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Immunology

Isolation, characterization, and transcription of the gene encoding mouse mast cell protease 7

(genomic cloning/tryptase/gene expression)

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ABSTRACT **A gene that encodes mouse mast cell protease (mMCP) 7 (also known as mouse mast cell tryptase 2) was isolated by genomic cloning with a cDNA that encodes mMCP-6, a tryptase in serosal mast cells. cDNAs encoding mMCP-7 were isolated from a bone-marrow-derived mast cell cDNA library. The mMCP-7 gene spans 2.3 kilobases and contains five exons rather than six, as found in the mMCP-6 and human mast cell tryptase I genes. Comparison of the 5′ end of the transcript with the genomic sequence indicated that the region corresponding to the first intron in the mMCP-6 and human tryptase I genes is not spliced during transcription of mMCP-7 mRNA because of a point mutation at the intron 1 acceptor splice site; this results in a 5′ untranslated region of 195 nucleotides, which is longer than that of any other known mast cell-specific transcript. mMCP-7 is 71–76% homologous with mMCP-6 and with dog and human mast cell tryptases, and it is the most acidic mast cell protease, with an overall net charge of −10. RNA blot analyses revealed that the mMCP-7 gene is transcribed in bone-marrow-derived mast cells but is not transcribed in mature serosal mast cells or in mucosal mast cell-enriched intestinal tissue of *Trichinella spiralis*-infected mice. Transcription of the mMCP-7 gene by differentiating bone-marrow-derived mast cells occurred within 1 week of bone-marrow culture but decreased dramatically after 3 weeks. Thus, the mMCP-7 gene displays a number of unusual structural characteristics and is distinctive in its transient and selective expression in immature mast cells maintained in interleukin 3-enriched medium.**

The secretory granules of mouse mast cells contain a family of serine proteases termed mouse mast cell protease (mMCP) 1 through mMCP-6 (1). mMCP-1 (2-4), mMCP-2 (5), mMCP-4 (6), and mMCP-5 (7) have been designated as chymases because of the predicted chymotryptic-like substrate specificities of their translated gene products. mMCP-6 (8) has been designated a tryptase because of its predicted tryptic-like substrate specificity. The mouse mast cell chymases have overall net charges at pH 7 from +2 to +15, whereas mMCP-6 has an overall net charge of −5. Serosal mast cells selectively express mMCP-4, mMCP-5, and mMCP-6, whereas mucosal mast cells that proliferate in the intestines of helminth-infected mice selectively express mMCP-1 and mMCP-2. The mMCP-5 and mMCP-6 genes are transcribed in bone-marrow-derived mast cells (BMMCs) obtained by culturing bone-marrow cells in medium containing interleukin (IL) 3. Because the BMMCs reconstitute all populations of mast cells *in vivo* when injected into mast cell-deficient $WBB6F_1$-W/W^v mice (9), the mMCP-5 and mMCP-6 genes are termed early-expressed protease genes. BMMCs exposed to the fibroblast-derived cytokine, c-*kit* ligand, transcribe the mMCP-4 gene (10), whereas BMMCs exposed to IL-10 express the mMCP-1 and mMCP-2 genes (4, 11). IL-3 not only induces transcription of the two early-expressed genes but also dominantly suppresses transcription of the three late-expressed protease genes that are differentially induced by c-*kit* ligand and IL-10.

Previous DNA-blot studies revealed that the mouse genome contains a second gene that hybridizes to mMCP-6 cDNA (8, 12), suggesting that this gene encodes an additional mast cell tryptase. Recently, the amino acid sequence of a mature mouse mast cell tryptase distinct from mMCP-6 (termed mouse mast cell tryptase 2) was reported, and its three-dimensional structure was determined by molecular modeling (13). We have now isolated and characterized a gene [designated the mMCP-7 gene (12)] that is homologous to the mMCP-6 gene and that encodes a mature serine protease identical to mouse mast cell tryptase 2 (13).§ Structural characterization of the mMCP-7 gene and transcript indicates numerous features in the genomic organization and translated gene product that differ from mMCP-6 and the recognized human (14-16) and dog (17) tryptases. Furthermore, unlike the previously characterized tryptases, mMCP-7 is not expressed in mature serosal or mucosal mast cells and is expressed only transiently at an early stage of *in vitro* mast cell differentiation.

