

Human mouse mast cell protease 7-like tryptase genes are pseudogenes

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Background: α -Tryptase and β -tryptase are important clinical markers for mast cell-dependent disorders. A third family of tryptase genes on human chromosome 16 has been identified and called human mouse mast cell protease 7 (hmMCP-7)-like tryptase.

Objective: This study was designed to determine whether these tryptase genes are expressed by human mast cells.

Methods: A 2842-bp hmMCP-7-like tryptase gene was cloned and sequenced from a human placental genomic library. PCR and RT-PCR procedures, respectively, were used to determine whether this tryptase gene family was present in most genomes and whether it was expressed.

Results: The tryptase clone was almost identical to the hmMCP-7-like tryptase II and I genes, and therefore it was called hmMCP-7-like tryptase III. All such genes encode a Gln⁻³ like α -tryptase. They also terminate translation after amino acid 235, whereas α - and β -tryptase genes each encode a 275-amino acid protein. In this study, cell lines HMC-1, KU812, and Mono-Mac-6; mast cells derived in vitro from cord blood and fetal liver progenitors; and mast cell-enriched preparations of dispersed skin and lung cells contained hmMCP-7-like tryptases in their genomes by PCR with gene-specific primers. To identify whether such genes were transcriptionally active, RT-PCR revealed α - or β -tryptase products in all mast cell preparations and cell lines and in activated skin-derived mast cells, but no hmMCP-7-like tryptase products.

Conclusion: These results indicate hmMCP-7-like tryptase (I, II, III) genes are pseudogenes and unlikely to affect measurements of α - and β -tryptases. (*J Allergy Clin Immunol* 2001;107:315-21.)

Key words: Mast cell, RT-PCR, pseudogene, tryptase

Human tryptase molecules are encoded by at least 3 genes per haploid chromosome— α (I, II)-tryptase, β (I, II, III)-tryptase, and human mouse mast cell protease 7 (hmMCP-7)-like tryptase (I, II)—each of which resides on chromosome 16.¹⁻⁴ The product of the β -tryptase gene, β -preprotryptase, is processed to β -protryptase, β -pro¹tryptase, and β -tryptase.⁵ β -Tryptase is stored in mast cell secretory granules as an enzymatically active tetramer and is released during degranulation.⁶ It serves as a specific clinical marker of mast cell activation in diseases such as systemic anaphylaxis^{7,8} and asthma.^{9,10} In contrast, the

Abbreviations used

CPSR: Heat-inactivated control-processed serum-replacement medium

DIG: Digoxigenin

dNTP: deoxyribonucleotide triphosphate

hmMCP: Human mouse mast cell protease

mMCP: Mouse mast cell protease

MMLVRT: Moloney murine leukemia virus reverse transcriptase

product or products of the α -tryptase genes appear to be processed only to α -protryptase and then are spontaneously secreted by mast cells.^{5,6} As a result, α -protryptase levels in serum reflect the total body burden of mast cells and are elevated in subjects with systemic mastocytosis. Both of these genes are expressed in mast cells; much smaller amounts of tryptase mRNA are found in peripheral blood basophils, where primarily α -tryptase mRNA is detected.^{11,12} HmMCP-7-like genes,⁴ such as α - and β -tryptase genes, contain 6 exons, and like α -tryptase, they contain a Gln⁻³ in the putative autoprocessing site of the leader peptide. Unlike α - and β -tryptase genes, hmMCP-7-like I (1749 nucleotide) and II (2575 nucleotide) genes (GenBank AF099147 and AF098327, respectively; NIH, Bethesda, Md) have an extended intron 4, an exon 5 that more closely resembles the corresponding exon in mMCP-7, and a premature stop codon in exon 6. Intron 4 of hmMCP-7-like tryptase, like mMCP-7, is larger than the corresponding intron in human α -tryptase and β -tryptase genes, but the intron 4 sequences of hmMCP-7-like tryptase and mMCP-7 are not closely homologous.

In this study a third hmMCP-7-like gene was identified, cloned, and sequenced. However, no mRNA molecules corresponding to these hmMCP-7-like genes were found in extracts of mast cells derived in vitro from fetal liver and cord blood progenitors, of enriched mast cell preparations from lung and skin, of activated skin-derived mast cells, and of the leukemic cell lines HMC-1 (mast cell), KU812 (basophil), 1059SK (skin fibroblast), and Mono-mac-6 (Mac-6, monocyte), even though the sequence was detected in the genomes of these cells. These results indicate that the hmMCP-7-like genes are pseudogenes and unlikely contributors to serum tryptase measurements.

