol/L EDTA, conditions designed to prevent basophil activation. Total cell numbers (hemocytometry) and viabilities (Trypan blue exclusion test) were determined. Basophil purity was calculated after assessing at least 300 cells on cytospin preparations labeled with Giemsa stain.

Magnetic cell separation. Further purification was performed with the Magnetic Cell Separation (MACS) basophil isolation kit according to the manufacturer’s protocol by using negative selection with antibodies against CD3, CD7, CD14, CD15, CD16, CD36, CD45RA, and HLA-DR and an LS column. Unbound cells, mostly basophils, were washed out with 4× 3 mL of ice-cold PBS-BSA-EDTA. Basophil numbers, purities, and viabilities were evaluated as described above.

Cell lines, genomic DNA, and cellular RNA
HMC-1 cells were maintained in Isocoves medium supplemented with 2 mmol/L t-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, 0.25 μg/mL amphotericin D, 10% (vol/vol) heat-inactivated FCS, and 0.01% α-thioglycerol. Mac-6 cells were maintained in RPMI 1640 medium supplemented with 2 mmol/L t-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, 0.25 μg/mL amphotericin D, and 10% (vol/vol) heat-inactivated FCS. All cell types were cultured at 37°C in an incubator maintained at 6% CO₂.
Genomic DNA from cell lines and buffy coat cells was purified with a commercial genomic DNA purification kit. Total RNA from each basophil sample was extracted with a commercial RNA isolation kit, in each case according to the manufacturer’s instructions. Also, samples were treated with RNase-free DNase to remove genomic DNA from preparations of total RNA, dissolved in RNase-free water, and measured by means of absorbance at 260 nm.

α-Tryptase and β-tryptase in DNA and RNA samples from cell lines and basophils
PCR and RT-PCR reactions were performed, respectively, to assess the presence of α- and β-tryptase genes and mRNAs, as previously described, except that PCR products and fragments were analyzed by means of electrophoresis in a 1.5% agarose gel and visualized by staining with ethidium bromide. By using separate PCR or RT-PCR reactions for α- and β-tryptases, α/β-tryptase ratio determinations were imprecise because of variability among different amplification reactions. An alternative procedure was to use tryptase primers that would amplify both α- and β-tryptases but that contain distinct internal restriction sites to enable measurements of α- and β-tryptase—derived products from the same amplification reaction. However, for this technique to work well, heteroduplexes between α- and β-tryptases that form during annealing reactions should be eliminated from the analysis.

Hot-stop PCR was performed to eliminate α,β-tryptase heteroduplexes from the analysis of PCR products, as previously described. Antisense primers were labeled with 32P ATP by using T4 polynucleotide kinase, and then the labeled DNA was separated from unincorporated labeled nucleotides by means of chromatography on a ProbeQuant G-50 Micro-column. For genomic DNA, hot-stop PCR was performed with 50 ng of genomic DNA in a total volume of 50 μL containing 1× reaction buffer with 200 μmol/L dNTP and 5 pmol of each primer (sense, 5′−GGAGCAG-CACTCTTACTACC−3′; antisense, 5′−GGCGCCAAGTGTTGATT- TTTGGC−3′). For mRNA, cDNA was synthesized from 1 μg of total RNA by using Maloney-murine leukemia virus reverse transcriptase and oligo(dT) primers and subjected to PCR as above. In each case the sense and antisense primers were located in exons 4 and 5, respectively, and amplified 432-bp (α-tryptase) and 443-bp (β-tryptase) regions from genomic DNA and 316-bp regions from cDNA. The optimal MgCl₂ concentration for PCR was 0.5 mmol/L.
Hot-start reactions (95°C for 3 minutes) were initiated with 1 U of Taq DNA polymerase, followed by 35 cycles of amplification in a volume of 50 μL. Each cycle consisted of 1 minute at 94°C, 1 minute at 62°C, and 1 minute at 72°C. By adding the 32P-labeled antisense primer (5′−GGCGCCAAGTGTTGATTTTGGC−3′) before the final cycle of amplification, only homoduplexes incorporate radioactivity. Any heteroduplexes formed during prior cycles would be unlabeled. The corresponding PCR products derived from genomic DNA and mRNA were digested with EcoRV and BstEII restriction enzymes to digest α-tryptase and β-tryptase, respectively. EcoRV digests of the α-tryptase PCR products yield 341-bp and 91-bp fragments (genomic DNA) and 225-bp and 91-bp fragments (mRNA). BstEII digests the β-tryptase PCR products to yield 298-bp and 145-bp fragments (genomic DNA) and 171-bp and 145-bp fragments (mRNA). The
digested PCR products were subjected to electrophoresis in 12% polyacrylamide gels, followed by β scanning and analysis with Quantity One software (Bio-Rad Laboratories, Hercules, Calif).

