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SNARE complex-mediated degranulation in mast cells

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Abstract

Mast cell function and dysregulation is important in the development and progression of allergic and autoimmune disease. Identifying novel proteins involved in mast cell function and disease progression is the first step in the design of new therapeutic strategies. Soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) are a family of proteins demonstrated to mediate the transport and fusion of secretory vesicles to the membrane in mast cells, leading to the subsequent release of the vesicle cargo through an exocytotic mechanism. The functional role[s] of specific SNARE family member complexes in mast cell degranulation has not been fully elucidated. Here, we review recent and historical data on the expression, formation and localization of various SNARE proteins and their complexes in murine and human mast cells. We summarize the functional data identifying the key SNARE family members that appear to participate in mast cell degranulation. Furthermore, we discuss the utilization of RNA interference (RNAi) methods to validate SNARE function and the use of siRNA as a therapeutic approach to the treatment of inflammatory disease. These studies provide an overview of the specific SNARE proteins and complexes that serve as novel targets for the development of new therapies to treat allergic and autoimmune disease.

Keywords: allergy • autoimmune disease • degranulation • granules • mast cells • RNA interference • SNARES

Introduction

Activation of mast cell degranulation has been demonstrated to be an important mediator of allergic disease and more recently, as an initiator or contributor to autoimmune disease [1–4]. Mast cells are granulocytes that emanate from myeloid progenitors in bone marrow and play a critical role in innate immunity as vital sentinel cells that combat invading microorganisms at tissue/environment interfaces [1–4]. Mast cells are phagocytic and can directly destroy pathogens; they also release inflammatory mediators which promote inflammation by recruiting and activating other leukocytes. As regulators of adaptive immunity, mast cells promote antigen presentation, naive T cell differentiation into helper T cells, and induction of acquired immunity towards parasites *via* IgE/FcϵR binding [1–4].

The major contribution of mast cells to both immune function and dysfunction results from the release of a plethora of inflammatory mediators through a process known as regulated exocytosis [1–5]. This process occurs in many cell types and involves the storage of intracellular pools of inflammatory mediators, hormones or neurotransmitters in pre-formed granules/vesicles [5]. Upon activation of the cell, the mediators are released *via* a vesicle fusion mechanism with the plasma membrane. Fusion can be activated through receptor stimulation or by membrane depolarization *via* 2nd messengers, for example Ca^{2+} [6]. The transport, fusion and release of vesicle contents through exocytosis is mediated by a family of proteins known as the SNAREs [7–11]. Soluble N-ethylmaleimide-sensitive factor attachment protein receptors have been demonstrated to play a pivotal role in regulated exocytosis (degranulation) in mast cells [12–22] and represent a mechanical step involved in inflammatory mediators release that can be targeted for the design and development of therapeutics. We review the expression, localization and operation of various functional SNARE complexes in both murine and human mast cells. We evaluate the published functional data that has been used to implicate specific SNAREs and SNARE complexes as indispensable mediators of mast cell degranulation.

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SNARE function in membrane fusion events

Membrane fusion is absolutely essential for normal cell physiology. Vesicular trafficking of essential molecules between cellular compartments and into and out of cells is required for cell function and survival. In neurons, membrane trafficking is required for regulated neurotransmitter release. Neurotransmitter release is widely acknowledged as critical for the development and function of the nervous system in all higher organisms. The SNARE family of proteins mediates the highly regulated processes of vesicular assembly and disassembly [6, 8–10]. These processes are energy-dependent and require many different protein interactions.

Numerous proteins are involved in the formation and disassembly of active SNARE complexes during membrane fusion. ER/Golgi traffic and trans-Golgi traffic all require specific SNARE proteins. Each set of SNARE proteins act as a vesicle loading signal, a mechanical address (delivering the vesicle to the correct target membrane), and in the mechanical process of fusing two opposing membranes. The neuronal and immunological SNARE proteins have a another layer of complexity added to this paradigm, the vesicles are loaded with cargo, but dock and await a chemical fusion signal.

