Proteome analysis of mast cell releasates reveals a role for chymase in the regulation of coagulation factor XIIIA levels via proteolytic degradation

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Background: Mast cells are significantly involved in IgE-mediated allergic reactions; however, their roles in health and disease are incompletely understood.

Objective: We aimed to define the proteome contained in mast cell releasates on activation to better understand the factors secreted by mast cells that are relevant to the contribution of mast cells in diseases.

Methods: Bone marrow–derived cultured mast cells (BMCMCs) and peritoneal cell–derived mast cells were used as "surrogates" for mucosal and connective tissue mast cells, respectively, and their releasate proteomes were analyzed by mass spectrometry.

Results: Our studies showed that BMCMCs and peritoneal cell–derived mast cells produced substantially different releasates following IgE-mediated activation. Moreover, we observed that the transglutaminase coagulation factor XIIIA (FXIIIA) was one of the most abundant proteins contained in the BMCMC releasates. Mast cell–deficient mice exhibited increased FXIIIA plasma and activity levels as well as reduced bleeding times, indicating that mast cells are more efficient in their ability to downregulate FXIIIA than in contributing to its amounts and functions in homeostatic conditions. We found that human chymase and mouse mast cell protease-4 (the mouse homologue of human chymase) had the ability to reduce FXIIIA levels and function via proteolytic degradation. Moreover, we found that chymase deficiency led to increased FXIIIA amounts and activity, as well as reduced bleeding times in homeostatic conditions and during sepsis.

Conclusions: Our study indicates that the mast cell protease content can shape its releasate proteome. Moreover, we found that chymase plays an important role in the regulation of FXIIIA via proteolytic degradation. (J Allergy Clin Immunol 2017;139:323-34.)

Key words: *Mast cells, proteases, chymase, proteomics*

Mast cells are hematopoietic progenitor-derived, granule-containing immune cells that are widely distributed in tissues that interact with the external environment, such as the skin and mucosal tissues. Although it is well known that mast cells are significantly involved in IgE-mediated allergic reactions, recent studies have shown that mast cells and their granule proteases have pleiotropic regulatory roles in other immunological responses and diseases, such as in bacterial and parasite infections, sepsis, autoimmune disease, and cancer.[1] Many of these studies, however, were biased to investigate mouse models in which mast cells were hypothesized to play key roles on the basis of *in vitro* studies or conditions in which mast cell numbers or their mediators were increased, suggesting that our understanding of the contribution of mast cells to health and disease is limited. In this study, we adopted an unbiased approach with the potential to provide a more comprehensive assessment of how mast cells may influence biological processes by characterizing mast cell releasate proteomes via mass spectrometry analysis.

Mast cells express protease profiles that vary among species and different mast cell subsets. In humans, mast cells either express tryptase (α and β) only (known as the MC_T subclass) or tryptase, chymase, and mast cell carboxypeptidase A (CPA) (known as the MC_{TC} subclass). In mice, mast cells are divided into connective tissue (connective tissue mast cells [CTMCs]) and mucosal mast cell (MMC) subtypes. CTMCs predominantly express the β-chymase, mouse mast cell protease 4 (mMCP-4),

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Abbreviations used
BMCMCs: Bone marrow derived–cultured mast cells
CLP: Cecal ligation and puncture
CPA: Carboxypeptidase A
CTMC: Connective tissue mast cell
FXIIIA: Coagulation factor XIIIA
MMC: Mucosal mast cell
mMCP: Mouse mast cell protease
PCMCs: Peritoneal cell–derived mast cells

and the α-chymase mMCP-5, whereas MMCs predominantly express 2 different β-chymases, mMCP-1 and mMCP-2. In addition, in the C57BL/6 mouse background, CTMCs express a tetrameric tryptase (mMCP-6) as well as CPA.[2] We and others have shown that mast cell proteases can cleave certain mediators released by mast cells into inactive or active fragments. For example, we reported that mMCP-4, the mouse homologue of human chymase in substrate specificity,[3] prevents hyperinflammation in severe sepsis via proteolytic degradation of TNF.[4] Consequently, it is important to understand how mast cell phenotypes and their protease content may shape their mediator release profile on activation. Herein, we characterized the releasates generated by bone marrow derived–cultured mast cells (BMCMCs) and peritoneal cell–derived mast cells (PCMCs) following IgE-mediated activation as "surrogates" for MMCs and CTMCs, respectively.[5,6]

Our proteomics studies indicate that BMCMCs and PCMCs produced substantially different releasates. As a specific example, we observed that the transglutaminase coagulation factor XIIIA (FXIIIA) was one of the most abundant proteins in the IgE-mediated BMCMC releasate. In contrast, the PCMC releasate did not contain FXIIIA. Therefore, we investigated the involvement of CTMC-specific proteases in the downregulation of FXIIIA, and we observed that mast cell chymase could proteolytically degrade FXIIIA and diminish its function during homeostatic and septic states.