METHODS

Materials

HindIII, *SacII*, *SacI*, *SfiI*, *XhoI*, *BamHI*, *HincII*, *Apal* I, *EcoRI*, 100-bp ladder, deoxyribonucleotide triphosphate (dNTP) mix, PCR kit, ligation kit, oligo (dT)15 primer, rNasin, Taq polymerase, and β -actin primer pair (Promega Corp, Madison, Wis); Maloney

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murine leukemia virus reverse transcriptase (MMLVRT; Life Technologies, Inc, Gaithersburg, Md); agarose, Isocove's medium, L-glutamine, penicillin, streptomycin, amphotericin D, FCS, α -thioglycerol, and RPMI-1640 medium (Sigma Chemical Co, St Louis, Mo); digoxigenin (DIG) DNA Labeling and Detection Kit (Roche Molecular Biochemicals, Indianapolis, Ind); SYBR green I (FMC Bioproducts Co, Rockland, Me); the human fibroblast cell line 1059SK (American Type Culture Collection, Manassas, Va); and pBluescript SKII⁺ vector (Stratagene, La Jolla, Calif) were purchased as indicated. Other primers were prepared by the Nucleic Acid Core facility at Virginia Commonwealth University. Human genomic DNA was purified from whole blood with the Wizard genomic DNA purification kit (Promega Corp). The Mac-6 myelomonocytic leukemia cell line (Dr H. W. Ziegler-Heitbrock, University of Munich, Munich, Germany), the KU812 cell line (Dr Gunnar Nilsson, University of Uppsala, Sweden), and the HMC-1 cell line (Drs Gerald Gleich and Joseph Butterfield, Mayo Clinic, Rochester, Minn) were provided as indicated.

Cell lines, mast cells, and cellular RNA

HMC-1 cells were maintained in Isocove's medium supplemented with 2 mmol/L of L-glutamine, 100 U/mL of penicillin, 100 μ g/mL of streptomycin, 0.25 μ g/mL of amphotericin D, 10% (vol/vol) heat-inactivated FCS, and 0.01% α -thioglycerol. Mac-6 and KU812 cells were maintained in RPMI-1640 medium supplemented with 2 mmol/L of L-glutamine, 100 U/mL of penicillin, 100 μ g/mL of streptomycin, 0.25 μ g/mL of amphotericin D, and 10% (vol/vol) heat-inactivated FCS. Fibroblast 1059SK cells were maintained in Eagle's minimal essential medium with 2 mmol/L of L-glutamine, 1.5 g/L of sodium bicarbonate, 0.1 mmol/L of nonessential amino acids, 1 mmol/L of sodium pyruvate, and 10% FCS. All cell types were cultured at 37°C in an incubator maintained at 6% CO₂.

Mast cells were developed from fetal liver and cord blood cells essentially as described previously.^{13,14} In brief, human fetal livers (18–21 weeks of gestation) each were obtained at the time of a therapeutic abortion as approved by the human studies internal review board at Virginia Commonwealth University. Single cell suspensions were made by mincing the liver and filtering single cells through a stainless-steel mesh. Erythrocytes and cell debris were removed by density gradient centrifugation over Histopaque (density = 1.077; Sigma Chemical Co) at 1000g for 25 minutes. Fetal liver cells were collected from the interface, washed 3 times in PBS, and suspended in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated control-processed serum-replacement medium 1 (CPSR-1) (Sigma Chemical Co), 2 mmol/L of L-glutamine, 0.1 mmol/L of nonessential amino acids, 10 mmol/L of HEPES, 50 μ mol/L of 2-mercaptoethanol, 100 U/mL of penicillin, and 50 μ g/mL of streptomycin. Cells (1×10^6 /2 mL/well) were plated in 24-well plastic tissue culture plates (Costar, Cambridge, Mass) in the presence of recombinant human stem cell factor (50 ng/mL; a gift from Amgen, Thousand Oaks, Calif). Umbilical cord blood was obtained at the time of delivery and collected in heparin-treated tubes. Cord blood was diluted 1:1 in PBS, layered over Histopaque, and centrifuged at 1000g for 20 minutes at room temperature to remove erythrocytes. Cord blood mononuclear cells were collected from the interface, washed twice in PBS, and submitted to another density-dependent centrifugation step as described above. Cells at the interface were collected, washed 3 times in PBS, and suspended in RPMI-1640 supplemented with 10% CPSR-1, 2 mmol/L of L-glutamine, 0.1 mmol/L of nonessential amino acids, 10 mmol/L of HEPES, 50 μ mol/L of 2-mercaptoethanol, 200 U/mL of penicillin, and 100 μ g/mL of streptomycin. Cells were dispensed into 24-well plastic tissue culture plates at 10^6 cells per 2 mL per well in the presence of recombinant human stem cell factor (100 ng/mL). Fetal liver and cord blood cells were cultured at 37°C in a 6% CO₂ incubator.

Half of the culture medium was replaced once a week for cord blood cell cultures and twice a week for fetal liver cell cultures. After 5 weeks, the cells were harvested and washed. Also, skin and lung mast cells were dispersed from their corresponding tissue essentially as described previously.¹² Dispersed cells (3×10^6 cells/mL) were resuspended in RPMI-1640 containing 10% CPSR-1 and penicillin-streptomycin-amphotericin B, counted in a hemocytometer, assessed for viability by trypan blue exclusion, and for human mast cell (MC_{TC} [tryptase-positive, chymase-positive] and MC_T [tryptase-positive, chymase-negative]) percentages by immunocytochemistry of cytospin preparations.¹⁵ Total RNA was isolated by guanidinium-phenol extraction,¹⁶ dissolved in RNase-free water, and measured by absorbance at 260 and 280 nm.