The SNARE family of evolutionarily conserved proteins was first identified in the 1980s in yeast and a decade later in mammalian cells. SNAREs are found in most eukaryotic cells; 25 members have been identified in *Saccharomyces cerevisiae*, 54 members in *Arabidopsis thaliana* and $>$36 members in humans [6]. The proteins are composed of a simple domain structure highlighted by a SNARE motif, a stretch of 60–70 amino acids arranged in a heptad repeat [6–11]. Individual SNAREs on opposing membranes associate into core complexes *via* their SNARE motifs. Core complexes form stable structures, which are composed of four intertwined parallel α-helices contributed by three to four different SNARE members [6–11]. These complexes consist of a central core of three glutamine residues and one arginine residue bordered by hydrophobic stacked layers of side chains. Soluble N-ethylmaleimide sensitive factor attachment protein receptors can be classified on the basis of whether they contain a Q or R residue in their motif and are referred to as either a Qa, Qb, Qc, Qbc, or R-SNAREs based on the position of their contributing motif in the assembled SNARE complex. Each individual class of SNARE proteins contributes a motif to the functional core complex. The vesicle-associated membrane protein (VAMP) family of SNAREs are examples of the R-SNARE sub-type and are characterized by a single transmembrane domain, a SNARE motif and a N-terminal domain containing profilin-like folds [8, 10]. VAMP members contribute one SNARE motif to the SNARE complex. The syntaxin family of SNAREs is an example of the Qa or Qc sub-type and are characterized by a single transmembrane domain, a SNARE motif and a N-terminal domain made up of anti-parallel three-helix bundles [8, 10]. Syntaxin members also contribute one SNARE motif to the SNARE complex. The synaptosome-associated protein (SNAP) family of SNAREs is an example of the Qbc subtype and contain two SNARE motifs joined by a flexible linker and that is palmitoylated and therefore lacks a transmembrane domain [8, 10]. The SNAP family is unique in that they contribute two SNARE motifs to the SNARE complex. Soluble N-ethylmaleimide-sensitive factor attachment protein receptors were previously classified based on whether they localized to the vesicle membrane (v-SNARES; *e.g.* VAMPs) or to the target plasma membrane (t-SNARES; *e.g.* syntaxins and the SNAP families) but these classifications have subsequently been shown to have a number of exceptions.

Soluble N-ethylmaleimide-sensitive factor attachment protein receptors localized on opposing membranes [vesicle:vesicle or vesicle:plasma membrane] drive fusion of membranes using the free energy released during the formation of the stable four-helix bundle. SNARE motifs 'zipper' from the N- to the C- terminus, 'clamping' membranes and initiating fusion. Post-fusion SNARE proteins end up on the interior surface of the target membrane. These bundles are recycled *via* dissociation mediated by N-ethylmaleimide-sensitive factor (NSF) and other co-factors such as αSNAP (soluble NSF attachment protein). Mechanical models [6–11] all predict that SNAREs function to bring opposing membranes into close proximity initiating fusion events. The presence of Ca^{2+} is indispensable, acting to bridge the opposing membranes, which leads to the exclusion of water allowing lipid mixing, fusion and subsequent exocytosis.

Ca^{2+}-regulated SNARE complexes are involved in neurotransmitter release [8, 23]. Regulated exocytosis differs from other types of constitutive intracellular trafficking events. The neuronal SNARE complex was the first SNARE complex identified and the most vigorously dissected. Docked vesicles containing SNARE complexes composed of Syntaxin 1a and SNAP-25 on the plasma membrane and VAMP-2 on the vesicular membrane allow for the rapid (millisecond) release of neurotransmitters upon Ca^{2+} influx. Free, uncomplexed membrane SNAREs form *cis*-SNARE acceptor complexes on the plasma membrane in response to the actions of regulatory proteins. These acceptor complexes on the plasma membranes interact with SNAREs on opposing vesicular membranes and form trans-SNARE complexes that are fusion 'ready'. These 'docked' vesicles persist for a substantial period of time until Ca^{2+} influx triggers the final step of fusion and cargo (neurotransmitter) release through the actions of Ca^{2+} sensors such as synaptotagmin and complexins.