MATERIALS AND METHODS

Isolation of mMCP-7 Gene. Approximately 6×10^5 recombinants from a mouse DBA/2 genomic DNA library in EMBL-3 (ML1009d; Clontech) were screened with a full-length mMCP-6 cDNA (8) that had been radiolabeled with [α-^{32}P]dCTP (3000 Ci/mmol; DuPont/New England Nuclear; 1 Ci = 37 GBq) using a random priming kit (Stratagene). Of the three recombinants isolated, restriction enzyme analyses indicated that clone G-7.1 contained a gene that was homologous, but distinct, from the mMCP-6 gene. A 6.6-kilobase (kb) *Eco*RI–*Eco*RI fragment from this clone was subcloned into pBluescript II (Stratagene), and the nucleotide sequence of its 5′ end was determined by using the dideoxynucleotide chain-termination method of Sanger *et al.* (18) with modifications essential for double-stranded sequencing (19). Two additional genomic clones, G-7.2 and G-7.3, were obtained by screening a BALB/c mouse genomic library from H. Avraham (Harvard Medical School) with a 168-base-pair (bp) fragment derived from a region of clone G-7.1. By comparison with the mMCP-6 gene, this PCR-generated (20) frag-

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Abbreviations: BMMC, bone-marrow-derived mast cell; IL, interleukin; KiSV, Kirsten sarcoma virus; mMCP, mouse mast cell protease.

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§The sequences reported in this paper have been deposited in the GenBank data base (accession nos. L00653 and L00654).

ment was predicted to constitute the 3′-untranslated region of the gene. A 4.5-kb *Hin*dIII–*Hin*dIII DNA fragment was excised from clone G-7.3 and subcloned into pUC18 (Pharmacia); the complete nucleotide sequence of the portion that contained the mMCP-7 gene was then determined in both strands.

RNA Blot Analyses. Mouse serosal mast cells were collected from BALB/c mice (21). Mouse Kirsten sarcoma virus (KiSV)-immortalized mast cell lines MC5 and MC8 (22), mouse P815 mastocytoma cells [line TIB-64, American Type Culture Collection (ATCC)], WEHI-3 myelomonocytic cells (line TIB-68, ATCC), and Abelson murine leukemia virus-transformed mast cell line, ABFTL (23), were all cultured in enriched medium [RPMI 1640 medium supplemented with penicillin at 100 units/ml, streptomycin at 100 μg/ml, 2 mM glutamine, 0.1 mM nonessential amino acids, 5 μM 2-mercaptoethanol, and 15% fetal calf serum (GIBCO)]. Mouse MC9 mast cells (24) (line CRL 8306, ATCC) were cultured in 50% enriched medium/50% Con A-stimulated mouse splenocyte-conditioned medium. BMMCs were obtained by culturing BALB/c mouse bone-marrow cells in 50% enriched medium/50% WEHI-3 cell-conditioned medium for 3–6 weeks (25). To obtain mucosal mast cell-enriched intestinal tissue, BALB/c mice were infected with *Trichinella spiralis*, and the intestinal tissue was harvested 10 days later (4). Total (26) or poly(A)$^+$ RNA (27) was purified from cells and tissues. Samples of RNA were denatured in formaldehyde/formamide, electrophoresed in 1.3% formaldehyde-agarose gels, and transferred to MagnaGraph membranes (MSI, Westover, MA) (28). The resulting blots were incubated for 1 hr in Quikhyb buffer (Stratagene) at 68°C containing salmon sperm DNA at 100 μg/ml and the ^{32}P-labeled 168-bp mMCP-7 probe. Each radiolabeled RNA blot was then washed at 60–65°C in 15 mM NaCl/1.5 mM sodium citrate/0.1% SDS before autoradiography. The blots were then stripped and analyzed with gene-specific probes for mMCP-2 (5), mMCP-5 (7), mMCP-6 (8), and actin (29).

Isolation of mMCP-7 cDNAs and Determination of the Primary Transcription-Initiation Site. The radiolabeled 168-bp DNA fragment was used to screen a cDNA library prepared from BALB/c mouse BMMCs cultured in 50% WEHI-3 cell-conditioned medium. Three distinct cDNAs (cDNA-7.1, -7.2, and -7.3) were isolated and subcloned into pBluescript II; their nucleotide sequences were then determined. The 5′ end of the mMCP-7 transcript in ABFTL mast cells was defined by primer-extension analysis and RNA sequencing with a kit from Promega and an antisense 25-mer oligonucleotide(5′-TTGGACACAGTGAGGAGTAGTGCG-G-3′) complementary to nucleotides (nt) 2–26 of the sense strand of the mMCP-7 cDNA-7.1. The extension reaction was done in the presence or absence of dideoxynucleotides, and the resulting product was electrophoresed in an 8% polyacrylamide/7 M urea gel to determine the size and sequence of the extended primer.

