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Physiologic Exocytosis in Pancreatic Acinar Cells and Pathologic Fusion Underlying Pancreatitis

Herbert Y. Gaisano, Subhankar Dolai, Toshimasa Takahashi

Departments of Medicine and Physiology, University of Toronto, Toronto, Canada, M5S1A8

Correspondence to: Herbert Y. Gaisano, M.D., University of Toronto, Department of Medicine, Medical Sciences Building, Room 7342, 1 King's College Circle, Toronto, Ontario, M5S 1A8, Canada; e-mail: herbert.gaisano@utoronto.ca

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Abstract

The pancreatic acinar cell is one of the best-studied cell models for regulated exocytosis and pathologic fusion underlying pancreatitis. Physiologic stimulation of pancreatic acinar cells leads to secretion of inactive digestive enzyme precursors from zymogen granules (ZGs) undergoing exocytosis at the apical pole of this polarized cell. The pancreatic acinar cell can undergo a number of pathologic fusion events which contribute to pancreatitis, the best studied of which is apical exocytotic blockade along with redirection and ectopic fusion of ZGs with the basolateral plasma membrane, releasing zymogens into the interstitial space. Both physiologic apical exocytosis and pathologic basolateral exocytosis are mediated by distinct sets of SNARE [Soluble N-ethylmaleimide-sensitive factor (NSF) Attachment Protein (SNAP) Receptors] and associated accessory proteins. The other major cellular contributor to pancreatitis is the formation of large autophagic vacuoles where premature trypsinogen activation takes place. This perturbation of autophagy also involves SNARE protein-mediated fusion events, which is only beginning to be unraveled. Here, we review the work that has been done in elucidating these pathologic fusions in pancreatic acinar cells that underlie pancreatitis, which more recent work also includes pathologic fusions occurring in the endosomal trafficking and endocytosis.

I. Pancreatic Acinar Cell, an Important Model for Secretion

The pancreatic acinar cell possesses a robust protein synthetic machinery that synthesizes a large spectrum of enzymes that digest all components of ingested food. Proteinases and some lipases are synthesized as inactive proenzymes. All are sorted through the *trans*-Golgi, and then vectorially transferred via a still incompletely defined maturation process, to the zymogen granules (ZGs) (58). These mature ZGs, among the largest secretory vesicles (~1 micron), accumulate at the apical pole of the highly polarized acinar cell, and occupy ~30% of total cell volume. Ca^{2+}-mobilizing neural (acetylcholine) and endocrine (cholecystokinin: CCK) stimulation, and to lesser extent cAMP-acting agonists (vasoactive intestinal polypeptide: VIP, secretin), act on their respective plasma membrane G-protein coupled receptors to trigger two signaling cascades (74). The first is the inositol triphosphate (IP3)-mediated intracellular Ca^{2+} release partnered with

diacylglycerol (DAG) formation and protein kinase C (PKC) pathways; the second is elevation of cAMP levels partnered with protein kinase A-(PKA)-activated pathways; and with extensive crosstalk between these two cascades. Other chapters in this book deal with these topics. These cell signals act on the complex exocytotic fusion machinery discussed below that is comprised of a large number of molecules on the ZGs and the plasma membrane (PM) to culminate in fusion of ZGs with apical PM, releasing the ZG cargo of nutrient hydrolases (e.g. proteases, lipase, amylase) into the ductal lumen which then transit to the duodenum for a timely meeting with a food bolus whose entry into the duodenum from the stomach is metered by the pylorus.