Expression of SNAREs and SNARE complexes in mast cells

Various investigators have reported the expression of multiple SNAREs in murine and human mast cells [12, 13, 15, 17, 19–22, 24–31]. Composite RT-PCR data identifying SNARE mRNA in

Table 1 SNARE family mRNA expression in murine and human mast cells

Cell type	SNARE	Reference(s)
RBL-2H3 cells	Qb,c family: SNAP-23	13, 22, 24
	Qa family: Syntaxin 2, 3, 4,	13, 22, 24
	R family: VAMP-1, 2, 3, 7, 8	13, 22, 24
Human mast cells	Qb,c family: SNAP-23, SNAP-25	17
	Qa family: Syntaxin 1b, 2, 3, 4,	17
	Qc family: Syntaxin 6	17
	R family: VAMP-2, 3, 7, 8	17

Table 2 SNARE family protein expression (*via* immunoblotting) in murine and human mast cells

Cell type	SNARE	Reference(s)
RBL-2H3 cells	Qb,c family: SNAP-23	13, 15, 22, 28, 29, 32
	Qa family: Syntaxin 2, 3, 4,	15, 22, 28, 29, 32
	R family: VAMP-1, 2, 3, 7, 8	13, 22, 29
	Qc family: Syntaxin 6	32
Primary murine mast cells	Qb,c family: SNAP-23, SNAP-25	12, 18–22, 27
	Qa family: Syntaxin 2, 3, 4,	12, 18–20
	R family: VAMP- 2, 3, 4, 7, 8	12, 18–22
Human mast cells	Qb,c family: SNAP-23	17
	Qa family: Syntaxin 1b, 2, 3, 4,	17
	R family: VAMP-2, 3, 7, 8	17
	Qc family: Syntaxin 6	17

murine and human mast cells are presented in Table 1. SNAP-23 is the consensus Qb,Qc SNARE expressed in all mast cells tested. The neuronal SNARE SNAP-25 showed weak mRNA expression in human mast cells [17], but was not detected *via* Western blot in the same study. Multiple studies report the expression of several VAMP family representatives mRNA [VAMP-1, -2, -3, -7, -8 (R SNAREs)] and several members of the Syntaxin family [Syntaxin 1, 2, 3, 4 (Qa SNAREs)] as well.

Immunoblotting studies confirm that SNAP-23 is the consensus representative of the Qb,c family in both murine and human mast cells (see composite of protein expression data presented in Table 2). Interestingly, two groups have reported the expression of the neuronal SNAP-25 Qb,c SNARE protein in primary murine mast cells *via* immunoblot and immunohistochemistry [25, 27]. These data could not be recapitulated in published work by several groups in primary murine mast cells [12, 22] and the rat cell line RBL-2H3 [13, 22, 24]. The reason[s] behind this discrepancy is not clear, but may involve specificity of antibodies and/or limits of detection of signal. Protein expression of VAMP and Syntaxin family members correlate well with mRNA studies, as the majority of SNAREs detected *via* RT-PCR were also detected *via* immunoblot.

Immunohistochemistry studies in primary murine mast cells [12, 18, 21] have demonstrated that SNAP-23 and Syntaxin 4 localize to the plasma membrane, while Syntaxin 3, VAMP-2 and VAMP-8 appear to localize to secretory granules. Interestingly, as mentioned *vide supra*, Salinas *et al.* [27] detected SNAP-25 expression in the secretory granules of rat primary mast cells (RPMC). Similar immunohistochemistry results in RBL-2H3 cells [13, 14, 24, 29, 31] have been reported demonstrating plasma membrane localization of SNAP-23, Syntaxin 4 and Syntaxin 3; and secretory granule localization of Syntaxin 3, VAMP-2, -3, -7 and -8. In human mast cells, Sander *et al.* [18] demonstrated that SNAP-23 and Syntaxin 4 localize in the plasma membrane while VAMP-3, VAMP-7 and VAMP-8 are dispersed throughout the cytoplasm, suggesting granule localization. However, upon activation of the mast cell, only VAMP-7 and VAMP-8 appear to redistribute to the periphery of the cell, suggesting fusion and degranulation.

