Roles for Ca\textsuperscript{2+} mobilization and its regulation in mast cell functions

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INTRODUCTION

Mast cell degranulation represents one of the earliest described examples of a Ca\textsuperscript{2+}-dependent biological process in non-excitable cells (Foreman et al., 1973; Kanno et al., 1973). The capacity for IgE-mediated activation of this process via crosslinking of IgE receptor (FcεRI) complexes at the mast cell surface was established in the 1970s (Metzger, 1978). Since biochemical identification (Metzger et al., 1986) and cloning (Ravetch and Kinet, 1991) of the three different subunits of FcεRI in the 1980s, much has been learned about the signaling pathways activated via this prototypic multichain immune recognition receptor and their roles in stimulated Ca\textsuperscript{2+} responses leading to granule exocytosis (Siraganian et al., 2002; Kraft and Kinet, 2007). In the present review we focus on the mechanisms by which FcεRI signaling mediates elevation of cytoplasmic Ca\textsuperscript{2+}, and the consequences of this process in mast cell functional responses, including granule exocytosis, chemotaxis to antigen, and stimulated endosomal trafficking, including cytokine secretion and recycling endosomal exocytosis. We will emphasize contributions to this area from our own laboratory, and we will focus on subcellular spatiotemporal relationships where this information is available.

MECHANISMS OF Ca\textsuperscript{2+} MOBILIZATION IN MAST CELLS

Although the importance of stimulated Ca\textsuperscript{2+} influx for mast cell degranulation has been known for decades, the mechanisms by which FcεRI-mediated Ca\textsuperscript{2+} entry occurs and the role of this process in stimulating granule exocytosis are incompletely understood. Mast cells do not exhibit voltage gated Ca\textsuperscript{2+} influx, a property of excitable cells (Jarvis and Zamponi, 2007). However, release of Ca\textsuperscript{2+} from endoplasmic reticulum (ER) stores by antigen or thapsigargin (an irreversible inhibitor of the SERCA ATPase, which normally maintains high Ca\textsuperscript{2+} levels in the ER) is sufficient to activate Ca\textsuperscript{2+} influx necessary for degranulation from these cells (Wolfe et al., 1996). This Ca\textsuperscript{2+} influx mechanism is commonly referred to as store-operated Ca\textsuperscript{2+} entry (SOCE), and it was originally shown by Putney and colleagues to depend critically on depletion of luminal Ca\textsuperscript{2+} from the ER (Takemura et al., 1989). The Ca\textsuperscript{2+} channel that mediates SOCE, known as the calcium release activated calcium (CRAC) channel, was defined electrophysiologically about two decades ago by Hoth and Penner (1992) in mast cells and by Lewis and Cahalan (1989) in T cells. However, its molecular identity remained unknown until 2006, when three papers from independent groups described cloning and functional reconstitution of the tetraspan channel protein Orai1/CRACM1 (Feske et al., 2006; Vig et al., 2006; Zhang et al., 2006).

In 2005, studies using large-scale siRNA screens to identify proteins important for SOCE discovered another protein, STIM1, that is critical for activation of Orai1 (Liou et al., 2005; Zhang et al., 2005). Unlike the CRAC channel protein, which is localized to the plasma membrane, STIM1 is an ER-localized Type 1 membrane protein that redistributes to discrete regions of the ER proximal to the plasma membrane upon Ca\textsuperscript{2+} depletion from ER stores (Liou et al., 2005; Wu et al., 2006). The mechanism of this redistribution, which appears to be critical for activation of Orai1/CRACM1, involves oligomerization of STIM1 that results from the loss of Ca\textsuperscript{2+} binding to EF-hand motifs in a luminal domain of this protein (Liou et al., 2007). Recent knockout studies demonstrated essential roles for STIM1 (Baba et al., 2008) and Orai1/CRACM1 (Vig et al., 2008) in mast cell degranulation and other processes in the allergic response (reviewed in Di Capite et al., 2011).

Our laboratory developed an imaging-based method to monitor the time-dependence of stimulated association of Orai1 with...
STIM1 that utilizes fluorescence resonance energy transfer (FRET) from monomeric Aequorin to STIM1-mRFP co-expressed in RBL mast cells (Calloway et al., 2009). Using this method, we found that thapsigargin stimulates much more extensive association of Orai1 with STIM1 than does antigen-mediated crosslinking of IgE receptors in the presence of extracellular Ca\(^{2+}\). However, removal of extracellular Ca\(^{2+}\) results in antigen-stimulated FRET that is comparable to that stimulated by thapsigargin in the presence of Ca\(^{2+}\), indicating that antigen-stimulated association of Orai1 with STIM1 is highly regulated in these cells (Calloway et al., 2009). Never the less, this more highly regulated coupling can still achieve robust SOCE in response to antigen (Parekh et al., 1997; Vasudevan et al., 2009). Interestingly, G protein-coupled receptors typically activate transient Ca\(^{2+}\) responses in mast cells in response to ligands such as adenosine or prostaglandin E2, but they fail to activate SOCE (Gilfillan and Beaven, 2011).

