Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 3230–3234, April 1990
Biochemistry

Different mouse mast cell populations express various combinations of at least six distinct mast cell serine proteases

(tryptase/chymase/secretory granule/granzyme)

DALE S. REYNOLDS*†, RICHARD L. STEVENS*, WILLIAM S. LANE‡, MATTHEW H. CARR*, K. FRANK AUSTEN*, AND WILLIAM E. SERAFIN*

*Department of Medicine, Harvard Medical School, and Department of Rheumatology and Immunology, Brigham and Women's Hospital, Boston, MA 02115; and ‡Harvard Microchemistry Facility, Harvard Biological Laboratories, Cambridge, MA 02138

Contributed by K. Frank Austen, February 7, 1990

ABSTRACT **Mouse serosal mast cells (SMCs) and Kirsten sarcoma virus-immortalized mast cells store large amounts of mast cell carboxypeptidase A and serine proteases in their secretory granules. Secretory granule proteins from 2.6×10^6 purified SMCs were separated by $NaDodSO_4$/PAGE, transblotted to poly(vinylidine difluoride) membranes, and subjected to amino-terminal amino acid sequencing. Four distinct mast cell serine proteases were identified. With mast cell carboxypeptidase A, these serine proteases comprise the major proteins of mouse SMC secretory granules. Each of the four SMC serine proteases was distinct from the two serine proteases present in mucosal mast cells in the intestines of helminth-infected mice. The secretory granules of a Kirsten sarcoma virus-immortalized mast cell line contained three of the SMC-derived serine proteases and one of the mucosal mast cell-derived serine proteases. Thus, the family of mouse mast cell secretory granule serine proteases has at least six distinct members that can be expressed in different combinations in different mast cell populations.**

Serine proteases are major constituents of the secretory granules of mouse (1–3), rat (4–6), dog (7,8), and human (9, 10) mast cells, and two or three distinct serine proteases have been identified in the mast cells of each species. Rat mast cell protease (RMCP) I, the first mast cell secretory granule protease to be biochemically characterized, was called "chymase" because of its chymotrypsin-like substrate specificity (4, 11, 12). RMCP-I was isolated from rat serosal mast cells (SMCs), which are found unattached in the peritoneal cavity. By several criteria, SMCs resemble connective tissue-type mast cells found in skin and muscle (13). A second chymotrypsin-like enzyme, termed RMCP-II, was subsequently isolated from the intestines of helminth-infected rats and shown by immunohistology to be localized in intestinal mucosal mast cells (MMCs) (14). The MMC population is greatly expanded in the intestinal mucosae of animals with intestinal worm infections (15, 16). Based on the amino acid sequence of the isolated protein (17) and the deduced amino acid sequence of the gene (18), RMCP-II is predicted to be translated as a preproenzyme. After removal of an 18-amino acid hydrophobic signal peptide, two amino acids (Glu-Glu) are cleaved from the amino terminus of the proenzyme, leaving the mature protease with an amino-terminal amino acid sequence of Ile-Ile-Gly-Gly. Tryptic enzymatic activity has also been detected in the secretory granules of rat SMCs (6). "Tryptase" has been purified from human and dog mast cells, and cDNAs that encode these two serine proteases have been isolated (8, 19). The mature form of tryptase from both human and dog mast cells has an amino-terminal amino acid sequence of Ile-Val-Gly-Gly (8, 19).

In the mouse, two distinct chymotrypsin-like serine proteases of 26 and 28 kDa, designated mouse mast cell protease (MMCP)-1 and MMCP-2, respectively, have been identified and shown to be selectively expressed in MMCs (2, 3, 20). A cDNA that encodes MMCP-2 has been isolated and characterized (3) from a Kirsten sarcoma virus-immortalized mast cell (KiSV-MC) line (21). Similar to RMCP-II, MMCP-2 is predicted to be synthesized as a preproenzyme that undergoes proteolytic processing at its amino terminus to form a mature enzyme with an amino-terminal amino acid sequence of Ile-Ile-Gly-Gly (3). Mouse SMC secretory granules contain the metallo-exopeptidase mast cell carboxypeptidase A (22, 23) and an undetermined number of serine proteases.