Immunoprecipitation (IP) pull-down studies in primary murine mast cells, RBL-2H3 cells, and human mast cells have identified SNARE complexes composed of SNAP-23 and Syntaxin 4 in complex with the R-SNAREs, VAMP-2 [13, 18, 28, 29], VAMP-8 [13, 15, 17, 18, 22]; VAMP-7 [17] and VAMP-3 [13]. In addition, complexes composed of SNAP-23, Syntaxin 3 and VAMP-8 also co-precipitated [15]. Other studies demonstrate association of Syntaxin 3 with VAMP-7 [14]. Paumet *et al.* [13] demonstrated that IP with anti-SNAP-23 antibody in RBL-2H3 cells pulled down Syntaxin 2, 3, 4 and VAMP-2, -3, -8. Interestingly, N-ethylmaleimide (NEM) treatment was required to observe VAMP-8 co-precipitation. It was also demonstrated that IP with anti-Syntaxin 4 resulted in the co-precipitation of SNAP-23, VAMP-2, VAMP-3 and VAMP-8, while IP with anti-Syntaxin 2 or Syntaxin 3 only pulled down SNAP-23. These data suggest that ternary complexes in RBL-2H3 cells consist of SNAP-23, Syntaxin 4 and a member of the R-SNAREs family, presumably VAMP-2, -3 or -8. Pombo *et al.* [15] showed that in RBL-2H3 cell lysates; Syntaxin 4 co-precipitated with SNAP-23 and VAMP-8 within and outside of lipid rafts; however, Syntaxin 3, SNAP-23 and VAMP-8 co-precipitates were found to be complexed only within lipid rafts. The implication of these two different complexes and their unequal distribution in the membrane remains unclear. Additional studies by Hepp *et al.* [28] demonstrated co-precipitated complexes of SNAP-23, VAMP-2 and Syntaxin 4 in RBL-2H3 cells and also showed that most of the SNAP-23 associated with VAMP-2 and Syntaxin 4 in these complexes is phosphorylated. An elegant study by Puri *et al.* [29] demonstrated that SNAP-23, Syntaxin 4, VAMP-2 complexes are present in lipid rafts and showed that SNAP-23 functions to recruit non-lipid raft-associated Syntaxin 4 into a functional complex. Once again it was demonstrated that a predominant proportion of the

Table 3 Functional data implicating SNARE proteins in mast cell degranulation

SNARES	Blocking rsSNAREs	Function-blocking Abs	Overexpression studies	RNA interference	SNARE-deficient phenotypes	Contra. data*
SNAP-23		12, 17, 19	14, 29, 33, 38	20, 22		
VAMP-2	16					17, 21 40
VAMP-3	16					17, 21
VAMP-7		17	14	22		16
VAMP-8	16	17	33	22	18, 21	
Syntaxin 4		17, 19	13	22, 39		

SNAP-23 in complexes was phosphorylated, implicating SNAP-23 phosphorylation as a key prerequisite to complex formation. Our group has demonstrated that in RBL-2H3 cells, SNAP-23 co-precipitates with Syntaxin 4 and VAMP-8; however, NEM treatment was needed to observe VAMP-8 association [22]. In human mast cells isolated from surgical tissues, Sander *et al.* [17] demonstrated that SNAP-23 co-precipitated with Syntaxin 4, VAMP-7 and VAMP-8, but not VAMP-2 and VAMP-3. In stimulated, NEM-treated murine bone marrow–derived mast cells, VAMP-8 was shown to associate preferentially with Syntaxin 4 and SNAP-23 [18], and to a lesser degree, VAMP-2. However, it appears that VAMP-2 and VAMP-3 [34] may act to substitute for VAMP-8 in VAMP-8-deficient cells, perhaps due to an unusual compensatory mechanism. Isolation of ternary complexes of SNARE proteins after cellular activation has proven anything but trivial. Previous data have demonstrated that only ~5% of the SNAP-23 present in RBL-2H3 cells is actually capable of forming a complex after activation [28]. Furthermore, it is suggested that SNARE complexes have limited lifespans after activation-induced formation [35, 36].

Using recombinant SNARE proteins, Foster *et al.* [37] demonstrated that SNAP-23 interacts with Syntaxin 4 and VAMP-2. However, the interaction of SNAP-23 and Syntaxin 4 was approx. five times stronger than SNAP-23 with VAMP-2 or Syntaxin 4 with VAMP-2. Similarly, Vaidyanathan *et al.* [38] demonstrated the *in vitro* association of the recombinant SNAREs, SNAP-23, VAMP-2 and Syntaxin 4 and further demonstrated that deletion of the amino terminus and the second coiled-coil domain of SNAP-23 inhibited binding to both VAMP-2 and Syntaxin 4.