In our initial study, we defined an acidic amino acid segment in a putative coiled-coil sequence at the C-terminus of Orai1 that is important for stimulated SOCE (Calloway et al., 2009; Figure 1). We subsequently showed that this acidic segment (E272–D291) couples to a short basic sequence (K382–R387) in the C-terminal segment of STIM1 to activate Ca\(^{2+}\) entry under conditions in which STIM1/Orai1 association is promoted (Calloway et al., 2010). This apparent electrostatic interaction, while necessary for Ca\(^{2+}\) entry, is not necessary for stimulated association of STIM1 with Orai1, which depends on a larger, ~110 amino acid sequence called the “CAD domain” that can constitutively activate CRAC channels when expressed as a cytoplasmic protein (Kawasaki et al., 2009; Muik et al., 2009; Park et al., 2009; Yuan et al., 2009). More recent studies have shown that the sequence comprising five basic amino acids within the CAD domain is inaccessible to Orai1 prior to ER store depletion because of a conformational restriction in STIM1 that sequesters it by intramolecular association with an acidic sequence (E302–D322) that is N-terminal to the CAD domain in the STIM1 cytoplasmic tail (Korzeniowski et al., 2010; Muik et al., 2011). By a mechanism that is still unclear, homo-clustering of STIM1 following depletion of ER luminal Ca\(^{2+}\) results in a conformational transition to a more extended C-terminus that readily couples to Orai1 to activate Ca\(^{2+}\) influx into the cytoplasm through a productive Orai1 channel.

Many ion channels are regulated by phosphoinositides in the plasma membrane, but the roles for these lipids in SOCE are controversial (Huang, 2007). Korzeniowski et al. (2009) demonstrated that activation of SOCE is sensitive to inhibition of phosphatidylinositol 4-kinase, but not prevented by acute depletion of phosphatidylinositol 4,5-bisphosphate (PIP\(_2\)) at the plasma membrane of COS-7 cells, suggesting a role for PI4P but not for PIP\(_2\). Walsh et al. (2010) showed that inhibition of multiple pathways of PIP\(_2\) generation is necessary to prevent thapsigargin-mediated translocation of STIM1 to the plasma membrane in HeLa cells, but that co-expression of Orai1 permits STIM1 to concentrate at ER–PM junctions even in the absence of PIP\(_2\). A previous study in our laboratory provided evidence that different PIP\(_2\)-kinase family members synthesize functionally different pools of PIP\(_2\) (Vasudevan et al., 2009). To explore whether these functionally different pools of PIP\(_2\) might be synthesized in detergent-resistant ordered lipid domains and detergent-sensitive disordered lipid domains (Lingwood and Simons, 2010), we used transient expression of an inositol 5'-phosphatase that is targeted to either ordered or disordered lipids in RBL mast cells, and we found that the thapsigargin-stimulated association of STIM1 with Orai1 is regulated by the balance between PIP\(_2\) associated with ordered lipids and PIP\(_2\) associated with disordered lipids, such that PIP\(_2\) in ordered lipid domains promotes this association and PIP\(_2\) in disordered lipid domains reduces it, with corresponding alterations in SOCE (Calloway et al., 2011). Furthermore, we found that over-expression of PIP5-kinase β, which enhances PIP\(_2\) levels in both ordered and disordered lipid domains, enhances the stimulated association of STIM1 with Orai1, whereas over-expression of PIP5-kinase γ, which enhances PIP\(_2\) levels only in the disordered lipid domains, has an inhibitory effect on this association. This regulation of STIM1/Orai1 coupling by PIP\(_2\) could be attributed to a basic sequence in the N-terminal segment of Orai1 and a basic sequence at the C-terminus of STIM1 because deletion of either of these prevents the effects of both P5-kinase over-expression and targeted inositol 5-phosphatase expression on stimulated FRET (Calloway et al., 2011). A simple model for these effects is illustrated in Figure 2, in which ER store depletion causes STIM1 to bind initially to PIP\(_2\) in ordered domains at the plasma membrane, followed by redistribution of Orai1 from disordered regions of the plasma membrane to its association with STIM1 in ordered lipid domains. We are currently testing this hypothesis.

