Mast cell secretory granules: armed for battle

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Abstract | Mast cells are important effector cells of the immune system and recent studies show that they have immunomodulatory roles in diverse processes in both health and disease. Mast cells are distinguished by their high content of electron-dense secretory granules, which are filled with large amounts of preformed and pre-activated immunomodulatory compounds. When appropriately activated, mast cells undergo degranulation, a process by which these preformed granule compounds are rapidly released into the surroundings. In many cases, the effects that mast cells have on an immune response are closely associated with the biological actions of the granule compounds that they release, as exemplified by the recent studies showing that mast cell granule proteases account for many of the protective and detrimental effects of mast cells in various inflammatory settings. In this Review, we discuss the current knowledge of mast cell secretory granules.

Tryptases
Serine proteases that have trypsin-like cleavage specificities — that is, they cleave peptide bonds on the carboxy-terminal side of arginine or lysine residues.

Chymases
Serine proteases that have chymotrypsin-like cleavage specificities — that is, they cleave peptide bonds on the carboxy-terminal side of aromatic amino acid residues.

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Mast cells are haematopoietic cells that arise from pluripotent precursors of the bone marrow[1–3]. After egression from the bone marrow, mast cell progenitors circulate in the blood before they enter various tissues and develop into mature mast cells under the influence of local growth factors, in particular stem cell factor (SCF; also known as KIT ligand) and interleukin-3 (IL-3)[2]. Mature mast cells are found in most tissues of the body and they are typically most abundant at sites that are close to host–environment interfaces, such as the skin and various mucosal tissues. Due to this anatomical location, mast cells are ideally situated to act during the first line of defence against external pathogens and other environmental insults[4,5].

The most distinguishing morphological feature of mast cells is their high content of electron-dense lysosome-like secretory granules (also known as secretory lysosomes) that occupy a major proportion of the cytoplasm of mature mast cells (FIG. 1). In fact, the presence of these secretory granules formed the basis for the discovery of mast cells in the late nineteenth century by the German scientist Paul Ehrlich, who observed connective tissue cells that appeared 'well fed' (the German word for which is *mastung*), which referred to the presence of filled secretory granules. Since then, the main criterion for the identification of mast cells is the presence of secretory granules, which are easily visualized with the use of various cationic dyes that produce the classical metachromatic staining of mast cells (FIG. 1a).

The secretory granules of mast cells are filled with a large panel of preformed compounds (TABLE 1). When mast cells are activated to degranulate, these compounds are released into the extracellular environment and can have a marked effect on any physiological or pathophysiological event. Mast cell degranulation can occur in response to various external stimuli — including, most notably, IgE receptor crosslinking — but they also degranulate in response to complement activation, neuropeptides and certain toxins[1,3]. However, it is important to note that, in addition to inducing the release of preformed granule constituents, mast cell activation leads to the *de novo* synthesis of many bioactive compounds, including lipid mediators — such as leukotriene C4, prostaglandin D2 and platelet-activating factor — as well as many cytokines and chemokines[1,3]. Of note, mast cell activation does not necessarily lead to degranulation. For example, exposure of mast cells to lipopolysaccharide can cause the release of cytokines without observable degranulation[3].

Traditionally, mast cells are best known for their detrimental impact on allergic reactions, with the most serious manifestation being anaphylaxis. However, mast cells have also been implicated in damaging responses in a large variety of additional disorders, including atherosclerosis, contact dermatitis, cancer and arthritis. Importantly though, mast cells also have numerous functions that are beneficial to the host, in particular, in bacterial infection, protection against envenomation and

allograft tolerance. Much of this knowledge is derived from studies on various mast cell-deficient animals such as the WBB6F1-*Kit*$^{W/W-v}$ and C57BL/6-*Kit*$^{W-sh/W-sh}$ ('sash') mice that have mutations in KIT (also known as SCFR), which is the cell surface receptor for the mast cell growth factor SCF[6]. More recently, additional mouse models have emerged in which mast cell deficiency is independent of alterations in KIT signalling[7,8]. Owing to the development of this new generation of mast cell-deficient mice, some of the proposed functions of mast cells have been questioned, whereas others have been confirmed[9,10].

Many of the physiological and pathophysiological functions of mast cells are closely associated with the biological actions of the preformed granule compounds. This notion is supported by a wealth of recent experimental evidence showing that the lack of specific granule compounds has a major impact on various immune conditions. So, the secretory granules of mast cells are emerging as important components in the regulation of numerous immune functions. Here, we review our current knowledge of the composition, biogenesis and maturation of mast cell granules, and our understanding of the biological functions of granule-derived compounds.

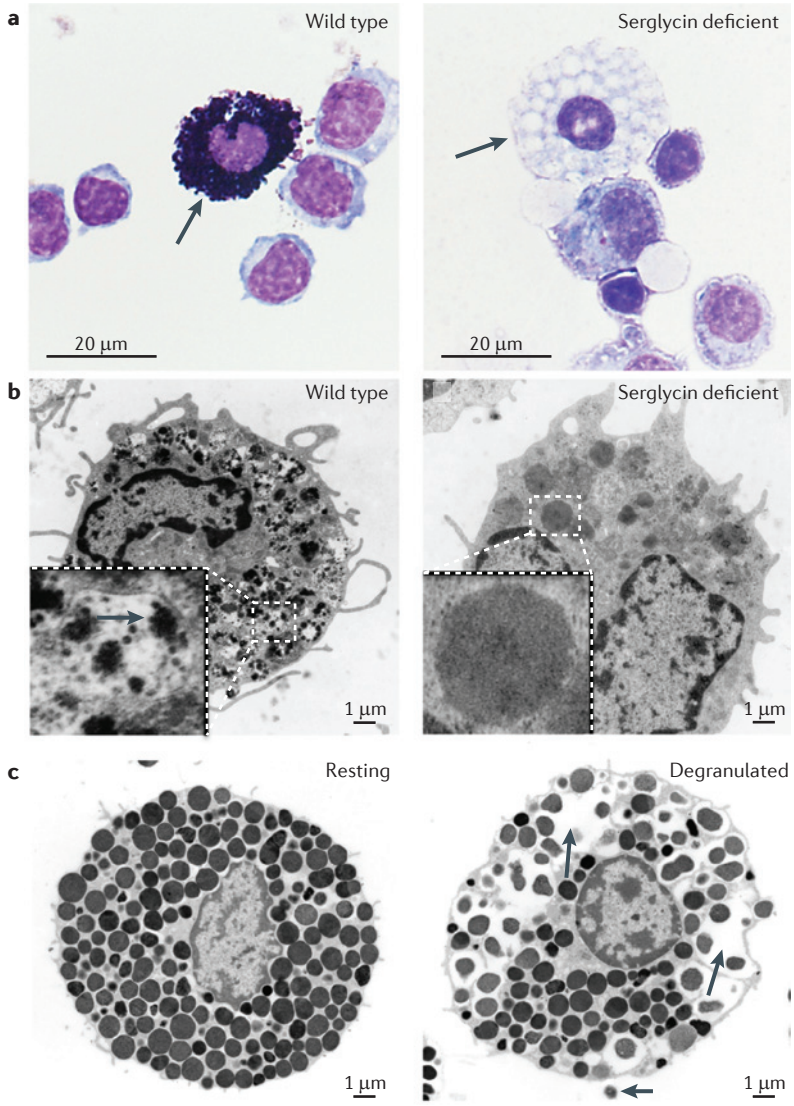


Figure 1 | **Mast cell granule morphology.** **a** | May–Grünwald Giemsa staining of wild-type and serglycin-deficient peritoneal cells (S.W. and G.P., unpublished observations). Metachromatic staining is strong in wild-type mast cells (indicated by the arrow; left panel) but is abolished in serglycin-deficient mast cells (indicated by the arrow; right panel). The morphology of cells other than mast cells is unaffected by the absence of serglycin. **b** | Transmission electron microscopy image of wild-type and serglycin-deficient bone marrow-derived mast cells. Granules in wild-type cells have a dense core (indicated by the arrow) that is interspersed with translucent areas (left panel), whereas granules from serglycin-deficient cells do not have this subdivision into distinct dense-core regions and adjacent translucent areas (right panel; S.W. and G.P., unpublished observations). Instead, the material is amorphous, with moderately electron-dense material that is evenly distributed throughout the entire volume of the granules. **c** | Resting and degranulating rat peritoneal mast cells. In resting mast cells, fully mature granules contain highly electron-dense material that is evenly distributed throughout the major part of each granule. During degranulation, 'degranulation channels' form by extensive granule–granule fusion (indicated by the arrows), and exocytosed membrane-free granule matrices — known as granule remnants — can be observed (indicated by the shorter arrow). Left panel of part **b** reprinted with permission from REF. 30, copyright © 2006 John Wiley and Sons. Part **c** reprinted with permission from REF. 154, Springer.