The pancreatic acinar cell, historically from the work of George Palade (Nobel Prize for Medicine or Physiology in 1999) and coworkers, had long served as the basic model to elucidate the complex processes of secretion (58). This was however largely overtaken by the neuron and endocrine fields that have exponentially accelerated as a result of the rapid identification of the exocytotic machinery (originally known as the SNARE Hypothesis) (63, 69) and membrane ion channel machinery that orchestrates plasma membrane Ca^{2+} channel opening with the ensuing Ca^{2+} influx as the major source of Ca^{2+} for secretory granule fusion. The rapid progress in the neural and endocrine fields was further supported by the rapid development of new technology, including genetic models, electrophysiology and microscopy imaging. The pancreatic acinar cell eventually followed in hot pursuit to initially show that it too has the fundamental components that support the SNARE Hypothesis (19). To the delight of neuroscientists, probably paralleled by the undeclared dismay of earlier workers in pancreatic acinar biology, was that the levels of the SNARE and associated proteins were at much higher levels in neurons than in acinar cells; this made the neuron a better model to isolate and characterize the large number of proteins of the exocytotic machinery (68). The acinar cell exocytotic machinery however does not simply mimic the neuron, and still lends itself to be an outstanding model for non-excitable cell secretory biology. For instance, the pancreatic acinar apical PM constitutes less than 10% of the total cell surface area even when maximally stimulated, therefore exocytosis of the few ZGs abutting the apical PM would be insufficient in exporting enough proteases for delivery into the duodenal lumen to efficiently digest the huge amount food continually being emptied from the stomach during a meal. Instead, the pancreatic acinar cell has a very different secretory granule architecture from the neuronal synapse, which enables an orderly and rapid (within 1 minute) fusion of majority (>70%) of the ZGs within the apical pole, termed sequential exocytosis (56), whereby fusion pores between homotypically-fused ZGs (also called compound exocytosis) remained open for very long periods (>20 min) (56, 71) to ensure efficient emptying of zymogen cargo from the deepest ZG layers within the apical pole. This provides an exquisitely regulated and metered machinery that can match hydrolase outputs to the varying amounts of food bolus along the entire duration of the meal. Similar exocytotic machineries can be greatly accelerated in mast cells and neutrophils during an allergic reaction or to mount an effective kill against invading bacteria (3, 77). Furthermore, the exocytotic machinery of the acinar cell can be misdirected (i.e. ectopic exocytosis at the basolateral PM) by disease-causing stimulation such as alcohol, as a major contributing mechanism to alcoholic pancreatitis (21, 70).

II. SNARE Hypothesis for Membrane Fusion and Exocytosis

The 2013 Nobel Prize in Physiology or Medicine was awarded to James Rothman, Randy Schekman, and Thomas Südhof for their discoveries of the machinery regulating vesicle traffic. This was actually the convergence of four fields (reviewed in (59)). James Rothman and coworkers were reconstituting membrane traffic events to show fusion of donor vesicles with target

membranes using cell-free assays. Randy Schekman and coworkers were studying the secretory pathway in yeast which led to the identifying SEC genes and their mutants that accounted for accumulation of various intermediated vesicles along the ER to the Golgi. Thomas Südhof cloned a large number of synaptic vesicle proteins found to mediate neuro-exocytosis, the latter actually was pivotally shown by a fourth field of coworkers that found that the synaptic proteins Südhof cloned were actually the substrates for proteolytic cleavage by tetanus and botulinum neurotoxins known to block neurotransmission.

The SNARE hypothesis as originally proposed by above-mentioned major contributors stated that cytoplasmic N-ethylmaleimide-sensitive fusion proteins (NSF) and soluble NSF attachment proteins (SNAP) bind SNAP receptors (SNAREs) on the donor vesicle (v-SNARE) and target membrane (t-SNARE) to form a multi-molecular complex capable of mediating fusion between the two membranes (63, 69). The v-SNAREs are VAMPs (vesicle-associated membrane protein) and t-SNAREs are Syntaxins (STX) and SNAP25 (synaptosomal associated protein of 25kDa), which form *trans*-SNARE complexes to constitute the minimal machinery for membrane fusion. The original neuronal SNARE proteins (68) were found to belong to large families that included numerous non-neuronal isoforms which have very broad tissue distribution (43), and with each cell containing multiple isoforms. Compartmental specificity of membrane fusion was initially thought to be encoded by the specific pairing of these SNARE proteins (48). This thinking turned out to be inaccurate as promiscuity was increasingly found (i.e. VAMP8 discussed below); and that compartmental specificity actually required many accessory proteins that serve to spatially target and bind distinct SNARE complexes, including Sec1/Munc18 (SM), Munc13 and RIM proteins, which further act to prime the SNARE complex to become fusion-ready (68, 69). For more exquisite regulation of fusion that achieves a higher level of precision and speed, these primed SNARE complexes would pull the cognate fusing membranes together only up a hemi-fused state, whereby complete fusion is prevented by fusion clamps (i.e. complexins, synaptotagmins) that also act as Ca^{2+} sensors that are ready to be activated by Ca^{2+} to trigger fusion (68, 69). Many of these proteins turned out to be the precise substrates for specific cell signals, including DAG (Munc13), PKC (SM proteins), Ca^{2+} (SNARE complex, synaptotagmins), and even cAMP/PKA (RIMs and EPACs) (37, 68). These fusion clamps also act to super-prime the SNARE complex, which is required to ensure speed and precision for neuroexocytosis (37, 68), but probably the full complement of these proteins is not required for the less efficient and less precise secretory cells, such as acinar cells (37). Nevertheless, the pancreatic acinar cell has been an ideal epithelial cell model to examine these tenets of the SNARE Hypothesis, wherein many isoforms of these proteins (i.e. SNARE and SM proteins, complexin, synaptotagmins, EPAC) were indeed reported (discussed below), whereas others that were found in neurons (i.e. Munc13, RIM) have not been yet found in the acinar cell.

