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Biochemical and Biophysical Research Communications 303 (2003) 320–325

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# Mouse mast cells express the tryptic protease neuropsin/Prss19☆

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Received 7 February 2003

**Abstract**

The only tryptic enzymes identified so far in mouse mast cells (MCs) are three members of the chromosome 17A3.3 family of neutral proteases. Sequence analysis of a cDNA library revealed that BALB/c mouse bone marrow-derived MCs express neuropsin, a member of the chromosome 7B2 family of tryptic kallikreins. Kinetic studies revealed that neuropsin is expressed relatively early in MC development. As assessed immunohistochemically, the MCs residing in numerous connective tissues store neuropsin in their secretory granules. The finding that the neuropsin transcript is maximally expressed in the intestine at the height of a helminth infection indicates that MC-committed progenitors selectively increase their expression of neuropsin as they develop into mature mucosal MCs. This is the first report documenting the expression of neuropsin in an immune cell. Thus, it is now apparent that mouse MCs store at least two distinct families of tryptic-like proteases in their secretory granules.


*Keywords:* Mast cells; Neuropsin; Prss19; Tryptases; Kallikreins; Serine proteases

Mast cells (MCs) are strategically located at the mucosal surfaces of vascularized tissues to enable these immune cells to control bacterial, parasitic, and other infections. MCs have also been implicated in nerve development and function. In the context of disease, MCs play a prominent role in asthma, rheumatoid arthritis, and other inflammatory disorders. As much as 50% of the weight of a mature mouse MC consists of varied combinations of at least 13 different serine proteases [designated mouse MC protease (mMCP)-1 to mMCP-10, transmembrane tryptase (TMT)/tryptase γ, granzyme B, and cathepsin G]. The genes that encode the tryptases mMCP-6 [1], mMCP-7 [2], and TMT [3] are clustered on chromosome 17A3.3, whereas the genes that encode the other known MC granule serine proteases are clustered on chromosome 14C1.

With regard to the MC tryptases, it is now clear that these related proteases evolved to carry out different functions. The amino acid sequences of mMCP-6 and mMCP-7 are 71% identical, yet exocytosed mMCP-6 is selectively retained for hours in extracellular matrices around activated MCs [4]. Screening of phage-display peptide libraries and a variety of chromogenic substrates revealed that mMCP-6, mMCP-7, and TMT have different substrate specificities [3,5–7]. Whereas fibrinogen is a physiologic substrate of mMCP-7, mMCP-6 cannot degrade this protein effectively in the presence of serum. The MCs in the peritoneal cavity express mMCP-6 [8] and peritoneal cavity MCs are essential for the host's survival if the cecum is punctured [9–11]. mMCP-6 induces neutrophil extravasation into the peritoneal cavity [6,12]. mMCP-6 (and its human ortholog tryptase βI) also induce a prominent and selective extravasation of neutrophils into the lung that allows MC-deficient C57BL/6-Kit$^{W\text{-}v}$ (W/W$^{v}$) mice to combat *Klebsiella pneumoniae* infections efficiently [13]. The accumulated data suggest that mMCP-6 and mMCP-7 are beneficial tryptases that work in concert in MC-mediated inflammatory reactions to allow efficient and selective extravasation of granulocytes into bacteria-infected tissues.

☆ *Abbreviations:* IL, interleukin; mBMMC, mouse bone marrow-derived MC; MC, mast cell; mMCP, mouse MC protease; Prss19, protease, serine S1 family member 19; TMT, transmembrane tryptase/tryptase γ.

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doi:10.1016/S0006-291X(03)00339-5

Unlike its other two family members, TMT possesses a membrane-spanning domain at its C terminus that causes this tryptase to be retained at the cell's surface when MCs degranulate [3,7]. TMT is therefore a novel exocytosed surface mediator that can alter gene expression in those cell types that are in close proximity to activated MCs. TMT induces airway hyperresponsiveness in normal mice but not in transgenic mice that lack STAT6 or the cytokine receptor that recognizes both interleukin (IL) 4 and IL-13. On the basis of these and other data, TMT is now considered an exocytosed MC neutral protease that can be harmful to the lung because of its ability to activate IL-13/IL-4Rα/STAT6-dependent pathways.