Mast cell granule composition

As detailed in TABLE 1, mast cell granules have been shown to contain a plethora of preformed constituents. Among these, histamine is probably the most well known but additional amines, such as serotonin, are also found in granules. In accordance with their lysosome-like properties[11,12], mast cell granules contain a large number of lysosomal hydrolases, and it is also known that mast cells have the unique ability to store certain preformed cytokines and growth factors, such as tumour necrosis factor (TNF) and vascular endothelial growth factor (VEGF). Various mast cell-specific proteases, including tryptases, chymases and carboxypeptidase A3 (CPA3) (BOX 1) are major constituents of mature mast cell granules[13]. In addition to the mast cell-specific proteases, the granules contain a number of proteases that are not expressed solely by mast cells. Proteoglycans of the serglycin species are also major constituents of mature mast cell granules[14] (BOX 2). By virtue of their attached glycosaminoglycan (GAG) chains, serglycin proteoglycans have a remarkably high anionic charge density and are thus able to interact electrostatically with other granule compounds that have a corresponding positive charge. In addition, the serglycin proteoglycans account for the typical strong staining of mast cells with various cationic dyes (FIG. 1a). Mast cell granules also contain various membrane-associated proteins, many of which have a role in the process of exocytosis.

Although the granules of a mature mast cell seem to be highly homogeneous and appear to be morphologically similar (FIG. 1), there is evidence to suggest that the granules are heterogeneous in their composition and morphology. In one study, it was suggested that there is a subdivision of granules in mouse bone marrow-derived mast cells (BMMCs); certain granules contain serotonin and cathepsin D, whereas others contain histamine and TNF[15]. The notion of granule heterogeneity is also supported by several independent studies[16–18] but has not been confirmed *in vivo*. There are also observations of granule heterogeneity at the ultrastructural level,

Table 1 | **Features and functions of mast cell granule constituents**

Granule constituent	Key features	Proposed or potential function	Refs
Lysosomal enzymes*			
β-hexosaminidase	• Enzyme involved in turnover of carbohydrates • Routinely used as a marker for mast cell degranulation responses	Probable role in normal lysosomal degradation processes	155–157
β-glucuronidase, *N*-acetyl-β-glucosaminidase and β-D-galactosidase	Enzymes involved in turnover of carbohydrates	Probable roles in normal lysosomal degradation processes	155–157
Arylsulphatase A	Enzyme involved in turnover of glycosphingolipids	Probable role in normal lysosomal degradation processes	155,156
Cathepsin B	Cysteine protease	• Implicated in pro-tryptase processing • Probable role in normal lysosomal degradation processes	158
Cathepsin C	Cysteine protease	• Crucial for processing of pro-chymases • Probable role in normal lysosomal degradation processes	159
Cathepsin L	Cysteine protease	• Implicated in pro-tryptase processing • Probable role in normal lysosomal degradation processes	158
Cathepsin D	Aspartic acid protease	• Unknown function in mast cells • Probable role in normal lysosomal degradation processes	158
Cathepsin E	Aspartic acid protease	• Role in processing of pro-CPA3 • Probable role in normal lysosomal degradation processes	61
Biogenic amines			
Histamine	Present in all subtypes of mast cells in all species	• Induces bronchoconstriction, vasodilation and vascular permeability • Potential role in mast cell-mediated signalling to nerve endings	160,161
Serotonin	• High levels in rodent mast cells • Low levels in human mast cells	• Neurotransmitter • Potential role in mast cell-mediated signalling to nerve endings	161–163
Dopamine	• Low levels • Not found in human mast cells	• Neurotransmitter • Potential role in mast cell-mediated signalling to nerve endings	164
Polyamines (for example, spermidine and spermine)	Ubiquitous components of mammalian cells	Regulate granule ultrastructure and storage of histamine, serotonin and β-hexosaminidase	17,55
Cytokines and growth factors			
TNF	First cytokine shown to be stored in mast cell granules	Pro-inflammatory activity	165
IL-4	Released by IgE receptor crosslinking	Potential role in mast cell-directed T_H2 cell polarization	166
bFGF	Seems to be associated with heparin	Implicated in pro-angiogenic effects of mast cells	167
VEGF	Released by IgE receptor crosslinking	Implicated in pro-angiogenic effects of mast cells	168,169
TGFβ	Released during degranulation	Implicated in pro-fibrotic and anti-inflammatory effects of mast cells	170
Nerve growth factor	Released by IgE receptor crosslinking	Implicated in potential interactions between mast cells and peripheral nerve endings	171
IL-5	• Present in the cytoplasm • Presence within granules unclear	Potential role in mast cell-directed recruitment and activation of eosinophils	172
IL-6	• Present in the cytoplasm • Presence within granules unclear	Pro-inflammatory activity	172
IL-15	Associated with granules but not released upon mast cell activation	Suppresses antibacterial activity of mast cells	130
Stem cell factor	• Present in the cytoplasm • Not released upon IgE receptor crosslinking	Major growth factor for mast cells	173
Proteoglycans			
Serglycin	• High expression in mast cells • Also expressed by other haematopoietic cells and by endothelial cells	• Major role in promoting storage of proteases and amines • Accounts for the metachromatic staining of mast cells	46

Table 1 (cont.) | **Features and functions of mast cell granule constituents**

Granule constituent	Key features	Proposed or potential function	Refs
Mast cell-specific proteases			
Tryptases	• Serine proteases • Highly expressed by mast cells • Low expression in basophils	Either protective or detrimental functions in inflammatory settings	174
Chymases	• Serine proteases • Highly expressed by mast cells	Either protective or detrimental functions in inflammatory settings	175
CPA3	• Metalloproteinase • Highly expressed by mast cells • Low expression in basophils	Essential for protection against certain toxins	176
Non-mast-cell-specific proteases			
Cathepsin G	• Serine protease • Major product of neutrophils	May contribute to antibacterial effects of mast cells	177
MMP9	• Metalloproteinase • Activated by chymase	May contribute to mast cell-mediated effects on the ECM	178
Active caspase 3	• Cysteine protease • Present in granules of viable mast cells	Unknown	179,180
ADAMTS5	Metalloproteinase	Implicated in aggrecan degradation	181
Granzyme B	• Serine protease • Major product of cytotoxic lymphocytes	Implicated in pro-apoptotic effects of mast cells on target cells	182
Renin	Aspartic acid protease	Implicated in mast cell-mediated angiotensin II generation and regulation of blood pressure	183
Granule membrane-associated proteins			
VAMP2, VAMP3, VAMP7 and VAMP8	v-SNAREs	• VAMP8 is essential for granule fusion events during degranulation of mouse mast cells • In human mast cells, both VAMP7 and VAMP8 have a functional effect on degranulation	15,68,69, 71,184,185
Syntaxin 3	t-SNARE	Contributes to both granule–granule and granule–plasma membrane fusion	68,69,71, 184,185
Synaptotagmin II and synaptotagmin III	Accessory protein in SNARE-mediated granule fusion events	• Calcium sensors • Synaptotagmin III has a role in regulating granule size	18,28,75
MUNC18-2	Accessory protein in SNARE-mediated granule fusion events	Provides a link between granules and microtubules	186
MUNC13-4	Accessory protein in SNARE-mediated granule fusion events	Implicated as a calcium sensor that promotes SNARE complex formation	77
SCAMP1 and SCAMP2	Accessory proteins in SNARE-mediated granule fusion events	Contribute to granule fusion events	187
CD63	Tetraspanin	Part of the degranulation machinery	188
RAB3D, RAB5, RAB7, RAB9A, RAB19, RAB27A, RAB27B, RAB42 and RAB43	Belong to a family of GTPases that regulates membrane trafficking	• RAB27B regulates degranulation • RAB5 regulates granule size in RBL-2H3 cells	25,74, 189,190
LC3-II	Autophagy-related protein	Implicates a role for autophagy in mast cell degranulation	191
MHC class II	Presents antigens mainly derived from extracellular sources	Suggests a role for mast cells in antigen presentation	192
Other			
Heparanase	Endoglycosidic enzyme that degrades heparin and heparan sulphate	May regulate heparin structure within granules, leading to ultrastructural effects and effects on protease storage	49,193
LL37	Antimicrobial peptide	Implicated in antibacterial effects of mast cells	194
Eosinophil granule major basic protein	Appears in granules after uptake from environment	Unknown function in a mast cell context	40
Secretogranin III and chromogranin A	• Members of the granin protein family • Present in granules of neuroendocrine cells	Regulate granule numbers in RBL-2H3 cells	24

ADAMTS5, a disintegrin and metalloproteinase with thrombospondin motifs 5; bFGF, basic fibroblast growth factor; CPA3, carboxypeptidase A3; ECM, extracellular matrix; IL, interleukin; LC3-II, lipidated light chain 3 (also known as MAP1LC3); MMP9, matrix metalloproteinase 9; SCAMP, secretory carrier-associated membrane protein; SNARE, soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor; TGFβ, transforming growth factor-β; T_H2 cell, T helper 2 cell; TNF, tumour necrosis factor; t-SNARE, target-SNARE; VAMP, vesicle-associated membrane protein; VEGF, vascular endothelial growth factor; v-SNARE, vesicle-SNARE.
*Unknown impact on extracellular events following degranulation.