Competitive RT-PCR

Competitive RT-PCR for measuring tryptase and G3PDH mRNAs was performed as previously described. Relative mRNA values were normalized to the percentage of basophils. Thus the amount of tryptase mRNA per basophil is the principal determinant of the relative mRNA value.

Protein extraction

Basophil-enriched pellets were vortexed in 300 µL of MES buffer (10 mmol/L MES, pH 6.5, and 1 mol/L NaCl) and then sonicated on ice with a Sonicator cell disrupter, Model W-225R (Plainview, NY; power 4, 10 pulses ×2). After ensuring complete lysis of the cells by means of phase-contrast microscopy, the concentration of each sample was adjusted to 3 × 10^6 cells/mL and microcentrifuged at 12,000g for 30 minutes. The supernatants were collected, and 300 µL of MES buffer was again added to each of the remaining pellets, which were again vortexed, sonicated, and microcentrifuged. The 2 supernatants were assayed separately for tryptase. Greater than 96% of the tryptase was recovered in the first extract for all samples, except for one sample from which 94% was recovered. The amounts of tryptase in both extracts were used to calculate immunoreactive tryptase levels per basophil.

Tryptase immunoassays

Tryptase protein levels were measured by using a sandwich ELISA technique with the B12 mAb for capture and biotin-G4 (total tryptase) and biotin-G5 (mature tryptase) mAbs for detection, as previously described. Purified human lung recombinant α-tryptase (2.1 ng/mL) and recombinant β-tryptase (2.0 ng/mL) were used as a standard because a prior study reported previously by human genome resources to indicate that the product spanned an intron. In addition, the genomic DNA of all 20 subjects had β-tryptase genes (Fig 1, A, upper panel), whereas the DNA from only 13 of the subjects contained an α-tryptase gene (Fig 1, B, upper panel). The 35% prevalence of α-tryptase gene deficiency in this small population was somewhat higher than the 26% to 29% prevalences reported previously in larger populations. As expected, Mac-6 cells, but not HMC-1 cells, contained the α-tryptase gene.

RT-PCR with primers specific for β-tryptase (Fig 1, A, lower panel) showed the presence of this tryptase mRNA in all 20 subjects. Similarly, RT-PCR with primers specific for α-tryptase showed this type of mRNA to be present in all of the subjects who had the corresponding gene and in none of the 7 subjects who were deficient in the α-tryptase gene (Fig 1, B, lower panel).

For the 13 subjects with at least one copy of the α-tryptase gene per diploid set of chromosomes, genomic DNA samples were further analyzed. Ratios of α-tryptase to β-tryptase genes were measured as described in the Methods section by using a hot-stop PCR technique to eliminate heteroduplexes from the analysis of PCR products. As shown in Fig 1, C, tryptase genotyping of samples known to have both α-tryptase and β-tryptase fell into 2 groups. In one group the mean ± SD α/β ratio was 1.03 ± 0.13 (n = 6), and in the other the ratio was 3.11 ± 0.21 (n = 7). PCR products derived from 2 of the genomic DNA samples with both α- and β-tryptase genes were not completely digested with EcoRV and BsrEI1 because of a polymorphism (A421G in exon 4; Thr141Ala) in a sequence that was otherwise identical to β-tryptase by means of DNA sequencing. This polymorphism disrupts the BsrEI cleavage site and has been reported previously by human genome resources to associate with both βI and βII tryptase, whereas a possible association with βIII tryptase had not been examined. The PCR product examined in the current study does not distinguish between the βI, βII, and βIII forms of tryptase. The effect of the conservative Thr to Ala change on tryptase function has not been assessed but is likely to be minimal. Both of the polymorphism-containing samples yielded a 3:1 gene ratio of β/α tryptase. Thus among the 20 genomic samples fully analyzed, assuming a total of 4 α/β tryptase genes per diploid chromosome set and no more than one α-tryptase gene per haploid chromosome, 7 were β/β, 7 were β/β/α, and 6 were β/α/α.

Tryptase protein expression

Total and mature levels of tryptase protein were measured in extracts of peripheral blood basophils by using immunoassays, as previously described. The mean ± SD level of total tryptase was 0.084 ± 0.077 pg per basophil, with levels ranging from 0.007 to 0.335 pg per basophil. The mean level of mature tryptase, 0.082 ± 0.081 pg per basophil, was not significantly different, with levels ranging from 0.004 to 0.351 pg per basophil. As shown in Fig 2, A, a plot of mature tryptase levels against total tryptase levels, when analyzed by means of linear regression, after excluding one outlier, yields a slope of 1.0 (P < .001). The outlier, derived from
a sample with a 3:1 $\beta/\alpha$-tryptase gene ratio, had less mature (0.06 pg per basophil) than total (0.15 pg per basophil) tryptase. On the basis of these immunoassay results, in 19 of the 20 samples, nearly all of the tryptase residing in peripheral blood basophils is mature.