Here we describe the identification and amino-terminal amino acid sequencing of four serine proteases that are major protein constituents of the secretory granules of mouse SMCs. The amino-terminal amino acid sequences of these SMC-derived proteases are distinct from the sequences of the two known mouse MMC-derived serine proteases. Furthermore, a KiSV-MC line was found to express three of the SMC serine proteases and one of the MMC serine proteases. Thus, different populations of mouse mast cells can contain various combinations of at least six distinct serine proteases in their secretory granules.

MATERIALS AND METHODS

Isolation and Culture of Mast Cells. Serosal mast cells were obtained from BALB/c mice (The Jackson Laboratory) by peritoneal lavage and were purified to >97% (24, 25). Interleukin 3-dependent mouse bone marrow-derived mast cells (BMMCs) were obtained by culturing BALB/c mouse bone marrow cells for 3–6 weeks in 50% (vol/vol) enriched medium [RPMI 1640 medium containing 10% (vol/vol) heat-inactivated fetal bovine serum, penicillin (100 units/ml), streptomycin (100 μg/ml), 2 mM L-glutamine, and nonessential amino acids (0.1 mM)]/50% (vol/vol) WEHI-3 conditioned medium (26) containing 5 mM Hepes. KiSV-MC lines MC4, MC5, MC8, and MC9 were cultured in enriched medium (21). Alcian blue/safranin color scores were based on a value of 1 for all blue (safranin$^-$) granules and a value of 5 for all red (safranin$^+$) granules (21). BMMCs and KiSV-MC4, KiSV-MC5, KiSV-MC8, and KiSV-MC9 cells had color scores of 1, 1, 4.5, 1.5, and 3.5, respectively. The color

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: BMMC, bone marrow-derived mast cell; DFP, diisopropyl fluorophosphate; KiSV-MC, Kirsten sarcoma virus-immortalized mast cell; MMCP, mouse mast cell protease; MMC, mucosal mast cell; RMCP, rat mast cell protease; SMC, serosal mast cell.

†To whom reprint requests should be addressed at: Harvard Medical School, The Seeley G. Mudd Building, Room 624, 250 Longwood Avenue, Boston, MA 02115.

scores for KiSV-MC4 and -MC8 are lower than those reported for these cell lines (21) due to a gradual change in these lines to a less-mature phenotype during 3 years in continuous culture.

Measurement of Chymotryptic and Tryptic Esterase Activities in Mast Cell Sonicates. Samples were assessed for chymotryptic (27) and tryptic (28) esterase activities by incubating 1×10^5 sonicated mast cells in 1 ml of the appropriate reaction solution. Chymotryptic activity was measured at 22°C by the cleavage of benzoyl-L-tyrosine ethyl ester (0.54 mM) in 25% (vol/vol) methanol/50 mM $CaCl_2$/0.5 M NaCl/40 mM Tris·HCl, pH 7.2, with continuous monitoring of absorbance at 256 nm. Tryptic activity was measured at 22°C by the cleavage of tosyl-L-arginine methyl ester (1.0 mM) in 2% (vol/vol) ethanol/0.15 M NaCl/2 mM $CaCl_2$/40 mM Tris·HCl, pH 7.0, with continuous monitoring of the absorbance at 247 nm. One unit of chymotryptic or tryptic activity was defined as the amount of enzyme that cleaves 1 μmol of substrate per min. The enzymatic activity for each cell line is expressed in units per 1×10^6 mast cells and is the average of duplicate determinations.

