2-22-2018

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HHS Public Access

Author manuscript

Biochem Soc Trans. Author manuscript; available in PMC 2019 April 17.

Published in final edited form as:

Biochem Soc Trans. 2018 April 17; 46(2): 235–247. doi:10.1042/BST20170450.

Diverse exocytic pathways for mast cell mediators

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Summary

Mast cells play pivotal roles in innate and adaptive immunities but are also culprits in allergy, autoimmunity and cardiovascular diseases. Mast cells respond environmental changes by initiating regulated exocytosis/secretion of various biologically active compounds called mediators (e.g., proteases, amines and cytokines). Many of these mediators are stored in granules/lysosomes and rely on an intricate degranulation process for release. Mast cell stabilizers (such as sodium cromoglicate) which prevent such degranulation process have therefore been clinically approved to treat asthma and allergic rhinitis. However, it has become increasingly clear that different mast cell diseases often involve multiple mediators, which seem to rely on overlapping but distinct mechanisms for release. This review highlights the evidence for diverse exocytic pathways and discusses strategies to identify unique molecular components in these pathways which could serve as new drug targets for more effective and specific treatments against mast cell-related diseases.

Keywords

membrane fusion; exocytosis; SNARE; Munc18; mast cell; degranulation

Introduction

Mast cells are best known for their ability to release a diverse range of biologically active compounds (aka mediators) when the cell surface receptors bind to their specific ligands [1]. The eventual targets of signaling cascades include the transcriptional machinery (i.e., to produce cytokines etc.), the cytoskeleton network (i.e., to facilitate granule translocation) and the membrane fusion machinery (i.e., to promote vesicular/granular fusion) [2, 3]. Much progress has been made in understanding the upstream events in the intricate signaling cascades [4, 5]. This review, however, intends to draw readers' attention to the interface between signaling and the exocytic fusion machinery, because different modes of activation at the cell surface are shown to generate different secretory profiles (Fig. 1) [6, 7]. This has

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important implication to mast cell-related diseases that involve different mediators as well as different modes of activation.

Mast cell mediators in health and disease

Mast cell mediators can be divided into two groups, preformed (including histamine, tryptase and β-hexosaminidase, etc.) and newly synthesized (cytokines, chemokines, prostaglandin and leukotriens, etc.). Some (such as TNFα) belong to both categories. For detailed description of different mediators please consult recent reviews [8, 9]. It is important to note that the *de novo* synthesized cytokines and chemokines are thought to undergo constitutive secretion [10], which means their release is regulated at the transcription level, contrary to the preformed the mediators.

Native immunity

Mast cells from human and rodent sources have the capacity to directly respond to the challenge of pathogens and their products by releasing preformed mediators, newly synthesized mediators, or both [11]. For example, *E.coli* induces the secretion of mediators in both categories [12–15] but *S.pneumonia* only elicits the release of preformed mediators [16, 17]. Meanwhile, HIV [18, 19], Dengue virus [18, 19], and cholera toxin [18, 19] seem to exclusively impact the newly synthesized mediators. Thus far, it is not clear whether specific subsets of the mediators within either group are selectively released to combat against different pathogens.

Allergic inflammation

In respiratory disorders, mast cells are known for their accidental or mistaken activation via cross-linking of surface-bound IgE which leads to rapid degranulation, mediator release (e.g., histamine, PDT2, tryptase, Cys-LTs) and manifestation of an acute phase allergic reaction [20]. Apart from their pro-inflammatory actions, mast cells have an impressive capability to down-regulate immunological responses, by releasing the anti-inflammatory cytokine IL-10 [21]. Another anti-inflammatory action is through the release of mast cell granule proteases to degrade and neutralize key cytokines such as TNFα, IL-4, IL-13 and IL-33 [22, 23]. Thus, mast cells act as local immune modulators which coordinate the delicate balance between pro- and anti-inflammatory responses of the host.

Autoimmunity

Mast cells are associated with a variety of autoimmune diseases ranging from multiple sclerosis (MS), rheumatoid arthritis (RA), to bullous pemphigoid (BP) [24]. Studies of murine models of MS (EAE, or Experimental autoimmune encephalomyelitis), RA and BP have revealed common underlying mechanisms of mast cell influence on these diseases [25]. For instance, in primary progressive EAE, mast cell-derived TNFα and tryptase are intimately associated with disease onset and development [26–30]. Similarly in BP, the exocytosis of preformed mediators including tryptase, histamine, and TNFα from skin mast cells result in an accumulation of neutrophils and skin blistering [31–35]. In RA, the *de novo* synthesis of TNFα by mast cells results in IL-1β release from macrophages, and subsequent increase in inflammatory cell infiltration in synovial joints [36]. Synovial inflammation can

also be augmented by mast cell-derived tryptase that promotes synovial fibroblasts to express neutrophil-recruiting chemokines [37].

Mast cell activation in autoimmune diseases such as RA likely involves several pathways, including autoantibodies, Toll-Like Receptor ligands and cytokines, each via a distinct cell surface receptor [38]. These pathways are thought to cooperate to create the pro-inflammatory environment which eventually results in tissue destruction. The development of biologic agents that target various immune mediators and their receptors has dramatically improved the patient prognosis. To date, established and approved therapies for rheumatoid arthritis are designed specifically to block cytokine responses toward TNFα and IL-6 [39].

Cardiovascular diseases

Cardiac mast cell activation/infiltration has been reported in a number of cardiac conditions including idiopathic cardiomyopathy [40], atherosclerosis [41], myocarditis [42] and ischemic heart disease [40]. The release of mast cell mediators (histamine, TNFα, IL-6, platelet activating factor and reactive oxygen species, etc.) leads to an inflammatory cascade that is detrimental to myocardial contractile function, tissue integrity and electrophysiological activity, and as expected, treatment with mast cell stabilizers has been shown to reduce the extent of cellular injury [43]. Interestingly, both cardiovascular disease risk factor endothelin-1[44] and cardioprotector adrenomedullin [45] were shown to induce cardiac mast cell degranulaiton [43]. Whether these two peptides impose opposite effects on cardiovascular diseases by eliciting distinct degranulation secretory pathways is currently not known.

Cancer

Mast cells promote tumorigenesis and tumor progression via a number of mechanisms. Mast cells may stimulate tumor expansion by releasing cytokines and growth factors (e.g., FGF-2, NGF, PDGF, IL-10 and IL-8) in the tumor stroma[46]. Mast cells also provide histamine, which induces tumor cell proliferation through H1 receptors while suppressing the immune system through H2 receptors [47]. Additionally, mast cell-derived angiogenic factors [48, 49] and matrix metalloproteinases [50, 51] induce tumor vascularization and metastasis. Furthermore, mast cells cause immunosuppression through the release of IL-10 and TNFα [52, 53], which facilitates tumorigenesis and progression. By contrast, mast cells play inhibitory roles in tumor cell growth, apoptosis, and inflammation by releasing cytokines such as IL-1, IL-4, IL-6, MCP-3, MCP-4, TGF, and chymase [46]. Other anti-cancer mediators include chondroitin sulphate which inhibits tumor cell diffusion, and tryptase which causes tumor cell disruption [54]. To control the differential release of these pro- and anti- cancer mediators may become strategically important in the fight against cancer.

Fusion machinery underlying mast cell exocytosis

Like other eukaryotic fusion events along the secretory and endocytic pathways, mast cell exocytosis depends on the specific interaction of SNAREs that are conserved from yeast to human. Anchored to apposed membranes, cognate SNAREs form a fusogenic 4-helical bundle, the so-called trans-SNARE complex [55–57]. SNAREs can be sorted into the Q- and

R- classes, depending on whether they contribute a glutamyl (Q) or an arginyl (R) side chain at the center of the helical bundle [58, 59]. A functional/fusogenic trans-SNARE complex is typically formed by three Q-SNAREs (Qa, b, c) that are emanated from one membrane and one R-SNARE emanated from the other [55]. It is largely accepted a different set of SNAREs are required for distinct fusion events, and that the specificity of vesicular transport is in part encoded by the specific interaction of the cognate SNAREs.