III. Physiologic Apical Exocytosis in the Pancreatic Acinar Cell

A. SNARE proteins

The pancreatic acinar cell was one of the first non-neuronal cells to show the multiple isoforms of each SNARE protein, along with the accessory proteins described above for the neuron (19). In fact, the investigation of SNARE-mediated exocytosis in the pancreatic acinar cell somewhat followed the developments reported in neurons as depicted below and shown in Figure 1. First, we showed the cellular locations of v-SNARE (VAMPs) and t-SNAREs (syntaxins (STX) and SNAP-25) that mapped out to different exocytotic membrane compartments. The three syntaxin isoforms for each of the three exocytotic compartments include syntaxin 2 (STX-2) for the apical PM, syntaxin 3 (STX-3) for the ZG

membrane, and syntaxin 4 (STX-4) for the basolateral PM (20). SNAP-23 was the ubiquitous non-neuronal SNAP-25 isoform that is present in ZGs, apical and basolateral PM (25). Of the first two VAMPs reported, VAMP-2 was first thought to be the main one for regulated exocytosis, whereas VAMP-3 might have a role in constitutive secretion (23). To assess the function of these SNARE proteins, we first used the tetanus and botulinum neurotoxins. However, unlike neurons, pancreatic acinar cells do not have PM receptors to internalize these neurotoxins, prompting us and others to employ cell permeabilization strategies (i.e. streptolysin O) to enable internalization of the neurotoxins, showing that tetanus toxin and botulinum neurotoxin C1 selectively cleaved pancreatic acinar VAMP-2 and syntaxins (STX-2 and STX-3 only), respectively, which blocked Ca^{2+}-evoked enzyme release (23, 28). However, tetanus toxin, while completely cleaving VAMP-2, resulted in only ~30% inhibition of acinar secretion (23) .

