Dipeptidyl Peptidase I Is Essential for Activation of Mast Cell Chymases, but Not Tryptases, in Mice*

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Dipeptidyl peptidase I (DPPI) is the sole activator in vivo of several granule-associated serine proteases of cytotoxic lymphocytes. In vitro, DPPI also activates mast cell chymases and tryptases. To determine whether DPPI is essential for their activation in vivo, we used enzyme histochemical and immunohistochemical approaches and solution-based activity assays to study these enzymes in tissues and bone marrow-derived mast cells (BMMCs) from DPPI+/+ and DPPI−/− mice. We find that DPPI−/− mast cells contain normal amounts of immunoreactive chymases but no chymase activity, indicating that DPPI is essential for chymase activation and suggesting that DPPI−/− mice are functional chymase knockouts. The absence of DPPI and chymase activity does not affect the growth, granularity, or staining characteristics of BMMCs and, despite prior predictions, does not alter IgE-mediated exocytosis of histamine. In contrast, the level of active tryptase (mMCP-6) in DPPI−/− BMMCs is 25% that of DPPI+/+ BMMCs. These findings indicate that DPPI is not essential for mMCP-6 activation but does influence the total amount of active mMCP-6 in mast cells and therefore may be an important, but not exclusive mechanism for tryptase activation.

Granule-associated serine proteases of cytotoxic lymphocytes, neutrophils, and mast cells (i.e. granzymes, elastase, cathepsin G, and chymases) are structurally related (1). They have a two-amino acid propeptide (also referred to as the “activation dipeptide”) and an isoleucine at the NH₂ terminus of the activated enzyme (2). The activation dipeptide maintains the protease in an inactive state. After removal of the dipeptide, the new NH₂-terminal isoleucine moves from its position on the surface of the protease to a region in the interior of the enzyme, where it interacts with an aspartate residue near the catalytic center (3). These movements are thought to change the enzyme substrate binding cleft and catalytic apparatus to a catalytically competent conformation.

Initial studies investigating the activation of the granule-associated serine proteases focused on the cysteine protease dipeptidyl peptidase I (DPPI), 1 also known as cathepsin C (2, 4–8). DPPI was a logical candidate activator of these proteases because it is found in the same cells and is a promiscuous exopeptidase that hydrolyzes most NH₂-terminal dipeptides (9). Studies using the DPPI inhibitor Gly-Phe-diazomethyl ketone reported that DPPI inhibition reduces granzyme A, neutrophil elastase, and cathepsin G activity in the cells (2). However, because the inhibitor is not entirely specific for DPPI and does not eliminate the activity of the proteases in question, the possibility remained that another protease was responsible for their activation. This possibility was tested in a DPPI knockout mouse (10). Cytotoxic lymphocytes of these animals produce normal amounts of granzymes A and B, but both enzymes are inactive and present in their pro-forms. These findings suggest that DPPI is the sole activator of granzymes A and B in vivo.

In mast cells, the two major types of serine proteases are chymases and tryptases. Chymases are grouped into α and β types based on structural and functional differences. Humans express α-chymase only. In contrast, mice express five known chymases: the α-chymase mouse mast cell protease-5 (mMCP-5) and the β-chymases mMCP-1, -2, -4, and -9 (11). Chymases possess an acidic activation dipeptide similar to that of most granule-associated serine proteases. Studies using recombinant human pro-α-chymase suggest that DPPI can activate prochymase in vitro by removing the activation dipeptide (6). Tryptases also are a diverse group of structurally related proteases that include α, β, and γ isomers in humans (12, 13) and two or three tryptases (mMCP-6, mMCP-7, and transmembrane/ψ) in mice (14). MMCP-7 is not expressed in C57BL/6 mice, and thus, its expression is strain-dependent (15). Available evidence suggests that activation of tryptases is more complicated than that of chymases and other granule-associated serine proteases. In vitro studies indicate that, after removal of a signal peptide by signal peptidase, human β-tryptase requires two additional, sequential cleavages of its propeptide for activation (7). The first such cleavage is thought to be by trypsin itself, which leaves a two-amino acid activation dipeptide to be removed by DPPI. Whether this sequence of events occurs in vivo is not known. Furthermore, the mechanism of activation of γ-tryptase, which appears to lack an activation dipeptide, may differ from that of the other tryptases.