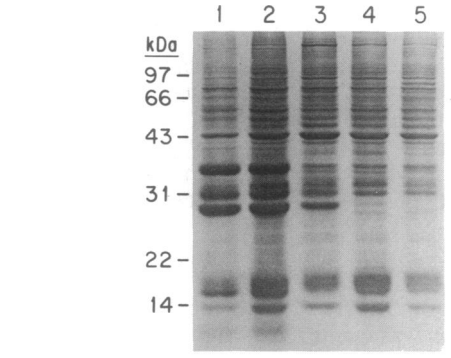
NaDodSO$_4$/PAGE Resolution and Amino-Terminal Amino Acid Sequencing of Mast Cell-Derived Serine Proteases. Whole mast cell lysates were subjected to standard NaDodSO$_4$/PAGE under reducing conditions (29). Rabbit muscle phosphorylase *b* (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumin (42.7 kDa), bovine carbonic anhydrase (31 kDa), soybean trypsin inhibitor (21.5 kDa), and hen egg white lysozyme (14.4 kDa) (Bio-Rad) were used as molecular mass markers. For preparative resolution of mast cell secretory granule proteins for amino-terminal amino acid sequencing, secretory granules were prepared (4, 30) from 1.5×10^7 KiSV-MC5 cells and electrophoresed in three parallel lanes. In a second gel, proteins from the secretory granules of 2.6×10^6 purified SMCs were resolved in a single lane. For comparative purposes, secretory granule proteins from 2.5×10^6 KiSV-MC5 cells were also resolved in a lane parallel to the SMC secretory granule proteins. Preparative NaDodSO$_4$/PAGE of the mast cell granule proteins was performed with the Tricine [*N*-tris(hydroxymethyl)methylglycine] electrophoresis buffer system of Schägger and von Jagow (31), which provided greater resolution of the proteins in the 28- to 32-kDa range when prolonged electrophoresis times were used. A 1.5-mm-thick 4% polyacrylamide stacking gel and a 13% polyacrylamide resolving gel were used in a Bio-Rad Protean gel electrophoresis apparatus. All samples were made 5% (vol/vol) in 2-mercaptoethanol and were heated at 65°C for 15 min before electrophoresis. The gels were polymerized the day before being used and were stored overnight at 4°C. The upper reservoir buffer was made 0.1 mM in sodium thioglycollate (Sigma) before electrophoresis to eliminate free radicals remaining from the polymerization reaction (32). The Tricine/NaDodSO$_4$/polyacrylamide gels were electrophoresed for 1 hr at 30 V and then at 110 V for 250% of the time needed for the tracking dye (Serva blue G; Serva) to reach the bottom of the resolving gel (≈22 hr) to maximize the separation of proteins in the 28- to 32-kDa range. After electrophoresis, the resolved proteins in the gel were electroblotted to a poly(vinylidine difluoride) membrane (0.45-μm pore size; Millipore) at 150 mA for 8 hr at 4°C in a Bio-Rad Transblot Cell apparatus (33). The transfer buffer contained 10% methanol and 10 mM 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS; Sigma), pH 11.3. The protein blots were stained for 3 min in 50% methanol/50% H_2O containing 0.1% Coomassie blue, partially destained for 5 min in acetic acid/methanol/H_2O, 10:45:45 (vol/vol), and dried between Whatman 3MM filter papers. The prominent 28- to 32-kDa Coomassie blue-stained bands in each lane were excised. The resulting 2 × 9-mm membrane strips were feathered with a clean razor blade, placed in the reaction cartridge of an Applied Biosystems model 470A gas-phase protein sequencer, and subjected to automated Edman degradation according to the manufacturer's standard program 03RPTH with product resolution after each cycle by high performance liquid chromatography.

Identification of [^{3}H]Diisopropyl Fluorophosphate ([^{3}H]-DFP)-Binding Mast Cell Proteins. In one experiment to radiolabel serine proteases, a whole cell sonicate of 5×10^6 KiSV-MC5 cells was prepared in 0.5 ml of distilled water. The sonicate (100 μl) was mixed with 100 μl of 0.15 M NaCl/20 mM Tris·HCl, pH 7.4, containing 25 μCi of [^{3}H]DFP (4 Ci/nmol; 1 Ci = 37 GBq; Amersham) and incubated for 15 min at 37°C. The reaction was quenched by the addition of DFP in isopropanol to a final concentration of 4 mM and incubated for 5 min at 37°C (1). The [^{3}H]DFP-labeled proteins were recombined with the remainder of the cell sonicate, and the proteins were separated from free [^{3}H]DFP by precipitation in 10% (wt/vol) trichloroacetic acid for 30 min at 4°C and centrifugation at 16,000 × *g* for 30 min. After two washes with acetone, the pellet was resuspended in NaDodSO$_4$/PAGE sample buffer (31) containing 5% 2-mercaptoethanol and was incubated at 100°C for 5 min. Tricine/NaDodSO$_4$/PAGE was performed as described above on 1×10^6 cell equivalents of labeled material, except that sodium thioglycollate was not included in the upper reservoir buffer. To localize the [^{3}H]DFP-labeled proteins, the gel was stained with Coomassie blue, and the lane was sectioned at 1-mm intervals. The gel slices were dissolved at 65°C for 1 hr in 28% (vol/vol) H_2O_2 containing 1.1% (vol/vol) NH_4OH (34), and the radioactivity in each gel slice was quantitated by liquid scintillation counting.