Because of the beneficial and harmful roles of the tryptic-like proteases of the MC, it is important to identify every tryptic-like granule protease that is expressed by this immune cell. We now report that numerous populations of mouse MCs differentiated in vitro and in vivo express the tryptic-like serine protease neuropsin which is a member of the chromosome 7B2 family of kallikreins.

## Materials and methods

*Isolation of neuropsin cDNAs from mBMMCs and evaluation of the expression of this tryptic protease in mouse MCs at the mRNA level.* Clones were arbitrarily isolated and sequenced from a BALB/c mBMMC cDNA library [14] by standard molecular biology procedures. The mBMMCs used to create the library had been cultured for >6 week to ensure that no contaminating cell types were present. As noted below, two of the sequenced clones corresponded to neuropsin. RNA blot and RT-PCR approaches were therefore used to determine when the neuropsin transcript is expressed in developing MCs. For these experiments, total RNA was isolated from BALB/c, 129/Sv, C57BL/6, and W/W$^v$ (Jackson Laboratory, Bar Harbor, ME) mBMMCs cultured in IL-3-enriched medium in the presence or absence of c-*kit* ligand for 3–4 weeks. The RT step was carried out at 55 °C for 30 min. Thirty-five cycles of PCR were performed with the primers 5′-GATCATAGCCTCCAGAGCAGAGATCAG-3′ and 5′-CTTCTTGATCCAGGTAGTGTAGCGGCAG-3′. Each cycle consisted of a 5-s denaturing step at 94 °C, a 5-s annealing step at 60 °C, and a 45-s extension step at 72 °C. The resulting PCR products were fractionated in 1% agarose gels, purified, and subjected to nucleotide sequencing. For a more quantitative analysis, blots were prepared with total RNA isolated from various mouse cell lines and from BALB/c mBMMCs obtained by culturing progenitors in IL-3-enriched medium for 2–9 week. Blots also were prepared with total RNA isolated from the small intestines of BALB/c mice 7, 14, and 40 days after these animals were infected with *Trichinella spiralis*, as described previously [15]. RNA (10–15 μg) was applied to each lane of the 1.2% agarose-formaldehyde gels. The gels were subjected to electrophoresis for 17 h, the separated RNA was transferred to nylon membranes (Schleicher and Schuell, Keen, NH), and the resulting blots were analyzed with a radiolabeled cDNA probe that corresponds to nucleotides 738–1248 in the full-length mouse neuropsin transcript.

*Histochemistry and immunohistochemistry*. Cells from the peritoneal lavage of BALB/c mice were pelleted and fixed in 4% paraformaldehyde in PBS at 4 °C. The preparations were washed twice with PBS containing 2% dimethyl sulfoxide, then dehydrated, and embedded in JB4 glycomethacrylate according to manufacturer's instructions (Polysciences, Warrington, PA). Sections were cut on a Reichert–Jung Supracut microtome (Leica, Deerfield, IL) at 5-μm thickness and picked up on glass slides. The first section was stained with a 1% solution of toluidine blue in methanol to identify MCs; the second section was incubated with a rat monoclonal antibody (designated mAbB5) specific for mouse neuropsin [16]. Immunohistochemical analyses also were performed on cytospins of BALB/c mBMMCs.

## Results and discussion

More than 2000 clones from a BALB/c mBMMC cDNA library were arbitrarily sequenced to identify those transcripts that are expressed in abundance in mouse MCs. No immune cell has been found that expresses neuropsin [also called protease, serine S1 family member 19 (Prss19); GenBank LocusID 259277] which is a member of the kallikrein family of tryptic-like serine proteases. Surprisingly, two of the sequenced cDNAs encoded this neuronal protease. For reference, 1, 20, and 8 of the sequenced cDNAs encoded mTMT, the chymase mMCP-5, and the housekeeping enzyme glyceraldehyde-3-phosphate dehydrogenase, respectively (Yang and Stevens, unpublished findings). None of the sequenced clones encoded mMCP-6 or mMCP-7. The accumulative data suggest that the steady-state level of the neuropsin transcript in BALB/c mBMMCs often is greater than that of the other tryptase transcripts previously identified in this cell. Neuropsin mRNA also was detected in 3-week old mBMMCs developed from 129/Sv, C57BL/6, and W/W$^v$ mice (Fig. 1B). Thus, unlike the tryptic MC granule proteases mTMT and mMCP-7 [3], neuropsin does not appear to be expressed in mouse MCs in a strain-restricted manner. The GeneChips we previously used to evaluate transcript expression in BALB/c mBMMCs lacked a probe set that recognizes neuropsin. This deficiency highlights the importance of the cDNA sequencing approach carried out in the present study to identify those transcripts that are abundant in mouse MCs.