To establish whether DPPI is essential for activating mast cell chymases and tryptases in vivo, we examined these en-

1 The abbreviations used are: DPPI, dipeptidyl peptidase I; mMCP, mouse mast cell protease; mMCP, rat mast cell protease; BMMC, bone marrow-derived mast cell; CAE, naphthol AS-D chloroacetate esterase; CAP, 3-(cyclohexylamino)propanesulfonic acid; PBS, phosphate-buffered saline; TBS, Tris-buffered saline.
zymes in DPPI-null mice. We found that mast cells from these mice contain normal amounts of at least two chymases (mMCP-4 and -5), but they and other chymases are completely inactive as determined by enzyme histochemistry and solution-based assays. In contrast, the mast cells contain reduced amounts of normally processed, active tryptase (mMCP-6). Thus, DPPI is required for the processing and activation of chymases, but not tryptases, in mouse mast cells. These findings improve our understanding of the regulation of serine protease activity in mast cells and have important implications for understanding their biological functions in vivo.

**EXPERIMENTAL PROCEDURES**

**Materials**—All chemicals were from Sigma unless otherwise noted. Rabbit anti-rat mast cell protease I (MCP-I) was from Morefun Scientific Limited, Penicuik, Scotland, rabbit anti-MCP-5 and rabbit anti-mMCP-6 were gifts from Richard Stevens, Harvard University, Cambridge, MA, and monoclonal anti-dinitrophenyl IgE and anti-β-actin were from Sigma.

**Experimental Animals—**DPPI+/− mice were originally developed by homologous recombination in the 129/C57BL/6 background (19). For our studies, we used DPPI+/− mice and DPPI+/+ or DPPI+/− littersmates as controls. All experimental procedures were performed in mice older than 8 weeks of age and were approved by the University of California San Francisco Committee on Animal Research.

**Tissue Preparation**—Harvested tissues were washed in PBS before being fixed for 6–18 h in PBS containing 4% paraformaldehyde. Tissues were then embedded directly in paraffin or incubated in PBS containing 30% sucrose for 18 h at 4 °C before freezing at −70 °C in Tissue-Tek OCT compound (Miles, Elkhart, IN), then sectioned (5 μm). Before use, paraffin-embedded sections were de-paraffinized in xylene, hydrated through graded alcohols, and equilibrated in PBS. Cryosections were washed in PBS. To visualize mast cells, tissue sections were incubated in 0.1% methylene blue for 10 s, rinsed with water, dehydrated in 100% ethanol, mounted under coverslips with Histomount (Zymed Laboratories Inc. Laboratories, South San Francisco, CA), and photographed.

**Enzyme Histochemistry**—To detect active chymase in tissue sections, naphthol AS-D chloroacetate esterase (CAE) histochemistry was performed as described (16). Slides were then washed with water, counterstained in 0.5% eosin Y, dehydrated in 100% ethanol, and mounted with Histomount. Active tryptase was detected using the method of Valchanov and Proctor (17). Briefly, tissue sections were incubated at 20 °C in 0.1% phosphate buffer (pH 7.5) containing 0.3 mg/ml of benzoyl-Gly-L-Pro-Arg-4-methoxy-2-naphthylamide (Enzyme Systems Products, Dublin, CA) and 3 mM Fast Blue B salt. After incubation for 1–5 min, the slides were rinsed in water and photographed.