Functional studies implicating specific SNAREs or SNARE complexes in mast cell degranulation

Although immunolocalization and IP studies identify the individual SNARE proteins and their complexes associated with mast cell function, functional studies aimed at disrupting SNARE complex formation/action provide a practical method to dissect the role of these complexes in mast cell degranulation. SNARE proteins also function to mediate constitutive trafficking events through both endocytic and secretory pathways; reviewed in Hong *et al.* [11]; it is therefore important that functional degranulation studies are utilized.

Previously published functional data have implicated the SNAREs SNAP-23, Syntaxin 4, VAMP-2, VAMP-3, VAMP-7 and VAMP-8 in mast cell degranulation in studies using streptolysin-O-permeabilized cells and inhibitory recombinant SNARE proteins [16], SNARE neutralizing antibodies [12, 17, 19]; overexpression studies [13, 14, 22, 29, 38], RNA interference methods [20, 22, 39] and SNARE-deficient mice [18, 21]. A compilation of functional data implicating the various SNARE proteins is presented in Table 3.

The most compelling evidence for the role of SNARE proteins in mast cell degranulation is presented in data describing VAMP-8-deficient mice [18, 21]. Puri *et al.* [21] demonstrated that mast cells derived from the bone marrow of these mice [BMMC] had a 50% decrease in their ability to release β-hexosaminidase and serotonin but had normal histamine and TNF-α release. Tiwari *et al.* [18] showed that BMMC from VAMP-8-deficient mice resulted in a 50% decrease in β-hexosaminidase and histamine, but no changes in cytokine/chemokine release. Our group has shown *via* siRNA that we could demonstrate about a 50% knockdown in RBL-2H3 degranulation after targeting VAMP-8 mRNA/protein [22]. Interestingly, Puri *et al.* [21] also showed that VAMP-2 and VAMP-3 do not play a role in mast cell degranulation as BMMC derived from VAMP-3-deficient animals and mast cells derived from VAMP-2-deficient stem cells showed normal degranulation. These data are confirmed by studies with tetanus toxins [17, 40], which are known to cleave and inactivate VAMP-2 but do not have any effect on mast cell degranulation. Supporting evidence for the role of VAMP-8 in mast cell degranulation comes from Sander *et al.* [17], who demonstrated that neutralizing anti-VAMP-8 antibodies inhibit degranulation in streptolysin-permeabilized human mast cells by ~60%. Data from this report also demonstrated a role for VAMP-7 (~50% inhibition) but not VAMP-2 and

Table 4 Mast cell phenotype of SNARE knockout animals/cells

SNARE	Phenotype	Reference(s)
VAMP-2	Perinatal lethal: spleen/liver derived mast cells from 18d embryos displayed no defects in antigen or PMA/ionomycin-induced degranulation	41, 21
VAMP-3	Phenotypically normal: bone marrow derived mast cells displayed no defects in antigen or PMA/ionomycin-induced degranulation	42, 21
VAMP-8	(1) Mast cells display a ~50% reduction in *in vitro* antigen-induced degranulation with no effect on cytokine production and a decrease in serum histamine *in in vivo* passive systemic anaphylaxis model [19]. (2) Approximately, 50% decrease in *in vitro* antigen-induced degranulation (defect limited to serotonin and Cathepsin D release; not histamine or cytokine release; Ref. [22]).	43, 18, 21
Syntaxin 4	Embryonic lethal	44
SNAP-23	Embryonic lethal	45

*These reports provide evidence that these SNARES do not participate in the mast cell degranulation process.

VAMP-3. Similarly, Lippert *et al.* [16] demonstrated that recombinant VAMP-8 inhibited Ca^{2+}-/GTPγS-mediated degranulation in permeabilized RBL-2H3 cells by ~30%.