Box 1 | **Mast cell-specific proteases**

Tryptases

The main tryptases in humans are the βI-, βII- and βIII-tryptases and the enzymatically inactive α-tryptase. Alleles of α-tryptase and βI-tryptase are encoded at one genetic locus, whereas the βII and βIII allelic variants are encoded at a neighbouring locus. In mice, the dominating tryptases of mast cell granules are mouse mast cell protease 6 (mMCP6) and mMCP7, of which mMCP6 is probably the most homologous to human β-tryptases. All of these tryptases have a unique tetrameric organization, in which the active sites face a narrow central pore that is inaccessible to all known endogenous protease inhibitors. In addition to these tetrameric tryptases, both humans and rodents express a monomeric transmembrane tryptase (γ-tryptase).

Chymases

Humans express only one mast cell chymase (which is encoded by *CMA1*). By contrast, the chymase locus of rodents has expanded considerably and encodes the four major chymases that are expressed by mature mouse mast cells: mMCP1, mMCP2, mMCP4 and mMCP5. On the basis of tissue location, substrate specificity and affinity for proteoglycans, mMCP4 is probably the functional homologue of human chymase, although mMCP5 has a closer phylogenetic relationship to human chymase. However, unlike human chymase and mMCP4, which both have chymotrypsin-like substrate specificity, mMCP5 has elastase-like cleavage properties.

Carboxypeptidase A3

In both humans and rodents, a single carboxypeptidase A3 (*CPA3*) gene is expressed. CPA3 is a zinc-containing exopeptidase of the metalloproteinase family.

Protease expression in mast cell subclasses

The profile of protease expression has been used to define subsets of mast cells. In humans, mast cells can be subdivided into the MC_T subset, which expresses tryptases only, and the MC_{TC} subset, which expresses tryptases, chymase and CPA3 (see the table). In rodents, mast cells are classified as either connective tissue mast cell (CTMC) or mucosal mast cell (MMC) types on the basis of the expression profile of mast cell-specific proteases (see the table). However, although this system of mast cell classification has been useful, it is most probably too simplistic. For example, a tryptase^{hi}CPA3^{hi}chymase^{low} intraepithelial mast cell population has been identified in the lungs of patients with asthma[153] and it is known that mouse mast cells can display mixed protease expression profiles in a strain-dependent and tissue-dependent manner.

Mast cell subset	Chymase	Tryptase	Carboxypeptidase A3
Human			
MC_{TC}	CMA1 (*CMA1*)*	• α/βI tryptase *(TPSAB1)* • βII/βIII tryptase *(TPSB2)*	CPA3 (*CPA3*)
MC_T	–	• α/βI tryptase *(TPSAB1)* • βII/βIII tryptase *(TPSB2)*	–
Mouse			
CTMC	• mMCP4 (*Mcpt4*) • mMCP5 (*Mcpt5*; also known as *Cma1*)	• mMCP7 *(Tpsab1)* • mMCP6 *(Mcpt6*; also known as *Tpsb2)*	CPA3 (*Cpa3)*
MMC	• mMCP1 (*Mcpt1)* • mMCP2 (*Mcpt2)*	–	–

*Gene names are provided in parentheses.

whereby they can be subdivided into scroll-, crystal- or particle-containing granules[19]. Such ultrastructural motifs can be mixed within individual granules[19] and, interestingly, it was shown that tryptases are preferentially located in crystalline structures, whereas chymase is predominantly located in electron-dense areas[20].

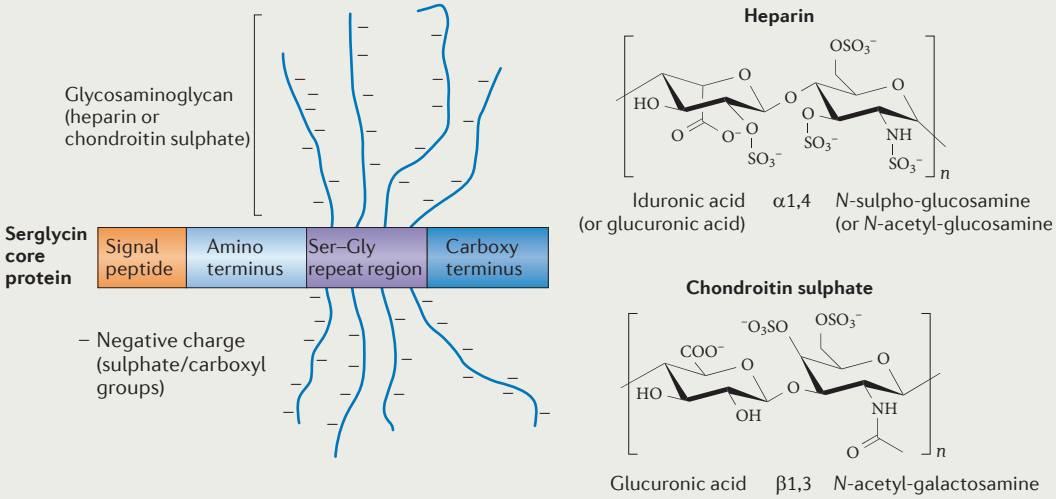
Mast cell granule biogenesis

In general, the mechanisms of secretory granule biogenesis in mast cells are relatively poorly characterized. However, it is reasonable to assume that the basic principles of mast cell granule biogenesis are similar to corresponding processes in other cell types that contain secretory granules, such as neuroendocrine cells and cytotoxic T lymphocytes[12]. The biogenesis of secretory granules is initiated at the *trans*-Golgi network, from which clathrin-coated vesicles bud off[11]. In mast cells, it has been shown that these vesicles — termed 'progranules' — are small and uniformly sized[21,22] (FIG. 2). Subsequently, extensive progranule fusion events occur, which results in the generation of immature granules that then undergo maturation. Interestingly, there is evidence to suggest that the size of the mature secretory granules is a direct function of the number of progranules that were fused during their biogenesis[21,23].

The molecular events that regulate these processes in mast cells mostly remain elusive. However, it has been shown that secretogranin III has an important role in promoting secretory granule formation in mast cells, as shown by an increase in the number of granules in RBL-2H3 cells that overexpress secretogranin III[24]. In addition, a role for the small GTPase RAB5 (also known as RAB5A) in regulating mast cell granule size has been suggested[25]. It is also of interest to note that

Box 2 | **Proteoglycans in mast cells**

Proteoglycans are comprised of a 'core protein' to which polysaccharide chains of the glycosaminoglycan (GAG) type are covalently attached through glycosidic bonds to serine residues that are present within Ser–Gly repeat regions of the respective core protein. Proteoglycans are ubiquitous components of most tissues, and are present in the extracellular matrix, on cell surfaces and intracellularly. In mast cells, proteoglycans of the serglycin type (see the figure) are the dominant species and they are found in large quantities within mast cell granules. The gene that encodes the serglycin core protein (*Srgn*) is expressed at remarkably high levels and is one of the most highly expressed of all genes in mast cells (together with the genes that encode the various mast cell-specific proteases). In connective tissue mast cells, GAGs of the heparin type dominate, whereas in mucosal mast cells chondroitin sulphate is the main GAG species on the serglycin core protein. Heparin is exclusively synthesized by mast cells and is formed from repeating units of iduronic/glucuronic acid–glucosamine disaccharide (see the figure), in which most of the uronic acid residues (predominantly iduronic acid) are 2-*O*-sulphated, and the majority of the glucosamine residues are *N*-sulphated and 6-*O*-sulphated. Heparan sulphate — a GAG that is expressed by numerous other cell types — has the same basic structure as heparin but has a higher proportion of glucuronic acid and has much less overall sulphation than heparin. Chondroitin sulphate is formed from glucuronic acid–*N*-acetyl-galactosamine disaccharide repeats (see the figure), in which the *N*-acetyl-galactosamine residues can be either 4-*O*-sulphated, 6-*O*-sulphated or 4,6-di-*O*-sulphated. GAGs are initially synthesized as non-sulphated disaccharide backbones but are subsequently modified by various enzymes, including uronic acid epimerases, *N*-deacetylase/*N*-sulphotransferases and a variety of *O*-sulphotransferases. Typically, the extent of sulphation of GAGs that are synthesized by mast cells is considerably higher than the sulphation of those that are synthesized by any other cell type.



granules are enlarged in beige mice[26,27] and in mast cells that have reduced expression of synaptotagmin III[28]. An important event that accompanies secretory granule biogenesis is the acidification of the granule lumen. This process is important for the condensation of prospective granule content and is initiated in the *trans*-Golgi network, which has a slightly acidic pH (approximately pH 6). During the maturation of granules, this acidification process continues, resulting in a final pH of ~5.5 in mature mast cell granules. The mechanism of acidification involves proton pumping from the cytosol into the granules, which is a process that depends on vacuolar ATPase (V-ATPase).