In mast cells at least seven exocytic SNAREs have been identified [60–68], which include two Qa-SNAREs (syntaxin3, 4), one Qb,c-SNARE (SNAP23; contributing two helices), and four R-SNAREs (VAMP2, 3, 7, 8). Our lab has recently reported that these Q- and R-SNAREs could form 8 distinct fusogenic trans-SNARE complexes in reconstitution [69], suggesting that mast cells could exploit distinct exocytic fusion machineries to release its mediators.

Heterogeneity of exocytic fusion in mast cell - from a theoretical perspective

A close look at the different type of exocytic events in mast cell helps explain the requirement for different sets of SNAREs. Both constitutive and regulated secretions are known to occur in mast cells [10]. Constitutive secretion depends on Golgi-derived vesicles that fuse directly with the plasma membrane. Newly synthesized cytokines are thought to get released via this route, and probably require at least one unique set of SNAREs. Regulated secretion applies to cargos which are pre-stored in granules. In response to extracellular cues, mast cells may undergo signaling-dependent anaphylactic degranulation (AND) [70], in which granules fuse homotypically with each other and heterotypically to the plasma membrane. These two types of fusion may require different molecular machineries. In fact, localization studies of resting murine mast cells indicate that secretory granules are decorated with R-SNAREs VAMP2, 3, 7, 8 [61, 62, 67, 68, 71, 72] and Qa-SNARE syntaxin3 [62, 67, 68, 71], whereas the plasma membrane is enriched with Qa-SNARE syntaxin4 [68, 73] and Qb,c-SNARE SNAP23 [61, 68]. During mast cell compound degranulation, syntaxin3 relocates from the granules to the plasma membrane [64], whereas SNAP23 relocates from the plasma membrane to the secretory granules [68]. These observations implicate syntaxin3 in homotypic fusion and syntaxin4 in heterotypic fusion. In contrast to AND described above, piecemeal degranulation (PMD) has been observed via ultra-structural studies [74]. In PMD, granule-associated materials are packaged into small vesicles that subsequently travel to and fuse with cell surface. Interestingly, both AND and PMD could happen within the same mast cell [75].

What further confounds our understanding of the regulation of mast cell exocytosis is the existence of granule subpopulations. At the ultrastructural level, human mast cell granules display scrolls, crystalline arrays, particles, or variable mixtures of any combination of two or all of these patterns [76]. Mast cells with these granule substructural patterns can be found in any human tissue [77]. Other granule patterns occur much less frequently, which include completely homogeneously dense, finely granular and reticular/beaded granules [76, 77]. Meanwhile, granules can also be classified into different groups based on their protein

contents and accessibility to different tracers [78]. Moon et al [10] recently proposed three types of secretory granules in mast cells: type I granules contain MHC class II, β-hexosaminidase, LAMP-1, LAMP-2, and M6PR but not serotonin (resembling a classical lysosome); type II granules contain the same set as mentioned above plus serotonin (perhaps a late secretory lysosome); type III granules contain β-hexosaminidase and serotonin but not MHC class II. Although mast cell degranulation is a term often linked to type II granules, the extent of participation of the other two types of granules in mast cell exocytosis has not been resolved.

Finally, it is important to note that even in PMD, different types of vesicles might be formed, containing different cargo and SNAREs. These different types of vesicles may form and fuse in response to different regulatory signals. This could be yet another mechanism used in the differential release of mast cell mediators, a topic to be discussed in the next section.

Functional Evidence for distinct degranulation pathways

Distinct pathways for newly synthesized mediators

The traditional assumption which has over relied on the release of β-hexosaminidase (which is present in all three types of granules [10, 78, 79]) as the hallmark for degranulation [80], has delayed the comparative investigation of the selective discharge of key mediators. However, pioneering studies by Blank and colleagues using bone marrow-derived mast cells (BMMCs) isolated from VAMP8 null mice indicate that preformed mediators (e.g., histamine and β-hexosaminidase) and newly synthesized cytokines and chemokines (IL-6, MIP-1α) exploit distinct set of SNAREs for IgE/FcεRI-mediated release [62].

More recently, human mast cells (isolated from intestine) were used to examine the IgE/FcεRI-dependent release of *de novo* synthesized chemokines (e.g., CXCL8, CCL2, CCL3, and CCL4) [81]. Partially permeabilized cells were incubated with antibodies to inhibit individual SNAREs before stimulation. While inhibition of syntaxin4 (Qa-SNARE) or VAMP8 (R-SNARE) resulted in a reduced release of CXCL8 (but not of CCL2, CCL3, or CCL4), inhibition of syntaxin6 (Qc-SNARE) attenuated the release of CXCL8 and CCL2, and inhibition of VAMP7 (R-SNARE) that of CCL3. In contrast, syntaxin3 (Qa-SNARE) and SNAP23 (Qbc-SNARE) are crucial for the release of all 4 chemokines, which suggests, in accordance with the 3Q: 1R rule, certain chemokines (e.g., CXCL8 and CCL2) involve at least 2 sets of SNAREs for release. How these 2 sets of SNAREs are differentially regulated in a signaling-dependent fashion is not well understood. Nevertheless, these observations are consistent with the finding that eosinophils – granulocytes related to mast cells – are capable of releasing different cytokines in a highly selective manner [82, 83].

Distinct pathways for preformed mediators

To-date there has been a lack of systematic effort to characterize the different degranulation pathways for preformed mediators. Published studies of human mast cells initially showed that histamine release requires syntaxin4 (Qa-SNARE), SNAP23 (Qbc-SNARE), and both VAMP7 (R-SNARE) and VAMP8 (R-SNARE) [65]. However, whether VAMP8 indeed plays a crucial role in histamine release from rodent cells has become controversial because

BMMCs isolated from VAMP8 knockout mice demonstrated modest reduction of histamine release in one study [62] while no reduction at all in another [72]. Furthermore, depletion of VAMP8 using siRNA, which inhibits β-hexosaminidase release, fails to affect histamine release from RBL-2H3 cells [60]. We favor a model which suggests that VAMP8 is required for the regulated release of serotonin rather than histamine because i) serotonin secretion is completely blocked in VAMP8 knockout mast cells [72]; ii) histamine and serotonin are enriched in distinct populations of granules (with only 20% overlap) [72]; and iii) regulated secretion of serotonin without comparable histamine release has been observed in rat mast cells [84, 85]. TNFα secretion on the other hand, is processed via a VAMP8-independent pathway [62, 72]. Based on the co-localization data [62] as well as functional studies of TNFα secretion from human synovial sarcoma cells [86], we propose the degranulation pathway for TNFα utilizes a distinctive set of SNAREs that includes VAMP3.

Differential regulation of distinct pathways

Addition fusion factors in exocytosis

Although SNAREs are at the core of exocytic fusion, exocytosis is an intricate process that required multiple factors to regulate three successive steps: 1) docking, 2) priming, and 3) merger of lipid bilayers [87–89]. Docking refers to the retention of transport vesicles to the target membrane (e.g., plasma membrane) [90], which is facilitated by membrane-tethering factor exocyst [91]. Exocyst is a multi-subunit protein complex that brings vesicles and target membranes into proximity by exploiting interactions with lipids and/or membrane-anchored proteins (e.g., Rab GTPase) [92]. Priming renders docked vesicles competent for Ca^{2+}-triggered exocytosis, through the activities of Munc13/CAPS and other factors including NSF [87, 93–95]. Priming results in partially assembled trans-SNARE complexes. In the final step of exocytosis, Ca^{2+} influx is thought to activate synaptotagmin, which relieves the inhibition imposed by complexin while cooperating with the trans-SNARE complex to merge the apposed lipid bilayers [96, 97]. The activities of all these factors could be modulated via signaling-dependent modification (e.g., reversible phosphorylation) to provide temporarily regulation for mediator release. However, emerging evidence suggests that the differential release of mast cell mediators is likely controlled by the different sets of SNAREs and through their interactions with Munc18 proteins [62, 65, 69, 72, 81].