**Immunohistochemistry**—Tissues were equilibrated in PBS, incubated in blocking solution (PBS containing 5% dehydrated milk, 3% nonimmune goat serum, 0.1% Triton X-100, and 1% glycine) at 20 °C for 1 h, washed in PBS. To visualize mast cells, tissue sections were incubated in blocking solution (PBS containing 5% dehydrated milk, 3% nonimmune goat serum, 0.1% Triton X-100, and 1% glycine) at 20 °C for 15 min, then incubated overnight at 4 °C with a 1:200 dilution of rabbit anti-mCP-I. Tissues were then washed in PBS-Tween, and the bound antibody was detected with fluorescein isothiocyanate-conjugated goat anti-rabbit IgG (Vector Laboratories, Burlingame, CA).

**Culture of Mast Cells from Bone Marrow**—Mouse BMMCs were cultured in WEHI-3B-conditioned medium as described (18). The cells were then cultured in WEHI-3B-conditioned medium as described (18). The cells were used after 4 weeks in culture, at which time the cell populations consisted of >95% mast cells as assessed by the presence of metachromatic granules in toluidine blue-stained cells.

**Protease Activity Assays**—BMMCs were harvested by centrifugation, washed in PBS, and resuspended in 10 mM Tris buffer (pH 6.1) containing 2 mM NaCl at 40 × 10^6 cells/ml. Cells were then lysed by sonication, debris was pelleted by centrifugation, and the supernatant was recovered. DPPI activity was measured spectrophotometrically by monitoring hydrolysis of L-Ala-Ala-p-nitroanilide. 20 μl aliquots of cell lysates were incubated for 5 min in 500 μl of activation buffer (100 mM Na_2HPO_4 buffer, 20 mM NaCl, 1 mM EDTA, 4 mM cysteine (pH 6.1)) followed by the addition of 400 μl of substrate buffer (100 mM Na_2HPO_4, 20 mM NaCl, 1 mM EDTA, 125 μM L-Ala-L-Ala-p-nitroanilide, 1% dimethylsulfoxide (pH 6.1)). Tryptase activity was measured using benzoyl-Gly-L-Pro-Arg-p-nitroanilide. 1 μl of enzyme solution was incubated in 1 ml of 0.06 M Tris-HCl (pH 7.8) containing 0.4% dimethylsulfoxide, 30 μg/ml heparin, and 80 μg/ml substrate at 37 °C. Chymase activity was measured by the addition of 20 μl of enzyme solution to 1 ml of 0.45 M Tris-HCl (pH 8.0) containing 1 μM succinyl-L-Ala-Ala-Pro-Phe-p-nitroanilide, 1.8 mM NaCl, and 1% dimethylsulfoxide. Release of free nitroaniline was measured spectrophotometrically at 410 nm for 5–10 min in all assays.

**Immunoblotting**—Cell lysates were subjected to SDS-polyacrylamide gel electrophoresis under reducing conditions and transferred to polyvinylidene difluoride membranes (PerkinElmer Life Sciences Products, Boston, MA) containing 25 mM Tris, 200 mM NaCl, and 15% methanol for 1 h at 4 °C. The membrane was washed with 50 mM Tris-HCl containing 0.5 mM NaCl, 0.01% Tween 20 (PBS; pH 7.5) and incubated for 1 h in TBS containing a 1:1,000 dilution of antibody. The membrane was then washed with TBS, incubated in TBS for 30 min containing a 1:2,000 dilution of horse radish peroxidase-conjugated goat anti-rabbit IgG (New England Biolabs, Beverly, MA), and visualized (1 g/ml) again. Immunoblotting was detected using the phototope horseradish peroxidase detection kit (New England Biolabs). After detection of the mast cell protease signal, the immunoblots were probed a second time with monoclonal anti-β-actin as a loading control.

