

Development, significance, and heterogeneity of mast cells with particular regard to the mast cell-specific proteases chymase and tryptase

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Abstract: Mast cells are one of the major effector cells in the pathogenesis of the immediate-type hypersensitivity reaction in a number of non-allergic immune disorders as well as in normal physiological processes. In addition, it has been shown recently that mast cells also play a significant role in a life-saving host response to bacterial reactions. But as much as the immunopathological role of mast cells has been acknowledged, these cells have also aroused much controversy and confusion. By now it is clear that one explanation for the sometimes even contradictory opinions on mast cell function arise from mast cell heterogeneity. This heterogeneity can express itself as differences in histochemical, biochemical, and functional characteristics. In vitro systems provided a powerful tool for the investigation of the basic mechanisms for mast cell development and differentiation and helped to demonstrate that mast cell heterogeneity can be traced back to certain cytokine patterns that are present in different microenvironments. In this context it has also been shown that the growth factors required for human mast cell differentiation are somewhat different than those for rodents. In rodents, the atypical, T cell-dependent mucosal type mast cell can be distinguished from the T cell-independent connective tissue-type mast cell. In humans, the strict classification into mucosal and connective tissue-type mast cells is not possible and the content of mast cell-specific proteases chymase and tryptase is the main criterion for mast cell subtypes in humans. The large quantities of tryptase and chymase that are synthesized by mast cells suggest and emphasize the significance of these proteinases in mast cell function and stimulated investigations about the biological properties of these mast cell-specific proteases. Comparing their biological activities it becomes clear that they share some activities. On the other hand, tryptase seems to participate in proinflammatory mast cell function, whereas chymase seems to be more involved in inflammatory reactions. This review provides a short overview of the discovery, origin, development, and biological significance of mast cells and will then concentrate on mast cell heterogeneity in rodents and humans with respect to the mast cell proteases tryptase and chymase and their function. *J. Leukoc. Biol.* 61: 233–245; 1997.

Key Words: murine mast cells · human mast cells · function

INTRODUCTION

Mast cells are found in almost all major organs and tissues of the body. It is estimated that if all mast cells in the body were assembled in one organ, their mass would equal that of the spleen [1]. They play a preeminent role in the immunopathology of immediate- and delayed-type hypersensitivity reactions as well as in the pathogenesis of chronic inflammation. Activation of mast cells, either by immunoglobulin E (IgE)-dependent or IgE-independent agonists, leads to the release of a variety of preformed and newly synthesized mediators, which then act on distinct effector cells to produce the symptoms of allergy and anaphylaxis [2, 3]. However, although the immunopathological role of mast cells has been acknowledged, controversy and confusion about these cells has also been aroused. It is clear by now that one explanation for the sometimes even contradictory opinions on mast cell function arises from mast cell heterogeneity. This heterogeneity can express itself as differences in histochemical, biochemical, and functional characteristics. Recent advances in the studies of mast cell proteases have led to a rapid expansion of knowledge concerning the biological role of mast cells.

Abbreviations: IgE, immunoglobulin E; IL-3, interleukin-3; SCF, stem cell factor; PGD₂, prostaglandin D₂; LTB₄, leukotriene B₄; IFN- γ , interferon- γ ; TGF- β , transforming growth factor- β ; TNF- α , tumor necrosis factor α ; MIP-1, macrophage inflammatory protein 1; GM-CSF, granulocyte-macrophage colony-stimulating factor; MMC, mucosal-type mast cells; CTMC, connective tissue-type mast cells; RMCP, rat mast cell protease; MMCP, mouse mast cell protease; MCT, mast cells containing tryptase; MCTC, mast cells containing tryptase and chymase; HMC-CP, human mast cell carboxypeptidase; HHMC, human heart mast cell; HLMC, human lung mast cell; HMMK, high-molecular-mass kininogen; VIP, vasoactive intestinal peptide; PHM, peptide-histidine methionine; CGRP, calcitonin gene-related peptide; MMP, metalloproteinases; VSMC, vascular smooth muscle cells.

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DISCOVERY, ORIGIN, AND DEVELOPMENT

In 1879 Paul Ehrlich [4] was the first to recognize and describe what he called mastung (i.e., overfed) cells with cytoplasmic basophilic granules in the connective tissue of humans. The monomorphic cytoplasmic granules of the round to long-streched cells with the oval to reniform nucleus show a strong purple-red metachromasia after staining with basic dyes such as methylene or toluidine blue.

The origin of the tissue mast cell remained unclear for a long time. They were suggested to be derived from T cells, fibroblasts, or macrophages [5–7]. However, the studies of Kitamura and his group [8, 9] have conclusively shown that mast cells are derived from pluripotent hematopoietic stem cells in the bone marrow. They transplanted bone marrow from a mouse mutant with cytoplasmic large granules (C57BL/6 bg^l/bg^l), respectively normal littermates (WBB6_{F1}-+/+) to genetically mast cell-deficient mouse mutants (WBB6_{F1}-W/W^V). As a consequence of this bone marrow transplant the mast cell-deficient mouse developed either mast cells that carried large granules as a phenotypic marker or normal mast cells. Further experiments using cells from colony-forming units injected into mouse skin demonstrated that mast cells originate from cells less differentiated than those precursors committed to either the neutrophil-macrophage or erythroid lineage [10].