METHODS

For detailed methods, including the mice used, experimental protocols and procedures, and statistical analysis, please see the Methods section in this article's Online Repository at www.jacionline.org.

RESULTS

Proteome profiling of mast cell releasates

To define the BMCMC and PCMC releasates following IgE-mediated activation, we used a mass spectrometry shotgun proteomics approach. Specifically, IgE-2, 4-dinitrophenyl–sensitized BMCMCs and PCMCs were stimulated with 2, 4-dinitrophenyl-human serum albumin antigen for 6 hours. Then, the supernatants were collected, concentrated, and subjected to LC-MS/MS to identify the IgE-mediated differentially produced proteins from these mast cells (Fig 1, *A*). We identified 91 proteins that were unique to PCMCs (Table I; see Table E1 in this article's Online Repository at www.jacionline.org), 390 proteins that were unique to BMCMCs (Table II; see Table E2 in this article's Online Repository at www.jacionline.org), and 674 proteins that were extracellularly produced by both BMCMCs and PCMCs (Table III; see Table E3 in this article's Online Repository at www.jacionline.org) (Fig 1, *B*). We associated the proteins identified in the releasates with functional annotations. By gene ontology analysis, the commonly enriched biological process categories in the BMCMC and PCMC releasates were linked to metabolic processes (eg, carbohydrate/protein metabolism and glycolysis) as well as intracellular protein trafficking, protein folding, cell structure and motility, and endocytosis (Table III; see Table E4 in this article's Online Repository at www.jacionline.org). Synthesis of newly formed mediators and mediator secretion are 2 main mast cell functions

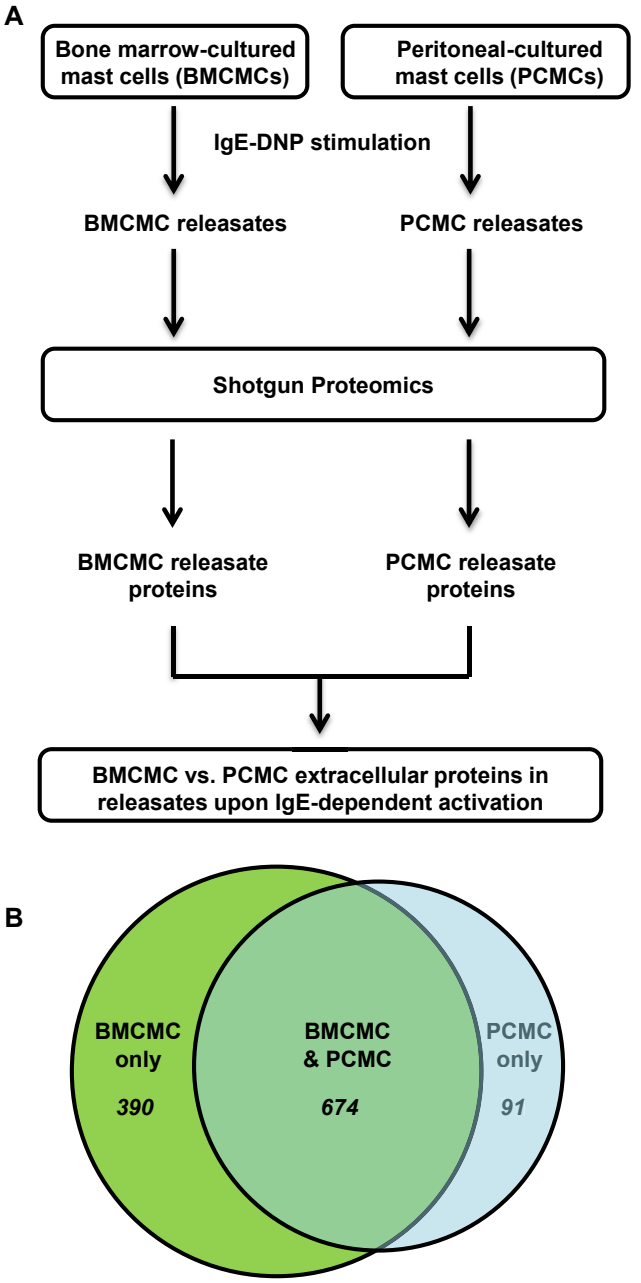

FIG 1. Differentially produced extracellular proteins from bone marrow compared with peritoneal-derived mast cells. **A,** A schematic overview of the approach used to define the releasates generated by BMCMCs and PCMCs following IgE-mediated activation. **B,** A Venn diagram showing the number of shared and exclusive proteins among the BMCMC and PCMC releasates. *DNP,* 2, 4-Dinitrophenyl.