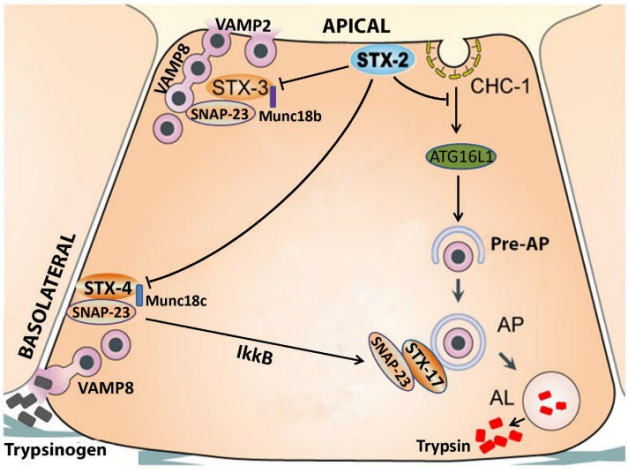

Figure 1. Diagram of pancreatic acinar cell showing the exocytotic machinery for apical exocytosis (Munc18b/STX-3/SNAP23/VAMP2 and VAMP8) and basolateral exocytosis (Munc18c/STX-4/SNAP-23/VAMP8) that have been well characterized, and the more recent findings of the roles of STX-2 and SNAP-23 in distorting autophagy in pancreatitis. Apical and pathologic basolateral exocytosis release trypsinogen and other ZG contents into the ductal lumen and interstitial space, respectively. STX-2 act as an inhibitory SNARE to block apical and basolateral exocytosis, and binds ATG16L1 to block the latter from binding clathrin heavy chain (CHC)-1 that would have contributed to pre-autophagosome (Pre-AP) formation that drives autophagosome (AP) biogenesis. APs fuses with lysosomes to from autolysosomes (ALs), wherein pancreatitis stimuli distort lysosome and/or AL function to cause premature trypsinogen activation into trypsin, which when released into the cytosol would promote acinar cell injury. How trypsinogen is activated into trypsin in the interstitial space to contribute to injury is not yet known. A most recent work showed that SNAP-23 is a substrate for IKKβ (a major inflammatory signal in pancreatitis)-mediated phosphorylation which enables SNAP-23 translocation from the basolateral plasma membrane to the AP where SNAP-23 would bind and modify syntaxin-17 (STX-17) actions and its SNARE complex in promoting an excess of AP-lysosome fusion.

It was much later that we discovered a third VAMP, VAMP-8, which when genetically-deleted, blocked most of the apical exocytosis (7). This finding was unexpected as VAMP8 is prominent in endosomal vesicles in most cells and was originally known as endobrevin (76). Here, VAMP-8 accounted for the multiple layers of compound ZG-ZG fusions within the apical pole, whereas exocytosis of the few ZGs with the apical PM is mediated by VAMP-2 (1, 7, 73). Using a perifusion assay to measure amylase release from acini of VAMP8-KO mice and adenovirus-tetanus toxin-infected acini that acutely cleaved VAMP2, VAMP2 was shown to mediate an early immediate phase of secretion, whereas VAMP8 regulates a later prolonged phase of secretion (51). These findings matched the exocytosis imaging results wherein VAMP-2-mediated first phase secretion corresponds to the fusion of the fewer ZGs abutting the apical PM, and VAMP8-mediated latter phase secretion corresponds to the continued emptying of the much larger number of sequentially fused ZGs that lie behind then fusing with the apical PM-fuse ZGs that would provide a more sustained second phase secretion (1, 7, 73). STX-2 was initially thought to mediate apical exocytosis (60), which was misled by its location on the apical PM (20, 60). It turned out that STX-2 actually acts as an inhibitory SNARE capable of competing and blocking STX-3 and STX-4 from forming their SNARE complexes, thus blocking apical and basolateral exocytosis (see below), respectively (12). We had reasoned that this inhibitory SNARE protein STX-2 may be a deliberate mechanism to reduce the efficiency of membrane fusion to a much slower secretory rate

(than neurons) of digestive enzyme release over a period of hours during a meal to ensure optimal nutrient digestion (12). Some SNARE proteins were distinguished by their resistance to botulinum neurotoxin cleavage, of which VAMP8 and also SNAP-23 are two examples. We therefore initially assessed SNAP-23 function with a dominant-negative carboxyl-terminal deleted SNAP-23, which would form non-functional SNARE complexes, and indeed this SNAP-23 mutant inhibited acinar secretion (33). A recent report unequivocally confirmed that SNAP-23 deletion in mice indeed blocked apical exocytosis (38).

B. Accessory proteins involved in the apical exocytotic machinery

Although neuronal Munc18a is not present in acinar cells, Munc18b and Munc18c are (7), and were found to bind and prime the predicted SNARE complexes shown to mediate apical exocytosis, ZG-ZG fusion and basolateral exocytosis. Specifically, Munc18b binds STX-3/SNAP-23 with VAMP2 to mediate exocytosis of ZGs with apical PM, and with VAMP8 to mediate sequential ZG-ZG fusion (7); whereas Munc18c binds STX-4/VAMP8/SNAP-23 to mediate basolateral exocytosis (discussed below). Of the several isoforms of synaptotagmins (Syt 1, 3, 6 and 7) found in pancreatic acinar cells, Syt-1 found on ZGs and apical PM was the only one involved in enzyme secretion (16). Pancreatic acinar cells also expressed complexin 2 in its apical pole (15), and apical VAMP2 could bind both Syt-1 and complexin 2, and their truncated mutants introduced into permeabilized acini inhibited enzyme secretion (15, 16). It is therefore plausible that Syt-1 and complexin 2 may be the fusion clamps and calcium sensors for physiologic exocytoses (49). However, gene deletion or depletion of Syt-1 or complex 2 in pancreatic acinar cells will be required to definitively show this. Cab45 (the 45kDa calcium-binding protein) was found to specifically bind the Munc18b/STX-3 complex and also acts as a Ca^{2+} sensor for exocytosis as shown by Cab45 antibody blockade of enzyme secretion by a remarkable 90% (40). cAMP-binding protein, EPAC (exchange protein directly activated by cyclic AMP) partially reversed supramaximal carbachol and CCK-induced inhibition of secretion (4). While this suggests a role for EPAC in either enhancing apical exocytosis or reversing the apical blockade, further work will be needed to elucidate how EPAC interacts with the apical exocytotic machinery to accomplish these effects.