Whereas most descendants of pluripotent stem cells, like erythrocytes, neutrophils, basophils, or eosinophils, do not leave the hematopoietic tissue until their differentiation is completed, mast cells invade the connective or the mucosal tissue as morphologically unidentifiable mast cell progenitors. There they differentiate into phenotypically identifiable mast cells [11–13]. In vitro studies have allowed the isolation of specific mast cell growth factors, which give rise to mast cell proliferation from their precursors. These include interleukin-3 (IL-3), IL-4, stem cell factor (SCF), IL-9, and IL-10 [14–16].

As opposed to the other mature hematopoietic cells, mast cells are still carrying a proliferative potential, although to date, mast cell mitoses have never been observed in tissue. The recovery of isolated human lung mast cells after IgE-induced degranulation during 48 h in vitro was observed, but no mitoses in these cells could be demonstrated [17]. Kuriu et al. [18] could show that, prior to proliferation, mast cells degranulate, losing their differentiated phenotype, only to differentiate afterward back into mast cells.

BIOLOGICAL SIGNIFICANCE

Mast cells can be found in large numbers wherever the body is in contact with its environment, e.g. the skin, the respiratory system, and the digestive tract. This selective accumulation at tissue sites where foreign material attempts to invade the host suggests that mast cells are among the first cells to initiate defense mechanisms. Their role as major effector cells in the pathogenesis of the immediate-

type hypersensitivity reaction such as bronchial asthma, allergic rhinitis, and urticaria is well established.

However, mast cells do not only play a major role in allergic diseases but also in a number of non-allergic immune reactions. An increased number of mast cells can be found in scar tissue, especially in keloids, in callus tissue, in osteoporosis-linked diseases, and in various neuropathies. Mast cell participation has also been reported in various types of inflammatory reactions, like interstitial pneumonia, ulcerative colitis, intestinal helminthosis, and cases of ectodermal parasitosis. In connection with pigment storage in fibrosis, as well as with infiltration in many tumors, the participation of mast cells can be observed. In such cases an increase in the number of mast cells as well as a mast cell degranulation, most likely complement-regulated, can occur [19]. Furthermore they are involved in such diverse skin diseases as psoriasis, atopic dermatitis, bullous pemphigoid, lichen planus, wound healing, and cancer [20–25]. Recently it has been shown that mast cells, which can be deadly in anaphylaxis, also play a significant role in a life-saving host response to bacterial reactions [26, 27]. Apart from the above-mentioned pathological conditions, mast cells also play a role in normal physiological processes. The mast cell mediators histamine and heparin have been shown to enhance vascularization and endothelial cell proliferation [28, 29]. Furthermore, the two-way communication between mast cells and sensory nerve cells may provide a homeostatic function such as the monitoring of cutaneous blood flow [30]. Finally, mast cell cytokines may influence other cells within the microenvironment [31].

The immunological activity of mast cells can, in principle, be explained by the release of mediators that follow their activation. In this context distinction must be made between mediators that are already stored within the granules, and mediators that are produced *de novo*, following an IgE-dependent activation process [32]. Among the stored mediators are the biogenic amines histamine and serotonin, as well as the proteoglycans heparin and chondroitinsulfate E, hydrolases, oxidative enzymes, chemotactic factors, and the proteinases tryptase, chymase, carboxypeptidase, and cathepsin G [33]. Newly generated lipid mediators synthesized on mast cell activation from membrane phospholipids include prostaglandin D₂ (PGD₂), leukotriene C₄ (LTC₄), platelet-activating factor, and to a lesser extent, LTB₄ [34].

The synthesis and storage mechanisms of the mast cell-produced cytokines are not yet completely clarified but it is well established that they have a Th-2-type cytokine-producing profile [35, 36]. Rodent mast cells have been shown to produce several cytokines, including IL-1, IL-3, IL-4, IL-5, IL-6, IL-8, interferon- γ (IFN- γ), transforming growth factor- β (TGF- β), tumor necrosis factor α (TNF- α), macrophage inflammatory protein 1 (MIP-1), and granulocyte-macrophage colony-stimulating factor (GM-CSF) [31]. Human mast cells have been reported to produce and store TNF- α [37], IL-4 [38, 39], IL-5 [39], and IL-6 [39], of which at least TNF- α and IL-4 can be rapidly released from preformed stores on degranulation [37, 38].

and with more prolonged kinetics from transcripts produced in response to appropriate activation of the cell [40, 41]. The consequences of cytokine release from mast cells remains to be determined in detail, although it can be anticipated that they play an important role in a number of physiological, pathological, and immunological processes including wound repair, tissue remodeling, pathological fibrosis, angiogenesis, blood clotting, host reactions to bacterial infections, certain neoplasms, hematopoiesis, and antibody production [26, 27, 42].

To analyze the reaction pattern of mast cells, it is necessary to distinguish between mast cell degranulation and mast cell hyperplasia. Hypersensitivity reactions of the immediate-type are characterized by mast cell degranulation. These are either IgE-mediated, complement-independent, as in the case of anaphylactic reactions, or alternatively, the degranulation is complement-dependent, as in immunocomplex-mediated hypersensitivity reactions. Mast cell hyperplasia without degranulation can be observed in immunoreactions of the delayed-type. This accumulation of mast cells is preceded by an aggregation of T-lymphocytes [43]. Mast cell-derived IL-1 [44] and TNF- α may contribute to this lymphocyte infiltration [31, 41].