It is well-established that antigen-stimulated Ca\(^{2+}\) mobilization in mast cells is initiated by activation of phospholipase C (PLC) γ-1 and γ-2, which depends on tyrosine phosphorylation of specific residues in these proteins by Syk kinase and/or by the Tec kinase BTK (Gilfillan and Rivera, 2009; Ma and Beaven, 2009). These lipases hydrolyze PIP\(_2\) to produce inositol 1,4,5-trisphosphate (IP\(_3\)) and diacylglycerol, and it is IP\(_3\)-mediated release of Ca\(^{2+}\) from ER stores that activates the association of STIM1 with Orai1 to form productive CRAC channels as discussed above. Using fast confocal imaging of the genetically encoded Ca\(^{2+}\) sensor, GCaMP2, and proximal antigen delivery via a micropipette, we found that antigen-stimulated Ca\(^{2+}\) responses usually initiate as a fast Ca\(^{2+}\) wave that begins at the tips of extended membrane protrusions, which are common on well-attached cells (Figure 3; Cohen et al., 2009). Not surprisingly, this Ca\(^{2+}\) wave initiation depends on PLC activity, but it does not utilize CRAC channels because it is not inhibited by Gd\(^{3+}\), which selectively blocks these channels in RBL mast cells (Cohen et al., 2009; Calloway et al., 2011). In the absence of extracellular Ca\(^{2+}\), wave initiation occurs more frequently at other sites along the cell body, suggesting that Ca\(^{2+}\) entry is important for its initiation from protrusions. A previous study provided evidence that certain six transmembrane-structured TRPC cation channel proteins can contribute to antigen-stimulated Ca\(^{2+}\) entry in RBL mast cells (Ma et al., 2008). Our results from shRNA knockdown experiments indicated that wave initiation from protrusions depends on Ca\(^{2+}\) entry via two particular TRPC channels, TRPC1 and TRPC3, particularly at limiting concentrations of antigen (Cohen et al., 2009).
FIGURE 1 | Molecular features of STIM1–Orai1 interactions. Prior to activation, acidic residues of the cytosolic coiled-coil domain 1 (CC1) of STIM1 electrostatically bind to and mask the basic residues in the CAD domain that are critical for CRAC channel gating. Activation by depletion of luminal ER Ca\(^{2+}\) causes conformational changes in both luminal and cytosolic segments of STIM1. Changes in the luminal EF-hand motifs and the sterile A motif (SAM) results in clustering of STIM1 molecules (not shown) that leads to alterations in the cytosolic region: Intramolecular electrostatic interaction between CC1 and CAD are broken, and the positively charged sequence in the CAD domain is free to interact with the acidic domain within the C-terminal domain of Orai1 (A) to activate the CRAC channel formed by Orai1. STIM1 also interacts with the N-terminus of Orai1 (B), and the C-terminal polybasic sequence of STIM1 associates with PIPs at the plasma membrane (C). STIM1 and Orai1 monomers are depicted for simplicity; interactions involve oligomers of these proteins in a multimeric complex. Modified from Wang et al. (2010).
FIGURE 2 | Model for the sequence of events that initiate coupling of the ER sensor protein STIM1 to the CRAC channel protein Orai1. Activated STIM1 in the ER (not shown) binds initially via its C-terminal polylysine sequence to PIP2 (blue/violet) in ordered lipids (yellow) at the inner leaflet of the plasma membrane. Concentration of STIM1 at this site facilitates its coupling to Orai1 that redistributes from disordered lipids (red) to ordered lipids. A basic polyarginine sequence in the N-terminus of Orai1 mediates its electrostatic interactions with PIPs and STIM1.

CONSEQUENCES OF Ca2+ MOBILIZATION
DEGRANULATION

In recent experiments, we found that Ca2+ wave initiation from protrusions confers a spatial preference for granule exocytosis from sites along these protrusions, particularly at the same low doses of antigen that enhance wave initiation from the tips of these structures (Cohen et al., 2012). This spatial and temporal comparison of granule exocytosis and Ca2+ mobilization is possible because of the implementation of fast confocal imaging of fluorescein isothiocyanate (FITC)–dextran-labeled granules, together with Fura Red–indicated Ca2+ responses. These methods allow us to detect individual exocytosis events as bursts of FITC fluorescence and to compare the spatial and temporal relationships of these events to stimulated Ca2+ oscillations in these cells. Consistent with an earlier study that used amperometry to compare the temporal relationship between granule exocytosis and Ca2+ oscillations (Kim et al., 1997), we find that exocytosis events monitored by FITC–dextran bursts occur primarily at the peaks, or just following the peaks, of sustained Ca2+ oscillations that depend on SOCE (Cohen et al., 2009).