Sorting of granule compounds. A central event in the biogenesis of secretory granules is the sorting of prospective granule compounds into the granules. Two central models have been proposed for this process[11]. In the 'sorting-by-entry' model, each granule compound has a sorting signal that interacts with a corresponding receptor in the *trans*-Golgi membrane. An alternative to this model is the 'sorting-by-retention' hypothesis, in which multiple compounds are initially enclosed in the immature granules and granule maturation is accomplished by the subsequent removal of selected compounds[11]. A notable example of the sorting-by-entry model is the mannose 6-phosphate (M6P) system, in which glycosylated proteins acquire a M6P group that interacts with corresponding M6P receptors in the Golgi membrane, and thereby only glycosylated proteins are selected for entry into granules. This system is widely used for the sorting of lysosomal hydrolases and it is therefore reasonable to assume that the M6P system is also operative for these compounds in the context of mast cells, although there is currently little experimental evidence to support this notion. Notably, however, there are indications that the sorting of TNF into mast cell granules depends on *N*-glycosylation, thus potentially implicating the M6P system[29]. By contrast, the mechanisms that underlie the sorting of other major mast cell granule compounds — such as chymases, tryptases and CPA3 — remain elusive, although the limited evidence that is available suggests a role for serglycin in the retention of some of these compounds in granules[30]. Interestingly, it

Beige mice
A strain of mice with beige hair and a mutation in the gene that encodes lysosomal trafficking regulator (*Lyst*). These mice have an autosomal recessive disorder that is characterized by hypopigmentation and immune cell dysfunction. The phenotype of beige mice results from aberrant lysosomal trafficking and is similar to that of patients with Chediak–Higashi syndrome.

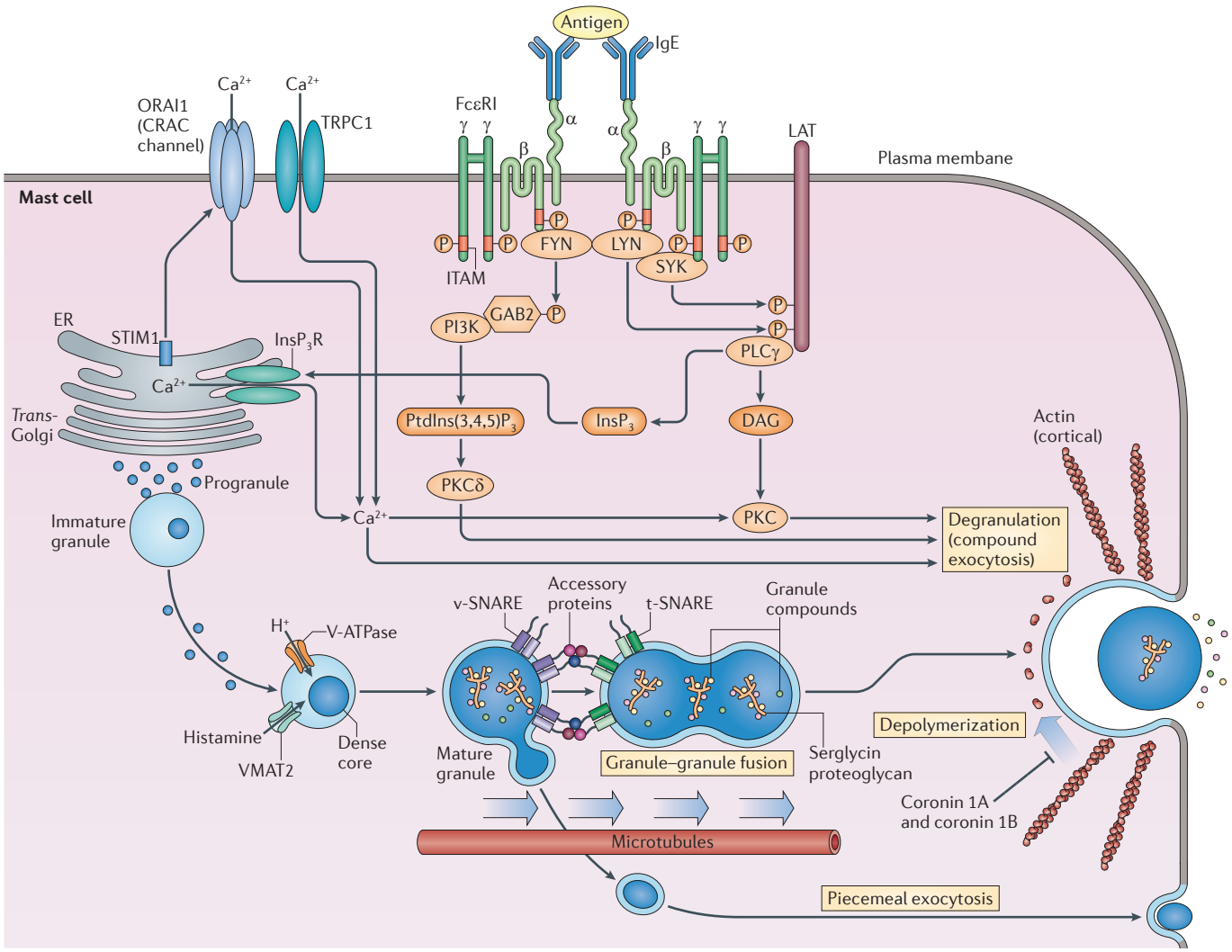


Figure 2 | **A highly simplified scheme for mast cell granule biogenesis, maturation and degranulation.** Mast cell secretory granule biogenesis is initiated at the *trans*-Golgi, where small vesicles (progranules) bud off and then undergo multiple fusion events leading to the formation of immature granules in which dense core formation is minimal. Granules then undergo maturation, a process in which dense core formation increases along with the gradual filling of granules with compounds such as proteases, bioactive amines and cytokines. In accordance with their lysosome-like properties (mast cell granules are also referred to as secretory lysosomes), granules are also filled with a large panel of lysosomal hydrolases. These compounds enter granules either by delivery from *trans*-Golgi-derived progranules or by pumping from the cytosol (for example, histamine). Granule maturation is strongly dependent on the presence of proteoglycans of the serglycin type. Mast cells express the high-affinity receptor for IgE (FcεRI) on their surface. FcεRI is a heterotetrameric receptor composed of an antigen-binding α-subunit, a β-subunit that contains an immunoreceptor tyrosine-based activation motif (ITAM) and two disulphide-linked γ-subunits that also contain ITAMs. When FcεRI-associated IgE molecules bind multivalent antigen (allergen), FcεRI receptors aggregate and this causes the LYN-dependent phosphorylation of ITAMs and the activation of additional tyrosine-protein kinases (FYN and SYK). This leads to the phosphorylation of several adaptor proteins, such as linker for activation of T cells (LAT) and GRB2-associated binding protein 2 (GAB2), followed by the recruitment and activation of many signalling molecules, including phospholipase Cγ (PLCγ) and phosphoinositide 3-kinase (PI3K). The activation of these signalling molecules results in the generation of second messenger molecules such as inositol-1,4,5-trisphosphate ($InsP_3$), diacylglycerol (DAG) and phosphatidylinositol-3,4,5-trisphosphate ($PtdIns(3,4,5)P_3$), which leads to the activation of protein kinase C (PKC) and the release of Ca^{2+} from the endoplasmic reticulum (ER). The release of Ca^{2+} from the ER leads to stromal interaction molecule 1 (STIM1)-mediated opening of the store-operated Ca^{2+} channel ORAI1, which leads to the influx of extracellular Ca^{2+}. The influx of Ca^{2+} is amplified by an additional mechanism that is mediated by short transient potential Ca^{2+} channel 1 (TRPC1). The increase in intracellular Ca^{2+} levels and the activation of PKC triggers the degranulation machinery so that granules translocate from the cell interior towards the plasma membrane in a microtubule-dependent manner. This is followed by cortical actin depolymerization, which is regulated by coronin 1A and coronin 1B. Degranulation is preceded by extensive granule–granule fusion events, which are mediated by various soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs), including vesicle (v)-SNARE and target (t)-SNARE proteins, that are assisted by several accessory proteins. Likewise, the degranulation event depends on a distinct set of v-SNARE, t-SNARE and accessory proteins. Following degranulation, many of the preformed granule compounds (such as histamine) are released in a soluble form, whereas others are retained in matrices (known as granule remnants) in which proteoglycans are most probably the main components, and which can contain proteoglycan-binding compounds, such as chymase, carboxypeptidase A3 (CPA3) and tumour necrosis factor (TNF). In addition to the degranulation mechanism, mast cells can also release granule-contained material by piecemeal exocytosis, a process by which vesicles bud off from the granules. $InsP_3R$, $InsP_3$ receptor; V-ATPase, vacuolar ATPase; VMAT2, vesicular monoamine transporter 2.

has been demonstrated that synaptotagmin IX may have an important function in the sorting of secretory granule compounds by segregating granules from the endocytic recycling compartment[31]. Histamine seems to enter granules through vesicular monoamine transporter 2 (VMAT2; also known as SLC18A2)[32].