Activation of skin-derived mast cells was performed with 22E7, an anti-Fc ϵ RI α mAb,¹⁷ or an isotype-matched negative control mAb as described,¹¹ except that AIM-V tissue culture medium¹⁸ was used in place of Tyrode's buffer to improve cell viability. Tryptase levels retained and released from the cells were measured by a tryptase ELISA¹⁹ after a 15-minute incubation with mAbs at 37°C. RNA was extracted from cells after incubation with mAb for 0, 1, 3, 6, 12, and 24 hours. Total cell viability was greater than 80% at each time point.

Isolation, subcloning, and sequencing of a genomic hmMCP-7-like gene

A human placenta genomic library (Clontech Laboratory Inc, Palo Alto, Calif) in EMBL3 SP6/T7 was screened with a DIG-labeled 225-bp PCR product amplified from the exon 4 portion of a human β II-tryptase cDNA template.² The sense (5'-GGAG CAGCACCTCTACTACC-3') and antisense (5'-TCATTGTCCA-CATCGCCCCAGC-3') primers were used at 1.0 mmol/L magnesium with Taq DNA polymerase at 95°C for 5 minutes (hot start), followed by 30 cycles at 95°C for 1 minute, 62°C for 1 minute, 72°C for 1 minute, and a final extension reaction at 72°C for 10 minutes. The PCR product was purified by electrophoresis in agarose gel and labeled with DIG according to a protocol provided by the manufacturer. Approximately 450,000 plaques were screened, and positive clones were visualized by use of the immunologic detection protocol provided by the manufacturer. Positive clones were plaque purified and rescreened. Phage DNA was purified by the plate lysate method. The presence of a tryptase gene was confirmed by Southern blotting with the 225-bp DIG-labeled probe and by restriction digests with *Sac*II of the 86-bp PCR product described below. After subcloning of a 4.7-kb *Xho*I/*Hinc*II restriction fragment containing a tryptase genomic clone into the pBluescript KS(+) vector, a portion of the insert was sequenced with tryptase-specific and vector-specific oligonucleotide primers. For sequence comparisons, BLAST provided by the Genetic Computer Group (Madison, Wis) was used.

PCR analyses of genomic DNA

Genomic DNA was analyzed for the presence of an hmMCP-7-like tryptase gene by PCR. Purified genomic DNA (100 ng) was amplified in a reaction mixture containing 20 pmol of specific hmMCP-7-like tryptase gene primers (sense primer, 5'-GGAGCAGCACCTCTACTACC-3' within exon 4; antisense primer, 5'-ACAGCTTTCAAATCGTCCGC-3' within exon 5) capable of recognizing hmMCP-7-like tryptases I, II, and III; 1 $\times$ PCR buffer; 200 μ mol/L of dNTP mix; 0.5 mmol/L of MgCl₂; and 1.5 U of Taq DNA polymerase. PCR was performed for 35 cycles; each cycle included a hot start (95°C, 5 minutes), denaturation (95°C, 1 minute), annealing (55°C, 1.5 minutes), extension (72°C, 1.5 minutes), and then a final extension (72°C, 10 minutes). After amplification of each DNA sample, the corresponding PCR product (1350 bp) was digested with *Hind*III to confirm the hmMCP-7-like