Recent studies have demonstrated that mast cells respond differentially to certain activating agents once they have been primed by contact with other substances. In a study investigating the histamine and tryptase release of nasal polyps on immunological activation, it was observed that epithelial mast cells were relatively unresponsive to IgE-mediated activation, have a low mediator content, and showed signs of activation at the electronmicroscopic level. Thus it appeared that the epithelial mast cell was chronically activated, resulting in a form of anergy to further activation. This study has important implications for the evaluation of mediator release data obtained from mast cells in the context of chronic disease [45]. A clinical study has suggested that nasal polyp patients may be less responsive to allergen exposure *in vivo* [46]. In contrast to studies suggesting down-regulation of mast cell function in chronic inflammation, some groups suggest that inflammatory mediators and cytokines can enhance mast cell function. The overall effect of these processes depends to a large extent on the chronicity of the challenge. The ability of substances produced during the inflammatory process to alter later mast cell responses may have important implications for the chronicity of the disease process [47].

HETEROGENEITY

Early workers like Maximow in 1906 [48] already suggested that mast cells represent a heterogeneous cell population because mast cells in the intestinal tract of several species were smaller and less granulated than those elsewhere. The pioneering work of Enerbäck [49, 50] on the distribution and staining properties of mast cells in the gastrointestinal tract of the rat showed that they require special fixation techniques for an adequate demonstration by thi-

azine or copper phthalocyanine dyes and stimulated studies on properties and heterogeneity of mast cells in rodents and humans.

By now it is apparent that mast cells exist *in vivo* and *in vitro* as a heterogeneous family of effector cells. Since the recognition of mast cell heterogeneity in rodent, and more recently in humans, there has been an increasing awareness of the potential roles of mast cells' granule serine proteases and exopeptidases in IgE-mediated immune responses.

In addition to their identification by histochemical or biochemical criteria, mast cell subpopulations may also be distinguished by their functional activity. Lack of appreciation of this point undoubtedly led to much confusion in earlier literature. Rodent and human mast cells exhibit functional heterogeneity in response to a variety of secretagogues and various drugs [2], a finding of potential clinical importance that may lead to the development of more efficacious drugs influencing only defined subpopulations of mast cells. A morphological equivalent to the functional heterogeneity has been demonstrated by electronmicroscopic studies. Mast cell degranulation after IgE-mediated reactions are characterized by a very rapid swelling and fusion of mast cell granules and an abrupt mediator release. In certain chronic disease states (e.g., bullous pemphigoid), there is piecemeal degranulation with a more gradual mediator release, indicating that these different patterns could reflect granule heterogeneity with attendant different responses to IgE-mediated stimuli [51].

Like the functional significance of mast cell heterogeneity, their origins have been discussed for a long time. Whereas Caughey et al. [52] have supposed that the various mast cell subtypes represent different states of a single cell line, Irani et al. [53] as well as Czarnetzki et al. [54] have preferred the concept that they originate from different precursor cells. Kitamura and co-workers have favored the theory that the development of various phenotypes is dependent on the anatomical microenvironment where the final differentiation takes place. Their view is supported by combined *in vitro* and *in vivo* studies which demonstrated that mucosal-type mast cells (MMC) can differentiate into the connective tissue type (CTMC) and vice versa [55, 56], suggesting that the mast cell phenotype is determined by the surrounding tissue. *In vitro* systems provided a powerful tool for the investigation of the basic mechanisms for mast cell development and differentiation and helped to demonstrate that mast cell heterogeneity can be traced back to certain cytokine patterns that are present in different microenvironments [13, 57–60]. The results of transfer experiments in mice suggest that mast cell subsets can change in phenotype from MMC to CTMC or from CTMC to MMC, through the influence of factors within the microenvironment [56, 61], including IL-3 [62] and SCF [63].

Murine mast cells cultured in the presence of IL-3 acquire the mucosal-type phenotype, whereas those cultured in the presence of SCF or fibroblasts with or without exogenous IL-3 resemble CTMCs [64]. The growth factors required for human mast cell differentiation are somewhat different than those for rodents. Thus, although IL-3 pro-

notes the proliferation of bone marrow-derived MMC and acts as growth factor for human basophils, it has no apparent effect on human mast cells [65, 67]. Consistent with these results is the presence of IL-3 receptors on the surface of human basophils but not on human mast cells [68]. Similarly, IL-4 acts as a mast cell growth factor in the rodent [69] but lacks substantial activity on the growth of human mast cells. On the other hand, SCF has been demonstrated to promote the development of tryptase-containing human mast cells [70]. However, using a co-culture system with murine 3T3 fibroblasts, the main source for SCF, and cord blood mononuclear cells, it could be demonstrated that after 8 weeks of culture more than 90% of the cells appeared to be mature tryptase- and chymase-containing mast cells. These experiments strongly suggest that factors other than SCF are present in the fibroblast-conditioned medium [70–72] and are required for the development of mast cells containing both neutral proteases.

MAST CELL SUBTYPES IN RODENTS

The use of modified fixation and the alcian blue/safranin histochemical staining sequence helps to distinguish rat mast cell subpopulations. Although the distinction by using these methods is not absolute, it remains the most widely employed criterion for defining rodent mast cell phenotypes [49, 50, 73, 74]. Due to different fixative and staining properties, two mast cell subtypes in rodents have been distinguished. First, there are the atypical, T cell-dependent MMC, which are mainly found in the mucosa of the gastrointestinal tract and in the lamina propria of the respiratory tract. The T cell-dependent proliferation of MMC has been shown to be mediated by IL-3, -4, -9, and -10 [75]. Second, there are T cell-independent CTMC that occur mostly in the submucosa of the gastrointestinal tract, in the skin, and in the peritoneum. Fibroblast-derived factors,

e.g. SCF, appear to mediate the development of this subtype [75].