Mast cell granule maturation and homeostasis

Following their initial biogenesis, mast cell granules undergo extensive maturation whereby the granules are gradually filled with mediators. This process has been studied *in vivo*, either by analysing granule development in rat peritoneal mast cells over time[33,34] or by studying granule recovery after inducing mast cell degranulation[35,36]. Granule maturation can also be studied by following the development of mouse bone marrow precursor cells into mast cells — that is, the development of BMMCs. As shown by these studies, there is a continuous, overall increase in granule size as mast cells mature, which most probably reflects the constant addition of progranules to pre-existing granules[23]. In the early phases of mast cell maturation, the levels of mast cell proteases and bioactive amines are low or undetectable. However, as granules mature, histamine and serotonin levels increase[33,34,37] and mast cell-specific proteases start to accumulate[30,38]. There is also a continuous increase in the expression of serglycin and of enzymes that are involved in the sulphation of serglycin[39].

It is likely that the bulk of granule content is a result of endogenous biosynthesis. However, it cannot be ruled out that certain components are present in mast cell granules as a result of uptake from the extracellular space. In support of this notion, it is known that eosinophil granule major basic protein (also known as BMPG)[40], histamine[41], dopamine[42], eosinophil peroxidase[43] and TNF[44] can be endocytosed by mast cells.

Proteoglycans in granule maturation. Dense core formation is a key step in secretory granule maturation and there is strong evidence that serglycin proteoglycan (with heparin or chondroitin sulphate side chains; see BOX 2) is crucial for this process in mast cells. In wild-type BMMCs, granules are typically composed of distinct dense core regions that are interspersed with electron-translucent material[45] (FIG. 1b). By contrast, granules from serglycin-deficient BMMCs are almost completely devoid of dense core regions and instead contain amorphous material that is evenly distributed within the granules[45] (FIG. 1b). Importantly, regardless of the absence or presence of serglycin, granules are of about equal size and are present in approximately equal numbers. Hence, serglycin is essential for dense core formation but does not take part in the actual biogenesis of secretory granules. In addition to its crucial role in promoting dense core formation, serglycin has a key role in promoting the storage of diverse granule compounds, including chymases, tryptases, CPA3, histamine and serotonin[45,46]. This effect most probably depends on electrostatic interactions between the sulphated, and thereby negatively charged, GAG side chains of serglycin with a corresponding positive charge displayed by the respective serglycin-dependent granule compounds. In support of this notion, abolished sulphation of the serglycin-associated heparin chains — as accomplished by the knockout of the gene encoding bifunctional heparan sulphate *N*-deacetylase/*N*-sulphotransferase 2 (NDST2) — caused a marked defect in the ability of mast cells to store several mast cell proteases, as well as histamine[47,48]. Along the same lines, it has been demonstrated that the overexpression of heparin-degrading enzymes causes a distortion of granule homeostasis and composition[49], and that defective synthesis of chondroitin sulphate leads to the reduced storage of tryptases and CPA3 in mast cells[50]. However, serglycin does not have a universal role in promoting the storage of mast cell granule compounds, as several — such as β-hexosaminidase, the chymase mast cell protease 1 (mMCP1; also known as MCPT1) and the tryptase mMCP7 (also known as TPSAB1) — are stored equally effectively in wild-type and serglycin-deficient mast cells[30,45].

Although BMMCs have been extensively used as models of mast cell function, it is important to emphasize that BMMCs are relatively immature in terms of granule maturation compared with *in vivo*-derived mature mast cells that are found in tissues. Typically, granules of BMMCs are highly heterogeneous and individual granules have varying content of high and low electron-dense material; granules with uniform high electron density are not normally seen (FIG. 1b). Moreover, a typical feature of BMMC granules is that they represent multivesicular bodies — that is, they contain exosomes — and there is evidence to suggest that such exosomes can activate other immune cell populations[51]. By contrast, granules of mature peritoneal mast cells are almost completely filled with electron-dense material (FIG. 1c) and thus they do not display the subdivision of electron-dense and translucent regions that is seen in BMMCs.

Interrelationships between granule components. In addition to the crucial role of sulphated proteoglycans in promoting granule maturation and in maintaining granule homeostasis, there are several other mechanisms that control these processes. In a study of mast cells that were deficient in histamine — owing to a lack of histidine decarboxylase (HDC) expression — it was observed that the storage of proteoglycans was reduced compared with wild-type controls[52]. This implies that there is an interrelationship between histamine and proteoglycans in regulating granule homeostasis. Moreover, it was shown that the absence of histamine led to a reduction in the storage of proteases[52]. In a subsequent study, these findings were extended by showing that histamine can have an effect on the expression levels of various mast cell granule compounds and enzymes that are involved in proteoglycan synthesis[53]. It is also of interest to note that the absence of histamine is associated with a corresponding increase in serotonin levels in granules[54], thus implying that these two bioactive amines can compensate for each other. In further support of a role of amines in regulating granule homeostasis, it has been demonstrated that a reduction in the levels of granule-contained polyamines

causes a decrease in histamine levels[55]. It has also been shown that the absence of multiple positively charged proteases in mast cells causes a reduction of the ability of mast cells to store proteoglycans[56].

These studies, together with studies of mast cells with a serglycin or NDST2 deficiency, imply that granule homeostasis is the result of a dynamic electrostatic balance between granule compounds of opposite electric charge. Hence, a reduction in negative charge (as imposed by the absence of serglycin or NDST2) results in a decrease in the ability of granules to accommodate positively charged compounds. Conversely, a reduction in positively charged compounds (as imposed by the absence of proteases or histamine) results in a corresponding decrease in the ability of granules to accommodate negative charge.

In addition to the electrostatic effects described above, there is also evidence of homeostatic mechanisms that are not necessarily of an electrostatic nature, as shown by the absence of stored mMCP5 in CPA3-deficient mast cells[57] and, vice versa, the absence of stored CPA3 in mast cells that lack mMCP5 (REFS 58,59). However, the nature of the interrelationship between mMCP5 and CPA3 remains to be clarified.

Processing of mast cell proteases. An important step in the maturation of mast cell granules is the processing of inactive protease precursors into active enzymes. Cathepsin C (also known as DPPI) has been shown to be essential for the processing of pro-chymases[60], whereas the processing of pro-CPA3 is, in part, dependent on cathepsin E[61] and possibly on cysteine cathepsins[62]. Cathepsin L (also known as cathepsin L1) and cathepsin B have been implicated in the processing of pro-tryptases[63]. Considering that the cathepsins implicated in these processes all depend on an acidic pH for optimal activity and that they are found within granules, it is likely that the processing steps occur within the granular compartment. Indeed, there is some experimental support for this scenario[62,64].

Granule exocytosis

Mast cell degranulation can occur in response to various stimuli, such as IgE receptor ligation, complement components (such as C3a and C5a), neuropeptides (such as substance P) and various other peptides from either endogenous sources (such as endothelin 1) or exogenous sources (such as venom-derived and bacteria-derived peptides)[3]. Of these, mast cell degranulation in response to IgE receptor ligation is the best characterized and has been extensively reviewed[65,66]. Briefly, the high-affinity receptor for IgE (FcεRI) is heterotetrameric and is composed of one α-subunit, one β-subunit and two γ-subunits, of which the α-subunit is the IgE-binding moiety and the β- and γ-subunits contain immunoreceptor tyrosine-based activation motifs (ITAMs) (FIG. 2). Upon crosslinking of FcεRI-bound IgE molecules by multivalent antigen, the IgE receptors aggregate, which leads to ITAM phosphorylation and the activation of tyrosine protein kinases such as FYN, LYN and SYK. Downstream effects of these events include the phosphorylation of adaptor proteins — such as linker for activation of T cells (LAT) and GRB2-associated binding protein 2 (GAB2) — followed by the activation of phospholipase Cγ (PLCγ) and phosphoinositide 3-kinase (PI3K). This results in the production of diacylglycerol (DAG), inositol-1,4,5-trisphosphate ($InsP_3$) and phosphatidylinositol-3,4,5-trisphosphate ($PtdIns(3,4,5)P_3$), which leads to Ca^{2+} mobilization and the activation of protein kinase C, thereby unleashing the degranulation machinery.