RESULTS

Isolation of mMCP-7 Gene. The deduced nucleotide sequence of genomic clone G-7.1 indicated that it contained the 3′ half of a serine protease gene that was homologous to, but distinct from, the mMCP-6 gene. This gene has previously been designated the mMCP-7 gene (12). A 168-bp fragment from clone G-7.1 that was predicted to reside in the 3′ untranslated region of the mMCP-7 gene differed significantly from the corresponding region in the mMCP-6 gene. When a genomic DNA blot was probed with the 168-bp fragment under high-stringency conditions, hybridization to only one band was seen (data not shown), confirming that this fragment could serve as a gene-specific probe for mMCP-7. This probe was then used to screen a second mouse genomic library to isolate two additional clones that both contained a 4.5-kb *Hin*dIII–*Hin*dIII fragment that included the entire mMCP-7 gene. The nucleotide sequence of the mMCP-7 gene in clone G-7.3 is depicted in Fig. 1.

Transcription of mMCP-7 Gene in Mast Cells and Isolation of mMCP-7 cDNA. To determine which, if any, population of mouse mast cells transcribed the mMCP-7 gene, blots containing total RNA from various mast cell populations were analyzed with the 168-bp, gene-specific probe. Fig. 2 shows that an ≈1.3-kb transcript was detected in nontransformed BMMCs cultured in 50% WEHI-3 cell-conditioned medium (lane 2) and in the transformed KiSV-MC8 (lane 6) and ABFTL (lane 8) mast cell lines. Prolonged exposure of the autoradiograph indicated that the KiSV-MC5 cell line also contained a small amount of mMCP-7 mRNA (data not shown). The MC9 (lane 7) and P815 (lane 9) mast cell lines and the myelomonocytic WEHI-3 cell line (lane 10) did not contain mMCP-7 mRNA. No mMCP-7 mRNA was detected in serosal mast cells (lane 1), in intestinal tissue from normal mice (lane 4), or in mucosal mast cell-enriched intestinal tissue from mice infected with *T. spiralis* (lane 3). mMCP-6 mRNA and mMCP-2 mRNA were present in abundance in lanes 1 and 3, respectively, indicating that these RNA samples contained serosal and mucosal mast cell-specific transcripts.

To determine the kinetics of transcription of the mMCP-7 gene during differentiation of BMMCs, bone-marrow progenitor cells were cultured for 6 weeks in 50% WEHI-3 cell-conditioned medium, and samples were taken from the cultures at weekly intervals for RNA analyses. mMCP-7 mRNA was detected after 1 week of culture along with mMCP-6 and mMCP-5 (Fig. 3). The amount of mMCP-7 mRNA increased up to the third week of the culture and then decreased dramatically, whereas the amount of mMCP-5 mRNA remained constant, and the amount of mMCP-6 mRNA decreased slightly.

A cDNA library prepared from BMMC RNA was screened with the gene-specific mMCP-7 probe to isolate 10 recombinant phage clones. Of the three that were subcloned and analyzed further, cDNA-7.1 contained an open reading frame of 819 bp, commencing with an ATG translation-initiation codon preceded by 26 bp of 5′-untranslated sequence. The 3′-untranslated region consisted of 176 bp and included a polyadenylylation signal sequence followed by a poly(A) tail. cDNA-7.2 and cDNA-7.3 were mMCP-7 cDNAs that were truncated at their 3′ and 5′ ends, respectively. The nucleotide sequences of the mMCP-7 cDNA (Fig. 4) and the exons of the mMCP-7 gene (Fig. 1) were identical.