C. Other proteins involved in other steps of apical exocytosis

Coordinated interactions of the Rho and Rab family of small G proteins (i.e. Rab3D, Rab 27A/B) (5, 31, 34, 64, 75) and Synaptotagmin-like proteins (65), and their subsequent concerted actions on primary exocytotic fusion proteins, including SNARE and accessory proteins (i.e. Munc18b) (18), appear to assist in ZG exocytosis perhaps by their potential ZG-tethering actions. Of these, the stronger evidence seems to building up for Rab27, localized to ZGs, wherein dominant-negative Rab27 constructs inhibited secretion in pancreatic and parotid acini (5, 34). Whereas Rab27A was shown to be involved only on a minor secretory (lysosomal/endosomal) pathway (31), Rab27B was found to be more important for ZG exocytosis in pancreatic and parotid acini (5, 30, 34). Synaptotagmin-like proteins (SLPs) include various isoforms (Slp1 to Slp5, and rabphilin), of which Slp1 and Slp4 were shown to regulate amylase release in pancreatic and parotid acini in part by their binding to Rab27B (18, 65).

The actin cytoskeleton also plays multiple roles in apical exocytosis, including actin coating of ZGs during acinar stimulation (72) that serves in part to support ZG-ZG fusion (57) and also fusion pore opening (36); and the actin-myosin complex that facilitates the expulsion of ZG contents (46). Interestingly, small G-proteins RhoA and Rac1 were shown to also regulate actin rearrangement in pancreatic acinar cells to support secretion (2).

IV. Pathologic Basolateral Exocytosis Contributing to Pancreatitis

The basolateral PM accounts for 90% of the pancreatic acinar cell surface area where little or no exocytosis normally occurs. Nevertheless, the acinar basolateral PM possesses an intact SNARE fusion machinery consisting of t-SNARE proteins (STX-4, SNAP-23) and SM protein Munc18c, which would indicate that exocytosis can potentially occur (22), as shown in Figure 1. In fact, early studies had shown that supramaximal CCK or cholinergic stimulation, capable of inducing pancreatitis *in vivo*, caused apical exocytotic blockade and redirected exocytosis to the lateral PM (66), releasing enzymes into the interstitial space to cause interstitial pancreatitis (29). This contribution to pancreatitis that was largely ignored, was shown to be of importance when we elucidated the exocytotic machinery mediating this pathologic fusion event [reviewed in (21)]. Using various fluorescence imaging tools (FM143, syncollin-pHluorin), we were able to observe basolateral exocytosis in rat pancreatic acini in response to supramaximal CCK or carbachol stimulation; and after clinical alcohol sensitization by physiologic CCK or carbachol stimulation (8, 17, 39) that mimicked alcoholic pancreatitis. We further showed that not only clinical concentrations of ethanol but also clinical concentrations of ethanol metabolites, acetaldehyde, ethyl palmitate and ethyl oleate, reduced the formation of apical exocytotic complexes with consequent blockade of CCK-8-stimulated apical exocytosis in rat pancreatic acini; and furthermore, acetaldehyde and ethyl oleate redirected VAMP8-containing ZGs towards and fusing with the basolateral PM, along with an increased formation of basolateral PM fusion complexes (Munc18c/STX-4, SNAP-23, VAMP8) (11). It is remarkable that despite only a few ZGs normally close to the basolateral PM, supramaximal stimulation could induce VAMP-8-labeled ZGs to move from the apical pole towards the lateral and basal PM (7). Mechanistically, these pancreatitis stimuli caused Munc18c on the basolateral PM to be phosphorylated by PKC-α which induced its binding and activation of the STX-4/VAMP-8/SNAP-23 complex (8) to become receptive to binding VAMP-8 of the approaching ZGs, whereby this complete SM/SNARE complex would then mediate basolateral exocytosis (7). Eventually, when Munc18c-depleted mice (Munc18c$^{+/-}$) became available, and along with Munc18c knockdown in human pancreas by lenti-Munc18c-shRNA treatment, we confirmed that depletion of Munc18c in pancreatic acini still exhibited normal apical exocytosis during physiologic CCK-8 stimulation, but upon supraphysiologic CCK-8 stimulation, there was reduction in basolateral exocytosis resulting from a decrease in STX-4 SNARE complexes, with consequent alleviation of caerulein pancreatitis (13). Very recently, we reported that SNAP-23 depletion in rat and human pancreatic acini blocked not only apical exocytosis, but also blocked basolateral exocytosis; the latter also led to reduction in pancreatitis severity (14). The Munc18c phosphorylation also caused Munc18c displacement from the basolateral PM into the cytosol where it is rapidly depleted by the cytosolic proteolytic cleavage (22). This Munc18c displacement from the PM may be of clinical relevance, as in a human case of quiescent chronic alcoholic pancreatitis, Munc18c was similarly displaced into the cytosol of residual intact acinar cells, suggesting that this might be a contributing mechanism predisposing to the clinically-observed recurrent pancreatitis (24). Finally, we showed that VAMP-8 deletion in mice completely blocked basolateral exocytosis, which resulted in reducing not only caerulein pancreatitis but also alcoholic pancreatitis, the latter shown in alcohol diet-fed mice stimulated with postprandial carbachol stimulation that mimicked clinical alcoholic pancreatitis (7). Whereas the mechanism for pathologic basolateral exocytosis of ZGs is now clear, how the zymogens particularly trypsinogen that are emptied into the interstitial space are activated into damaging trypsin is not and requires further investigation. In a number of our more recent reports, we have performed these studies directly on the human exocrine pancreas using a slice technique that allowed preservation of secretory function over at least four days (42), enabling us to not only overexpressed candidate