Four isoforms of neuropsin that result from differential splicing of the precursor transcript have been identified in humans [17,18]. Transcript variants 1, 2, 3, and 4 encode proteins that contain 260, 305, 119, and 32 amino acids, respectively. Nucleotide sequence analysis of the BALB/c mBMMC cDNA and its subsequent RT-PCR products (Figs. 1A and B) revealed that the major neuropsin transcript present in MCs encodes the 260-residue, enzymatically active form of this protein. Blot analysis was carried out on RNA isolated from mBMMCs cultured for 2–9 weeks in the presence of IL-3. The neuropsin transcript was expressed relatively early in these developing MCs (Fig. 1C). In contrast to the transiently expressed mMCP-6 and mMCP-7 transcripts [2], neuropsin continued to be expressed at weeks 8 and 9 of the culture. To eliminate the possibility that neuropsin was expressed by a contaminating cell, we

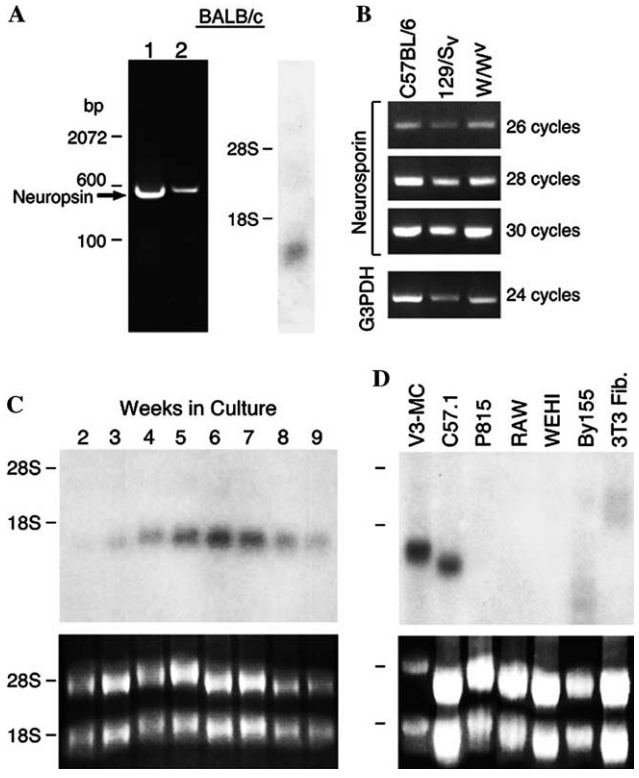


Fig. 1. Expression of neuropsin mRNA in mBMMCs and MC lines. RT-PCR (A,B) and RNA blot (C) analyses were carried out on BALB/c (A,B,C), C57BL/6 (B), 129/Sv (B), and W/W$^v$ (B) mBMMCs derived by culturing progenitors in the presence of IL-3 with (A, lane 1) or without (A, lane 2; B,C) c-*kit* ligand to evaluate the expression of neuropsin at the mRNA level in non-transformed MCs developed from different mouse strains. (D) RNA blot analysis also was carried out on a variety of mouse cell lines. V3, C57.1, and P815 are MC lines; RAW and WEHI are macrophage cell lines; By155 is a T cell line, and 3T3 is a fibroblast cell line.

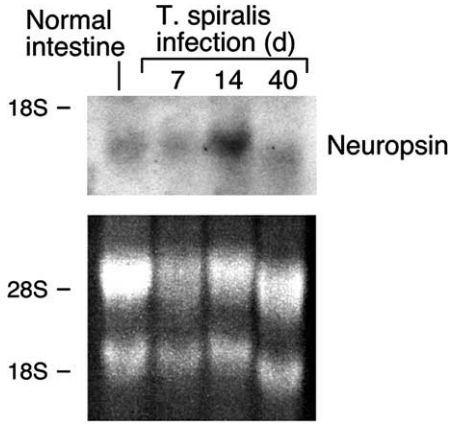


Fig. 2. Up-regulation of neuropsin transcripts in the intestines of *Trichinella spiralis*-infected BALB/c mice. At days 7, 14, and 40 post-infection, total RNA was isolated from the infected mice and RNA blot analysis was carried out to quantitate the expression of neuropsin transcripts in the intestine relative to that in normal, uninfected mice.

also examined a variety of mouse MC lines for their expression of neuropsin by RNA blot analysis (Fig. 1D). While the immature MC line P815 failed to express neuropsin, this protease transcript was expressed in abundance in the V3 and C57.1 MC lines. The steady-state level of the neuropsin transcript was below detection in macrophage, T cell, and fibroblast cell lines.