Munc18 proteins act at various steps of exocytic fusion by exploiting different modes of association with the fusion machinery [98]. All three Munc18 isoforms specific for regulated exocytosis in mammals are implicated in mast cell degranulation, particularly Munc18b. Munc18b knockdown or overexpression in RBL-2H3 cells inhibited IgE/FceRI-mediated β-hexosaminidase release [73, 99]. Co-immunoprecipitation and immunofluorescence studies showed that Munc18b associated with syntaxin3 on both granules and the plasma membrane [73, 99, 100]. Munc18c, on the other hand, appears to interact specifically with syntaxin4 on the plasma membrane [99], although the functional relevance of the interaction has yet to be established. Munc18a was thought to function mainly in neurotransmission, but has now been identified in non-neuronal tissues [101–103]. Recently, a double knockdown of Munc18a and Munc18b in RBL cells was found to eliminate β–hexosaminidase release, yet the phenotype was effectively rescued by reintroducing Munc18a alone [104]. Using

reconstitution, we and others have shown that Munc18a and Munc18c effectively and specifically promote VAMP2- and VAMP3- mediated fusion reactions [69, 105], but the cognate Munc18 proteins for VAMP7- or VAMP8- based reactions have yet to identified. We propose that either SNARE and/or Munc18 modification might be necessary to kick start VAMP7- and VAMP8- mediated exocytosis in activated mast cells.

SNARE phosphorylation in exocytosis

The importance of SNARE phosphorylation in exocytosis has been recognized for decades [106, 107]. Biochemically, these modifications either regulate SNARE interactions with their binding partners including cognate SNAREs or Munc18 proteins (Table 1). In mast cells, site-specific phosphorylation of exocytic SNAREs has been reported for VAMP8, SNAP23 and sytnaxin3. PKC-dependent phosphorylation of VAMP8 targets residues inside the SNARE domain, and appears to reduce both reconstituted fusion and the regulated release of β-hexosaminidase from RBL-2H3 cells [108]. IKK2-dependent phosphorylation of SNAP23 at Ser95 and Ser120 increases SNAP23 binding to syntaxin4 and VAMP2 *in vitro*, and is required for optimal degranulation *in vivo* [66]. On the other hand, CaMKII-dependent phosphorylation of sytnaxin3 at Thr14 negatively regulates mast cell exocytosis, likely by inhibiting syntaxin3-Munc18b interaction [109]. Meanwhile, we have preliminary data suggesting that both VAMP7 and syntaxin4 are also phosphorylated in RBL-2H3 cells (Xu, unpublished). It is rather conceivable that these phosphorylation events play key roles in the differential regulation of mediator release.

Munc18 phosphorylation in exocytosis

Reversible phosphorylation of Munc18 has been widely exploited to connect signaling cascades with the fusion apparatus. In neurotransmission and chromaffin cell exocytosis, PKC-dependent phosphorylation of Munc18a at Ser306 and Ser313 reduces its affinity for syntaxin1 and changes the kinetics of transmitter/vesicle release [110, 111]. In contrast, Dyrk1A-dependent phosphorylation of Munc18a at Thr479 in embryonic kidney cells enhances Munc18a binding to syntaxin1 [112]. Polarized secretion in rabbit gastric parietal cells requires CDK5-dependent phosphorylation of Munc18b at Thr572 (Thr573 in rat), which promotes the assembly of the Munc18b/VAMP2/syntaxin3/SNAP25 tetracomplex [113]. For GLUT4 exocytosis in adipocytes and muscle cells, Insulin receptor-mediated Munc18c phosphorylation at Y521 appears to facilitate SNARE complex formation between VAMP2, syntaxin4, and SNAP23 [114]. Collectively, these studies indicate that i) signaling-dependent Munc18 phosphorylation is a prevalent regulatory mechanism in exocytosis and ii) Munc18 phosphorylation drives conformational changes to modulate their affinity for binding partners. Besides these site-specific studies, proteomic discovery-mode mass spectrometry has uncovered scores of Munc18 phosphorylation sites from various tissues and cell lines (Fig. 2). However, the functional significance of these sites in mast cell exocytosis has yet to be clarified.

To-date there is no direct evidence connecting Munc18 phosphorylation to mast cell exocytosis. However, mast cell degranulation requires activated PKC pathways [115–118], which are shown to phosphorylate Munc18a [110, 111] and Munc18c [119–121] in a variety of secretory cells. In addition, cGMP-dependent protein kinase (PKG) has been found

critical for degranulation, targeting a number of fusion components including Munc18c [122]. We hypothesize that signaling-dependent phosphorylation of Munc18 proteins provide an important way to regulate the functions of these Munc18 isoforms in distinct exocytic fusion events in activated mast cells.

Future Perspective

Tremendous progress has been made over the decades in understanding the complexity of mast cell exocytosis, and the diverse roles of mast cell mediators in health and disease. The increasing appreciation of the differential release of mast cell mediators will likely lead to more focused investigation on the specific regulation of individual mediators, particularly at the interface between signaling and exocytic fusion. It may also lead to better integration of cell-based secretion assays with “omics” studies, which will generate global insights on the diverse secretory pathways in mast cells. We expect the combination of these approaches to accelerate the development of “smart” drugs to treat mast cell related diseases (e.g., inhibiting the release of pro-inflammatory mediators while promoting the release of anti-inflammatory mediators) in the near future.

Acknowledgments

This work was supported by the National Institute Of Allergy and Infectious Diseases Grant 1R15AI133430-01 to H.X. and S.S.

References

1. Gilfillan AM, Beaven MA. Regulation of mast cell responses in health and disease. Crit Rev Immunol. 2011; 31:475–529. [PubMed: 22321108]
2. Metcalfe DD, Baram D, Mekori YA. Mast cells. Physiol Rev. 1997; 77:1033–1079. [PubMed: 9354811]
3. Nishida K, Yamasaki S, Ito Y, Kabu K, Hattori K, Tezuka T, Nishizumi H, Kitamura D, Goitsuka R, Geha RS, Yamamoto T, Yagi T, Hirano T. Fc{epsilon}RI-mediated mast cell degranulation requires calcium-independent microtubule-dependent translocation of granules to the plasma membrane. J Cell Biol. 2005; 170:115–126. [PubMed: 15998803]
4. Kalesnikoff J, Galli SJ. New developments in mast cell biology. Nat Immunol. 2008; 9:1215–1223. [PubMed: 18936782]
5. Draber P, Halova I, Polakovicova I, Kawakami T. Signal transduction and chemotaxis in mast cells. Eur J Pharmacol. 2016; 778:11–23. [PubMed: 25941081]
6. Gilfillan AM, Tkaczyk C. Integrated signalling pathways for mast-cell activation. Nat Rev Immunol. 2006; 6:218–230. [PubMed: 16470226]
7. Theoharides TC, Alysandratos KD, Angelidou A, Delivanis DA, Sismanopoulos N, Zhang B, Asadi S, Vasiadi M, Weng Z, Miniati A, Kalogeromitros D. Mast cells and inflammation. Biochim Biophys Acta. 12; 1822:21–33. [PubMed: 21185371]
8. Wernersson S, Pejler G. Mast cell secretory granules: armed for battle. Nat Rev Immunol. 2014; 14:478–494. [PubMed: 24903914]
9. Lundequist A, Pejler G. Biological implications of preformed mast cell mediators. Cell Mol Life Sci. 2011; 68:965–975. [PubMed: 21069421]
10. Moon TC, Befus AD, Kulka M. Mast cell mediators: their differential release and the secretory pathways involved. Front Immunol. 2014; 5:569. [PubMed: 25452755]
11. Shelburne CP, Abraham SN. The mast cell in innate and adaptive immunity. Adv Exp Med Biol. 2011; 716:162–185. [PubMed: 21713657]