In addition to their T cell-dependent and -independent proliferation, respectively, the two mast cell subtypes can be distinguished by size and proteoglycan and histamine content. MMC granules consist of a relatively soluble matrix containing chondroitinsulfate and little histamine. Their anionic site is blocked by aldehyde and therefore special conditions of fixation and staining are required to reveal this cell type. After fixation in some common formalin-based fixatives the granules of the MMC may become resistant to metachromatic staining [76]. Fixatives that rapidly penetrate the cell and strongly acid staining conditions are ideal for this cell type. CTMC contain heparin and large amounts of histamine, which imparts the metachromatic staining characteristics of mast cells when imposed to basic dyes such as toluidine blue. The most important properties of rat mast cells are summarized in Table 1.

A further distinctive criterion for the subpopulations of rodent mast cells is the expression of different mast-cell-specific serine proteases. Mast cell proteases are enzymatically active at neutral pH and often represent more than 50% of the total protein content of mature mast cells in mouse [74, 77–83] and rat [84–87]. Mast cell proteases are stored in the secretory granules in an enzymatically active state. Although the concentration of the proteases in the granules is extremely high, they are not rapidly catabolized because they are bound to serglycin proteoglycans. The importance of protease packaging is underscored by the fact that the serglycin gene is expressed before any of the mast cell proteases [88, 89].

Rat mast cells contain rat mast cell protease (RMCP), whereas in the mouse they contain mouse mast cell protease (MMCP), which can be distinguished biochemically. However, Befus et al. [90] could demonstrate clearly that rat peritoneal but not intestinal MMC express MMCP-5 and carboxypeptidase A as well. But these proteinases

TABLE 1. Characteristics of Rat MMC and CTMC^a

Characteristic	MMC	CTMC
Staining with alcian blue/safranin sequence	blue	red or blue and red
Fixation	critical, aldehyde blocking	no aldehyde blocking
Appearance	small, variable shape, variable-size granules	larger, uniform shape, uniform-size granules
Behavior	migratory	nonmigratory
Dependent on	T cells, fibroblasts	fibroblasts
Lifespan	short: half life <40 days	long: half life >6 months
Histamine content	low: <2 pg/cell	high: <35 pg/cell
Proteoglycans		
Heparin	–	+
Chondroitinsulfate di-B	+	–
Chondroitinsulfate A	+	–
Chondroitinsulfate E	+	+

^aThis table was modified from Enerbäck [174] and Huntley [3].

TABLE 2. Predominating Granule Mediators of Rodent Mast Cells^a

Mast cell type	Biogenic amine	Neutral protease	Proteoglycan
Mouse			
Mucosal	histamine	MMCP-1, -2	chondroitinsulfate E
Connective tissue	histamine	MMCP-3, -4, -5, -6, -7	heparin
	serotonin	carboxypeptidase	
Rat			
Mucosal	histamine	RMCP-2, MMCP-5, carboxypeptidase ^b	chondroitinsulfates di-B, A, E
Connective tissue	histamine	RMCP-1	heparin
	serotonin	carboxypeptidase	chondroitinsulfate E

^aThis table was modified from Schwartz [133].^bOnly in peritoneal MMC.

appear to be restricted in their distribution to selected MMC populations.

The relative molecular mass of these proteases is in the range of 27,000–32,000 *M_r*. According to their cleavage specificity, mast cell serine proteases have been classified as chymases or tryptases. Chymases have a chymotrypsin-like cleavage specificity and are stored in their active form in the granules as basically charged monomers. All serine proteases that have so far been found in rats and the great majority of those found in mice are chymases. Members of the mouse chymase family include MMCP-1 [91], MMCP-2 [92], MMCP-4A [93], MMCP-4B [91], MMCP-L [93], and MMCP-5 [91, 94]. The genes for mouse mast cell chymases are clustered together with genes for the cytotoxic T cell granzymes and T cell receptor A/D gene locus on chromosome 14 [92].

Tryptases have a trypsin-like cleavage specificity and have been characterized in mouse, dog, and human. Tryptases have a net negative charge and the catalytically active form present in mast cell granules is tetrameric [95]. The two mast cell tryptases that have been characterized in mice are MMCP-6 [96–98] and MMCP-7 [80]. The predominating granule mediators of mouse and rat mast cells are summarized in Table 2.

MAST CELL SUBTYPES IN HUMANS

In humans the strict classification into mucosal- and connective tissue-type mast cells is not possible. Unlike in rodent mast cells, histochemical heterogeneity, based on the presence or absence of heparin proteoglycan, results in formalin resistance and hence does not appear to be a useful marker for distinguishing different subtypes of human mast cells. An ultrastructural analysis using antithrombin III gold labeling revealed that all human mast cells contain heparin [99]. Nevertheless, to a minor extent it is also possible in humans to define mast cell subtypes according to different staining and fixation properties [100]. As in rodents the dominant protein components of human mast cell secretory granules are neutral proteinases, enzymes that catalyze the cleavage of peptide bonds and perform best

near neutral pH [101]. Since the extensive investigations of Irani et al. [102], the content of the mast cell-specific proteases chymase and tryptase as the main criterion of differentiation for mast cells in humans is established, and mast cells containing only tryptase (MC_T) can be distinguished from mast cells containing both tryptase and chymase (MC_{TC}).