RESULTS

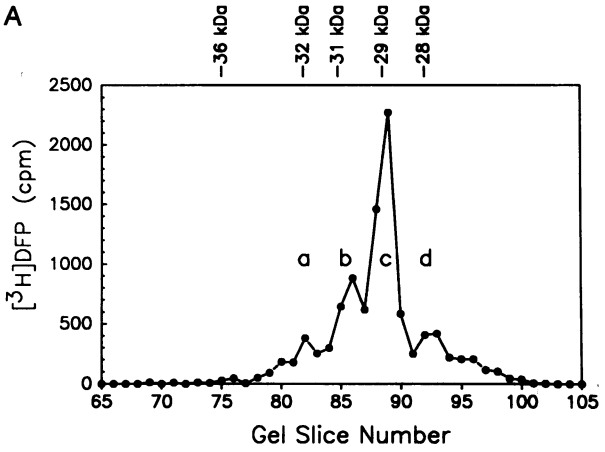
Identification of Serine Proteases in the Secretory Granules of KiSV-MCs. Fifteen KiSV-MC lines were shown (21) to represent mouse mast cells at various stages of differentiation, based on their different degrees of safranin staining and expression of biochemical markers such as histamine, heparin proteoglycans, and mast cell carboxypeptidase A. When four of the KiSV-MC lines were examined for chymotryptic and tryptic esterase activities, cell lines that more closely resembled SMCs in their alcian blue/safranin histochemistry (color score >3) had the highest levels of these enzyme activities (Fig. 1, lanes 1 and 2). NaDodSO$_4$/PAGE analysis of whole cell sonicates showed that the KiSV-MC lines containing the highest chymotryptic and tryptic esterase activities also contained the largest amounts of several prominent proteins that migrated at 28–32 kDa under reducing conditions (Fig. 1). To identify putative serine proteases, a whole cell sonicate of KiSV-MC5 cells was incubated with the [^{3}H]DFP and subjected to Tricine/NaDodSO$_4$/PAGE under reducing conditions. The prominent 28- to 32-kDa proteins in KiSV-MC5 (Fig. 1) were thus resolved into four Coomassie blue-staining bands at 28, 29, 31, and 32 kDa. The [^{3}H]DFP localized to four areas of the lane (peaks a–d, Fig. 2*A*), corresponding to the 28-, 29-, 31-, and 32-kDa Coomassie blue-stained proteins. No other peaks of radioactivity were observed.

Amino-Terminal Amino Acid Sequencing of Mouse Mast Cell Serine Proteases. Secretory granules were purified from 1.5×10^7 KiSV-MC5 cells and subjected to preparative Tricine/NaDodSO$_4$/PAGE under reducing conditions. The secretory granule proteins were then transblotted onto a poly(vinylidine difluoride) membrane and stained with Coomassie blue. Under these conditions, the prominent 28- to 32-kDa proteins identified previously in whole cell sonicates of KiSV-MC5 cells copurified with the secretory granules and again resolved as four discrete bands at 28, 29, 31, and 32 kDa (Fig. 2*B*, lane 1). To determine which of the prominent KiSV-MC secretory granule proteins were also



	MC5	MC9	MC8	MC4	BMMC
Color Score	4.5	3.5	1.5	1.0	1.0
Chymotryptic Activity (Units/10^6 Cells)	0.23	0.26	0.09	0.03	0.02
Tryptic Activity (Units/10^6 Cells)	0.10	0.12	0.05	0.02	0.00

FIG. 1. $NaDodSO_4$/PAGE analysis of four KiSV-MC lines (lanes 1–4) and mouse BMMCs (lane 5) that differ in their alcian blue/safranin staining properties and their contents of chymotryptic and tryptic enzymatic activities. Color scores: 1, all granules blue; 3, half granules blue and half granules red; 5, all granules red. Chymotryptic and tryptic activities were determined from whole cell sonicates. The molecular mass markers are indicated to the left.