Primer-extension analysis and RNA sequencing were done with RNA from ABFTL cells to determine the primary transcription-initiation site of the mMCP-7 gene. A total of 171 nt were extended onto the primer (data not shown). Thus, the major mMCP-7 transcript in this mast cell line extends an additional 169 nt 5′ of cDNA-7.1, indicating that the 5′ untranslated region spans 195 nt. Direct sequencing of this region of the transcript indicated that the sequence of the 5′ end of mMCP-7 mRNA was identical to the genomic sequence immediately 5′ of the translation-initiation site (data not shown). In addition, a 30-mer antisense oligonucleotide probe complementary to a region 40–70 nt upstream of the 5′ end of cDNA-7.1 hybridized to the mature mMCP-7 transcript in an RNA blot (data not shown), confirming that the 5′-untranslated region and the protein-coding portion of the transcript are contiguous.

Analysis of the consensus sequence of the cDNA indicates that mMCP-7 is initially translated as a protein of 273 amino acids with a predicted molecular size of 30.3 kDa. Based upon the rules of von Heijne (30), the N-terminal 18 amino acids compose a hydrophobic signal peptide that is predicted to be cleaved at Ala^{11}-Ala^{10} in the endoplasmic reticulum (Fig. 4).

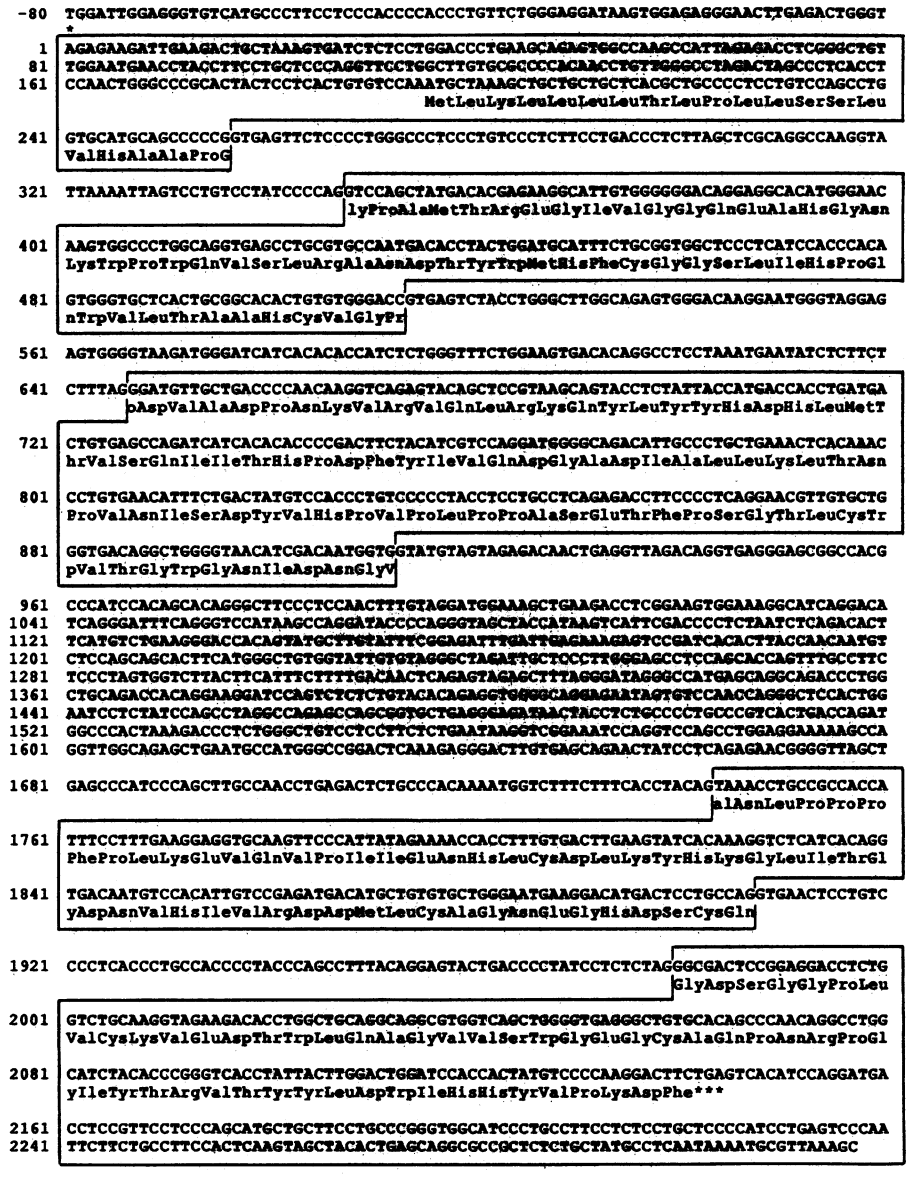

FIG. 1. Exon/intron organization and nucleotide sequence of the mMCP-7 gene. Exons are boxed, and predicted translated amino acids are shown below the nucleotide sequence. Numbering of nucleotides begins at the transcription-initiation site (*).