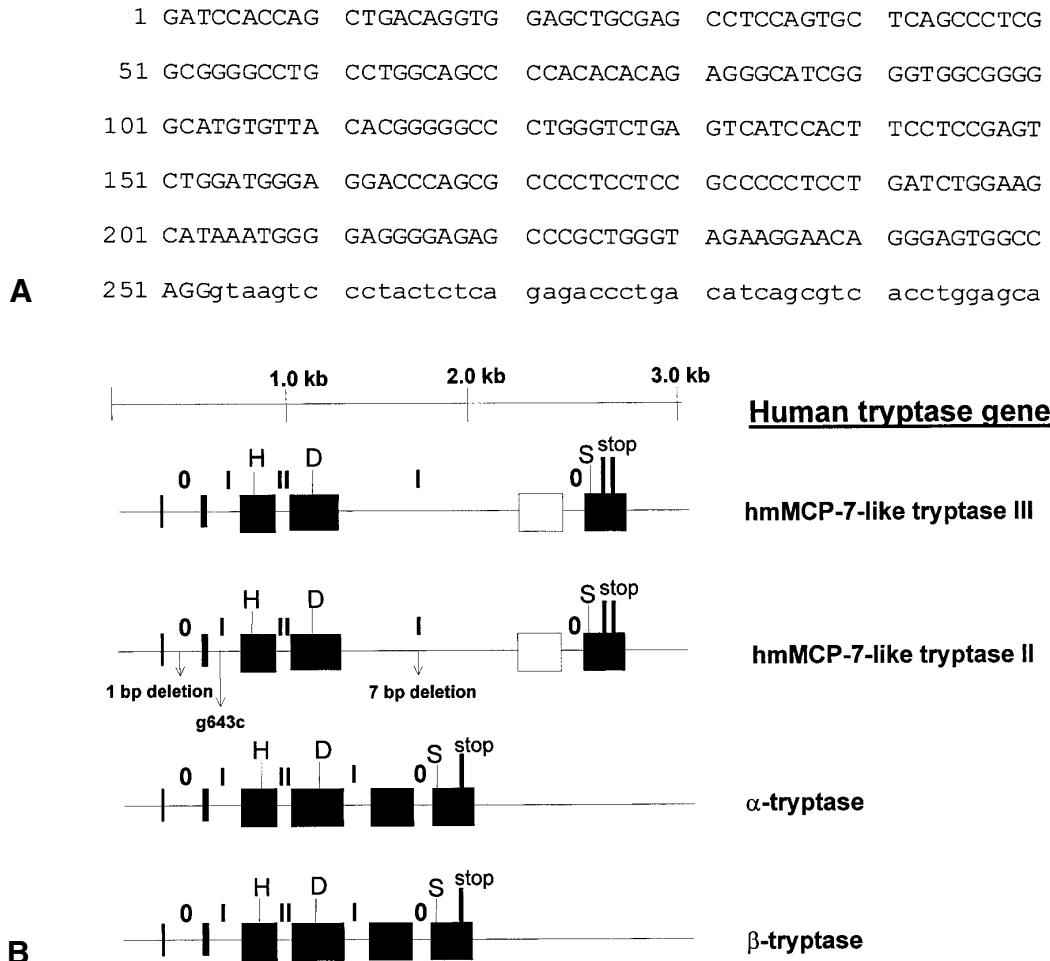


FIG 1. HmMCP-7-like tryptase III gene. **A**, HmMCP-7-like tryptase III 5' nucleotide sequence. Reported nucleotide sequences for hmMCP-like tryptases I and II begin at nucleotides 295 and 272, respectively, of that of hmMCP-like tryptase III. Consequently, only the initial 300 nucleotides of the hmMCP-III gene are displayed. *Uppercase letters* include nucleotides within exon 1; *lowercase letters* include those within intron 1. The entire sequence has been deposited in GenBank (AF318074). **B**, Organization of tryptase genes. α / β -Tryptase and hmMCP-7-like tryptases I, II, and III genes are each drawn to approximate scale. Except for hmMCP-7-like tryptase I, for which the sequence is incomplete, each is composed of 6 exons (black and white boxes), 5 introns, and identical exon/intron phase transitions noted by *Roman numerals* over each intron. Codons encoding the catalytic triad residues, histidine (H), aspartic acid (D), and serine (S), are indicated. Only the nucleotide differences between hmMCP-7-like tryptases II and III are shown (arrows), as indicated under the nucleotide sequence of hmMCP-7-like tryptase III.

nature of this gene because only hmMCP-7-like gene products are digested by this endonuclease (yielding 747-bp, 596-bp, and 6-bp fragments for hmMCP-7-like tryptase III and 747-bp and 602-bp fragments for hmMCP-7-like tryptases I and II).

RT-PCR analyses

For reverse transcription of RNA, 1 μ g of total RNA combined with 20 pmol of oligo (dT)15 primer was heated to 70°C for 10 minutes and then cooled on ice. Reaction mixture components (200 μ mol/L dNTP mix, 10 mmol/L dithiothreitol, 1 $\times$ first-strand buffer, 5 U rRNasin, and 1 μ L MMLVRT) were then added in a final volume of 20 μ L and incubated at 37°C for 2 hours, essentially as recommended by Life Technologies Inc. The cDNA synthesized by reverse transcription was diluted 5-fold to 100 μ L with water and stored at -20°C. A portion (5 μ L) of the diluted cDNA was amplified by PCR in a reaction mixture containing 20 pmol of universal

tryptase primers (sense, 5'-TGGCAGGTGAGCCTGAGAGT-3', and antisense, 5'-CCAGTGGGTGCTGACCGC-3', to yield an 86-bp product; sense, 5'-GGAGCAGCACCTCTACTACC-3', and antisense, 5'-CCCTGGTGTGCAAGGTGAAT-3', to yield a 427-bp product) of hmMCP-7-like tryptase gene-specific primers (sense, 5'-GGAGCAGCACCTCTACTACC-3', and antisense, 5'-GCGGACGATTTGAAAGCT-3', to yield a 347-bp product) or of β -actin primers (sense, 5'-TCATGAAGTGTGACGTTGACATC-CGT-3', and antisense, 5'-CCTAGAAGCATTTCGGTGCAC-GATG-3', to yield a 285-bp product), 1 $\times$ PCR buffer, 200 μ mol/L dNTP mix, and 1.5 U Taq DNA polymerase. PCR was initiated with a hot start (95°C, 5 minutes) and continued for 35 cycles of denaturation (95°C, 1 minute), annealing (55°C, 1 minute), and extension (72°C, 1 minute), before a final extension (72°C, 10 minutes). One tenth of the total PCR reaction volume was subjected to electrophoresis in a 10% polyacrylamide gel for the 86-bp product and