**Mast Cell Degranulation—**5 × 10^5 BMMCs were cultured overnight in the presence of monoclonal anti-dinitrophenyl IgE (25 μg). These sensitized cells were harvested by centrifugation, washed twice in Ca^2+− and Mg^2+−free PBS, and resuspended in RPMI 1640 at 20 × 10^6 cells/ml. Cells were then incubated alone or with 400 ng/ml dinitrophenyl-conjugated bovine serum albumin for 60 min. Aliquots were removed at 0 and 60 min then centrifuged immediately. Degranulation supernatants were separated from cell pellets, which were resuspended in the same volume as the supernatant and lysed by repeated cycles of freezing and thawing. Cell pellets were pelleted at 15,000 × g. Recovered supernatants were assayed for histamine content using an enzyme-linked immunosorbent assay kit (Research Diagnostics, Flanders, NJ) according to the manufacturer’s instructions. The net percent of histamine released was calculated as previously described (19).

**Activation of Mouse Pro-chymases—**5 μl of DPPI+/− BMMC lysate (equivalent to 2 × 10^6 BMMCs) was added to 50 μl of the DPPI activation buffer containing 0.3 μg of pre-activated, purified, dog DPPI (20). The mixture was incubated at 37 °C for 2 h, then assayed for chymase activity using the method noted.

**Tryptase Purification and Sequencing—**Cell lysates from 40 × 10^6 DPPI+/− BMMCs were loaded onto a 1 × 1-cm column of benzamidine-agarose (Sigma) equilibrated in high salt buffer (10 mM bis-Tris (pH 6.1) containing 2 mM NaCl), washed extensively in high salt buffer, and then eluted with high salt buffer containing 150 mM benzamidine. Fractions containing eluted protein were checked for the presence of mMCP-6 by immunoblotting. Purity was checked by SDS-polyacrylamide gel electrophoresis. A separate column was used to purify trypstatse from 40 × 10^6 DPPI+/− BMMCs. For sequencing, 5 μg of trypstatse purified from DPPI+/− BMMCs were subjected to SDS-polyacrylamide gel electrophoresis, transferred to polyvinylidene difluoride membrane in 10 mM CAPS buffer containing 10% methanol for 1 h at 4 °C, then stained with Coomassie Blue. Protein bands at Mr, 35,000 were cut from the membrane and subjected to Edman degradation using a 470A gas phase sequencer with an on-line 120A phenylthiohydantoin analyzer (Applied Biosystems, Foster City, CA) by the Biomolecular Resource Center at the University of California at San Francisco.

**RNA Blotting—**Total RNA was isolated from DPPI+/− and −/− BMMCs using Tri-reagent (Invitrogen, Carlsbad, CA). Denatured RNA was size-fractionated by agarose gel electrophoresis and transferred to Nytran Plus nylon membrane (Schleicher and Schuell). Vacuum-baked membranes were prehybridized at 42 °C for 2 h and hybridized with an [α-32P]dCTP (Amersham Pharmacia Biotech) random prime-labeled, mouse MCP-6 probe (obtained by cloning a 310-base pair cDNA polymerase chain reaction product corresponding to base pairs 235–545 of the mMCP-6 open reading frame) at 42 °C overnight. After two washes at room temperature and two at 37 °C, the membrane was exposed to film for 48 h, then developed. After removal of previously bound probe, the membrane was hybridized with an α-actin-labeled probe to control for possible variations in signal intensity due to differing amounts of mRNA loaded per lane.

**RESULTS**

**Identification of Active Chymase in Mouse Tissues**—We detected active chymase in tissue sections via esterase activity using CAE enzyme histochemistry. In tissue sections from DPPI+/− mice, we identified CAE-positive cells in ear, tongue, stomach, and trachea. CAE-positive cells had a distribution, number, and morphology typical of mast cells. In contrast, DPPI−/− mice had no CAE-positive staining cells (Fig. 1).
Nonetheless, DPPI −/− tissues contained mononuclear cells staining metachromatically with methylene blue (Fig. 1), indicating that the absence of CAE activity is not due to a lack of mast cells. Furthermore, mast cells in sections of ear and tongue from DPPI −/− mice contain chymase because they are immunoreactive when stained with anti-rMCP-I, which recognizes its mouse orthologue mMCP-4 (Fig. 2) (21). These findings suggest that the mast cells of DPPI −/− mice contain inactive mMCP-4, which is the principal extractable chymase isoform in mouse skin (21).