Tryptase was purified to homogeneity from dispersed human lung mast cell preparations [103]. In Carnoy's fixed sections of human lung, skin, and small intestine, sequential double labeling with alcian blue followed by immunohistochemistry with a murine monoclonal antitryptase antibody showed a one-to-one correlation between cells stained metachromatically with alcian blue and cells reacting with the antitryptase antibody [104]. These results indicate that tryptase is present in all mast cells identified by classic histochemistry where metachromasia occurs with acid dyes. Furthermore, because only mast cells stained with antitryptase antibody, tryptase was shown to be a selective marker for human mast cells [105]. Tryptase immunoreactivity in formalin-fixed tissue is diminished compared with Carnoy's fluid-fixed tissue, leading to an apparent decrease in total numbers of mast cells in lung and small intestine, but not in skin [24].

Chymase was purified from human skin and localized to human skin mast cells by immunofluorescence using a polyclonal rabbit antichymase antibody [106, 107]. Chymase immunoreactivity is lost in formalin-fixed tissues.

Human mast cell carboxypeptidase (HMC-CP) was detected in lysates of unstimulated human lung and skin mast cells [108]. Using a simultaneous double-labeling technique, a one-to-one correlation was demonstrated between chymase-positive mast cells and mast cells containing HMC-CP [109].

The presence of a cathepsin G-like protease was demonstrated in MC_{TC} but not in MC_T mast cells by immunohistochemistry and by enzymatic activity. Because cathepsin G-like protease is also present in neutrophils and monocytes it is not specific for mast cells [110].

Elastase is a neutral protease found in neutrophil leukocytes, monocytes, and macrophages [111] and the detection of elastase in dispersed human lung mast cells by immunoassay and enzymatic assay was reported [112]. In contrast, immunohistochemical studies using a polyclonal rabbit anti-elastase antibody demonstrated the absence of elastase from all mast cells in human skin, lung, and small intestine [113]. Based on these contradictory reports the presence of mast cell elastase still has to be confirmed.

Immunoelectronmicroscopic studies revealed that MC_T and MC_{TC} can also be differentiated ultrastructurally. MC_{TC} have secretory granules with grating and lattice substructures, whereas MC_T have granules containing discrete scrolls [114], which also have occasionally been detected in the peripheral regions of MC_{TC} granules [115]. Bradding et al. [116] could show that, in addition to exhibiting heterogeneity with respect to neutral protease content and morphology of the secretory granules, human mast cells are also heterogeneous with respect to cytokine content. Al-

TABLE 3. Characteristics of Human MC_{TC} and MC_T Cells^a

Characteristic	MC _{TC} (pg/cell)	MC _T (pg/cell)
Protease	tryptase (35) chymase (4.5) carboxypeptidase (10) cathepsin G (+)	tryptase (10)
Histamine	1.9	1.5
Proteoglycans	heparin chondroitinsulfates A, E	heparin chondroitinsulfates A, E
Granule morphology	grating/lattice complete scroll-poor	complete scroll-rich
T lymphocyte-dependence	+	—
Cytokine content	IL-4	IL-4, IL-5, IL-6

^aData collected from Irani and Schwartz [175], Schwartz [176], and Bradding [116].

+, Detectable but not quantified.

though IL-4 was distributed among both mast cell phenotypes, it was expressed preferentially by the MC_{TC} subset (overall: 85% MC_{TC}, 15% MC_T). In contrast, IL-5 and IL-6 were restricted almost exclusively to the MC_T subset. This suggests that the different biological functions of mast cell subsets differ also as a result of their capacity to generate and release different cytokine profiles. The main characteristics of human mast cell types are shown in Table 3.

In mast cells that contain both neutral proteases, the enzymes can be found in the same cytoplasmic granules. Independent confirmation of these results was provided using the synthetic substrates Suc-Ala-Ala-Phe-MNA and *N*-acetyl-L-methionine- α -naphthyl ester to demonstrate the presence of chymase in mast cells by enzyme histochemistry [117]. Contradicting these previous reports, Weidner and Austen discovered in 1993 a third mast cell type at several body sites using immunofluorescence staining with monoclonal antibodies. This third type contains only chymase and carboxypeptidase (MC_C) but no tryptase. The third suggested mast cell type, the MC_C, can be found in the submucosa of the gastrointestinal tract and in lymph nodes. However, since many investigators have failed to detect the MC_C type [114, 118, 119], its presence in human mast cells awaits confirmation. The tissue distribution of the mast cell subtypes according to their tryptase and chymase content, including the results of Weidner and Austen, is depicted in Table 4. In contrast to most reports, Aldenborg and Enerbäck [120] found, using monoclonal antibodies, the MC_{TC} to be the predominant mast cell type in both mucosa and submucosa of normal small and large intestine. Mast cells that contain tryptase alone are preferentially located in the mucosa. Colonic but not small intestinal mucosa displayed great individual variations in their numbers of MC_T. Matin et al. [121] found that the percentage of chymase-containing mast cells differs strikingly between bronchial tissue compartments, although the absolute number of chymase-containing mast cells was similar in all compartments.