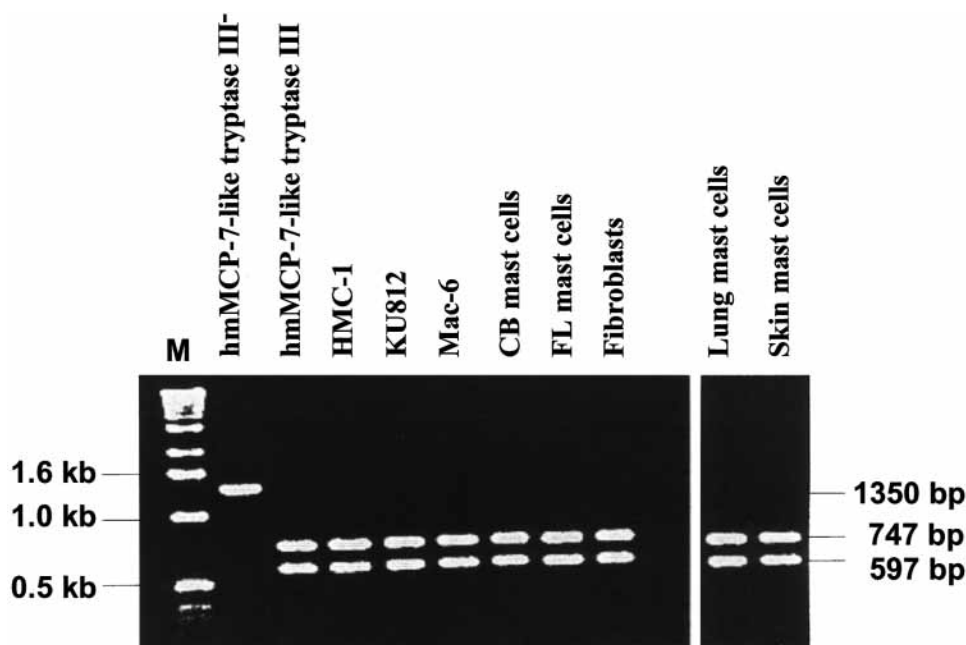


FIG 2. HmMCP-7-like tryptase is present in the normal genome. Genomic DNA from various cell lines, mast cell preparations, and fibroblasts were amplified by PCR with primers specific for hmMCP-7-like tryptase. Each lane is labeled with the origin of the DNA. The molecular weight marker (*M*) is 1-kb DNA ladder. All PCR products were digested with *HindIII* before being subjected to electrophoresis, except for hmMCP-7-like tryptase III⁻, which was not digested. Although each sample represents a single preparation, 2 fetal liver, 2 cord blood, 2 skin, and 2 lung preparations were examined and found to exhibit the same results.

a 1.5% agarose gel for the other RT-PCR products and visualized by staining with SYBR green I (1:10,000 dilution of stock) and ethidium bromide, respectively. Negative controls were routinely performed by omission of the template for both PCR and RT-PCR and omission of the MMLVRT for RT-PCR, and they did not reveal a product in the experiments considered in this study.

Identification of hmMCP-7-like tryptase genes

Two methods were used to identify hmMCP-7-like tryptase cDNA molecules after reverse transcription with an oligo (dT)15 primer. First, with universal tryptase primers, *SacII* cleaves the 86-bp PCR product obtained from exon 3 of hmMCP-7-like tryptases into 62-bp and 24-bp fragments, but it does not cleave the corresponding products from either α -tryptase or β -tryptase cDNA species. Second, the hmMCP-7-like tryptase-specific primers do not yield a PCR product from α -tryptase and β -tryptase cDNA molecules. Although the universal 86-bp product does not distinguish genomic DNA from mRNA-derived cDNA, the 347-bp product from putative hmMCP-7-like tryptase is smaller than that derived from genomic DNA (1350 bp).

RESULTS

Cloning, subcloning, and sequencing of an hmMCP-7-like tryptase gene

Among approximately 450,000 plaques screened, 2 positive clones were identified. The presence of a tryptase gene or genes in these clones was confirmed by Southern blotting with the tryptase probe used for screening the library and by PCR with universal tryptase primers (data not shown). One of the 2 clones revealed only β -like tryptase by the restriction digest patterns of the corresponding PCR products

obtained with universal tryptase primers (data not shown) and was not further evaluated in this study. Phage DNA from the other clone was isolated and digested with *XhoI* to release insert DNA, which was about 20.0 kb in length by agarose gel electrophoresis. *SacII* restriction digests of the 86-bp PCR product obtained with universal tryptase primers suggested the presence of hmMCP-7-like tryptase, but neither α - nor β -like tryptases. A 6.5-kb (*XhoI* $\times$ *HincII*) fragment of the 20-kb insert was subcloned into pBluescript SKII⁺. This fragment was further digested with *XhoI* and *SacI* to create a 4.7-kb fragment that contained the hmMCP-7-like tryptase gene, which in turn was subcloned and sequenced over a span of 2842 bp, from both the 5' and 3' directions, including 463 bp upstream of putative exon 2 (translation-initiation codon) and 177 bp downstream from the stop codon after amino acid 275 in α - and β -tryptases. The initial 300 nucleotide are shown in Fig 1, A.