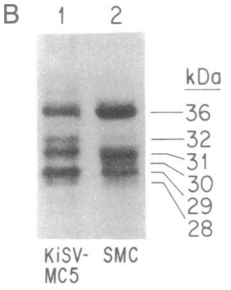


FIG. 2. (*A*) Identification of [^{3}H]DFP-binding proteins in a [^{3}H]DFP-labeled KiSV-MC5 sonicate analyzed by Tricine/$NaDodSO_4$/PAGE. The locations of the prominent 28-, 29-, 31-, and 32-kDa proteins are indicated. Incorporated [^{3}H]DFP was detected in four peaks (a–d). (*B*) Tricine/$NaDodSO_4$/PAGE analysis of secretory granule proteins from mouse KiSV-MC5 cells and SMCs. Proteins from the granules of 2.5×10^6 KiSV-MC5 cells (lane 1) and 2.6×10^6 SMCs (lane 2) were resolved by Tricine/$NaDodSO_4$/PAGE, transblotted to a poly(vinylidine difluoride) membrane, and stained with Coomassie blue. The locations of prominent 28- to 32-kDa serine proteases are indicated. Note that the 30-kDa SMC-derived protein was not detected in KiSV-MC5 granules. The position of mast cell carboxypeptidase A (36 kDa) is also indicated.

expressed in SMCs, 2.6×10^6 SMCs were purified from the peritoneal cavities of 100 mice. When the SMC secretory granule proteins were resolved, five discrete protein bands of 28, 29, 30, 31, and 32 kDa were observed (Fig. 2*B*, lane 2). The 36-kDa protein present in the secretory granules of both SMCs and KiSV-MC5 cells was identified (23) as the metallo-exopeptidase mast cell carboxypeptidase A.

The amino-terminal amino acid sequences of the resolved 28- to 32-kDa secretory granule proteins from KiSV-MC5 cells and from SMCs are depicted in Table 1. Each sequenced protein had an amino terminus beginning with Ile-(Ile or Val)-Gly-Gly, typical of serine proteases. The 28-kDa protease in KiSV-MC5 cells was identified and designated as MMCP-2 (3). A similar-sized 28-kDa serine protease was present in SMC granules, but its amino-terminal amino acid sequence was distinct from that of MMCP-2. The 28-kDa SMC protein was thus designated MMCP-3. The amino-terminal amino acid sequences of the 29-, 31-, and 32-kDa serine proteases in KiSV-MC5 granules revealed each to be a distinct protein, and they were designated MMCP-4, MMCP-5, and MMCP-6, respectively. MMCP-4, MMCP-5, and MMCP-6 were also present in SMC granules, since the

Table 1. Amino-terminal amino acid sequences of mouse mast cell secretory granule serine proteases

Serine protease	M.M., kDa	Mast cell type	Amino acid residue 1 5 10 15 20 25 30
MMCP-1	26	MMC	I I G G V E A R P H S R P Y M A H L K I I T D R G S E D R C*
MMCP-2	28	KiSV-MC5	I I G G V E A K P H S R P Y M A Y L K F T T†
		MMC	
MMCP-3	28	SMC	I I G G V E S R P H S R P Y M A T L E I T‡
MMCP-4	29	KiSV-MC5	I I G G V E S R P H S R P Y M A H L E I T[T E]
	29	SMC	I I G G V E S R P H S R P Y M A H L E I T T E
MMCP-5′	30	SMC	I I G G T E X I P H S R P Y M A Y L E I
MMCP-5	31	KiSV-MC5	I I G G T E X I P H S R P Y M A Y L E I V T S E
	31	SMC	I I G G T E X I P H S R P Y M A Y L E I V T S E N Y L[S]A[R]
MMCP-6	32	KiSV-MC5	I V G G H E A S E S K W P W Q V S L R F K L N
	32	SMC	I V G G H E A S E[S]K X P X Q V

Letters in brackets are probable amino acids. The single-letter amino acid code is used. Amino acid positions 1–30 are indicated. X, no residue could be determined; M.M., molecular mass.

*Data are from LeTrong *et al.* (20).

†Conclusion that MMCP-2 (3) is expressed in MMCs is based on the preferential expression of the gene that encodes this enzyme in the intestines of helminth-infected mice.

‡Because the amino-terminal amino acid sequences of MMCP-3 and MMCP-4 differed by only a single residue (position 17), secretory granule proteins were again isolated from 3×10^6 purified SMCs. The second sequence determined was identical to the first sequence determined, indicating that MMCP-3 and MMCP-4 are distinct proteins.

similarly migrating proteins in SMC granules had identical amino-terminal amino acid sequences. The distinct 30-kDa serine protease of SMCs (designated MMCP-5′) was not detected in KiSV-MC5 cells. MMCP-5′ had an amino-terminal amino acid sequence identical to that of the 31-kDa protein, MMCP-5 (Table 1).