Based on its homology to mMCP-6 and human mast cell tryptase I, mMCP-7 is predicted to contain a 10-amino acid activation peptide that is cleaved at Gly^{-1}-Ile^{1} to provide a mature enzyme consisting of 245 amino acids with a molecular size of 27.4 kDa. Asp^{188} indicates that mMCP-7 possesses tryptic-like substrate specificity. The protein core contains two potential N-glycosylation sites at Asn^{21} and Asn^{102}. The presence and location of eight cysteine residues are homologous to that in other tryptases and imply that mMCP-7 has four intramolecular disulfide bonds. The net charge of mMCP-7 at neutral pH is −10 (15 arginine plus lysine residues versus 25 glutamate plus aspartate residues). The deduced amino acid sequence of mature mMCP-7 is identical to that of mature mast cell tryptase 2 (13).

DISCUSSION

The existence of multiple mast cell tryptase genes was known in the dog (17) and human (14-16) and implied in the mouse when the mMCP-6 tryptase gene was cloned (8). Because the mMCP-6 cDNA cross-hybridized with a second fragment in a genomic DNA blot (8), it was used to isolate a gene that we have designated the mMCP-7 gene. Although clone G-7.1 contained only the 3′ half of the mMCP-7 gene, a 168-bp fragment from the predicted 3′-untranslated region was used to screen a second genomic library to isolate clones G-7.2 and G-7.3, which contained complete copies of this gene.

Transcription of the mMCP-7 gene by mast cells was confirmed by RNA blot analysis with the 168-bp gene-specific probe (Figs. 2 and 3). Because BMMCs express the mMCP-7 transcript, cDNAs encoding mMCP-7 were isolated from a mouse BMMC cDNA library (Fig. 4). The consensus nucleotide sequence of the mMCP-7 cDNA was identical to the predicted exons of the genomic sequence (Fig. 1). Whereas cDNA-7.1 spanned ≈1 kb, the mMCP-7 transcript detected by RNA-blot analysis was ≈1.3 kb (Figs. 2 and 3), suggesting the presence of an extended 5′-untranslated region. Primer-extension analysis of mMCP-7 mRNA confirmed that the transcript extended an additional 169 nt 5′ of the isolated cDNA, indicating that the transcription-initiation site is located 195 nt upstream of the translation-initiation site. No mast cell-specific transcript has been found to contain such a large 5′-untranslated region, which is considerably larger than the 23 nt in both mMCP-6 (8) and human tryptase I (16) transcripts. Comparison of genomic sequence (Fig. 1) with mRNA sequence indicated that the 5′-

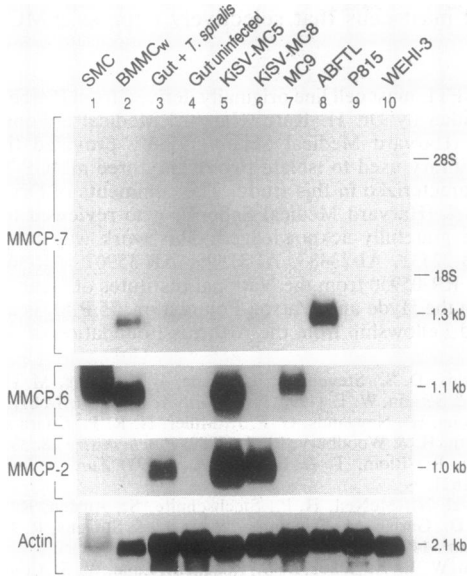

FIG. 2. mMCP-7 mRNA levels in various mouse mast cells. A blot containing total RNA from serosal mast cells (SMC) (≈2 μg, lane 1), BMMCs cultured in 50% WEHI-3 cell-conditioned medium for 3 weeks ($BMMC_W$) (10 μg, lane 2), KiSV-MC5 cells (10 μg, lane 5), KiSV-MC8 cells (10 μg, lane 6), MC9 cells (10 μg, lane 7), ABFTL cells (10 μg, lane 8), P815 cells (10 μg, lane 9), WEHI-3 cells (10 μg, lane 10), and poly(A)$^+$ RNA from the intestines of *T. spiralis*-infected mice (≈2 μg, lane 3) or uninfected mice (≈5 μg, lane 4) were probed with gene-specific fragments for mMCP-7, mMCP-6, mMCP-2, and actin. Sizes of various transcripts are indicated on right.