Chymase Activity in BMMCs—To confirm the enzyme histochemical results, we measured protease activities in mast cells cultured from DPPI +/+ and −/− marrow. We used DPPI +/+ animals as controls for these and all subsequent experiments because pilot studies revealed that protease activities are similar in extracts of DPPI +/+ and DPPI +/− tissues. DPPI −/− BMMCs develop normally and are similar to DPPI +/+ BM-MCs in gross morphology and granularity (Fig. 3A). Cell lysates of DPPI +/+ BMMCs contain DPPI and chymase activity (Table I). In contrast, DPPI −/− BMMC lysates lack detectable DPPI or chymase activity. The relative amount of α-chymase in DPPI +/+ and −/− BMMCs was determined by immunoblotting using an anti-mMCP-5 antibody. Immunoblots of DPPI +/+ and DPPI −/− BMMC lysates, normalized for total protein, demonstrate equal immunoreactive bands (Fig. 3B). The higher apparent size of immunoreactive mMCP-5 in DPPI −/− cell extracts is consistent with the pro-form of the enzyme (22). At least some of the pro-chymase in DPPI −/− cell extracts remains activable, as suggested by our ability to generate active chymase (0.38 kU mg protein; Table I) in DPPI −/− extracts incubated with DPPI.

Mast Cell Degranulation—Prior studies using chymase (Fab)2 fragments (23), nonspecific inhibitors (24), or added chymase (25) suggested that chymase plays a role in mast cell degranulation. To determine if endogenous chymase regulates this process, we compared IgE-mediated degranulation of DPPI +/+ and −/− mast cells, finding that DPPI +/+ and −/− BMMC histamine release is indistinguishable after IgE receptor activation (Fig. 3C). These findings suggest that endogenous chymase does not play a major role in the degranulation of mouse BM-MCs.

Identification of Active Tryptase in Mouse Tissues—The enzyme histochemical substrate benzoylarginine-7-amino-4-methoxy-2-naphthylamide detected active tryptase in situ in mouse tissues. Ear, tongue, and stomach tissue sections obtained from DPPI +/+ mice possess multiple, red-brown, tryptase-positive cells (Fig. 4). A comparable number of tryptase-positive cells were seen in tissue sections from DPPI −/− mice, indicating that active tryptase is present in these animals as well. No red-brown staining cells are present in...
control sections treated in the absence of substrate (data not shown).

**Tryptase Activity in BMMCs**—Because tryptase enzyme histochemistry is qualitative, not quantitative, the possibility remained that levels of active tryptase differ between DPPI +/- and +/- mast cells. To test this possibility, we measured tryptase activity in DPPI +/- and +/- BMMC lysates normalized for total protein. Although tryptase activity is present in both lysates, the activity in DPPI +/- mast cell lysates is only 25% that of DPPI +/- lysates (Table I). Seeking an explanation for the 75% reduction of active tryptase in DPPI +/- BMMCs, we quantified the relative amounts of mouse tryptase (mMCP-6) protein and mRNA in DPPI +/- and +/- BMMCs. We found reduced mMCP-6 immunoreactivity in the DPPI +/- cell lysates compared with +/- controls (Fig. 5A). PromMCP-6 or mMCP-6 degradation fragments were not detected in either lysate. The relative amounts of mRNA are equal in DPPI +/- and +/- mast cells (Fig. 5B). MMCP-6 purified from DPPI +/- mast cells possesses an NH2-terminal amino acid sequence (IVGGHEAS) corresponding to normally processed, active mMCP-6. No sequences corresponding to protryptase were found. Furthermore, no mMCP-6 immunoreactivity was detected in the benzamidine column flow-through fractions. These findings suggest that the 75% reduction of active mMCP-6 in DPPI +/- mast cell extracts stems from decreased amounts of active protein, suggesting that the reduction is due to post-transcriptional events.