DISCUSSION

The secretory granules of mouse KiSV-MCs were found to contain four prominent proteins of 28, 29, 31, and 32 kDa (designated MMCP-2, -4, -5, and -6, respectively) (Fig. 2*B*) that differed in their amino-terminal amino acid sequences (Table 1). These proteins were serine proteases by four criteria: they bound the serine protease inhibitor [^{3}H]DFP (Fig. 2*A*); their molecular weights were in the range of other mast cell serine proteases (Figs. 1 and 2*B*); they each had an amino-terminal amino acid sequence of Ile-(Ile or Val)-Gly-Gly (Table 1); and increased levels of tryptic and chymotryptic esterase activities were found in KiSV-MC lines that had the highest contents of these proteins (Fig. 1). Furthermore, the complete amino acid sequence of MMCP-2 deduced from its cDNA (3) contains the His/Asp/Ser charge relay triad that is characteristic of all serine proteases (14).

Like KiSV-MCs, the secretory granules of SMCs contained proteins of 28, 29, 31, and 32 kDa (Fig. 2*B*). The amino-terminal amino acid sequences of the 29-, 31-, and 32-kDa proteins in SMCs were identical to the sequences of the corresponding proteins in KiSV-MC5 cells (MMCP-4, -5, and -6, respectively). However, the 28-kDa protein from the KiSV-MC granules (MMCP-2) had an amino-terminal amino acid sequence distinct from that of the 28-kDa protein from the SMC granules (MMCP-3) (Table 1). Except for position 17, the amino-terminal 21-amino acid sequence of MMCP-3 was identical to that of the 29-kDa serine protease MMCP-4. This single difference in the amino-terminal amino acid sequences of the two proteins is unlikely to be due to a sequencing error because identical and unambiguous results were obtained when a second cell preparation was analyzed. This difference between MMCP-3 and MMCP-4 is not due to allelic variability because the SMCs, which contained both proteins, were obtained from inbred BALB/c mice (35). An additional protein of 30 kDa (designated MMCP-5′) was detected in the granules of SMCs but not KiSV-MC5 cells. The amino-terminal sequence determined for 19 amino acids of MMCP-5′ was identical to that of the 31-kDa serine protease, MMCP-5, which was present in both KiSV-MC5 cells and SMCs. Therefore, it is possible that MMCP-5 and MMCP-5′ represent the same protein that has undergone differing degrees of posttranslational processing. Neither KiSV-MC5 cells nor SMCs had detectable quantities of MMCP-1, the mouse MMC serine protease characterized by LeTrong *et al.* (20). This family of at least six mouse mast cell serine proteases is similar in its complexity to the distinct but homologous family of serine proteases that has been identified in the granules of mouse cytotoxic T lymphocytes (36–39).

Previously described mast cell serine proteases have been classified as chymotryptic or tryptic based on their substrate specificities and predicted substrate-binding regions. Based on their known complete amino acid sequences, MMCP-1 (20) and MMCP-2 (3) strongly resemble both of the rat mast cell chymotryptic proteases RMCP-I and RMCP-II in their predicted substrate binding regions and their amino termini. The amino-terminal amino acid sequences of MMCP-3, -4, and -5 presented here have 95%, 100%, and 74% identity, respectively, with RMCP-I. Thus, it is likely that MMCP-1, -2, -3, -4, and -5 are chymotryptic in their substrate specificities. The amino terminus of MMCP-6 has 70% identity to human lung mast cell tryptase (19) and has no more than 33% identity with MMCP-1, -2, -3, -4, or -5. Thus, it is likely that MMCP-6 is a mouse homologue of the human (19) and dog (8) mast cell tryptases.