Our group's siRNA studies also implicate SNAP-23 and Syntaxin 4 as essential for RBL-2H3 degranulation, as knockdown of these SNAREs resulted in 30% inhibition of degranulation when compared to control siRNA treatment. In support of our data, Salinas *et al.* [19] demonstrated that in permeabilized RPMC, neutralizing antibodies to SNAP-23 and Syntaxin 4 resulted in 25% and 65% inhibition of histamine release. In addition, Sander *et al.* [17] demonstrated that neutralizing antibodies to SNAP-23 and Syntaxin 4 inhibited histamine release from permeabilized human mast cells with inhibition reaching ~90% and ~40%, respectively. Earlier studies by Guo *et al.* [12] demonstrated that anti-SNAP-23 neutralizing antibody decreased RPMC degranulation, although this inhibition required high antibody concentrations (~500 μg/ml). Recently, Suzuki *et al.* [20] demonstrated that a shRNA to SNAP-23 knocked down degranulation in stimulated RBL-2H3 cells by ~30%, similar to the maximal inhibition we have observed for SNAP-23. In addition, Liu *et al.* [39] demonstrated that treatment of RBL-2H3 cells with Syntaxin 4 siRNA resulted in a decrease in antigen-induced histamine and β-hexosaminidase release. Interestingly, various groups [13, 14, 29, 37] have implicated Syntaxin 4, VAMP-7 and SNAP-23 in mast cell degranulation *via* overexpression studies. Overexpression of Syntaxin 4, VAMP-7 and SNAP-23 all had an effect on degranulation in RBL-2H3 cells, although the effects were manifested as either an augmentation or inhibition of degranulation. The exact mechanism leading to augmentation or inhibition has yet to be elucidated but may involve competition for free SNARE proteins or competition for regulatory proteins. Because many of the SNARE-deficient transgenic mice are embryonic lethal (Table 4), siRNA methods may represent the only efficient way to characterize degranulation pathways in both *in vitro* and *in vivo* models of anaphylaxis and autoimmune disease.

RNA interference studies, SNARES and mast cells

There are a number of studies published recently that utilize siRNA to validate the role of individual SNAREs or SNARE complexes mediating trafficking events in various cell types [46–54]. However, an interesting report published recently [55] eloquently suggests that the abundance of SNARE-member isoforms and therefore the overall increase in SNARE redundancy may play a major role in the lack of SNARE siRNA cellular efficacy. In addition, the authors describe the phenomenon that many cell types appear to express a sizeable reserve of SNARE proteins not required for normal physiological cellular processes (a so-called 'SNARE reserve').

Interestingly, the literature is scant with respect to siRNA studies characterizing SNARE-protein function in mast cells. No small molecule inhibitors of mast cell SNAREs have been identified to date. In addition, as SNAREs are intracellular targets, the use of biotherapeutics such as recombinant proteins or neutralizing antibodies is not possible as these large proteins cannot as of yet be targeted to the inside of the cell. Therefore, interfering RNA offers an exciting new approach for the development of a SNARE-directed therapy. To date, several siRNA-based therapies have initiated clinical trials for the treatment of viral diseases, cancer and macular degeneration [56]. Using siRNA modulation we achieved a 50% knockdown in RBL-2H3 degranulation by targeting VAMP-8. In addition, our siRNA data identifies SNAP-23 and Syntaxin 4 as essential for RBL-2H3 degranulation, and knockdown of these SNAREs resulted in 30% inhibition of degranulation when compared to control siRNA treatment. Several other studies corroborate these finding [20, 39]. These studies reflect the effect that a SNARE siRNA therapeutic could have as we utilized whole cells stimulated with physiologic stimuli. However, additional studies focused on *in vivo* delivery of these siRNA and testing in animal models of disease are warranted.