untranslated region of mMCP-7 mRNA is contiguous with the protein-coding region of exon 1, whereas an intron separates these two domains in both mMCP-6 and human tryptase I genes (8, 16). A comparison of these two genes with the mMCP-7 gene in this region (Fig. 5) shows that although all three genes contain an intron donor splice sequence beginning 24 bp after the transcription-initiation site, the mMCP-7 gene lacks a consensus acceptor splice sequence in the region immediately upstream of the translation-initiation site. This finding suggests that the extended 5′-untranslated sequence in mMCP-7 is a consequence of a mutation in the 3′ acceptor site. Thus, the mMCP-7 gene spans 2.3 kb but only contains five exons (Fig. 1). The other exon/intron junctions conform to the consensus sequences defined by Breathnach and Chambon (31) and are in identical locations to those in the mMCP-6 and human tryptase I genes. The 5′-flanking se-

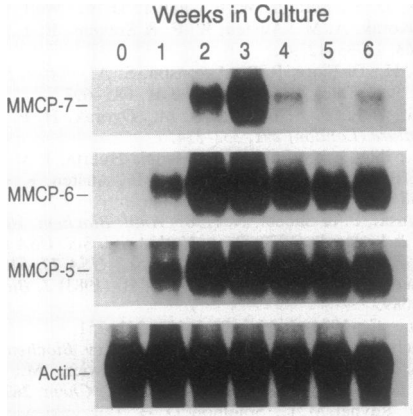

FIG. 3. Kinetics of expression of mMCP-7, mMCP-6, mMCP-5, and actin mRNAs in BMMCs. A blot containing total RNA from bone-marrow cells (0 weeks) and from cells cultured for 1–6 weeks in 50% WEHI-3 cell-conditioned medium was probed with gene-specific fragments for these transcripts.

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                                        **
[-28]                                   MetLeuLysLeuLeuLeuLeuThrLeuProLeu
  1  CCCGCACTACTCCTCACTGTGTCCAA ATGCTAAAGCTGCTGCTGCTCACGCTGCCCCTC

                                        |.....................|  $
[-17] LeuSerSerLeuValHisAlaAlaProGlyProAlaMetThrArgGluGlyIleValGly
 60   CTGTCCAGCCTGGTGCATGCAGCCCCCGGTCCAGCTATGACACGAGAAGGCATTGTGGGG

                                                              ‡
 [4] GlyGlnGluAlaHisGlyAsnLysTrpProTrpGlnValSerLeuArgAlaAsnAspThr
120  GGACAGGAGGCACATGGGAACAAGTGGCCCTGGCAGGTGAGCCTGCGTGCCAATGACACC

[24] TyrTrpMetHisPheCysGlyGlySerLeuIleHisProGlnTrpValLeuThrAlaAla
180  TACTGGATGCATTTCTGCGGTGGCTCCCTCATCCACCCACAGTGGGTGCTCACTGCGGCA

     ▬▬
[44] HisCysValGlyProAspValAlaAspProAsnLysValArgValGlnLeuArgLysGln
240  CACTGTGTGGGACCGGATGTTGCTGACCCCAACAAGGTCAGAGTACAGCTCCGTAAGCAG

[64] TyrLeuTyrTyrHisAspHisLeuMetThrValSerGlnIleIleThrHisProAspPhe
300  TACCTCTATTACCATGACCACCTGATGACTGTGAGCCAGATCATCACACACCCCGACTTC

                                                              ‡
     ▬▬
[84] TyrIleValGlnAspGlyAlaAspIleAlaLeuLeuLysLeuThrAsnProValAsnIle
360  TACATCGTCCAGGATGGGGCAGACATTGCCCTGCTGAAACTCACAAACCCTGTGAACATT

[104] SerAspTyrValHisProValProLeuProProAlaSerGluThrPheProSerGlyThr
420   TCTGACTATGTCCACCCTGTCCCCCTACCTCCTGCCTCAGAGACCTTCCCCTCAGGAACG