None of the SMC-derived serine proteases has an amino acid sequence identical to the two MMC enzymes (MMCP-1 and -2) (3, 20), and, therefore, mouse MMCs and SMCs can be distinguished by their serine protease contents. We have reported (3) that the gene that encodes MMCP-2 is not expressed in interleukin 3-dependent mouse BMMCs, even though BMMCs can give rise to MMCs when injected into W/W^v mast cell-deficient mice (40). This finding indicated that MMCP-2 is probably expressed late in MMC differentiation. The mouse splenocyte-derived cell line KiSV-MC5 resembles mouse SMCs in histochemistry, histamine content, biosynthesis of [^{35}S]heparin proteoglycans, mast cell carboxypeptidase A content (21), and content of MMCP-4, -5, and -6 (Table 1). However, KiSV-MC5 cells differ from SMCs in that they do not express MMCP-3 (Table 1) but rather express the MMC protease MMCP-2 (3). Because a well-differentiated mast cell line can prominently express both SMC and MMC proteases, it is possible that mouse mast cells *in vivo* display a greater diversity of phenotypes than has been recognized to date.

Classification of mast cell populations based on immunohistology with a limited number of anti-protease antibodies may underestimate mast-cell diversity. The amino-terminal amino acid sequences of the identified mouse mast cell serine proteases have revealed substantial regions of sequence identity between the various proteases [e.g., residues 9–16 of MMCP-1, -2, -3, -4, -5, and -5′ (Table 1)]. When the complete amino acid sequences of MMCP-1 and MMCP-2 were compared, >65% overall sequence identity was found (3, 20). In studies of rat mast cell protease expression, Gibson and Miller (41) demonstrated that antisera to RMCP-I and RMCP-II must be cross-absorbed to prevent cross-reactivity. Therefore, the demonstration of a high degree of specificity of anti-mast cell protease antibodies will be necessary for future studies of mouse mast cell protease expression and mast cell phenotype. The development of a panel of nucleic acid probes for these mast cell secretory granule enzymes will likewise require demonstration of the gene specificity of each probe. The characterization of a large number of mouse mast cell secretory granule proteases that are differentially expressed in different mast cell populations should eventually permit studies addressing the molecular mechanisms of mast cell protease gene expression and of mast cell phenotypic heterogeneity.

This work was supported by Grants AI-22531, AI-23401, AI-23483, AR-35907, CA-09141, and HL-36110 from the National Institutes of Health and by the Irvington Foundation for Medical Research. R.L.S. is an Established Investigator of the American Heart Association. W.E.S. is a Burroughs Wellcome Investigator in the Immunopharmacology of Allergic Diseases.

1. DuBuske, L., Austen, K. F., Czop, J. & Stevens, R. L. (1984) *J. Immunol.* **133,** 1535–1541.
2. Newlands, G. F. J., Gibson, S., Knox, D. P., Grencis, R., Wakelin, D. & Miller, H. R. P. (1987) *Immunology* **62,** 629–634.
3. Serafin, W. E., Reynolds, D. S., Rogelj, S., Lane, W. S., Conder, G. A., Johnson, S. S., Austen, K. F. & Stevens, R. L. (1990) *J. Biol. Chem.* **265,** 423–429.
4. Lagunoff, D. & Pritzl, P. (1976) *Arch. Biochem. Biophys.* **173,** 554–563.
5. Yurt, R. & Austen, K. F. (1977) *J. Exp. Med.* **146,** 1405–1419.
6. Kido, H., Fukusen, N. & Katunuma, N. (1985) *Arch. Biochem. Biophys.* **239,** 436–443.
7. Caughey, G. H., Viro, N. F., Ramachandran, J., Lazarus, S. C., Borson, D. B. & Nadel, J. A. (1987) *Arch. Biochem. Biophys.* **258,** 555–563.