12. Malaviya R, Ikeda T, Ross E, Abraham SN. Mast cell modulation of neutrophil influx and bacterial clearance at sites of infection through TNF-alpha. Nature. 1996; 381:77–80. [PubMed: 8609993]
13. Church MK, Norn S, Pao GJ, Holgate ST. Non-IgE-dependent bacteria-induced histamine release from human lung and tonsillar mast cells. Clin Allergy. 1987; 17:341–353. [PubMed: 2441900]
14. Brzezinska-Blaszczyk E, Wasiela M. Vaginal bacterial flora activates rat peritoneal mast cells. Int J Immunopathol Pharmacol. 2002; 15:233–238. [PubMed: 12575924]
15. Malaviya R, Ross EA, MacGregor JI, Ikeda T, Little JR, Jakschik BA, Abraham SN. Mast cell phagocytosis of FimH-expressing enterobacteria. J Immunol. 1994; 152:1907–1914. [PubMed: 8120397]
16. Di Nardo A, Vitiello A, Gallo RL. Cutting edge: mast cell antimicrobial activity is mediated by expression of cathelicidin antimicrobial peptide. J Immunol. 2003; 170:2274–2278. [PubMed: 12594247]
17. Barbuti G, Moschioni M, Censini S, Covacci A, Montecucco C, Montemurro P. Streptococcus pneumoniae induces mast cell degranulation. Int J Med Microbiol. 2006; 296:325–329. [PubMed: 16530009]
18. Brown MG, King CA, Sherren C, Marshall JS, Anderson R. A dominant role for FcgammaRII in antibody-enhanced dengue virus infection of human mast cells and associated CCL5 release. J Leukoc Biol. 2006; 80:1242–1250. [PubMed: 16940332]
19. King CA, Anderson R, Marshall JS. Dengue virus selectively induces human mast cell chemokine production. J Virol. 2002; 76:8408–8419. [PubMed: 12134044]
20. Lei Y, Gregory JA, Nilsson GP, Adner M. Insights into mast cell functions in asthma using mouse models. Pulm Pharmacol Ther. 2013; 26:532–539. [PubMed: 23583635]
21. Ishizuka T, Okayama Y, Kobayashi H, Mori M. Interleukin-10 is localized to and released by human lung mast cells. Clin Exp Allergy. 1999; 29:1424–1432. [PubMed: 10520066]
22. Piliponsky AM, Chen CC, Rios EJ, Treuting PM, Lahiri A, Abrink M, Pejler G, Tsai M, Galli SJ. The chymase mouse mast cell protease 4 degrades TNF, limits inflammation, and promotes survival in a model of sepsis. Am J Pathol. 2012; 181:875–886. [PubMed: 22901752]
23. Waern I, Karlsson I, Thorpe M, Schlenner SM, Feyerabend TB, Rodewald HR, Abrink M, Hellman L, Pejler G, Wernersson S. Mast cells limit extracellular levels of IL-13 via a serglycin proteoglycan-serine protease axis. Biol Chem. 2012; 393:1555–1567. [PubMed: 23667909]
24. Xu Y, Chen G. Mast cell and autoimmune diseases. Mediators Inflamm. 2015; 2015:246126. [PubMed: 25944979]
25. Walker ME, Hatfield JK, Brown MA. New insights into the role of mast cells in autoimmunity: evidence for a common mechanism of action? Biochim Biophys Acta. 2012; 1822:57–65. [PubMed: 21354470]
26. Sayed BA, Christy AL, Walker ME, Brown MA. Meningeal mast cells affect early T cell central nervous system infiltration and blood-brain barrier integrity through TNF: a role for neutrophil recruitment? J Immunol. 2007; 184:6891–6900.
27. Secor VH, Secor WE, Gutekunst CA, Brown MA. Mast cells are essential for early onset and severe disease in a murine model of multiple sclerosis. J Exp Med. 2000; 191:813–822. [PubMed: 10704463]
28. Roznieckl JJ, Hauser SL, Stein M, Lincoln R, Theoharides TC. Elevated mast cell tryptase in cerebrospinal fluid of multiple sclerosis patients. Ann Neurol. 1995; 37:63–66. [PubMed: 7818259]
29. Lock C, Hermans G, Pedotti R, Brendolan A, Schadt E, Garren H, Langer-Gould A, Strober S, Cannella B, Allard J, Klonowski P, Austin A, Lad N, Kaminski N, Galli SJ, Oksenberg JR, Raine CS, Heller R, Steinman L. Gene-microarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomyelitis. Nat Med. 2002; 8:500–508. [PubMed: 11984595]
30. Johnson D, Seeldrayers PA, Weiner HL. The role of mast cells in demyelination. 1. Myelin proteins are degraded by mast cell proteases and myelin basic protein and P2 can stimulate mast cell degranulation. Brain Res. 1988; 444:195–198. [PubMed: 2451996]
31. Dvorak AM, Mihm MC Jr, Osage JE, Kwan TH, Austen KF, Wintroub BU. Bullous pemphigoid, an ultrastructural study of the inflammatory response: eosinophil, basophil and mast cell granule

changes in multiple biopsies from one patient. J Invest Dermatol. 1982; 78:91–101. [PubMed: 7035575]

32. Baba T, Sonozaki H, Seki K, Uchiyama M, Ikesawa Y, Toriisu M. An eosinophil chemotactic factor present in blister fluids of bullous pemphigoid patients. J Immunol. 1976; 116:112–116. [PubMed: 1107422]
33. Katayama I, Doi T, Nishioka K. High histamine level in the blister fluid of bullous pemphigoid. Arch Dermatol Res. 1984; 276:126–127. [PubMed: 6372708]
34. D'Auria L, Pietravalle M, Cordiali-Fei P, Ameglio F. Increased tryptase and myeloperoxidase levels in blister fluids of patients with bullous pemphigoid: correlations with cytokines, adhesion molecules and anti-basement membrane zone antibodies. Exp Dermatol. 2000; 9:131–137. [PubMed: 10772387]
35. Brockow K, Abeck D, Hermann K, Ring J. Tryptase concentration in skin blister fluid from patients with bullous skin conditions. Arch Dermatol Res. 1996; 288:771–773. [PubMed: 8950458]
36. Sandler C, Lindstedt KA, Joutsiniemi S, Lappalainen J, Juutilainen T, Kolah J, Kovanen PT, Eklund KK. Selective activation of mast cells in rheumatoid synovial tissue results in production of TNF-alpha, IL-1beta and IL-1Ra. Inflamm Res. 2007; 56:230–239. [PubMed: 17607547]
37. Shin K, Nigrovic PA, Crish J, Boilard E, McNeil HP, Larabee KS, Adachi R, Gurish MF, Gobezie R, Stevens RL, Lee DM. Mast cells contribute to autoimmune inflammatory arthritis via their tryptase/heparin complexes. J Immunol. 2009; 182:647–656. [PubMed: 19109198]
38. Suurmond J, van der Velden D, Kuiper J, Bot I, Toes RE. Mast cells in rheumatic disease. Eur J Pharmacol. 2016; 778:116–124. [PubMed: 25943290]
39. Smolen JS, Aletaha D, Koeller M, Weisman MH, Emery P. New therapies for treatment of rheumatoid arthritis. Lancet. 2007; 370:1861–1874. [PubMed: 17570481]
40. Patella V, Marino I, Arbustini E, Lamparter-Schummert B, Verga L, Adt M, Marone G. Stem cell factor in mast cells and increased mast cell density in idiopathic and ischemic cardiomyopathy. Circulation. 1998; 97:971–978. [PubMed: 9529265]
41. Pouchlev A, Youroukova Z, Kiprov D. A study of changes in the number of mast cells in the human arterial wall during the stages of development of atherosclerosis. J Atheroscler Res. 1966; 6:342–351. [PubMed: 5975281]
42. Estensen RD. Eosinophilic myocarditis: a role for mast cells? Arch Pathol Lab Med. 1984; 108:358–359. [PubMed: 6546856]
43. Walsh SK, Kane KA, Wainwright CL. Mast cells, peptides and cardioprotection - an unlikely marriage? Auton Autacoid Pharmacol. 2009; 29:73–84. [PubMed: 19566747]
44. Yammine L, Kang DH, Baun MM, Meininger JC. Endothelin-1 and psychosocial risk factors for cardiovascular disease: a systematic review. Psychosom Med. 2014; 76:109–121. [PubMed: 24434952]
45. Wong HK, Cheung TT, Cheung BM. Adrenomedullin and cardiovascular diseases. JRSM Cardiovasc Dis. 2012:1.
46. Ribatti D. Mast cells as therapeutic target in cancer. Eur J Pharmacol. 2015; 778:152–157. [PubMed: 25917325]
47. Fitzsimons C, Molinari B, Duran H, Palmieri M, Davio C, Cricco G, Bergoc R, Rivera E. Atypical association of H1 and H2 histamine receptors with signal transduction pathways during multistage mouse skin carcinogenesis. Inflamm Res. 1997; 46:292–298. [PubMed: 9297573]
48. Norrby K, Jakobsson A, Sorbo J. Mast-cell secretion and angiogenesis, a quantitative study in rats and mice. Virchows Arch B Cell Pathol Incl Mol Pathol. 1989; 57:251–256. [PubMed: 2474890]
49. Marone G, Varricchi G, Loffredo S, Granata F. Mast cells and basophils in inflammatory and tumor angiogenesis and lymphangiogenesis. Eur J Pharmacol. 2015; 778:146–151. [PubMed: 25941082]
50. Tanaka A, Yamane Y, Matsuda H. Mast cell MMP-9 production enhanced by bacterial lipopolysaccharide. J Vet Med Sci. 2001; 63:811–813. [PubMed: 11503911]
51. Koskivirta I, Rahkonen O, Mayranpaa M, Pakkanen S, Husheem M, Sainio A, Hakovirta H, Laine J, Jokinen E, Vuorio E, Kovanen P, Jarvelainen H. Tissue inhibitor of metalloproteinases 4 (TIMP4) is involved in inflammatory processes of human cardiovascular pathology. Histochem Cell Biol. 2006; 126:335–342. [PubMed: 16521002]