MCs undergo a transient but pronounced hyperplasia in the jejunum during a *T. spiralis* infection. Many of the MCs that reside in the mucosa during the recovery phase of the infection express the tryptases mMCP-6 and mMCP-7 [19]. However, at the height of the infection at day 14, the only abundantly expressed proteases that have been identified in mucosal MCs to date are the chymases mMCP-1 and mMCP-2. As assessed by RNA blot analysis, the level of neuropsin mRNA is maximal at day 14 (Fig. 2). These data indicate that the expression of the neuropsin transcript is preferentially up-regulated in the jejunum during helminth infection. The data also support the mBMMC library results which indicated that the expression of neuropsin is not coordinately regulated with the transcripts that encode the other tryptic granule proteases of the MC.

An immunohistochemical approach was used to determine whether or not the neuropsin transcript is translated in MCs. mBMMCs differentiated in vitro stored appreciable amounts of neuropsin in their secretory granules (Fig. 3A). Histochemical and immunohistochemical analyses also were carried out on a serially sectioned pellet of a peritoneal cavity cellular exudate to evaluate whether or not mouse MCs differentiated in vivo contain neuropsin protein. The granulated, metachromatic MCs possessed immunoreactive neuropsin (Fig. 3B). The mouse neuropsin gene resides at chromosome 7B2 [20]. In contrast, the genes that encode the other three tryptic-like MC proteases (i.e., mMCP-6, mMCP-7, and mTMT) reside at chromosome 17A3.3 [3,21,22]. It is now apparent that mouse MCs express at least four members of two distinct families of tryptic-like proteases. The finding that mouse MCs express multiple tryptic proteases therefore indicates that caution should be exercised in interpreting data from studies that attempt to evaluate the role of a particular granule-localized protease exocytosed from an immunologically activated MC.

Neuropsin [23] is a 32-kDa, tryptic-like serine protease [24] whose crystal structure has been deduced [25]. Neuropsin initially was cloned from mouse brain [23]. The hippocampus and associated limbic structures express neuropsin, and in situ hybridization studies revealed that the hippocampus pyramidal neurons are the primary source of this protease in the central nervous system. The conclusion that neuropsin is an extracellular protease is based on its continuous release from insect cells and from Neuro 2a neuroblastoma cells [24]. Our immunohistochemical data suggest that mouse MCs preferentially store their neuropsin in their secretory