**DISCUSSION**

In this study, DPPI-deficient mice reveal that DPPI is essential for the in vivo activation of mast cell chymases. Surprisingly, DPPI is not essential for the activation of the tryptase mMCP-6, although it does influence the amount of active mMCP-6 in mast cells. These alterations of protease activity do not affect mast cell growth, maturation, tissue migration, or degradation, indicating that DPPI and chymase are not essential for these processes. These findings suggest a mechanism for the intracellular activation and regulation of a major class of mast cell preformed mediators.

Prior studies report that purified DPPI activates human pre-chymase in vitro (6, 26). Mice have five chymases, mMCP-1, -2, -4, -5, and -9 (11), all with similar measured or predicted activity and primary structure. mMCP-1, -2, -4, and -9 are predicted to have identical propeptides (EE) and mature NH2 termini (IIGG) (11). MMCP-5 also has a similar propeptide (EE) and mature NH2 termini (IIGG) (11). MMCP-1, -2, -4, -5, and -9 (11), all with similar measured or predicted activity and primary structure. MMCP-1, -2, -4, and -9 are predicted to have identical propeptides (EE) and mature NH2 termini (IIGG). Our results show that DPPI is essential for the activation of mouse chymases in BMMCs and mucosal and connective tissue mast cells in vivo. Furthermore, in the absence of DPPI, the chymases exist in an activable pro-form. In our immunodetection studies we focused on mMCP-4 and -5 because the former is the major class of mast cell preformed mediators.
isoform extracted from mouse skin (21), and the latter is the major product of mature BMPCs (27, 28). Because of the high degree of structural similarity of the chymases, it is likely that our findings are true for all chymases. Furthermore, the absence of CAE-positive cells in DPPI+/− mouse tissues containing mMCP-1, 2, 4, and 5 (28, 29) supports this assertion, since the CAE procedure is an activity-based histochemical approach that detects chymase activity in all known chymase-containing subsets of mast cells in a broad spectrum of mammalian tissues.

Our observation that DPPI+/− mast cells degranulate as well as DPPI+/+ mast cells indicates that endogenous DPPI and chymase play little or no role in the IgE-mediated release of histamine from BMPCs. These findings are contrary to expectations generated by reports that chymase directly degranulates or potentiates IgE-mediated degranulation (23–25, 30). These reports are based largely on experiments conducted in vitro using inhibitors that are not chymase-specific and potentially non-physiologic quantities of chymase. Also, in vitro findings may fail to predict in vivo behavior of chymases when conducted in the absence of natural chymase inhibitors or using a chymase that is unassociated with the proteoglycans to which chymases are bound to upon release from mast cell granules. Our studies overcome some of these limitations by comparing degranulation using mast cells with and without endogenously synthesized and packaged chymase. However, our findings in mouse BMPCs do not rule out the possibility that chymases modulate mast cell exocytosis in humans or other mammals or in subsets of mouse mast cells expressing a different profile of chymases.