Recent reports provide strong evidence of human mast cell heterogeneity with respect to their functional characteristics. Ninety percent of the human cardiac mast cells are

MC_{TC} primarily located in the appendage of the atrium. This mast cell type exhibits surface membrane antigen and functional properties similar to those of lung and uterine mast cells [122]. Patella et al. [123] investigated the immunological and biochemical characteristics of partially purified human heart mast cell (HHMC) and compared them with mast cells isolated from lung and skin tissues. Cross-linking of the high-affinity receptor for IgE (Fc ϵ RI) by a polyclonal anti-Fc ϵ antibody caused the release of preformed (histamine and tryptase) and de novo synthesized mediators (LTC₄ and PGD₂). The tryptase content of HHMC was lower than in skin mast cells and higher than in lung mast cells. Maximal stimulation of HHMC with anti-IgE led to the release of LTC₄ and PGD₂, whereas skin mast cells synthesized more PGD₂ and much less LTC₄. Recombi-

TABLE 4. Tryptase (T) and Chymase (C) Reactivity of Normal Human Mast Cells at Multiple Body Sites^a

Body site	% MC-T	% MC-TC	% MC-C
Lung			
Alveoli	91	8	1
Bronchi	78	10	12
Bronchial epithelium	100	0	—
Bronchial	75	25	—
Subepithelium			
Axillary lymph nodes	1	97	2
Breast parenchyma	1	99	0
Stomach			
Mucosa	52	39	9
Submucosa	0	73	27
Small intestine			
Mucosa	65/81	31/19	4
Submucosa	0/23	76/77	24/—
Colon			
Mucosa	53	37	10
Submucosa	0	96	4
Nasal mucosa	66	34	—
Conjunctiva	5	95	—

^aData from Weidner and Austen [177] and Irani and Schwartz [175]. Data are expressed as mean. —, No data are given; /, different values given by the authors.

nant human C5a anaphylatoxin and protamine induced histamine release from HHMC and skin mast cells, but not from lung mast cells (HLMC). Substance P and morphine selectively induced the release of histamine from human skin mast cells, but not from HHMC and HLMC. Compound 48/80 caused histamine release from HSMC and HHMC, but not from HLMC.

Other authors investigating mast cell heterogeneity defined, according to their immunohistochemical, functional, and structural investigations, that human uterine mast cells differ from lung and skin mast cells [124, 125]. Biochemical analysis revealed that the human uterine mast cell, unlike the skin mast cell, does not respond to substance P and does make significant quantities of LTC₄ in response to anti-IgE. After stimulation it responds to similar secretagogues and releases similar arachidonic acid metabolites than the lung mast cell. Ultrastructural analysis demonstrated that uterine mast cells share morphological features common to both skin and lung mast cells [124]. These findings are supported by Tainsh et al. [125], who found that the human uterine mast cells are neither histochemically nor functionally similar to those from skin but instead show significant similarity to the human lung mast cell. Uterine mast cells are formalin-sensitive and have a strong response after stimulation with anti-IgE. Furthermore, uterine mast cells exhibit functional heterogeneity with respect to their response to antiallergic drugs in comparison with skin and lung mast cells.

Guo et al. [126] found that human uterine mast cells express various surface antigens consistent with mast cells from other tissues. But unlike other mast cells, uterine mast cells expressed CD11c/CD18 and CD32.

TRYPTASE

The large quantities of tryptase and chymase that are synthesized by mast cells suggest and emphasize the significance of these proteinases in mast cell function [127]. This brings up the question of the biological properties of these mast cell-specific proteases.

Tryptase is the major protein constituent of human mast cells, where it is stored almost exclusively within the secretory granules as a fully active tetramer. Two tryptase genes (alpha and beta) are expressed by human mast cells at the level of mRNA and protein, each with a 30-amino-acid leader sequence. Alpha-tryptase is not stored in secretory granules, but instead is constitutively secreted by mast cells and is the predominant form of tryptase found in the blood of both healthy subjects and those with systemic mastocytosis under non-acute conditions. Conversion of the catalytic portion of the inactive monomeric form of beta-tryptase to the active homotetramer requires an acid pH and heparin. Thus, processing of tryptase to an active form should occur optimally only in cells that coexpress heparin proteoglycan, restricting this pathway to a mast cell lineage [128]. Once dissociated from heparin, the enzyme undergoes pro-

found conformational changes from a tetramer to a monomer and irreversibly loses activity [129]. The initial phase of this conversion appears to exhibit limited reversibility [106]. Tryptase is stored with chymase, carboxypeptidase, and a cathepsin G-like proteinase in the same granules, but differences in their subgranular distribution have been observed. Tryptase is bound to proteoglycan complexes that are smaller than those binding chymase and carboxypeptidase [130]. Electronmicroscopically, tryptase is localized in the electron-lucent areas of the granules, whereas chymase and a cathepsin G-like proteinase reside in the electron dense regions [131]. In vitro studies confirmed the presence of tryptase in the granules before the mast cells can be stained metachromatically [132]. In human mast cells histamine appears to regulate the stability of tryptase [133].

Studies in humans have attempted to document protease release in situ and to correlate the presence or activity of proteases with disease states. Until now, however, little definitive in vivo data exist that document specific protease-mediated mechanisms in disease. Several activities of potential biological interest have been examined in vitro, promising interesting in vivo results in the close future. Investigations attempting to identify protein and peptide substrates in vitro that are hydrolyzed by tryptase have shown that tryptase has a very restricted substrate specificity for proteins, leaving most uncleaved. The biological properties of tryptase are only restricted by the action of specific biological inhibitors for serine proteases [78].

Little and Johnson [134] have demonstrated that human mast cell tryptase exists in two different isoforms. Using high-molecular-mass kininogen (HMK) and vasoactive intestinal peptide (VIP) as substrates, these two forms of tryptase were found to differ with regard to specificity and rate of cleavage. So far, it could not be clarified whether these isoforms are products of the above-mentioned alpha and beta tryptase gene or the result of posttranslational modification.