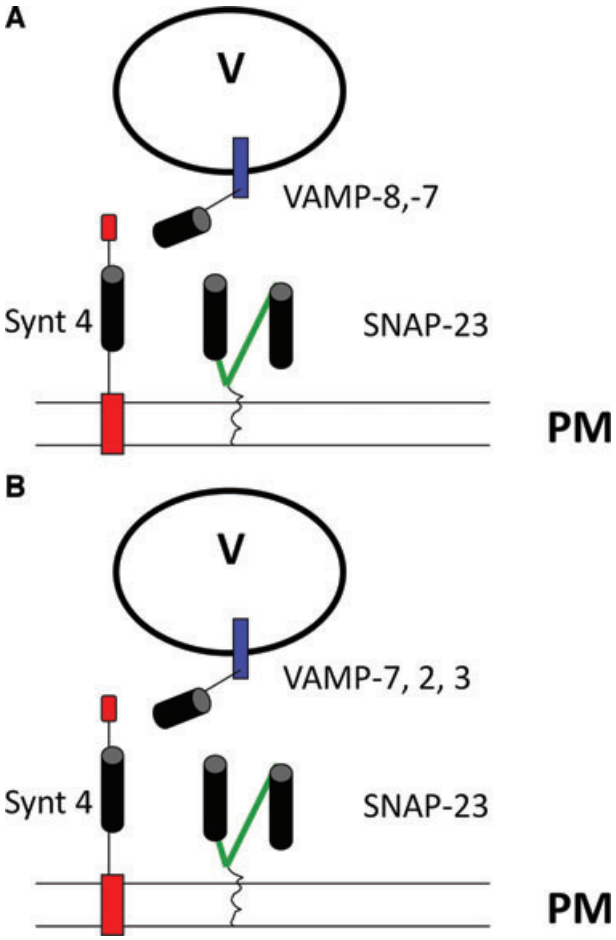

Fig. 1 Model of the mast cell SNARE complex mediating degranulation based on functional findings [12–14, 16–22, 29, 37–38]. (**A**) SNAP-23 and Syntaxin 4 represent the consensus plasma membrane SNAREs involved in mast cell degranulation. Under normal physiological conditions, VAMP-7 and/or VAMP-8 represent the secretory granule (vesicle) SNARE that interacts with SNAP-23 and Syntaxin 4 to form a functional ternary complex. (**B**) In the absence of VAMP-8, it appears that a compensatory mechanism may allow VAMP-2 and/or VAMP-3 to associate with SNAP-23 and Syntaxin 4 to mediate ternary complex formation and possible function.

Regulation of mast cell SNAREs

SNARE complex formation and function are regulated by several groups of accessory proteins. Sec1/Munc18 (SM) proteins are arch-shaped cytosolic proteins that have been demonstrated to bind syntaxins and regulate intracellular trafficking [57, 58]. Specifically, Munc18–2 has been demonstrated to play a role in mast cell degranulation [15, 26, 31]. SM proteins appear to regulate SNARE activity in several ways [57–60]. They can bind non-complexed syntaxins and act as negative regulators of SNARE assembly and interestingly, can also bind to trans-complexes and promote fusion. Members of the Munc13 family of accessory proteins also have been demonstrated to act as positive regulators of mast cell degranulation [60]. Complexins are cytosolic SNARE regulatory proteins that bind SNARE complexes and 'lock' the SNARE machinery into a primed state awaiting a final trigger of fusion events [57, 60]. Specifically, Complexin II has been demonstrated to function as a positive regulator of mast cell degranulation as siRNA to Complexin II attenuates IgE-induced degranulation [61]. Synaptotagmins are type I membrane calcium binding proteins that facilitate the formation of the SNARE–calcium–phospholipid complex that triggers final fusion and release of mediators from the cell [57, 60]. In mast cells, synaptotagmin II has been shown to be indispensible for mediator release [62]. The Rab family of GTPases has been implicated in the control of degranulation in mast cells [60]. Rab3D overexpression or constitutively active Rab3D inhibit RBL-2H3 antigen-induced degranulation [63]. The exact mechanism of Rab3D modulation of degranulation is poorly defined. Secretory carrier membrane proteins (SCAMPS) are accessory proteins that play a role in the regulation of mast cell degranulation [60]. SCAMP-1 and SCAMP-2 are postulated to function at the later stages of membrane fusion, in concert with phospholipase D, to form functional fusion pores [63]. Finally, as previously mentioned *vide supra*, SNARE disassembly is mediated by the proteins αSNAP and the ATPase NSF [60, 64]. αSNAP functions to capture the SNARE complex and allow the binding of NSF and the subsequent disassembly of the SNARE complex.

Conclusions

The important Qb,c and Qa SNAREs on the plasma membrane unequivocally appear to be SNAP-23 and Syntaxin 4, while the most likely R-SNARE partners on the vesicle membranes appear to be both VAMP-7 and VAMP-8 (Fig. 1). Functionally, there is growing evidence that ternary complexes of the above SNAREs are critically involved in the mast cell degranulation process. Disruption of these complex's formation or interactions with regulators may offer a window for therapeutic intervention and the development of novel small molecules for the treatment of allergic and autoimmune disease.

Conflict of interest

The authors confirm that there are no conflicts of interest.

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