Recombinant human β-proctryptase can be activated in vitro by two sequential steps of propeptide processing (7). First, β-proctryptase is autoprocessed to the inactive intermediate pro’-trypsin, which has a residual pro-dipeptide. The pro’-trypsin intermediate can then be fully activated by DPPI, which removes the dipeptide. Because of sequence similarities of the pro-region of mMCP-6 to β-trypsin, we expected that mMCP-6 would be inactive in DPPI+/− mice mast cells and that any immunoreactive protein would be in the pro’ form. To our surprise, enzyme histochemistry showed active trypsin in mast cells of tissues obtained from DPPI+/− mice. This activity is due in part to mMCP-6 because it exists entirely in its pro-form and is not processed, active form in DPPI+/− BMPCs. This finding is similar to that of granzyme C in DPPI+/− mice. In these animals, 50% of granzyme C is found in the pro-form, and 50% is found in the active form (10). These observations indicate that DPPI is not essential for the activation of all mast cell trypsinases or granzyme C. How mMCP-6 becomes activated and whether trypsin and granzyme C share a mechanism of activation in the absence of DPPI are unclear. One possibility is that mMCP-6 undergoes two-step processing by trypsin and a second protease, possibly an aminopeptidase (31), which compensates for DPPI. Alternatively, mMCP-6 may be activated directly by an endoprotease. Candidates include cathepsins B, L, and S, which are present in BMPCs.

Our observation that DPPI+/− BMPCs contain less mMCP-6 than DPPI+/+ control cells indicates that DPPI influences the total amount of active trypsin in BMPCs. Furthermore, this influence probably is exerted post-transcriptionally, giving the equivalence of steady state mRNA levels in the two types of cells. One explanation for our findings is that DPPI normally plays a role in two-step processing of proctryptase to an active, protease-resistant form. In the absence of DPPI, trypsinase exists in its pro- or pro’-form longer because the compensating protease is less efficient, thereby increasing the time of exposure to degradative enzymes as pro-tryptases traffic from the endoplasmic reticulum to the secretory granules. This explanation is consistent with prior observations that unprocessed granzymes are susceptible to degradation in DPPI+/− mice (10). Alternatively, less likely explanations are that DPPI processes proteins that regulate the quantity of degradative enzymes or that DPPI directly stabilizes trypsinase molecules during biogenesis.

Most proteases are synthesized as inactivezymogens that are activated by proteolytic removal of a propeptide. Some proteases autoactivate (e.g. cathepsins L and K), whereas others are activated by a different protease hydrolyzing a specific site (e.g. trypsin activation by enteropeptidase). In some instances, a series of proteases must be activated sequentially before activation of a protease. The best known example of this is the clotting cascade, where sequential activation of proteases culminates in conversion of prothrombin to thrombin. This activation cascade serves two purposes. First, because each active protease activates more than one molecule of zymogen at each stage of the cascade, the final signal (in this example, formation of a fibrin clot by thrombin) is greatly amplified. Second, the sequential activation generates multiple branch points at which the cascade can be regulated. Our finding that DPPI is the sole chymase activator establishes a cascade in mast cells in which DPPI activates chymase, which in turn activates gelatinase B (32). What remains to be determined is how DPPI itself is activated. Two possibilities are that it is auto-activated in a manner similar to that of the related cysteine proteases, cathepsins L and K (33, 34), or that it is activated by one or more other proteases. Defining a cascade of protease activation in mast cells is important because it provides an understanding of how the proteolytic activity and subsequent biological effects of the cascade are regulated.

Mutations in the DPPI gene resulting in a loss of DPPI activity are linked to the Papillon-Lefèvre syndrome (35, 36), a disease characterized by early periodontitis, palmoplantar hyperkeratosis, and a predisposition to bacterial infections (37). How the absence of DPPI activity produces the manifestations of the Papillon-Lefèvre syndrome is currently unknown. However, past studies in knockout mice suggest that the predisposition to bacterial infections may be due in part to altered levels of serine protease activity (10, 38, 39). The current studies allow us to predict that patients with Papillon-Lefèvre syndrome will have no active mast cell chymase and reduced levels of trypsinase.

In summary, this report establishes that DPPI is essential for the activation of mouse mast cell chymases in vivo. Furthermore, DPPI regulates the total amount of active trypsinase within mast cells, although it is not essential for trypsinase activation. These findings further our understanding of the biogenesis of the major preformed mediator proteins of mast cells and suggest a means of regulating their biologic functions in vivo.

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