52. Ullrich SE, Nghiem DX, Khaskina P. Suppression of an established immune response by UVA--a critical role for mast cells. Photochem Photobiol. 2007; 83:1095–1100. [PubMed: 17880504]
53. Grimbaldeston MA, Nakae S, Kalesnikoff J, Tsai M, Galli SJ. Mast cell-derived interleukin 10 limits skin pathology in contact dermatitis and chronic irradiation with ultraviolet B. Nat Immunol. 2007; 8:1095–1104. [PubMed: 17767162]
54. Ribatti D, Vacca A. The role of inflammatory cells in angiogenesis in multiple myeloma. Adv Exp Med Biol. 2014; 816:361–376. [PubMed: 24818730]
55. Jahn R, Scheller RH. SNAREs--engines for membrane fusion. Nat Rev Mol Cell Biol. 2006; 7:631–643. [PubMed: 16912714]
56. Rothman JE. The principle of membrane fusion in the cell (Nobel lecture). Angew Chem Int Ed Engl. 2014; 53:12676–12694. [PubMed: 25087728]
57. Han J, Pluhackova K, Bockmann RA. The Multifaceted Role of SNARE Proteins in Membrane Fusion. Front Physiol. 2017; 8:5. [PubMed: 28163686]
58. Fasshauer D, Sutton RB, Brunger AT, Jahn R. Conserved structural features of the synaptic fusion complex: SNARE proteins reclassified as Q- and R-SNAREs. Proc Natl Acad Sci U S A. 1998; 95:15781–15786. [PubMed: 9861047]
59. Kloepper TH, Kienle CN, Fasshauer D. An elaborate classification of SNARE proteins sheds light on the conservation of the eukaryotic endomembrane system. Mol Biol Cell. 2007; 18:3463–3471. [PubMed: 17596510]
60. Woska JR Jr, Gillespie ME. Small-interfering RNA-mediated identification and regulation of the ternary SNARE complex mediating RBL-2H3 mast cell degranulation. Scand J Immunol. 2011; 73:8–17. [PubMed: 21128998]
61. Paumet F, Le Mao J, Martin S, Galli T, David B, Blank U, Roa M. Soluble NSF attachment protein receptors (SNAREs) in RBL-2H3 mast cells: functional role of syntaxin 4 in exocytosis and identification of a vesicle-associated membrane protein 8-containing secretory compartment. J Immunol. 2000; 164:5850–5857. [PubMed: 10820264]
62. Tiwari N, Wang CC, Brochetta C, Ke G, Vita F, Qi Z, Rivera J, Soranzo MR, Zabucchi G, Hong W, Blank U. VAMP-8 segregates mast cell-preformed mediator exocytosis from cytokine trafficking pathways. Blood. 2008; 111:3665–3674. [PubMed: 18203950]
63. Tiwari N, Wang CC, Brochetta C, Scandiuzzi L, Hong W, Blank U. Increased formation of VAMP-3-containing SNARE complexes in mast cells from VAMP-8 deficient cells. Inflamm Res. 2009; 58(Suppl 1):13–14. [PubMed: 19274433]
64. Brochetta C, Suzuki R, Vita F, Soranzo MR, Claver J, Madjene LC, Attout T, Vitte J, Varin-Blank N, Zabucchi G, Rivera J, Blank U. Munc18-2 and syntaxin 3 control distinct essential steps in mast cell degranulation. J Immunol. 2014; 192:41–51. [PubMed: 24323579]
65. Sander LE, Frank SP, Bolat S, Blank U, Galli T, Bigalke H, Bischoff SC, Lorentz A. Vesicle associated membrane protein (VAMP)-7 and VAMP-8, but not VAMP-2 or VAMP-3, are required for activation-induced degranulation of mature human mast cells. Eur J Immunol. 2008; 38:855–863. [PubMed: 18253931]
66. Suzuki K, Verma IM. Phosphorylation of SNAP-23 by IkappaB kinase 2 regulates mast cell degranulation. Cell. 2008; 134:485–495. [PubMed: 18692471]
67. Hibi T, Hirashima N, Nakanishi M. Rat basophilic leukemia cells express syntaxin-3 and VAMP-7 in granule membranes. Biochem Biophys Res Commun. 2000; 271:36–41. [PubMed: 10777677]
68. Guo Z, Turner C, Castle D. Relocation of the t-SNARE SNAP-23 from lamellipodia-like cell surface projections regulates compound exocytosis in mast cells. Cell. 1998; 94:537–548. [PubMed: 9727496]
69. Xu H, Arnold MG, Kumar SV. Differential Effects of Munc18s on Multiple Degranulation-Relevant Trans-SNARE Complexes. PLoS One. 2015; 10:e0138683. [PubMed: 26384026]
70. Pickett JA, Edwardson JM. Compound exocytosis: mechanisms and functional significance. Traffic. 2006; 7:109–116. [PubMed: 16420520]
71. Puri N, Kruhlak MJ, Whiteheart SW, Roche PA. Mast cell degranulation requires N-ethylmaleimide-sensitive factor-mediated SNARE disassembly. J Immunol. 2003; 171:5345–5352. [PubMed: 14607937]

72. Puri N, Roche PA. Mast cells possess distinct secretory granule subsets whose exocytosis is regulated by different SNARE isoforms. Proc Natl Acad Sci U S A. 2008; 105:2580–2585. [PubMed: 18250339]
73. Tadokoro S, Kurimoto T, Nakanishi M, Hirashima N. Munc18-2 regulates exocytotic membrane fusion positively interacting with syntaxin-3 in RBL-2H3 cells. Mol Immunol. 2007; 44:3427–3433. [PubMed: 17408745]
74. Crivellato E, Nico B, Gallo VP, Ribatti D. Cell secretion mediated by granule-associated vesicle transport: a glimpse at evolution. Anat Rec (Hoboken). 2010; 293:1115–1124. [PubMed: 20340095]
75. Kaminer MS, Murphy GF, Zweiman B, Lavker RM. Connective tissue mast cells exhibit time-dependent degranulation heterogeneity. Clin Diagn Lab Immunol. 1995; 2:297–301. [PubMed: 7664175]
76. Dvorak AM. Human mast cells. Adv Anat Embryol Cell Biol. 1989; 114:1–107. [PubMed: 2658484]
77. Dvorak AM. Ultrastructural morphology of basophils and mast cells. In Blood Cell Biochemistry. 1991:67–99.
78. Raposo G, Tenza D, Mecheri S, Peronet R, Bonnerot C, Desaymard C. Accumulation of major histocompatibility complex class II molecules in mast cell secretory granules and their release upon degranulation. Mol Biol Cell. 1997; 8:2631–2645. [PubMed: 9398681]
79. Baram D, Adachi R, Medalia O, Tuvim M, Dickey BF, Mekori YA, Sagi-Eisenberg R. Synaptotagmin II negatively regulates Ca2+-triggered exocytosis of lysosomes in mast cells. J Exp Med. 1999; 189:1649–1658. [PubMed: 10330444]
80. Lorentz A, Baumann A, Vitte J, Blank U. The SNARE Machinery in Mast Cell Secretion. Front Immunol. 2012; 3:143. [PubMed: 22679448]
81. Frank SP, Thon KP, Bischoff SC, Lorentz A. SNAP-23 and syntaxin-3 are required for chemokine release by mature human mast cells. Mol Immunol. 2011; 49:353–358. [PubMed: 21981832]
82. Melo RC, Spencer LA, Dvorak AM, Weller PF. Mechanisms of eosinophil secretion: large vesiculotubular carriers mediate transport and release of granule-derived cytokines and other proteins. J Leukoc Biol. 2008; 83:229–236. [PubMed: 17875811]
83. Moqbel R, Coughlin JJ. Differential secretion of cytokines. Sci STKE. 2006; 2006:pe26. [PubMed: 16757734]
84. Theoharides TC, Bondy PK, Tsakalos ND, Askenase PW. Differential release of serotonin and histamine from mast cells. Nature. 1982; 297:229–231. [PubMed: 6176873]
85. Theoharides TC, Kops SK, Bondy PK, Askenase PW. Differential release of serotonin without comparable histamine under diverse conditions in the rat mast cell. Biochem Pharmacol. 1985; 34:1389–1398. [PubMed: 2581583]
86. Boddul SV, Meng J, Dolly JO, Wang J. SNAP-23 and VAMP-3 contribute to the release of IL-6 and TNFalpha from a human synovial sarcoma cell line. FEBS J. 2014; 281:750–765. [PubMed: 24373201]
87. Klenchin VA, Martin TF. Priming in exocytosis: attaining fusion-competence after vesicle docking. Biochimie. 2000; 82:399–407. [PubMed: 10865127]
88. Weimer RM, Richmond JE. Synaptic vesicle docking: a putative role for the Munc18/Sec1 protein family. Curr Top Dev Biol. 2005; 65:83–113. [PubMed: 15642380]
89. Jahn R, Fasshauer D. Molecular machines governing exocytosis of synaptic vesicles. Nature. 2012; 490:201–207. [PubMed: 23060190]
90. Toonen RF, Kochubey O, de Wit H, Gulyas-Kovacs A, Konijnenburg B, Sorensen JB, Klingauf J, Verhage M. Dissecting docking and tethering of secretory vesicles at the target membrane. EMBO J. 2006; 25:3725–3737. [PubMed: 16902411]
91. Baker RW, Hughson FM. Chaperoning SNARE assembly and disassembly. Nat Rev Mol Cell Biol. 2016; 17:465–479. [PubMed: 27301672]
92. Dubuke ML, Munson M. The Secret Life of Tethers: The Role of Tethering Factors in SNARE Complex Regulation. Front Cell Dev Biol. 2016; 4:42. [PubMed: 27243006]
93. James DJ, Martin TF. CAPS and Munc13: CATCHRs that SNARE Vesicles. Front Endocrinol (Lausanne). 2013; 4:187. [PubMed: 24363652]