8. Vanderslice, P., Craik, C. S., Nadel, J. A. & Caughy, G. H. (1989) *Biochemistry* **28,** 4148–4155.
9. Schechter, N. M., Choi, J. K., Slavin, D. A., Deresienski, D. T., Sayama, S., Dong, G., Lavker, R. M., Proud, D. & Lazarus, G. S. (1986) *J. Immunol.* **137,** 962–970.
10. Schwartz, L. B., Lewis, R. A. & Austen, K. F. (1981) *J. Biol. Chem.* **256,** 11939–11943.
11. LeTrong, H., Parmelee, D. C., Walsh, K. A., Neurath, H. & Woodbury, R. G. (1987) *Biochemistry* **26,** 6988–6994.
12. LeTrong, H., Neurath, H. & Woodbury, R. G. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 364–367.
13. Stevens, R. L., Katz, H. R., Seldin, D. C. & Austen, K. F. (1986) in *Mast Cell Differentiation and Heterogeneity*, eds. Befus, A. D., Bienenstock, J. & Denburg, J. A. (Raven, New York), pp. 183–202.
14. Woodbury, R. G., Gruzenski, G. M. & Lagunoff, D. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 2785–2789.
15. Enerbäck, L. (1966) *Acta Pathol. Microbiol. Scand.* **66,** 303–312.
16. Miller, H. R. P. & Jarrett, W. F. H. (1971) *Immunology* **20,** 277–288.
17. Woodbury, R. G., Katunuma, N., Kobayashi, K., Titani, K. & Neurath, H. (1978) *Biochemistry* **17,** 811–819.
18. Benfey, P. N., Yin, F. H. & Leder, P. (1987) *J. Biol. Chem.* **262,** 5377–5384.
19. Miller, J. S., Westin, E. H. & Schwartz, L. B. (1989) *J. Clin. Invest.* **84,** 1188–1195.
20. LeTrong, H., Newlands, G. F. J., Miller, H. R. P., Charbonneau, H., Neurath, H. & Woodbury, R. G. (1989) *Biochemistry* **28,** 391–395.
21. Reynolds, D. S., Serafin, W. E., Faller, D. V., Wall, D. A., Abbas, A. K., Dvorak, A. M., Austen, K. F. & Stevens, R. L. (1988) *J. Biol. Chem.* **263,** 12783–12791.
22. Serafin, W. E., Dayton, E. T., Gravallese, P. M., Austen, K. F. & Stevens, R. L. (1987) *J. Immunol.* **139,** 3771–3776.
23. Reynolds, D. S., Stevens, R. L., Gurley, D. S., Lane, W. S., Austen, K. F. & Serafin, W. E. (1989) *J. Biol. Chem.* **264,** 20094–20099.
24. Yurt, R. W., Leid, R. W., Jr., Austen, K. F. & Silbert, J. E. (1977) *J. Biol. Chem.* **252,** 518–521.
25. Razin, E., Stevens, R. L., Akiyama, F., Schmid, K. & Austen, K. F. (1982) *J. Biol. Chem.* **257,** 7229–7236.
26. Razin, E., Ihle, J. N., Seldin, D., Mencia-Huerta, J.-M., Katz, H. R., LeBlanc, P. A., Hein, A., Caulfield, J. P., Austen, K. F. & Stevens, R. L. (1984) *J. Immunol.* **132,** 1479–1486.
27. Hummel, B. C. W. (1959) *Can. J. Biochem. Physiol.* **37,** 1393–1399.
28. Walsh, K. A. (1970) in *Methods in Immunology*, eds. Colowick, S. P. & Kaplan, N. O. (Academic, New York), pp. 41–42.
29. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
30. Woodbury, R. G., Everitt, M. T. & Neurath, H. (1981) *Methods Enzymol.* **80,** 588–609.
31. Schägger, H. & von Jagow, G. (1987) *Anal. Biochem.* **166,** 368–379.
32. Hunkapiller, M. W., Lujan, E., Ostrander, F. & Hood, L. E. (1983) *Methods Enzymol.* **91,** 227–236.
33. Matsudaira, P. (1987) *J. Biol. Chem.* **262,** 10035–10038.
34. Stevens, R. L., Otsu, K. & Austen, K. F. (1985) *J. Biol. Chem.* **260,** 14194–14200.
35. Doolittle, D. P. (1987) *Population Genetics: Basic Principles* (Springer, New York).
36. Masson, D. & Tschopp, J. (1987) *Cell* **49,** 679–685.
37. Pasternak, M. S. & Eisen, H. N. (1985) *Nature (London)* **314,** 743–745.
38. Lobe, C. G., Finlay, B. B., Paranchych, W., Paetkau, V. H. & Bleackley, R. C. (1986) *Science* **232,** 858–861.
39. Gershenfeld, H. K. & Weissman, I. L. (1986) *Science* **232,** 854–858.
40. Nakano, T., Sonoda, T., Hayashi, C., Yamatodani, A., Kanayama, Y., Yamamura, T., Asai, H., Yonezawa, T., Kitamura, Y. & Galli, S. J. (1985) *J. Exp. Med.* **162,** 1025–1043.
41. Gibson, S. & Miller, H. R. P. (1986) *Immunology* **58,** 101–104.