Putative exon 1, as reported for α -tryptase and β -tryptase,^{3,4} is predicted to be untranslated. Translation is anticipated to begin at the ATG codon (nucleotides 464, 184, and 171 for hmMCP-7-like tryptases III, II, and I, respectively) encoding methionine at the beginning of exon 2. Putative catalytic triad residues His, Asp, and Ser are found in exons 3, 4, and 6, respectively, for all tryptases except hmMCP-7-like tryptase I, for which the known sequence extends only to intron 4. In contrast to α -tryptase and β -tryptase coding regions, the hmMCP-7-like tryptase described here, like hmMCP-7-like tryptase II, contains 2 in-frame stop codons in putative exon 6, one of which terminates translation 40 amino acids before the terminal

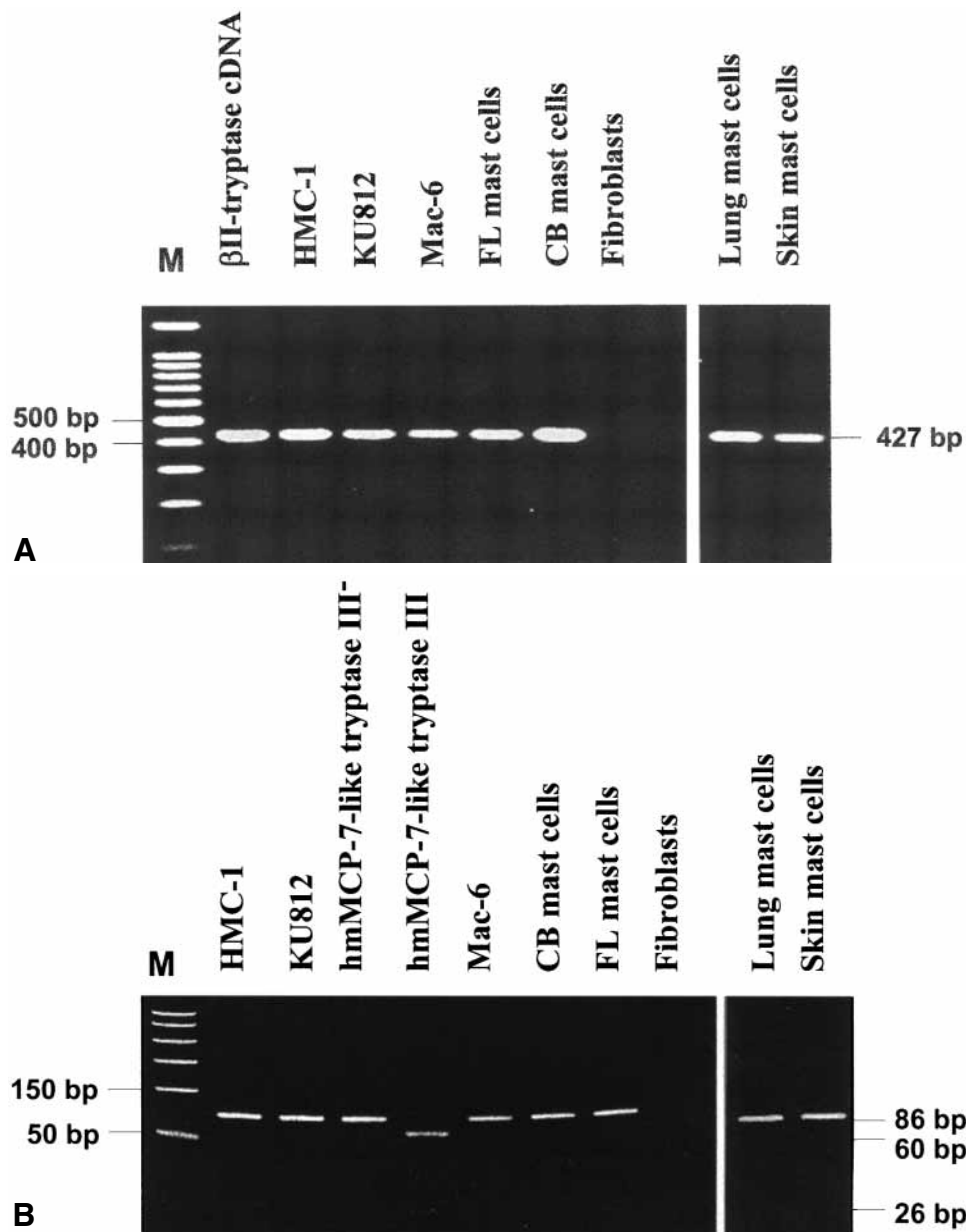


FIG 3. HmMCP-7-like tryptase is not expressed by human mast cells. **A**, RT-PCR with universal tryptase primers. The origin of the template is designated above each lane. CB, Cord blood derived; FL, fetal liver derived. The position of the expected 427-bp product of tryptase mRNA is shown to the right. **B**, RT-PCR with universal tryptase primers followed by digestion with *Sac*I in each case except lane *hmMCP-7-like tryptase III-*, which was not digested. The positions of the expected RT-PCR product (86 bp) and fragments (60 and 26 bp) are shown to the right. The molecular weight markers (M) in each panel are from a 100-bp DNA ladder. Although each lane represents the result from a single experiment, 2 fetal liver, 2 cord blood, 2 skin, and 2 lung preparations were examined, and each was found to exhibit essentially the same result.