proteins but also knockdown protein expression with shRNA adenoviruses, thereby providing direct translational value over the conventional use of genetic mouse models.

V. Dysregulated SNARE Protein-Mediated Fusion Underlying Abnormal Autophagy in Pancreatitis

A. Autophagy SNARE proteins

Autophagy is the major cellular process that removes damaged organelles and other cellular debris (41, 53). Autophagy is controlled by a series of autophagy-related proteins (ATGs) (41, 53, 54) but also involves membrane fusion events which are intrinsically regulated by SNARE proteins (44, 55) that also interact with ATGs (10, 44). Autophagy comprises four key stages. The first is phagophore nucleation induced by AMP kinase and PI3K-III complex-I (41, 53). Second is phagophore expansion to engulf damaged organelles (i.e. mitochondria by mitophagy, ER by ER-phagy, ZG by zymophagy). Phagophore expansion is by an aggressive recruitment of membranes from various sources (PM, ER, endosomes) which requires ATG16L1 complexes (41, 53) and fusion events mediated by a distinct SNARE complex [STX7/STX8/VAMP7] (55); and eventual closure of the phagophore ends involving LC3 to form the autophagosome. The third stage is autophagosome fusion with lysosome to form the autolysosome, which is mediated by two other SNARE complexes [STX17/SNAP29/VAMP8/ATG14 and STX7/SNAP29/YKT6] (35, 78). STX17 translocates from the cytosol to the autophagosome (35) to initiate fusion with lysosome by first recruiting SNAP29 and then to form a complete complex with lysosomal VAMP8; this SNARE complex is stabilized by ATG14 (44, 55). Lysosomal STX7 can also bind SNAP29, which then forms the complete SNARE complex with autophagosome v-SNARE YKT6 (47). The fourth and last stage is autolysosome maturation whereby its cargo is degraded for reuse. Defects in autophagy, particularly in autolysosome formation and maturation, the latter resulting in inadvertent trypsinogen activation, are now well known to occur and has become the current dogma for the cellular mechanism of pancreatitis (26, 27, 45). However, there is no work that directly addresses the role of the STX17 SNARE complex (STX17/SNAP29/VAMP8/ATG14] in autophagy, whose dysregulation could possibly contribute to the defective autolysosome formation or maturation in pancreatitis; and possibly some defects might also arise in phagophore formation and expansion that could be contributed by dysregulation of the STX7/STX8/VAMP7 SNARE complex.