Mature $\beta$-tryptase, when stabilized by heparin proteoglycan in its tetrameric form, is enzymatically active, whereas mature $\alpha$-tryptase, if it occurs in vivo, exhibits little if any enzymatic activity. Tryptase enzymatic activity was measured in basophil extracts in the presence of soybean trypsin inhibitor, which was added to neutralize most other trypsin-like proteases. Enzyme activity was detected in 14 of the 20 samples and correlated closely with immunoreactive levels of tryptase.

FIG 1. Tryptase genotype. Genomic DNA and RNA were examined for the presence of the $\beta$-tryptase (A) and $\alpha$-tryptase (B) genes (upper panels) and mRNAs (lower panels), respectively. In Fig 1, B (side panel), genomic DNA from the HMC-1 (H) and Mac-6 (M) cell lines were tested. Results are shown for 7 of the 20 samples analyzed (lanes 1-7). MW, Molecular weight standards; W, negative control without a DNA template. Among those samples with $\alpha$- and $\beta$-tryptase genes (C), the experimentally determined $\beta/\alpha$ gene ratios (y-axis) are plotted against their ultimate designation as a 2:2 or 3:1 genotype. Mean and SD error bars are shown for the 2 genotypes.
as shown for total tryptase levels in Fig 2, B. The relationship between enzyme activity and mature tryptase levels also was analyzed (Fig 2, C). Activity was not detected for the 6 samples having total tryptase levels of <0.022 pg per basophil. Of the 14 samples for which activity was detected, a mean of $1.0 \times 10^{-10} \pm 0.9 \times 10^{-10}$ U per basophil was observed. Excluding the single outlier in the plots of tryptase versus tryptase activity, the specific activity of basophil tryptase was 10.1 U/mg total tryptase when calculated from the slope of Fig 2, B, and 9.7 U/mg mature tryptase when calculated from the slope of enzyme activity versus mature tryptase. That outlier had a specific activity of 0.5 U/mg total tryptase and 1.2 U/mg mature tryptase.

The relationship between tryptase levels and tryptase genotype was analyzed. Fig 3, A, shows total tryptase levels per basophil plotted against the experimentally determined $\alpha/\beta$-tryptase gene ratio. All genotypes were associated with substantial variability in tryptase levels, and there was no significant difference between the median total tryptase values of each genotype. Similar results were found for mature tryptase (ie, no significant differences between median values associated with each genotype; data not shown). Fig 3, B, shows that the total tryptase-specific activities (where measurable and excluding the outlier noted above) associated with each genotype were similar to one another.

**Analysis of tryptase mRNA**

The competitive RT-PCR technique was applied to RNA isolated from peripheral blood basophils to measure relative levels of tryptase mRNA ($\alpha$ together with $\beta$). In each of the 17 samples available for this measurement, values were normalized to the corresponding level of G3PDH mRNA and corrected for the purity of basophils. These tryptase mRNA expression data were then compared with total and mature tryptase levels. However, no apparent relationship between mRNA levels and tryptase protein was discerned (data not shown). Furthermore, levels of tryptase mRNA did not vary among the 3 different tryptase genotypes. Thus levels of tryptase mRNA correspond neither to the amounts of tryptase protein in peripheral blood basophils nor to the tryptase genotype.

Relationships also were explored between the $\alpha/\beta$-tryptase mRNA ratios and both the tryptase genotype (Fig 4) and the amounts of tryptase protein per basophil. For an $\alpha/\beta$ genotype of 2:2, 3 of 5 samples had more $\alpha$-tryptase than $\beta$-tryptase mRNA, whereas for those associated with a genotype of 1:3, 4 of 7 had more $\alpha$-tryptase than $\beta$-tryptase mRNA. There was no significant difference between the median $\alpha/\beta$-tryptase mRNA ratios of the 2:2 and 1:3 $\alpha/\beta$-tryptase genotypes. Mean total tryptase levels from samples in which $\alpha$-tryptase mRNA was greater than $\beta$-tryptase mRNA ($0.05 \pm 0.05$ pg per basophil, $n = 7$), $\beta$-tryptase mRNA was greater than $\alpha$-tryptase mRNA ($0.12 \pm 0.03$ pg per basophil, $n = 5$), and only $\beta$-tryptase mRNA was present ($0.10 \pm 0.11$ pg per basophil, $n = 7$) were not significantly different from one another ($P = .28$, 1-way ANOVA). Analogous results were found with mature tryptase values ($P = .16$).