The above-described degradation of VIP, HMK, and in addition peptide-histidine methionine (PHM) and calcitonin gene-related peptide (CGRP), by tryptase might be the reason for the observed increased contractility of bronchial muscle after stimulation with tryptase [135]. These neuropeptides are known to relax smooth muscle and their destruction may result in increased contractility [136, 137]. A further action of mast cell tryptase on bronchial epithelial cells is the stimulation of DNA synthesis and therefore can be regarded as a potent mitogen.

In epithelial cells tryptase has been shown to stimulate a catalytic site-dependent release of IL-8, which was also associated with up-regulation of intercellular adhesion molecule-1 (ICAM-1) expression. Thus tryptase may play a critical role in epithelial repair and in the recruitment of granulocytes following mast cell activation [138]. This is supported by granulocyte recruitment occurring after injection of tryptase into guinea pig skin or mouse peritoneum [139].

In the epidermis tryptase releases and cleaves fibronectin [140] as well as collagenase type IV and thus may be involved in tissue remodeling and matrix degradation [141].

Metalloproteinases (MMP) are purported to degrade almost all connective tissue elements and are secreted by cells in the form of inactive precursors. So far, the mechanisms of MMP activation *in vivo* are poorly understood. *In vitro* it has been shown that tryptase activates MMP III and thus, indirectly, procollagenase [142]. This suggests that tryptase could contribute to local MMP activation and subsequent matrix degradation [143].

In blood coagulation, tryptase seems to play a role by way of fibrinogen cleavage and inactivation [144]. The observed increased permeability of blood vessels and the stimulation of leukocyte emigration [145] may be a result of the inhibition of thrombin's mitogenic effects on vascular smooth muscle cells (VSMC) by tryptase. In this context it has also been shown that this is not mediated by the destruction of thrombin itself. Furthermore, tryptase does not alter the mitogenic response of VSMC to purified growth factors such as platelet-derived-growth-factor, suggesting that tryptase inhibits thrombin-induced DNA synthesis without interfering with intracellular mitogenic signaling pathways activated by thrombin or other growth factors [146]. These findings contrast the stimulatory effect of tryptase on fibroblasts, in which synergy is observed with thrombin, with thrombin-receptor peptide, and with other growth factors. These data provide *in vitro* evidence that tryptase is a multifunctional growth factor with cell-specific actions. The main biological activities of tryptase are summarized in Table 5.

An earlier report of the participation in the complement

system, by forming C3a from C3 [147], could not be reproduced with immunoaffinity-purified tryptase [148].

Although the biological and pathobiological role of tryptase *in vivo* still needs to be clarified, these properties suggest an important participation of tryptase in proinflammatory mast cell function. Because basophils and mast cells share several mediators, e.g., histamine, LTC₄, and PGD₂, the assessment of mast cell involvement in clinical events has delayed the progress of research investigations for a number of years. Because mast cell tryptase is selectively localized in the secretory granules, it is a useful discriminating marker for the participation of mast cells in disease and a specific immunoassay was developed to quantify total amounts of tryptase in complex biological fluids [149]. With this assay, tryptase levels in normal serum (< 1 ng/mL) are undetectable, whereas elevated levels after mast cell activation can be detected [150].

CHYMASE

Chymase is also a serine endoprotease with a specificity for chymotrypsin-like substrates and various isoforms and for which interspecies variations exist. Although not all of the chymases have been characterized chemically and catalytically, they demonstrate wide variations in their properties as charge, solubility, proteoglycan binding, glycosylation, catalytic efficiency, and regulated expression in mast cell subsets. These differences predict that different chymases play different roles, a speculation at this point supported almost exclusively by data gathered *in vitro*. Presently there is neither an adequate functional explanation of this dissimilarity among chymases nor an explanation of the multiplicity of chymases in some individual species. However, in humans the situation may be simpler than in rodents, since current genetic and enzymological data are most consistent with the existence of just one major chymase [152]. The expression of human chymase is, as already mentioned, confined to a subset of mast cells [102, 107, 110] and perhaps, to certain vascular endothelial cells [153, 154]. Nevertheless, regarding chymase variety in other species, it is possible that additional human mast cell chymases are awaiting discovery. The closest known relatives of human chymase are dog chymase and mouse chymase 5 [94, 152, 155]. Surprisingly, neither of the two rat chymases are closely related to human chymase with respect to their amino acid sequence. This suggests that caution should be applied in using the results of experiments involving rat chymases to predict the role of chymase in humans [151].

The importance of granule proteoglycans in the biogenesis of chymases is not clear. Mast cell chymases vary greatly in surface charge and in their binding to negatively charged heparin- and chondroitinsulfate-containing proteoglycans. Several of these enzymes, especially canine and rat chymase 1, bind strongly to heparin proteoglycan [87, 156, 157]. However, the ability to express active chymase

TABLE 5. Biological Activities of Tryptase

Activity	Significance
Cleaves:	increase in bronchial muscle contractility
High-molecular-mass kininogen (HMMK)	
Vasoactive intestinal peptide (VIP)	
Peptide-histidine methionine (PHM)	
Calcitonin gene related peptide (CGRP)	
Stimulation of DNA synthesis on bronchial epithelial cells	mitogen for bronchial epithelial cells
Stimulates IL-8 release from epithelial cells, associated with up-regulation of ICAM-1	recruitment of granulocytes, epithelial repair
Activates: metalloproteinase III (MMP-3), indirectly procollagenase (pro-MMP-1); release and cleavage: fibronectin and collagenase type IV	tissue remodeling and matrix degradation
Cleaves: fibrinogen α -chains	local anticoagulant
Inhibition of thrombin mitogenic effect on vascular smooth muscles	increase of vessel permeability
Mitogen and co-mitogen for fibroblasts	role in tissue fibroses, wound healing

in COS cells [154], which probably contain little heparin, suggest that proteoglycans may not be essential for the generation of active enzyme.