B. Exocytotic SNARE proteins affecting autophagy

While we were investigating the role of the exocytotic SNARE proteins, we inadvertently ventured into their unexpected roles in autophagy that contributed to pancreatic acinar injury and pancreatitis. Likely as a result of the basolateral exocytotic blockade, Munc18c-depleted acini unexpectedly activated a component of the ER stress response which contributed to autophagy induction, resulting in accumulation of autophagic vacuoles and autolysosomes (13). We found that STX-2 deletion also led to an accumulation of autophagic vacuoles as a result of increased autophagic induction (12), but by a different mechanism. ATG16L1 translocates to the PM during intense autophagy to recruit more membranes to expand the phagophore, which is mediated by PM-bound clathrin heavy chain-1 (CHC-1) that forms a complex with pre-autophagosome–linked ATG16L1 (62). As depicted in Figure 1, we discovered that STX-2 also binds ATG16L1, which blocked ATG16L1 binding to clathrin in the PM; thus STX-2 deletion resulted in the increased binding of ATG16L1 to clathrin, which leads to increased formation of pre-autophagosomes that drives autophagosome biogenesis (12). This increased autophagy induction at least in part accounted for the CCK-8-induced increase in autolysosome formation and

activation of trypsinogen (12). As depicted in Figure 1, we also discovered a novel link between SNAP-23, autolysosome formation and their connection to IKK (inhibitor of NF-κB kinase) (14), the latter well-known to mediate NF-κB-mediated production of proinflammatory chemokines and cytokines that initiates and perpetuates the inflammatory response of pancreatitis (32). Pancreatitis stimuli of acini resulted in the translocation of SNAP-23 from its native location at the PM to the autophagosomes where SNAP-23 is able to perturb the STX-17 SNARE complex-mediated autophagosome-lysosome fusion (14). This SNAP-23 translocation was mediated by IKKβ-induced phosphorylation of SNAP-23, which was blocked by IKKβ inhibitors and IKKβ-insensitive mutants of SNAP-23 (14). Interestingly, these pathologic SNAP-23-mediated events would not occur during physiologic starvation (14), which in part explains why this was not previously discovered. SNAP-23 depletion in rats therefore resulted in reduced autolysosome formation with consequent reduction in trypsinogen activation that takes place in autolysosomes; which together with SNAP-23 depletion induced blockade of basolateral exocytosis contributed to a major reduction in pancreatitis severity (14). Of note, this is the first report that a single protein could serve as a mechanistic link of the three major cellular pathways for pancreatitis, including inflammatory signaling and the pathologic fusion events of dysregulated autolysosome formation and basolateral exocytosis. This therefore has major therapeutic implication in the targeting of SNAP23 for the treatment of pancreatitis.

VI. Future Directions in the Investigation of Pathologic Fusion Underlying Pancreatitis

While of interest, the above work on exocytotic SNAREs affecting autophagy are nevertheless not the primary SNARE proteins that mediate autophagy. Of note, STX-17 expression was recently reported to be reduced after cerulein pancreatitis (61). This reduction of STX-17 levels can conceivably perturb autolysosome formation or maturation, which could contribute to the acinar injury. However, there is no reported work on autophagy SNAREs or their role in pancreatitis. Another major pathologic fusion contributing to pancreatitis is the very large (>2.5 micron) trypsin-containing endocytic vacuoles enveloped by F-actin whose rupture inside the pancreatic acinar cell releases damaging trypsin (6, 9). The membrane fusion machinery that mediates this pathologic endocytosis has not been elucidated. VAMP8, originally discovered as an endosomal VAMP (76), was shown to play an additional and important role in endosomal trafficking by its interactions with early endosomal proteins D52 and Rab5, whereby VAMP8 depletion resulted in depletion of these endosomal proteins with consequent accumulation of activated trypsin in the autolysosomes (50-52). Remarkably, replenishment of D52 or Rab5 prevented the accumulation of activated trypsin (51). Finally, there is pathologic endocytosis of zymogens by the macrophages invading the acini during pancreatitis, which led to activation of macrophage NF-κB that in turn induced the production of inflammatory cytokines to perpetuate and accentuate pancreatitis injury (67). Much more work is needed to pursue these lines of investigation in elucidating the fusion machinery of each of these pathologic fusions that contribute to pancreatic acinar injury in pancreatitis; and from those insights, novel therapeutic strategies could then be devised to treat pancreatitis.

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