94. Lai Y, Choi UB, Leitz J, Rhee HJ, Lee C, Altas B, Zhao M, Pfuetzner RA, Wang AL, Brose N, Rhee J, Brunger AT. Molecular Mechanisms of Synaptic Vesicle Priming by Munc13 and Munc18. Neuron. 2017; 95:591–607e510. [PubMed: 28772123]
95. Wang S, Choi UB, Gong J, Yang X, Li Y, Wang AL, Brunger AT, Ma C. Conformational change of syntaxin linker region induced by Munc13s initiates SNARE complex formation in synaptic exocytosis. EMBO J. 2017; 36:816–829. [PubMed: 28137749]
96. Rizo J, Xu J. The Synaptic Vesicle Release Machinery. Annu Rev Biophys. 2015; 44:339–367. [PubMed: 26098518]
97. Lai Y, Lou X, Wang C, Xia T, Tong J. Synaptotagmin 1 and Ca2+ drive trans SNARE zippering. Sci Rep. 2014; 4:4575. [PubMed: 24694579]
98. Munson M, Bryant NJ. A role for the syntaxin N-terminus. Biochem J. 2009; 418:e1–3. [PubMed: 19159342]
99. Martin-Verdeaux S, Pombo I, Iannascoli B, Roa M, Varin-Blank N, Rivera J, Blank U. Evidence of a role for Munc18-2 and microtubules in mast cell granule exocytosis. J Cell Sci. 2003; 116:325–334. [PubMed: 12482918]
100. Pombo I, Rivera J, Blank U. Munc18-2/syntaxin3 complexes are spatially separated from syntaxin3-containing SNARE complexes. FEBS Lett. 2003; 550:144–148. [PubMed: 12935901]
101. Schraw TD, Lemons PP, Dean WL, Whiteheart SW. A role for Sec1/Munc18 proteins in platelet exocytosis. Biochem J. 2003; 374:207–217. [PubMed: 12773094]
102. Tellam JT, McIntosh S, James DE. Molecular identification of two novel Munc-18 isoforms expressed in non-neuronal tissues. J Biol Chem. 1995; 270:5857–5863. [PubMed: 7890715]
103. Zhang W, Efanov A, Yang SN, Fried G, Kolare S, Brown H, Zaitsev S, Berggren PO, Meister B. Munc-18 associates with syntaxin and serves as a negative regulator of exocytosis in the pancreatic beta -cell. J Biol Chem. 2000; 275:41521–41527. [PubMed: 11024017]
104. Bin NR, Jung CH, Piggott C, Sugita S. Crucial role of the hydrophobic pocket region of Munc18 protein in mast cell degranulation. Proc Natl Acad Sci U S A. 2013; 110:4610–4615. [PubMed: 23487749]
105. Yu H, Rathore SS, Lopez JA, Davis EM, James DE, Martin JL, Shen J. Comparative studies of Munc18c and Munc18-1 reveal conserved and divergent mechanisms of Sec1/Munc18 proteins. Proc Natl Acad Sci U S A. 2013; 110:E3271–3280. [PubMed: 23918365]
106. Gerst JE. SNAREs and SNARE regulators in membrane fusion and exocytosis. Cell Mol Life Sci. 1999; 55:707–734. [PubMed: 10379359]
107. Snyder DA, Kelly ML, Woodbury DJ. SNARE complex regulation by phosphorylation. Cell Biochem Biophys. 2006; 45:111–123. [PubMed: 16679567]
108. Malmersjo S, Di Palma S, Diao J, Lai Y, Pfuetzner RA, Wang AL, McMahon MA, Hayer A, Porteus M, Bodenmiller B, Brunger AT, Meyer T. Phosphorylation of residues inside the SNARE complex suppresses secretory vesicle fusion. EMBO J. 2016; 35:1810–1821. [PubMed: 27402227]
109. Tadokoro S, Shibata T, Inoh Y, Amano T, Nakanishi M, Hirashima N, Utsunomiya-Tate N. Phosphorylation of syntaxin-3 at Thr 14 negatively regulates exocytosis in RBL-2H3 mast cells. Cell Biol Int. 2016; 40:589–596. [PubMed: 26936588]
110. Barclay JW, Craig TJ, Fisher RJ, Ciufo LF, Evans GJ, Morgan A, Burgoyne RD. Phosphorylation of Munc18 by protein kinase C regulates the kinetics of exocytosis. J Biol Chem. 2003; 278:10538–10545. [PubMed: 12519779]
111. Genc O, Kochubey O, Toonen RF, Verhage M, Schneggenburger R. Munc18-1 is a dynamically regulated PKC target during short-term enhancement of transmitter release. Elife. 2014; 3:e01715. [PubMed: 24520164]
112. Park JH, Jung MS, Kim YS, Song WJ, Chung SH. Phosphorylation of Munc18-1 by Dyrk1A regulates its interaction with Syntaxin 1 and X11alpha. J Neurochem. 2012; 122:1081–1091. [PubMed: 22765017]
113. Liu Y, Ding X, Wang D, Deng H, Feng M, Wang M, Yu X, Jiang K, Ward T, Aikhionbare F, Guo Z, Forte JG, Yao X. A mechanism of Munc18b-syntaxin 3-SNAP25 complex assembly in regulated epithelial secretion. FEBS Lett. 2007; 581:4318–4324. [PubMed: 17716669]

114. Kioumourtzoglou D, Gould GW, Bryant NJ. Insulin stimulates syntaxin4 SNARE complex assembly via a novel regulatory mechanism. Mol Cell Biol. 2014; 34:1271–1279. [PubMed: 24469400]

115. Chakravarty N, Kjeldsen B, Hansen M, Nielsen EH. The involvement of protein kinase C in exocytosis in mast cells. Exp Cell Res. 1990; 186:245–249. [PubMed: 1688804]