Degranulation also involves multiple membrane fusion events, including both granule–granule fusion and the fusion of granules with the plasma membrane (reviewed in REF. 67) (FIG. 2). Such events are crucially dependent on the interaction between various v-SNAREs (vesicle soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors) that are present on the granule membrane and target (t)-SNAREs that are present on the target membrane (either on the plasma membrane or on another granule). Of the various v-SNAREs, mast cell granules seem to predominantly express functionally active vesicle-associated membrane protein 8 (VAMP8)[15,68] and VAMP7 (REF. 69). The t-SNAREs of primary functional significance in mast cells seem to be syntaxin 3 (REF. 70), syntaxin 4 (REFS 69,71) and synaptosomal-associated protein 23 (SNAP23)[69,72]. For membrane fusion to occur, several accessory proteins in addition to the SNARE proteins are required. These include the calcium sensor complexin II[73] and GTPases of the RAB family, of which RAS-related protein RAB27B has been shown to be crucial for degranulation responses in a mast cell context[74]. It is also known that synaptotagmins — especially synaptotagmin II — are crucial in the degranulation process, by acting as calcium sensors and by promoting membrane fusion events[18,75]. There is also recent evidence implicating the tetraspanin CD63 as an important component of the degranulation machinery[76]. In addition, it has been shown that granule fusion in mast cells depends on MUNC18 family members, which are proteins that interact with syntaxin SNAREs. Of these, MUNC18-2 (also known as STXBP2; binding partner to syntaxin 3) seems to be of particular importance for mast cell degranulation, partly by serving as a link between granules and the microtubules[70]. It has also been shown that MUNC13-4 (also known as UNC13D) is an important part of the degranulation machinery, possibly by acting as a binding partner for RAB27A[77].

During the degranulation process, granules translocate along microtubules from the interior of the cell towards the plasma membrane[78]. For degranulation to occur, the cortical actin needs to be depolymerized[79], which is a process that is regulated by coronin 1A and coronin 1B[80]. Before degranulation, there is extensive granule–granule fusion (FIGS 1c,2) — thereby generating a 'degranulation channel' — followed by fusion of granules with the cell membrane. This type of exocytosis is referred to as 'compound exocytosis'. Typically, mast cell degranulation occurs in a non-directional manner, which is in contrast to the polarized exocytosis that is seen in cytotoxic T lymphocytes, for example. In addition to compound exocytosis, it has been shown that mast cells can release granule compounds by 'piecemeal exocytosis', a process in which small vesicles are budded off from granules[81] (FIG. 2).

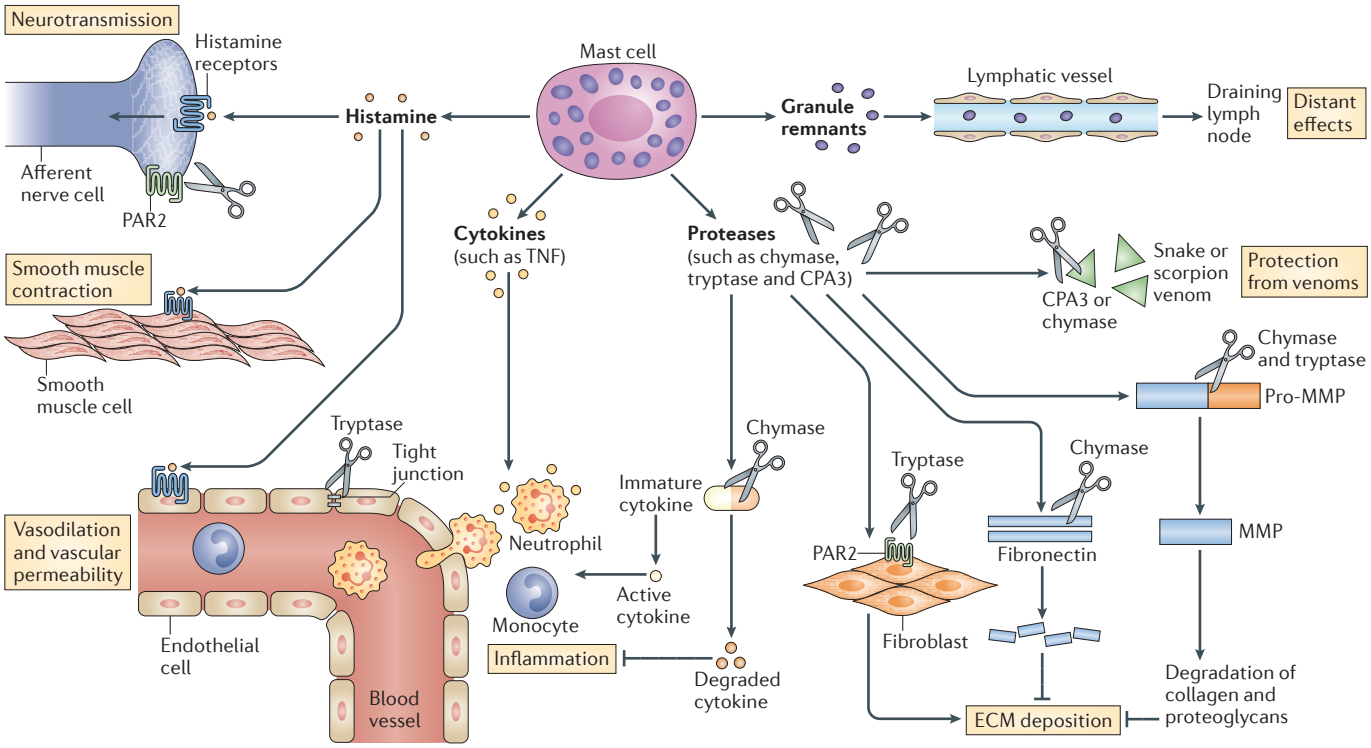


Figure 3 | **Biological effects of mast cell granule components.** The figure shows examples of the biological effects that are attributable to the granule compounds that mast cells release. Mast cell degranulation leads to a powerful pro-inflammatory response, which is, in part, mediated by preformed granule compounds such as histamine, various cytokines (such as tumour necrosis factor (TNF)) and mast cell-specific proteases (such as chymases, tryptases and carboxypeptidase A3 (CPA3)). Histamine has multiple effects that are mediated by binding to histamine receptors, including the stimulation of afferent nerve cells, stimulation of smooth muscle contraction (for example, leading to bronchoconstriction), and the induction of vascular permeability and vasodilation. The release of preformed cytokines, such as TNF, contributes substantially to the pro-inflammatory response but there is also a marked contribution of cytokines and lipid mediators that are synthesized *de novo* by mast cells following mast cell activation (not shown in the figure). Mast cell proteases can also contribute to the inflammatory response, for example, through the activation of protease-activate receptor 2 (PAR2), by degrading tight junction proteins or hemidesmosome proteins (not shown), or by inducing cytokine maturation through limited proteolysis. In other settings, mast cell proteases can have anti-inflammatory activities, mainly through the degradation of pro-inflammatory cytokines and chemokines. The granule compounds (in particular, the proteases) that are released can also have extensive effects on the extracellular matrix (ECM). For example, mast cell proteases can be pro-fibrotic and promote ECM deposition by activating PAR2 on fibroblasts (thereby inducing collagen synthesis) or by activating pro-fibrotic transforming growth factor-β (not shown). Mast cell proteases can also promote ECM disassembly, either directly through the degradation of ECM components, such as fibronectin, or indirectly by proteolytic activation of matrix metalloproteinase pro-enzymes (pro-MMPs) that subsequently degrade ECM components, such as collagen and proteoglycans. Mast cell proteases are essential for protection against noxious compounds, such as the various toxins that are present in the venoms of snakes and scorpions. Released granules can be transported through the lymphatic system to the draining lymph nodes, thereby acting as carriers for bioactive compounds (such as TNF).

Biological actions of released granule mediators

When considering the functional impact of mast cell degranulation, it is important to emphasize that mast cell granules have an exceptionally high content of bioactive substances and that all of the preformed granule compounds are stored in their active form. These observations imply that mast cell granules have evolved due to the need for a powerful arsenal of bioactive compounds that can rapidly aid in the protection of the host against diverse external insults[82] (FIG. 3).