Human chymase has been shown to convert angiotensin I to angiotensin II independent of angiotensin-converting enzyme in vivo and in vitro [158], thus playing a role in blood pressure regulation [159, 160]. It has been shown that in some situations chymase is at least as important as angiotensin-converting enzyme in activating angiotensin I [161]. But as mentioned before, chymase of various species exhibit different substrate specificities and generation of angiotensin II is therefore not a characteristic of chymases from all species [150].

Furthermore, chymase-like tryptase is capable of cleaving VIP [52, 162], resulting in increased bronchial muscle contractility. On the other hand chymase can hydrolytically inactivate bronchoactive peptides such as bradykinin and kallidin [163] as well as certain neuropeptides, such as Substance P and VIP [162, 164]. This enables chymase, unlike tryptase, to inactivate certain bronchoconstrictors, which possibly limits mast cell degranulation. Chymase strips components of the glycocalyx from the surface of cultured airway epithelial cells [165] and is a highly potent secretagogue for cultured airway submucosal gland serous cells [166].

In the skin, chymase is suspected to play a role in modulating inflammation. Chymase appears to be an important determinant of the duration of CGRP-induced vasodilatation after release from sensory nerves [167]. In addition it enhances histamine-induced cutaneous wheal formation in vivo [168]. IL-1 β precursor has been shown to be activated by human chymase [169]. Incubation of human skin strips with human skin chymase results in the loss of anchoring filaments and widening of the lamina lucida at the dermo-epidermal junction, followed by separation of the epidermis from the dermis at the lamina lucida [170]. Chymase has also been proposed, but not yet demonstrated, to cause the blistering seen occasionally in children with cutaneous mastocytosis [171].

Mast cell chymase and leukocyte elastase efficiently release but do not activate matrix-bound latent TGF- β 1 complexes. The release of TGF- β 1 from the matrix by leukocyte and mast cell enzymes may contribute to tissue inflammation [172].

Mast cell chymase was, like tryptase, shown to process and activate purified MMP-1 and MMP-3, contributing to local MMP activation and subsequent matrix degradation [143]. It was demonstrated that the activation of MMP-1 is time- and concentration-dependent. Heparin was found to increase markedly the rate at which chymase activates MMP-1, both by accelerating the cleavage of MMP-3 and also by preventing its further degradation. Furthermore, chymase activates MMP-1 directly by cleaving the Leu 83-Thr 84 bond, without formation of any intermediate species [173]. The main biological activities of chymase are summarized in Table 6. Comparing the biological activities of the major mast cell proteases, it becomes clear that

TABLE 6. Biological Activities of Chymase

Activity	Significance
Converts angiotensin I to II	blood pressure regulation
Cleaves: vasoactive intestinal peptide	increase in bronchial muscle contractility
Cleaves: bradykinin and kallidin substance P	inactivation of bronchoconstrictors; limits substance P induced mast cell degranulation
Stimulates secretion of mucopolysaccharides; strips components of glycocalyx in bronchial epithelium	secretagogue
Cleaves CGRP in skin	limits vasodilatation
Enhances histamine induced wheal formation; activates IL-1 β precursors; loss of anchoring filaments in the epidermis; releases matrix-bound TGF- β 1	tissue inflammation
Activates metalloproteinase III (MMP-3), indirectly procollagenase (pro-MMP-1); release and cleavage of fibronectin and collagenase type IV	tissue remodeling and matrix degradation

they share some activities. On the other hand chymase seems to be more involved in the inflammatory reaction.

CONCLUSIONS AND FUTURE DIRECTIONS

In conclusion, mast cells can no longer be regarded as cells that initiate acute allergic reactions through the release of rapidly metabolized mediators such as histamine and products of arachidonic acid oxidation. The past few years have brought an increased awareness of the potential range of mast cell functions and interactions with other parts of the immune response. By now it is well established that they can clearly orchestrate the infiltration of leukocytes into sites of mast cell activation, an effect that develops over a period of several hours.

The recognition of various mast cell types with distinct protease compositions is a crucial step toward better understanding of mast cell biology. However, the major difficulty with current mast cell nomenclature is that it fails to recognize the full extent of mast cell heterogeneity and essentially does no more than to divide these cells into extreme types. Research over the last decade has revealed that mast cell proteases have the potential for modulating humoral and cellular events in tissue, including regulation of vascular and airway tone, vasopermeability, inflammatory cell response, secretion, growth regulation, neuropeptide modulation, coagulation, tissue destruction, intercellular protein and peptide processing, and hydrolysis of inflammatory mediators. Understanding the role of these proteases in vivo will help determine how mast cells modify tissue in several disease states. Immunohistochemical technique

provides a tool for precisely evaluating the distribution of mast cell subtypes in tissue during both health and disease. The distribution can be markedly affected by pathological conditions.

So far there are still no conclusive data on the relative contribution of mast cells to inflammatory disease compared with other cell types. Careful studies of clinical material combined with new technical abilities should help to address this issue. It is also important to consider mast cell function both in the resting state and in the context of the stimuli encountered during chronic disease, such as priming agents and chronic activation. Increasing knowledge of mast cell development and function in disease should lead to the development of strategies to modulate mast cell behavior more effectively so as to benefit of patients with a variety of acute and chronic diseases.

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