residue of α - and β -tryptases. Also, intron 4 is substantially larger in all hmMCP-7-like tryptases than in α - and β -tryptases. The cloned tryptase gene described in this study is 99% or more identical to ones described previously and called hmMCP-7-like tryptases I and II.⁴ The current hmMCP-7-like tryptase gene differs from II and I by deletion of an *a* between nucleotides 310 and 311 in intron 1 and a 7-nucleotide insert between nucleotides 1742 and 1750 of intron 4; from II by substitution of c^{373} (II) to g^{643}

(III) in intron 2; and from I by substitution of $g^{214}c^{215}$ to $c^{507}g^{508}$ in exon 2 (R-16P amino acid change), of g^{223} (I) to t^{516} (III) in exon 2 (V-13G amino acid change), of a^{539} (I) to g^{832} (III) in exon 3 (no amino acid change), and of t^{1164} (I) to c^{1457} (III) in intron 4. All hmMCP-7-like genes encode a Q-3, like α -tryptase, suggesting processing at this site may occur suboptimally if at all. Because of the close homologies to hmMCP-7-like tryptases I and II, the current one is called hmMCP-7-like tryptase III. Exon 5 of

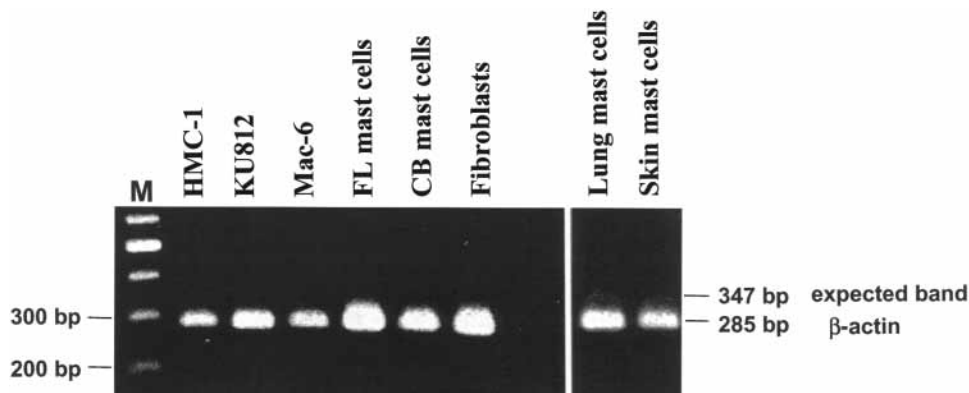


FIG 4. RT-PCR using hmMCP-7-like tryptase-specific primers and β -actin-specific primers. The origin of the template is shown above each lane. The positions of the expected RT-PCR products (285 bp, actin; 347 bp, hmMCP-7-like tryptase) are shown to the right. The molecular weight marker (M) is from a 100-bp DNA ladder. Although each lane represents the result from a single experiment, 2 fetal liver, 2 cord blood, 2 skin, and 2 lung preparations were examined, and each was found to exhibit essentially the same result.

this tryptase is more homologous to mMCP-7 (75% nucleotide sequence, 70% amino acid sequence) than to either α (I)-tryptase (71% nucleotide sequence, 60% amino acid sequence) or β (II)-tryptase (71% nucleotide sequence, 64% amino acid sequence). Using intron 4 and exon 5 of hmMCP-7-like tryptase as probes, we searched GenBank for homologous sequences using Blast. But no sequences with sufficient homology to be of interest were found other than hmMCP-7-like tryptases I and II.

The organization of the genes for hmMCP-7-like tryptases III and II, α -tryptase, and β -tryptase is summarized in Fig 1, B. HmMCP-7-like tryptase III shows similar intron sizes and exon/intron phase transitions to those of hmMCP-7-like tryptases II and I. Whether hmMCP-7-like tryptases are distinct genes or allelic variants remains to be determined.