When elucidating the functional impact of mast cell secretory granules in various pathophysiological situations, various knockout mice that lack one or more of the preformed granule compounds have been useful tools (TABLES 2,3). Strains that are relevant for this purpose include mice that are deficient in the chymases mMCP1 (REF. 83), mMCP4 (REF. 84) and mMCP5 (REF. 58), the tryptase mMCP6 (also known as TPSB2)[85,86], CPA3 (REF. 57), serglycin[46], NDST2 (REFS 47,48) and HDC[52]. As the expression of chymases, tryptases and CPA3 is essentially mast cell specific, the effects of the knockout of these enzymes are most probably limited to mast cells. By contrast, the expression of some granule components — such as histamine, serglycin and various cytokines — is not restricted to mast cells, and so it cannot be ruled out that the effects of their deficiency are related to their expression by other cell types. To account for this issue, strategies in which mast cell-deficient mice are

Table 2 | **Mouse strains relevant for studies of mast cell granule compound function**

Mouse strain	Mast cell granule phenotype	Refs
Mcpt1$^{-/-}$	• Lacks mMCP1 • Altered granule ultrastructure	83
Mcpt4$^{-/-}$	• Lacks mMCP4 • Normal granule phenotype • Abolished chymotrypsin-like activity in CTMCs	84
Mcpt5$^{-/-}$	• Lacks mMCP5 • Post-translational defect in CPA3 storage	58
Mcpt6$^{-/-}$	Lacks mMCP6	85,86
Cpa3$^{-/-}$	• Post-translational defect in mMCP5 storage • Reduced granular staining • Increased solubility of granule proteoglycans	57
Mcpt4$^{-/-}$*Mcpt6*$^{-/-}$*Cpa3*$^{-/-}$	• Lacks mMCP4, mMCP6 and CPA3 • Post-translational mMCP5 deficiency • Reduced granular staining • Reduced proteoglycan content	56
Cpa3^{Y356L,E378A}	• Expression of mutant CPA3 that lacks catalytic activity • Normal granules	126
C57BL/6	• Lacks mMCP7 due to a mutation in *Mcpt7* that introduces a premature stop codon • Normal expression of mMCP6	195
CMA1-tg	Overexpression of human chymase	196
Ndst2$^{-/-}$	• CTMCs unable to store mMCP4, mMCP6, CPA3 and mMCP5 (all post-translational defects) • CTMCs unable to store histamine • MMCs unaffected	47,48
Srgn$^{-/-}$	• CTMCs unable to store mMCP4, mMCP6, CPA3 and mMCP5 (all post-translational defects) • CTMCs unable to store histamine and serotonin • MMCs have reduced mMCP2 storage	45,46
Hpa$^{-/-}$	• Increased granule staining and proteoglycan content • Increased levels of proteases	49
Hpa-tg	• Overexpresses heparanase • Reduced granule staining and proteoglycan content • Distorted granule morphology • Reduced levels of proteases	49
Hdc$^{-/-}$	• Reduced staining and proteoglycan content • Reduced levels of proteases	52
Tph1$^{-/-}$	Not investigated	197
Rab27b$^{-/-}$	Mild clustering of granules	74
Ctsc$^{-/-}$	• Defective processing of chymase • Abrogated chymase activity in granules	60
Beige (mutation in *Lyst*)	• Enlarged granules • Reduced numbers of granules	26
Mcpt5-Cre	Allows mast cell-specific inactivation of genes	87

CMA1, human chymase; CPA3, carboxypeptidase A3; CTMCs, connective tissue mast cells; *Ctsc*, cathepsin C; *Hdc*, histidine decarboxylase; *Hpa*, heparanase; *Lyst*, lysosomal trafficking regulator; *Mcpt*, mast cell protease gene; MMCs, mucosal mast cells; mMCP, mouse mast cell protease; *Ndst2*, bifunctional heparan sulphate *N*-deacetylase/*N*-sulphotransferase 2; *Srgn*, serglycin; tg, transgenic; *Tph1*, tryptophan hydroxylase 1; *Tpsb2*, tryptase βII (gene that encodes mMCP6).

reconstituted with either wild-type mast cells or mast cells that lack expression of a gene of interest have been used frequently. In addition, a recently developed technology has enabled the generation of mice in which only mast cells lack the expression of a gene of interest[87]. In particular, these approaches have been used to study the role of various mast cell-derived cytokines. However, although a role for mast cell-derived cytokines has been defined, it has not been possible to distinguish between the involvement of preformed, granule-localized cytokines and cytokines that are newly synthesized following mast cell activation. For reasons described above, studies on various mast cell protease-knockout animals have been particularly informative in elucidating the biological actions that are specifically attributed to preformed mast cell granule constituents, and we therefore focus the following discussion on the function of mast cell proteases.

Table 3 | **Impact of mast cell granule compounds on disease models as determined by studies of knockout mice**

Disease model	Role of mast cells*	Role of mast cell granule compound	Proposed mechanism	Refs
Bacterial infection	Protective	• Protective: mMCP4 and mMCP6 • Detrimental: histamine	• mMCP4: TNF degradation • mMCP6: neutrophil recruitment • Histamine: suppression of phagocytosis	86,129,132
Venom defence	Protective	Protective: CPA3 and mMCP4	Degradation of toxic peptides	124–126
Kidney fibrosis (unilateral ureteral obstruction)	Protective	Protective: mMCP4	Degradation of fibronectin	145
Trichinella spiralis infection	Protective	Protective: mMCP1 and mMCP6	• mMCP1: promotes parasite expulsion • mMCP6: contributes to eosinophil recruitment	85,133
Post-traumatic spinal cord damage	Protective	Protective: mMCP4	Degradation of cytokines (including CCL2, IL-6 and IL-13)	119
Post-traumatic brain inflammation	Protective	Protective: mMCP4	Not known	118
Kidney inflammation or fibrosis (immune complex-mediated glomerulonephritis)	Protective	Detrimental: mMCP4	Angiotensin II formation	147
Abdominal aortic aneurysm formation	Detrimental	Detrimental: mMCP4 and mMCP6	• Pro-angiogenic effects • Elastase degradation • Activation of cysteine proteases • Cytokine induction	95
Experimental colitis	Detrimental	Detrimental: mMCP6	Stimulation of chemokine and cytokine expression	97
Asthma	Detrimental	• Protective: mMCP4 • Detrimental: histamine	Degradation of IL-33	113,114,198
Burn injury	Detrimental	Detrimental: mMCP4 and mMCP5	Degradation of tight junctions	58,94
Lung fibrosis (bleomycin)	Detrimental	Detrimental: mMCP4	Not known	96
Arthritis	Detrimental‡	Detrimental: mMCP6, mMCP4 and histamine	• mMCP6: aggrecan degradation (via MMP3 and MMP13 activation) • Effects of mMCP4 and histamine unknown	137–140,144
Experimental autoimmune encephalitis	Detrimental‡	Protective: histamine	Histamine deficiency reduces the production of pro-inflammatory cytokines and reduces brain inflammation	199
Skin blistering (bullous pemphigoid)	Detrimental	Detrimental: mMCP4	• Degradation of hemidesmosomes • MMP9 activation	104

CCL2, CC-chemokine ligand 2; CPA3, carboxypeptidase A3; IL, interleukin; mMCP, mouse mast cell protease; MMP, matrix metalloproteinase; TNF, tumour necrosis factor. *As determined by studies of mast cell-deficient mice. ‡The role of mast cells in autoimmune disease has recently been questioned based on studies of new generation (KIT-independent) mast cell-deficient mice.

Pro-inflammatory actions of granule constituents. In addition to the long-established pro-inflammatory effects of histamine (reviewed in REF. 88) and granule-localized cytokines, such as TNF, in allergic conditions and other pathological settings, accumulating evidence suggests that mast cell granule proteases account for a substantial proportion of the pro-inflammatory actions that are attributed to mast cells. Initial evidence for this came from studies in which purified mast cell tryptase or chymase was shown to produce inflammatory responses[89–91]. In keeping with these findings, mast cell protease inhibitors have been shown to be anti-inflammatory[92,93]. Further support for a pro-inflammatory role of mast cell proteases comes from studies of knockout mice. For example, the absence of chymases mMCP4 or mMCP5 weakens the inflammatory response after burn injury[58,94], the absence of mMCP4 results in an attenuated inflammatory reaction in conjunction with abdominal aortic aneurysm formation[95] and also results in reduced bleomycin-induced lung inflammation[96], and the absence of tryptase mMCP6 ameliorates experimental colitis[97].