**DISCUSSION**

Our results verify and expand on earlier observations that peripheral blood basophils express tryptase at the protein level, albeit at levels less than 1% of those found in mast cells (11 pg per lung mast cell and 35 pg per skin...
The mean ± SD level of total tryptase was 0.08 ± 0.08 pg per basophil, with levels ranging from 0.007 to 0.34 pg per basophil. The results are similar to the values of 0.04 pg per basophil seen previously. Also, as for mast cells, the major type of tryptase retained in basophils is mature and enzymatically active. Nearly equivalent levels of tryptase were detected by using ELISAs for mature and total (mature plus pro forms) tryptase. These results support the hypothesis that basophils, like mast cells, preferentially retain mature forms of tryptase.

Tryptase retained in basophils was previously shown to be releasable in parallel to histamine by means of degranulation. Whether basophil-derived tryptase might affect mature tryptase levels in serum during allergic reactions will depend on the magnitude of basophil numbers and degranulation, as well as the tryptase level per basophil. The ratio of mean tryptase (0.07 pg per basophil) to mean histamine (1.2 pg per basophil) levels in basophils, 0.06, is markedly lower than the ratios of 7 and 18 for lung- and skin-derived mast cells, respectively. One report suggested that basophils from patients with active asthma and those with drug allergies have increased tryptase levels, as determined by means of immunocytochemistry. However, a subsequent study found no significant difference in tryptase levels assessed by means of flow cytometry between subjects with allergic asthma (n = 18) and systemic mastocytosis (n = 22) and non-atopic healthy control subjects (n = 22). If all of the tryptase and histamine in basophils was released into the circulation and detected in plasma by using mean values per basophil and normal basophil concentrations (40,000/mL), the plasma histamine level would increase to 48 ng/mL and that of tryptase would increase to 2.8 ng/mL, again yielding a tryptase/histamine ratio of 0.06. During systemic anaphylaxis, peak levels of plasma histamine range from 2 to 1500 ng/mL and those of plasma or serum tryptase range from 1.8 to 4400 ng/mL. In the circulation peak levels of tryptase appear later than those of histamine, presumably because of its slower diffusion out of the tissues, where these mediators are first deposited by mast cells. This later appearance favors a mast cell origin for the tryptase. If these mediators had been released by circulating basophils, they should have appeared simultaneously in plasma, analogous to what is observed in vitro when suspensions of basophils are activated. Overall, the egress of tryptase into the circulation is considered to be less efficient than that of histamine. Also, histamine is removed from the circulation with a half-life of 1 to 2 minutes, far more rapidly than tryptase, which decreased with a half-life of 2 to 3 hours. In the largest series of severe anaphylaxis (n = 17), the ratio of the mean value for peak tryptase to that of histamine was about 3, 50-fold higher than the ratio of these mediators in basophils and 2- to 6-fold lower than in mast cells. Thus on the basis of the time course of the appearance of
histamine and tryptase and on the ratios and absolute amounts of histamine and tryptase detected free in the circulation during insect sting—induced systemic anaphylaxis, it appears that mast cells typically account for the vast majority of the mature tryptase released during insect sting—induced systemic anaphylaxis.

Among subjects with a detectable α-tryptase gene, β-tryptase to α-tryptase gene ratios were determined by means of a novel hot-stop technique, which eliminates confounding heteroduplexes from the analysis. This technique yielded ratios that grouped closely around either 2:2 or 3:1, which is consistent with the gene ratios previously predicted and reported.6,25,26 Among the 20 subjects examined in the current study, 7 were β/β/β, 7 were β/β/βα, and 6 were βα/βα. The percentage of α-tryptase—deficient subjects (35%) was somewhat higher than the 29% (n = 274),6 20% (n = 60),7 and 26% (n = 109) values previously reported, probably because of the small number of subjects examined.

The tryptase genotype had no significant effect on the total level of tryptase mRNA or on the tryptase protein content per basophil. Protryptase that is spontaneously secreted by mast cells in vitro can be derived from both α- and β-tryptase genes.8 The current study did not address whether basophils produce and spontaneously secrete immature forms of tryptase and whether the tryptase genotype affects this process. On the other hand, mature tryptase is likely to be derived exclusively from the β-tryptase gene.17 Nevertheless, the current study found no dramatic trend for increasing tryptase mRNA or mature protein per basophil in subjects with 2, 3, and 4 β-tryptase genes per diploid genome. However, sample numbers were relatively small and thus could not exclude small haplotype effects on basophil tryptase content. Other factors must regulate the magnitude of tryptase expression in the basophils of healthy subjects.

In summary, peripheral blood basophils from healthy subjects express mature and enzymatically active tryptase at levels of less than 1% of those of tissue mast cells unrelated to the α/β-tryptase genotype.

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