PCR amplification of genomic hmMCP-7-like tryptase DNA

Although hmMCP-7-like tryptases were cloned from genomic libraries, their routine presence in the normal genome needed to be assessed. Accordingly, genomic DNA was isolated from cell lines HMC-1, KU812, and Mac-6; from mast cells derived in vitro from cord blood and fetal liver progenitors; from dispersed cells enriched for mast cells from lung and skin; and from the skin fibroblast cell line 1059SK, each preparation reflecting a different individual. HmMCP-7-like tryptase-specific primers were used to amplify each DNA sample (1350-bp product), which was then digested with *Hind*III into 747-bp, 596-bp, and 6-bp fragments (not visualized) for hmMCP-7-like tryptase III and 747-bp and 602-bp fragments for hmMCP-7-like tryptase II, the 597-bp and 602-bp bands being indistinguishable from each other under the experimental conditions used. Fig 2 shows that all genomic DNA samples have hmMCP-7-like tryptase genes.

RT-PCR analyses with tryptases and hmMCP-7-like specific primers

RT-PCR was used to analyze whether the hmMCP-

7-like tryptase genes are transcriptionally active in the cells listed above. Prior studies indicated HMC-1 and KU812 cells express primarily β -tryptase mRNA, Mac-6 cells express primarily α -tryptase mRNA,^{12,20,21} and mast cells derived from skin and lung or from fetal liver progenitors express both α -tryptase and β -tryptase.^{11,12} Fig 3, A, shows the RT-PCR product (427-bp fragment between exons 4 and 6) with universal tryptase primers used for each cell preparation other than fibroblasts (lane 7). Next, RT-PCR with universal PCR primers was used to amplify an 86-bp portion of tryptase in exon 3 from the above cells and from cloned hmMCP-7-like tryptase III DNA (Fig 3, B). Products were then digested with *Sac*II to yield 60-bp and 26-bp fragments only in the case of hmMCP-7-like tryptase. As shown, only the hmMCP-7-like tryptase III positive control, but none of the cell-derived mRNA samples, yielded an hmMCP-7-like tryptase digestion pattern. This result suggests that hmMCP-7-like tryptases are not expressed in these cells. To confirm this result, we performed RT-PCR with hmMCP-7-like tryptase-specific primers recognizing complementary sequences in exons 4 and 5 that generate a 347-bp product from cDNA and a 1350-bp fragment (Fig 2) from genomic DNA. β -Actin-specific primers were used as positive comparative controls. As shown in Fig 4, a β -actin product (285 bp) was observed in each case, but no hmMCP-7-like tryptase product was detected.

To determine whether hmMCP-7-like tryptase mRNA might be induced by mast cell activation, skin-derived mast cells were exposed to 1 μ g/mL of 22E7 or a nonimmune isotype-matched control mAb for up to 24 hours. In the 2 experiments performed with different samples of skin, at 15 minutes spontaneous degranulation measured by tryptase protein release was 6% and 7%, whereas 22E7-stimulated release was 16% and 24%. However, after reverse transcription and 35 cycles of PCR with RNA extracted at 0, 1, 3, 6, 12, and 24 hours after activation were initiated, no hmMCP-7-like product was detected, even though the α/β -tryptase product was clearly discernible. Thus mast cell activation did not result in detectable induction of hmMCP-7-like gene transcription.

DISCUSSION

The presence of an hmMCP-7-like tryptase gene or genes in preparations of mast cells and in various cell lines in 12 different genomes without evidence for expression of corresponding mRNA indicates such genes are nonfunctional (ie, pseudogenes).²² They may have arisen by duplication of an ancestral mMCP-7-like tryptase gene because introns are present and the nucleotide sequence of exon 5 is more homologous to that of mMCP-7 than to those of either human α -tryptase or human β -tryptase. The hmMCP-7-like tryptase gene sequence reported here is slightly different than the 2 sequences reported previously. Neither the current nor previous studies have clarified whether there is more than 1 hmMCP-7-like tryptase gene in a normal chromosome. It is likely that there would be little evolutionary pressure to conserve this sequence because it appears to be a pseudogene. Thus polymorphisms might arise frequently.

The failure to detect hmMCP-7-like mRNA in various preparations of resting mast cells and cell lines and in activated skin-derived mast cells suggests that little, if any, is produced in vivo. On the basis of an estimate of the number of molecules of cloned hmMCP-7-like tryptase DNA detectable with the PCR method used and the mast cell equivalents of RNA from skin and lung examined by RT-PCR, and with assumption of an efficient reverse transcription step, the potential number of hmMCP-7-like tryptase mRNA molecules should be less than 1 per mast cell. It is therefore unlikely that such mast cells could produce enough hmMCP-7-like tryptase to affect the measurement of either α - or β -tryptases.

Even if hmMCP-7-like tryptase were transcribed and translated, it is unlikely the protein would be stable. The 40-amino acid truncation is likely to substantially impair formation of the substrate binding pocket, even though the catalytic triad residues would be expressed. It is also uncertain whether the proenzyme can be processed to a mature protein because there is a Q at position -3, similar to α -protryptase.⁵ Alternatively, this gene might be activated by cell types or conditions not tested in this study. However, expression by another cell type is unlikely to be a common event because, among normal cells and tissues, only mast cells and basophils are labeled with polyclonal and monoclonal antitryptase antibodies made against purified human tryptase.²³⁻²⁶ We conclude that mMCP-7-like tryptase is a pseudogene that will not contribute to the measurements of α and β types of tryptase.

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