The mechanisms underlying the pro-inflammatory effects of the mast cell proteases may be manifold, which reflects their relatively broad substrate specificities[98] and also reflects the multiple potential substrates that are available in distinct types of inflammatory condition and during different phases of any given inflammatory reaction. With regard to tryptases, several studies show that proteinase activated receptor 2 (PAR2) is one of the main substrates of tryptases[99,100], and there is also evidence to suggest that tryptases

degrade fibrinogen[101]. With regard to the mechanisms of chymase action, there is evidence that tight junction proteins (for example, claudin 4, claudin 5 and occludin)[94,102,103] or hemidesmosome proteins (for example, BP180 (also known as COL17A1)[104]) may be important as direct or indirect chymase substrates. Clearly, the degradation of such proteins may promote inflammation by facilitating the influx of plasma components and the transmigration of blood-borne leukocytes through the endothelium. In addition, chymase is implicated in the generation of angiotensin II[105], the conversion of big endothelin 1 to endothelin 1 (REF. 106) and in the limited proteolysis of various cytokines and chemokines that leads to their activation[107–110]. There is also recent evidence to suggest that heparin released from mast cells may enhance vascular permeability by promoting coagulation factor XII-dependent activation of bradykinin[111] and, possibly, by inducing the formation of lacunae in the endothelial cell layer[112].

Anti-inflammatory effects of granule compounds. Although mast cells are usually associated with pro-inflammatory functions, it is notable that individual granule compounds have, in many cases, been shown to be anti-inflammatory. A striking example of this is the anti-inflammatory effect of the chymase mMCP4 in models of allergic lung inflammation[113,114], despite the general pro-inflammatory effects of mast cells in such settings[115,116]. Although we cannot fully explain the apparent discrepancy between the phenotype of chymase-deficient and mast cell-deficient mice, it is plausible that chymase (and possibly other mast cell-derived compounds) prevents the damaging effects of excessive levels of pro-inflammatory mast cell products, such as TNF[117]. Chymase has also been shown to have an anti-inflammatory role in neuroinflammatory conditions[118,119]. The mechanisms underlying the anti-inflammatory effects of mast cell proteases seem to involve the degradation of various pro-inflammatory cytokines and chemokines, such as IL-6, IL-13, IL-33, CC-chemokine ligand 2 (CCL2), CCL3 and CCL5 (REFS 110,114,119–121), as well as alarmins[114,122].

Granule compounds in defence against toxins. One of the most striking biological effects of mast cell granule compounds is their role in protection against various toxins. In a hallmark study, Galli and colleagues[123] showed that mast cell-deficient mice were highly sensitive to the effects of endothelin 1 — an endogenous toxin that is produced during sepsis — whereas mast cell-sufficient mice were protected. In subsequent studies, it was shown that mast cells were also crucial for protection against various toxins that are present in the venoms of snakes, honeybees, Gila monsters (venomous lizards) and scorpions[124,125]. Remarkably, mast cell granule proteases were found to account for almost all of the protective effects that are attributable to mast cells. Specifically, it was shown that CPA3 had a major role in protection against endothelin 1 and snake venom sarafotoxins[124,126], whereas the chymase mMCP4 was crucial for protection against Gila monster and scorpion toxins, as well as for protection against the toxic effects of vasoactive intestinal peptide[125]. These findings support the notion that mast cell degranulation is of particular importance in situations that require a rapid protective response to various noxious substances[82].

Granule compounds in infection. Since the identification of a protective role of mast cells in sepsis[127,128] investigators have sought to identify the underlying mechanisms. Several studies have implicated mast cell-derived TNF but there is also evidence to suggest that granule-derived proteases make an important contribution, as mMCP6-deficient mice have increased susceptibility to *Klebsiella pneumoniae* infection compared with wild-type animals[86], and chymase mMCP4 contributes to survival in response to sepsis that is induced by cecal ligation and puncture[129]. The mechanism by which chymase mMCP4 confers protection was shown to involve the degradation of TNF, which thereby prevented the toxic effects of the cytokine[129]. There is also evidence that the chymase mMCP2 has a protective role during sepsis[130], and that serglycin contributes to the clearance of *K. pneumoniae*[131]. In contrast to the beneficial effects of mast cell proteases, histamine has been implicated as a detrimental factor in the clearance of *Escherichia coli* infection[132]. In addition to their role in bacterial infection, granule compounds have been implicated in host defence against parasites[85,102,133].

Granule compounds in autoimmune disease. Studies of various mast cell-deficient mouse models — especially the WBB6F1-*Kit*$^{W/W-v}$ strain — have suggested a prominent role for mast cells in autoimmune diseases, such as bullous pemphigoid[134], arthritis[135] and experimental autoimmune encephalitis (EAE)[136]. In subsequent studies, it was shown that mast cell granule proteases and histamine, at least in part, account for the detrimental actions of mast cells in these disease models[104,137–140]. However, the role of mast cells in autoimmune disease has recently been questioned following studies of the new generation of (KIT-independent) mast cell-deficient mice[7]. It is therefore unclear how the absence of individual mast cell granule compounds can have such prominent effects on disease, whereas global mast cell deficiency has little effect. One explanation for this apparent discrepancy could be that mast cells have a panel of activities that have the potential to influence disease progression in positive and negative ways — that is, they have both pro-inflammatory and anti-inflammatory effects. A total absence of mast cells will therefore eliminate all of these mast cell-dependent activities, whereas the absence of individual granule compounds may shift the balance such that, for example, pro-inflammatory mast cell-mediated events will dominate over corresponding anti-inflammatory mechanisms. Another possibility is that the total absence of mast cells may trigger compensatory mechanisms that mask the contribution of mast cells. Yet another explanation could be that mast cell proteases, under certain conditions, can be expressed outside of a mast cell context. However, arguing against the latter possibility, recent studies indicate that cell toxicity that

is driven by expression of Cre recombinase under the control of the promoters of mast cell proteases (CPA3 or mMCP5) seems to specifically eliminate mast cells, with only limited toxicity for other cell types[7,8].

Granule compounds in extracellular matrix remodelling and fibrosis. There is substantial evidence showing that granule compounds can affect processes that are relevant to extracellular matrix (ECM) remodelling, either by direct effects on ECM components or indirectly by activating ECM-remodelling enzymes. As an important example of an indirect role, chymases have been shown to have pro-collagenase activity[141] and they are important for processing the pro-enzyme for matrix metalloproteinase 9 (pro-MMP9) and pro-MMP2 into active enzymes[103,104,142,143]. Similarly, tryptases have been shown to activate pro-MMP3 and pro-MMP13 (REF. 144). Direct effects of mast cell proteases on ECM components include the processing of fibronectin by chymase, as the absence of chymase leads to substantial fibronectin accumulation both under normal conditions[143] and during renal fibrosis[145]. Furthermore, tryptases have been shown to degrade denatured collagen (gelatin)[146] and aggrecan[144]. Therefore, granule compound-mediated effects on the ECM could have important implications for fibrosis and, indeed, chymase has recently been implicated in models of fibrosis[96,145,147]. There is also evidence to suggest that mast cell proteases can exert pro-fibrotic activity by inducing fibroblast proliferation[148] and that chymase can activate transforming growth factor-β[141], thereby mediating pro-fibrotic activity. In line with these findings, chymase inhibitors may reduce fibrosis[149]. It is also possible that granule protease-mediated effects on the ECM could promote angiogenesis, which could be further amplified by the release of VEGF and basic fibroblast growth factor (bFGF; also known as FGF2) from preformed stores in granules.

Granules as entities. Intriguing insight into mast cell granule function comes from a study by Abraham and co-workers[150]. These authors showed that mast cell granule-derived, heparin-containing particles (most probably reflecting the 'granule remnants', which represent the proteoglycan-containing core that remains after dissociation of soluble mediators) can be transported to the draining lymph nodes, and thereby act as carriers for TNF[150]. In a subsequent report, the same group introduced the concept of using artificial granule-like matrices that encapsulate TNF as adjuvants during vaccination[151]. These studies suggest that secreted granules can act as entities, rather than merely being vehicles for the storage of various mediators that diffuse away in a soluble form following mast cell degranulation. Along the same lines, it has been shown that apparently intact mast cell granules or granule remnants can enter adjacent cells through a process known as 'transgranulation' (REF. 152).

Conclusions and perspectives

It has been known for several decades that mast cells have an important role in many pathological situations but it is only more recently that the underlying molecular mechanisms are becoming understood. Notably, as reviewed here, in many cases it is now established that various granule-derived compounds account for both the beneficial and the detrimental functions that are ascribed to mast cells. On this basis, it is likely that efforts to limit mast cell-driven pathology by using approaches that target mast cell granules or granule-derived compounds may be intensified in the near future. It is notable that recent research efforts have particularly focused on the function of one group of granule compounds — namely, the mast cell-specific proteases. However, it will be of utmost importance to also study the functions of those granule constituents for which expression is not limited to mast cells — for example, cytokines, histamine and non-mast-cell-specific proteases. To accomplish this will require the generation of mice with mast cell-specific deletions of the respective compounds, as has recently been done for IL-10 (REF. 8), and we foresee that this type of approach will be a major focus for mast cell biologists during the next decade. We also foresee that hitherto relatively unexplored areas of mast cell granule biology, such as the mechanisms of granule biogenesis, will receive increased attention.

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