116. Koopmann WR Jr, Jackson RC. Calcium- and guanine-nucleotide-dependent exocytosis in permeabilized rat mast cells. Modulation by protein kinase C. Biochem J. 1990; 265:365–373. [PubMed: 1689146]

117. Pernas-Sueiras O, Alfonso A, Vieytes MR, Botana LM. PKC and cAMP positively modulate alkaline-induced exocytosis in the human mast cell line HMC-1. J Cell Biochem. 2006; 99:1651–1663. [PubMed: 16823786]

118. Abdel-Raheem IT, Hide I, Yanase Y, Shigemoto-Mogami Y, Sakai N, Shirai Y, Saito N, Hamada FM, El-Mahdy NA, Elsisy Ael D, Sokar SS, Nakata Y. Protein kinase C-alpha mediates TNF release process in RBL-2H3 mast cells. Br J Pharmacol. 2005; 145:415–423. [PubMed: 15806111]

119. Imai A, Nashida T, Shimomura H. Roles of Munc18-3 in amylase release from rat parotid acinar cells. Arch Biochem Biophys. 2004; 422:175–182. [PubMed: 14759605]

120. Cosen-Binker LI, Lam PP, Binker MG, Gaisano HY. Alcohol-induced protein kinase Calpha phosphorylation of Munc18c in carbachol-stimulated acini causes basolateral exocytosis. Gastroenterology. 2007; 132:1527–1545. [PubMed: 17408632]

121. Reed GL, Houng AK, Fitzgerald ML. Human platelets contain SNARE proteins and a Sec1p homologue that interacts with syntaxin 4 and is phosphorylated after thrombin activation: implications for platelet secretion. Blood. 1999; 93:2617–2626. [PubMed: 10194441]

122. Nanamori M, Chen J, Du X, Ye RD. Regulation of leukocyte degranulation by cGMP-dependent protein kinase and phosphoinositide 3-kinase: potential roles in phosphorylation of target membrane SNARE complex proteins in rat mast cells. J Immunol. 2007; 178:416–427. [PubMed: 17182580]

123. Bennett MK, Miller KG, Scheller RH. Casein Kinase-Ii Phosphorylates the Synaptic Vesicle Protein-P65. Journal of Neuroscience. 1993; 13:1701–1707. [PubMed: 8463845]

124. Dubois T, Kerai P, Learmonth M, Cronshaw A, Aitken A. Identification of syntaxin-1A sites of phosphorylation by casein kinase I and casein kinase II. Eur J Biochem. 2002; 269:909–914. [PubMed: 11846792]

125. Rickman C, Duncan RR. Munc18/Syntaxin Interaction Kinetics Control Secretory Vesicle Dynamics. Journal of Biological Chemistry. 2010; 285:3965–3972. [PubMed: 19748891]

126. Hirling H, Scheller RH. Phosphorylation of synaptic vesicle proteins: Modulation of the alpha SNAP interaction with the core complex. Proceedings of the National Academy of Sciences of the United States of America. 1996; 93:11945–11949. [PubMed: 8876242]

127. Tian JH, Das S, Sheng ZH. Ca2+-dependent phosphorylation of syntaxin-1A by the death-associated protein (DAP) kinase regulates its interaction with Munc18. Journal of Biological Chemistry. 2003; 278:26265–26274. [PubMed: 12730201]

128. Liu XQ, Heidelberger R, Janz R. Phosphorylation of syntaxin 3B by CaMKII regulates the formation of t-SNARE complexes. Mol Cell Neurosci. 2014; 60:53–62. [PubMed: 24680688]

129. Tadokoro S, Shibata T, Inoh Y, Amano T, Nakanishi M, Hirashima N, Utsunomiya-Tate N. Phosphorylation of syntaxin-3 at Thr 14 negatively regulates exocytosis in RBL-2H3 mast cells. Cell Biol Int. 2016; 40:589–596. [PubMed: 26936588]

130. Risinger C, Bennett MK. Differential phosphorylation of syntaxin and synaptosome-associated protein of 25 kDa (SNAP-25) isoforms. Journal of Neurochemistry. 1999; 72:614–624. [PubMed: 9930733]

131. Burgo A, Casano AM, Kuster A, Arold ST, Wang G, Nola S, Verraes A, Dingli F, Loew D, Galli T. Increased activity of the Vesicular Soluble N-Ethylmaleimide-sensitive Factor Attachment Protein Receptor TI-VAMP/VAMP7 by Tyrosine Phosphorylation in the Longin Domain. Journal of Biological Chemistry. 2013; 288:11960–11972. [PubMed: 23471971]

132. Malmersjo S, Di Palma S, Diao JJ, Lai Y, Pfuetzner RA, Wang AL, McMahon MA, Hayer A, Porteus M, Bodenmiller B, Brunger AT, Meyer T. Phosphorylation of residues inside the SNARE

complex suppresses secretory vesicle fusion. Embo Journal. 2016; 35:1810–1821. [PubMed: 27402227]

133. Shu YL, Liu X, Yang Y, Takahashi M, Gillis KD. Phosphorylation of SNAP-25 at ser187 mediates enhancement of exocytosis by a phorbol ester in INS-1 cells. Journal of Neuroscience. 2008; 28:21–30. [PubMed: 18171919]
134. Shimazaki Y, Nishiki T, Omori A, Sekiguchi M, Kamata Y, Kozaki S, Takahashi M. Phosphorylation of 25-kDa synaptosome-associated protein - Possible involvement in protein kinase C-mediated regulation of neurotransmitter release. Journal of Biological Chemistry. 1996; 271:14548–14553. [PubMed: 8662851]
135. Kataoka M, Kuwahara R, Iwasaki S, Shoji-Kasai Y, Takahashi M. Nerve growth factor-induced phosphorylation of SNAP-25 in PC12 cells: A possible involvement in the regulation of SNAP-25 localization. Journal of Neurochemistry. 2000; 74:2058–2066. [PubMed: 10800949]
136. Nagy G, Matti U, Nehring RB, Binz T, Rettig J, Neher E, Sorensen JB. Protein kinase C-dependent phosphorylation of synaptosome-associated protein of 25 kDa at Ser(187) potentiates vesicle recruitment. Journal of Neuroscience. 2002; 22:9278–9286. [PubMed: 12417653]
137. Nagy G, Reim K, Matti U, Brose N, Binz T, Rettig J, Neher E, Sorensen JB. Regulation of releasable vesicle pool sizes by protein kinase A-dependent phosphorylation of SNAP-25. Neuron. 2004; 41:417–429. [PubMed: 14766180]
138. Gao J, Hirata M, Mizokami A, Zhao J, Takahashi I, Takeuchi H, Hirata M. Differential role of SNAP-25 phosphorylation by protein kinases A and C in the regulation of SNARE complex formation and exocytosis in PC12 cells. Cellular Signalling. 2016; 28:425–437. [PubMed: 26721188]
139. Horvath D, Tamas I, Sipos A, Darula Z, Becsi B, Nagy D, Ivan J, Erdodi F, Lontay B. Myosin phosphatase and RhoA-activated kinase modulate neurotransmitter release by regulating SNAP-25 of SNARE complex (vol 12, e0177046, 2017). Plos One. 2017; 12
140. Polgar J, Lane WS, Chung SH, Houng AK, Reed GL. Phosphorylation of SNAP-23 in activated human platelets. Journal of Biological Chemistry. 2003; 278:44369–44376. [PubMed: 12930825]
141. Hepp G, Puri N, Hohenstein AC, Crawford GL, Whiteheart SW, Roche PA. Phosphorylation of SNAP-23 regulates exocytosis from mast cells. Journal of Biological Chemistry. 2005; 280:6610–6620. [PubMed: 15611044]
142. Karim ZA, Zhang JC, Banerjee M, Chicka MC, Al Hawas R, Hamilton TR, Roche PA, Whiteheart SW. I kappa B kinase phosphorylation of SNAP-23 controls platelet secretion. Blood. 2013; 121:4567–4574. [PubMed: 23613522]
143. Wei Y, Wang D, Jin FF, Bian Z, Li LM, Liang HW, Li MZ, Shi L, Pan CY, Zhu DH, Chen X, Hu G, Liu Y, Zhang CY, Zen K. Pyruvate kinase type M2 promotes tumour cell exosome release via phosphorylating synaptosome-associated protein 23. Nature Communications. 2017:8.
144. Pieu Naskar NP. Phosphorylation of SNAP-23 regulates its dynamic subcellular localization and regulated exocytosis in mast cells. The Journal of Immunology. 2017:198.