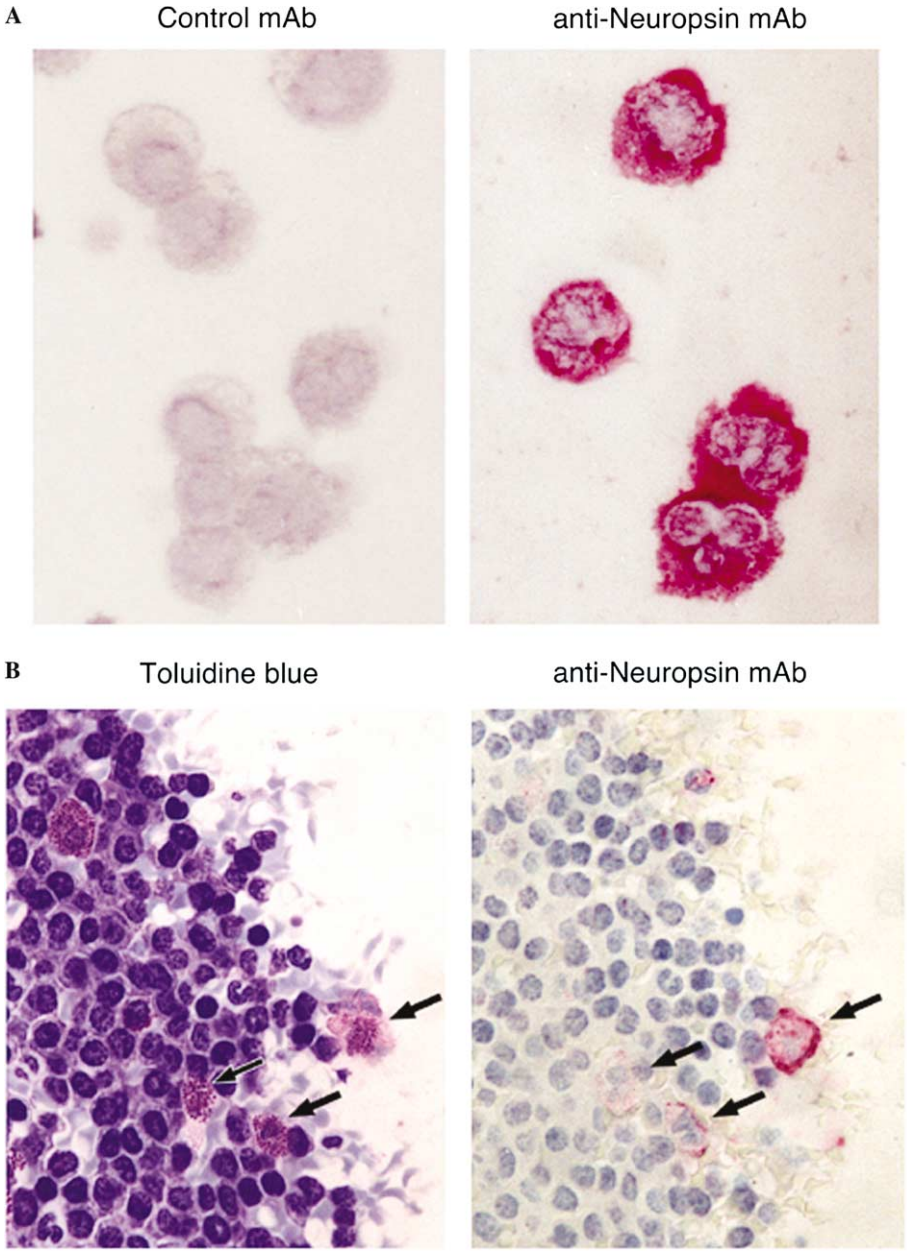


Fig. 3. Expression of neuropsin protein in MCs. Immunohistochemical analysis was carried out on 4-week-old BALB/c mBMMCs with a control monoclonal antibody (A, left panel) or a rat monoclonal antibody that recognizes mouse neuropsin (A, right panel). For evaluation of neuropsin expression in a population of MCs differentiated in vivo, the cells in a peritoneal cavity cellular exudate of a BALB/c mouse were collected and pelleted. Serial sections of the resulting cell pellet were stained with toluidine blue (B, left panel) to identify the highly granulated, metachromatic MCs in the exudate. Adjacent sections were incubated with anti-neuropsin antibody (B, right panel). Arrows in B highlight peritoneal MCs that contain neuropsin protein.

granules. It is presumed that this neutral protease is exocytosed when MCs degranulate in response to inflammatory stimuli. Serine proteinase inhibitor 3 and murinogobulin I are potent inhibitors of neuropsin [26]. The sequestering of the protease in the cell's secretory granules probably evolved so that short bursts of neuropsin could be delivered to adjacent cells and/or extracellular matrices when MCs degranulate.

Neuropsin is preferentially induced in the central nervous system following injury [27,28]. For example, the levels of the neuropsin transcript are increased >10-fold in the hippocampus of patients with Alzheimer's disease [29]. Oka and co-workers [30] concluded that neuropsin probably participates in neurite outgrowth and fasciculation during the development of the nervous system. Recombinant neuropsin can degrade myelin [28] in vitro. MCs are often in close proximity to nerves, and direct interactions between MCs and nerves have been noted in the jejunum and other sites [31]. MCs have also been implicated in demyelination diseases such as the

experimental allergic encephalomyelitis mouse model of multiple sclerosis [32]. These observations suggest that MC-derived neuropsin regulates nerve development and/or function during states of MC activation.

## Acknowledgments

This work was supported by NIH Grants HL-36110 and HL-63284. We thank Drs. Sadao Shiosaka and Kazumasa Matsumoto (Division of Structural Cell Biology, Nara Institute of Science and Technology, Nara, Japan) for providing the rat monoclonal antibody that recognizes mouse neuropsin.

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