[124] LeuCysTrpValThrGlyTrpGlyAsnIleAspAsnGlyValAsnLeuProProProPhe
480   TTGTGCTGGGTGACAGGCTGGGGTAACATCGACAATGGTGTAAACCTGCCGCCACCATTT

[144] ProLeuLysGluValGlnValProIleIleGluAsnHisLeuCysAspLeuLysTyrHis
540   CCTTTGAAGGAGGTGCAAGTTCCCATTATAGAAAACCACCTTTGTGACTTGAAGTATCAC

[164] LysGlyLeuIleThrGlyAspAsnValHisIleValArgAspAspMetLeuCysAlaGly
600   AAAGGTCTCATCACAGGTGACAATGTCCACATTGTCCGAGATGACATGCTGTGTGCTGGG

      ▬▬
[184] AsnGluGlyHisAspSerCysGlnGlyAspSerGlyGlyProLeuValCysLysValGlu
660   AATGAAGGACATGACTCCTGCCAGGGCGACTCCGGAGGACCTCTGGTCTGCAAGGTAGAA

[204] AspThrTrpLeuGlnAlaGlyValValSerTrpGlyGluGlyCysAlaGlnProAsnArg
720   GACACCTGGCTGCAGGCAGGCGTGGTCAGCTGGGGTGAGGGCTGTGCACAGCCCAACAGG

[224] ProGlyIleTyrThrArgValThrTyrTyrLeuAspTrpIleHisHisTyrValProLys
780   CCTGGCATCTACACCCGGGTCACCTATTACTTGGACTGGATCCACCACTATGTCCCCAAG

[244] AspPhe***
840   GACTTCTGAGTCACATCCAGGATGACCTCCGTTCCTCCCAGCATGCTGCTTCCTGCCCGG

900   GTGGCATCCCTGCCTTCCTCTCCTGCTCCCCATCCTGAGTCCCAATTCTTCTGCCTTCCA

960   CTCAAGTAGCTACACTGAGCAGGCGCCGCTCTCTGCTATGCCTCAATAAAATGCGTTAAA

1020  GCAAAAAAAAAA
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FIG. 4. Nucleotide sequence (lower line) and deduced amino acid sequence (upper line) of mMCP-7 cDNA. The translation-initiation codon (**), translation-termination signal (***), signal peptide (overlined), activation peptide (· · ·), potential N-linked glycosylation sites (‡), components of the catalytic triad (▬), 168-bp region used as a gene-specific probe (underlined), and polyadenylylation signal sequence (double underlined) are indicated. Nucleotides are numbered beginning at the 5′ end of cDNA-7.1. Amino acid numbering (brackets) begins with residue 1 of the mature protein (§). Arrows indicate the predicted cleavage sites for the hydrophobic signal peptide and the activation peptide.

quence of the mMCP-7 gene demonstrates homology with both the human tryptase I and mMCP-6 genes, including a putative TATA element ≈28 bp upstream of exon 1 (Fig. 5).

Based upon the structure of its gene and cDNA, mMCP-7 is translated as a 30.3-kDa zymogen that has an 18-residue hydrophobic leader sequence followed by a 10-amino acid activation peptide. The activation peptide ends in the sequence Arg-Glu-Gly, which is more similar to the Arg-Val-Gly sequence in mMCP-6 (8), human β-tryptase (15), human skin tryptases I to III (16), and dog tryptase (17), than to the Glu-Ala-Gly sequence in human α-tryptase (14). The mature form of mMCP-7 is predicted to commence at Ile-Val-Gly-Gly based upon a comparison with the known N-terminal amino acid sequence of mMCP-6 (1) and human pituitary tryptase (32); it consists of 245 residues and has a predicted molecular size of 27.4 kDa. The deduced amino acid sequence of mature mMCP-7 is identical to that of mouse mast cell tryptase 2 (13). mMCP-7 contains a putative N-linked glycosylation site at Asn^{102} that is conserved in mMCP-6, human α-tryptase, human skin tryptase I, and dog tryptase but is not conserved in human β-tryptase. Although only mMCP-7, human skin tryptase I, and human α-tryptase contain a second glycosylation site, this site resides at Asn^{21} in mMCP-7 and at Asn^{203} in the two human tryptases.