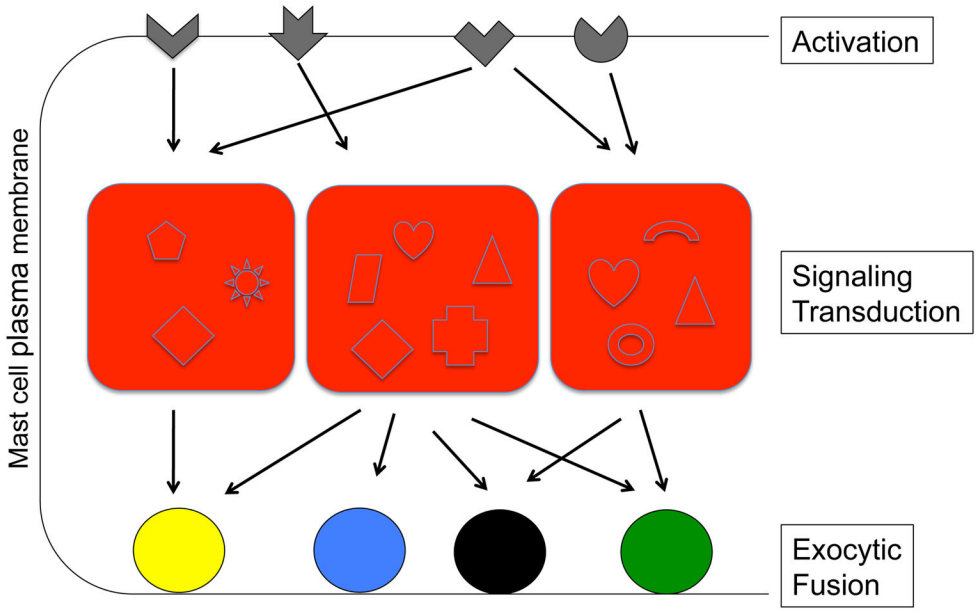

Fig. 1. Differentiation regulation of mast cell exocytosis

Different modes of mast cell activation exploit distinct cell surface receptors which can be broadly categorized into three groups: i) FcRI receptors (including FcεRI and FcγRI, ii) IL-1/TLR/ST2 family of receptors and pathogen recognition receptors (including toll-like receptors, the IL-33 receptor, and NOD-like receptors), and iii) G protein coupled receptors (including adenosine receptors, Prostaglandin E_2 receptors, and sphingosine1-phosphate receptors, C3A and chemokine receptors, neuropeptide receptors, and antimicrobial peptide receptors) [Gilfillan and Beaven (2011)]. Different receptors at mast cell surface seem to rely on distinct signaling transduction mechanisms (in red) to target exocytic fusion events. These different signaling transduction mechanisms may have overlapping signaling transducers.

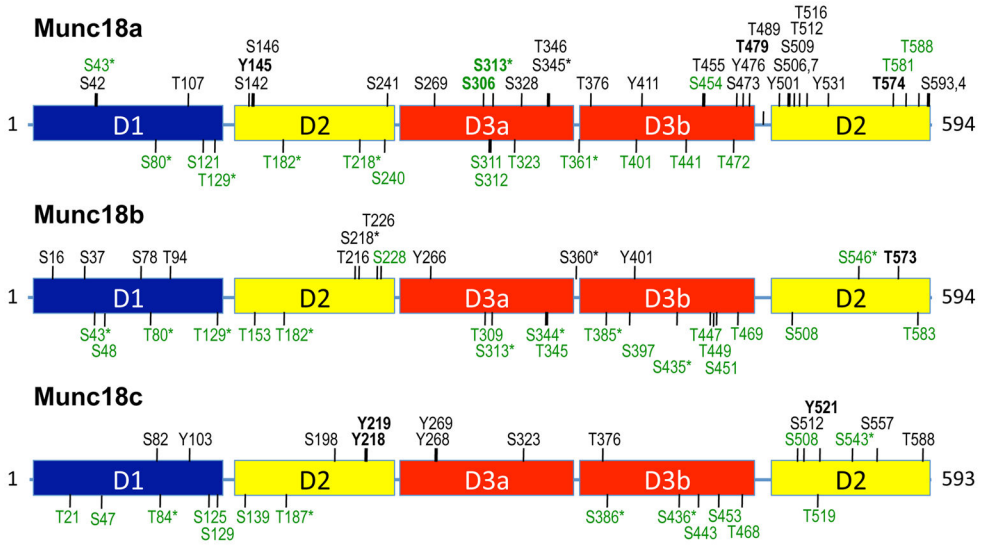

Fig. 2. Predicted or known phosphorylation sites in Munc18

Sites above each schematic representation of the domain structure of Munc18 were identified from various tissues/cells, by either proteomic discovery-mode mass spectrometry (www.phosphosite.org) or site-specific methods (in **bold**). In green are identified or predicted PKC sites (http://kinasephos2.mbc.nctu.edu.tw/). All sites underneath the domain structure are predicted PKC sites. Conserved or semi-conserved PKC sites are indicated by *, according to sequence alignment of rat Munc18 proteins using Clustal Omega. Note that Munc18 domain 2 is split by the insertion of domain 3.

Table 1

Phosphorylation of exocytic SNAREs in mammalian cells

Protein	Species	Phosphorylation site(s)	Responsible kinase(s)	Biochemical effects	Physiological effects	Ref.
Stx1	Rat	Ser14	Casein kinaseII	N/A	Regulates N-terminal interaction with Munc18-1	[123–125]
Stx1	Bovine	N/A	CaMKII	N/A	N/A	[126]
Stx1	Rat	Ser188	DAP kinase	Decreases syntaxin binding to Munc18a	N/A	[127]
Stx3	Mouse	Thr14	CaMKII	Stronger binding affinity for SNAP25	Modulates the assembly of the SNARE complex in ribbon synapses of the retina	[128]
Stx3	Rat	Thr14	CaMKII	Inhibits binding of Stx3 to Munc18b	Negatively regulates mast cell exocytosis	[129]
Stx4	Rat	N/A	Casein kinase II	Reduces interaction between SNAP25 and phosphorylated Stx4	N/A	[130]
VAMP2	Bovine	Ser61	CaMKII	N/A	N/A	[126]
VAMP7	Human	Tyr45	c-Src kinase	Promotes VAMP7 interaction with Q-SNAREs	Promotes insulin-induced exocytosis	[131]
VAMP8	Rat	Ser17, Thr47, Thr53, Ser54	PKC	Reduces SNARE complex formation	Reduces vesicle fusion *in vitro*; suppresses secretion in living cells after vesicle docking	[132]
SNAP25	Bovine	Ser187	PKC	N/A	Enhances exocytosis in bovine chromaffin cells and INS-1 cells	[133]
SNAP25	Bovine	N/A	CaMKII	N/A	N/A	[126]
SNAP25	Rat	Ser187	PKC	Decreases binding with Stx1	Regulates subcellular localization of SNAP25, accelerates vesicle recruitment after emptying	[134–136]
SNAP25	Rat	Thr138	PKA	N/A	Maintains a large number of release-ready primed vesicles	[137]
SNAP25	Rat	Thr138	PKA	Inhibits SNARE complex formation	Inhibits functional exocytosis in PC12 cells	[138]
SNAP25	Rat	Ser187	PKC	Enhances SNARE complex formation	Enhances functional exocytosis in PC12 cells	[138]
SNAP25	Mouse	Thr138	RhoA- activated kinase	Decreases interaction between SNAP25 and syntaxin	Decreases neurotransmitter release	[139]
SNAP23	Human	Ser23, Thr24	PKC	Inhibits Stx4 interaction	Modulates SNARE complex interaction during membrane trafficking and fusion	[140]
SNAP23	Rat	Ser95, Ser120, Ser161	IKK2	Increases binding to Stx4 and VAMP2	Optimal compound exocytosis	[141, 142]
SNAP23	Human	Ser95	Pyruvate kinase type M2	Enables the formation of the SNARE complex	Promotes tumour cell exosome release	[143]
SNAP23	Rat	Ser95, Ser120, Thr102	N/A	N/A	Regulates SNAP23 internal membrane association and subsequently mast cell exocytosis	[144]