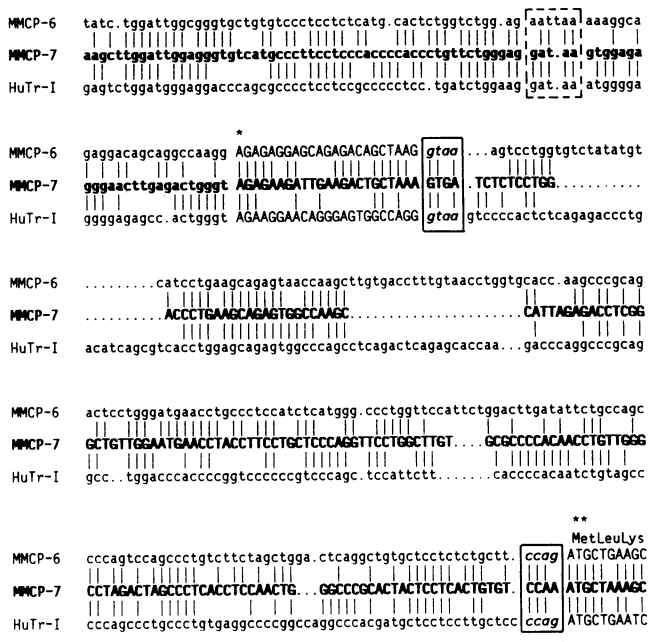

FIG. 5. Comparison of the 5′ end of the mMCP-7 gene with the mMCP-6 and human tryptase I (HuTr-I) genes. The nucleotides that compose exons are in uppercase letters, and those composing introns and 5′-flanking sequence are in lowercase letters. The transcription-initiation site (*), translation-initiation site (**), and putative TATA element (broken box) are indicated. The exon/intron donor and acceptor sequences in the mMCP-6 and human tryptase I genes, together with the corresponding sequence in the mMCP-7 gene, are highlighted in italics and closed boxes. Vertical lines indicate identical nucleotides. Periods indicate gaps introduced to optimize alignments.

mMCP-7 possesses an overall net charge of −10, making mMCP-7 the most acidic serine protease so far identified in mast cells of any species. mMCP-7 exhibits homologies with human β-tryptase, human skin tryptase I, human α-tryptase, mMCP-6, and dog tryptase of 76%, 76%, 74%, 71%, and 71%, respectively. In contrast, mMCP-7 is <29% homologous with the mouse mast cell chymases mMCP-1, mMCP-2, mMCP-4, and mMCP-5.

Because the mMCP-7 gene is transcribed in BMMCs in response to IL-3-enriched WEHI-3 cell-conditioned medium, it is an early-expressed protease gene along with the mMCP-5 and mMCP-6 genes. The findings that mMCP-7 mRNA is present only transiently in BMMCs cultured in 50% WEHI-3 cell-conditioned medium (Fig. 3) and is absent from serosal and mucosal mast cells (Fig. 2) were unexpected. This pattern of gene expression is unusual among protease genes transcribed by mouse mast cells. The suppression of transcription of a serine protease gene in human myeloid HL-60 cells induces differentiation of these immature human leukemic cells (33). Similarly, monocyte differentiation into macrophages is associated with suppression of transcription of the elastase and cathepsin G genes (34). Our previous studies (4, 10, 11) indicate that the expression of mast cell protease genes in BMMC is regulated in a complex fashion by specific cytokines. Therefore, the transient expression of the mMCP-7 gene during growth of BMMCs may be associated with their transition from progenitor cells through a differentiation pathway to immature mast cells. Alternatively, inasmuch as transformed (22) and nontransformed mast cells (4, 11) that transcribe combinations of protease genes distinct from those expressed by serosal and mucosal mast cells have been generated *in vitro*, additional subsets of mature tissue-localized mast cells that selectively express mMCP-7 may exist.

The ABFTL mast cell line originally derived from Pierce *et al.* (23) was provided by Dr. H. Katz (Harvard Medical School). Dr. H. Avraham (Harvard Medical School) kindly provided the mouse genomic library used to isolate two of the three mMCP-7 genomic clones characterized in this study. The comments of Drs. H. Katz and R. Jack (Harvard Medical School), who reviewed this manuscript, are gratefully acknowledged. This work was supported by Grants AI-22531, AI-23483, AI-31599, AR-35907, AR-36308, HL-36110 and RR-05950 from the National Institutes of Health and by a grant from the Hyde and Watson Foundation. H.P.M. is a recipient of a Heald Fellowship from the Arthritis Foundation of Australia.

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