We also find that granule exocytosis under these conditions is sensitive to knockdown of the TRPC1 Ca2+ channel protein, further supporting a role for Ca2+-dependent wave initiation via this channel in spatially directed granule exocytosis (Cohen et al., 2012).

Multiple lines of evidence point to the participation of protein kinase C (PKC) isoforms in mast cell granule exocytosis, but the mechanism has been unclear (Rivera and Beaven, 1997). Molecular genetic studies highlight a specific role for PKCβ (PKCB) in this process (Nechushtan et al., 2000). Our studies to understand PKC in stimulated granule exocytosis have utilized the effector domain (ED) of the ubiquitous MARCKS protein. MARCKS-ED is a 25-amino acid segment that contains 13 basic residues that mediate its very tight binding to polyphosphoinositides (PIPs) at the inner leaflet of the plasma membrane (Gambhir et al., 2004; Heo et al., 2006). Protein kinase C normally causes dissociation of MARCKS by phosphorylating three serine residues in the ED sequence, thus adding negative charges to reduce the electrostatic interaction of the ED and thereby increasing access to other PIP-binding proteins in processes such as granule exocytosis (Figure 4).

To explore the role of this PIP sequestration by MARCKS-ED in stimulated mast cell granule exocytosis, we over-expressed a ser→ala mutated form of this polypeptide, which lacks the capacity to be displaced by PKC phosphorylation. We found that this polypeptide construct effectively delays antigen-stimulated Ca2+ mobilization, including release of Ca2+ from intracellular stores, most likely because it reduces access to and hydrolysis of PIP2 by PLC. Despite this delay, the sustained phase of SOCE stimulated by antigen, and the Ca2+ response to thapsigargin, are not inhibited. However, the degranulation response to each of these stimuli is substantially delayed and reduced (Figure 5; Gadi et al., 2011). These results are consistent with a model in which the availability of PIPs is important for stimulated exocytosis at a step that is downstream of Ca2+ mobilization. Synaptotagmins are multidomain proteins known to play key roles in Ca2+-dependent exocytosis in neuronal and other cell types (Chapman, 2008). We hypothesize that PIP2-dependent binding of synaptotagmin to Ca2+ (Bai et al., 2004) is critically inhibited by mutated MARCKS-ED, although we cannot exclude a role for PIP2 in other SNARE-dependent steps (James et al., 2010). In general, the ser→ala mutated MARCKS-ED has proven to be a useful reagent for evaluating PIP participation in signaling processes based on the capacity of this polypeptide to sequester these key lipids in a manner that is not reversed by PKC phosphorylation.

Does PKC phosphorylation of endogenous MARCKS play a physiological role in the regulation of stimulated granule exocytosis? One indication of this possibility comes from a comparison that we made between the effects of over-expression of the wild type sequence of MARCKS-ED and the effects of the ser→ala mutated MARCKS-ED summarized above. In contrast to the inhibitory effects of mutated MARCKS-ED on stimulated granule exocytosis, we found that the wild type sequence does not inhibit this process, confirming that serine phosphorylation by PKC facilitates the dissociation of this sequence during cell signaling to facilitate subsequent events (Gadi et al., 2011).
MARCKS has long been known as a substrate for PKCs, but the physiological role of the full-length protein in cells has been elusive (Stumpo et al., 1995; Trifaro et al., 2008). To evaluate more directly the role of endogenous MARCKS in mast cell functional responses, we knocked down this protein expression using two different siRNA sequences. We were initially surprised by our results indicating that endogenous MARCKS exhibits a positive effect on antigen-stimulated Ca\(^{2+}\) mobilization: Both siRNA constructs cause a significant reduction in SOCE (31 ± 7% inhibition, \(n = 5\)), without inhibiting antigen-stimulated Ca\(^{2+}\) release from ER stores (Figure 6A). Consistent with this effect, we also found that these siRNA sequences inhibit antigen-stimulated granule exocytosis measured in suspended RBL cells with granule-incorporated FITC–dextran (Figure 6B; 30 ± 3% inhibition, \(n = 2\)). These results indicate that endogenous MARCKS normally facilitates antigen-stimulated Ca\(^{2+}\) and degranulation responses.

How might MARCKS contribute to enhancement of stimulated SOCE that supports enhanced exocytosis? One possibility is that MARCKS dissociates from the plasma membrane and associates with Ca\(^{2+}\)-bound calmodulin under stimulating conditions. This would occur upon elevation of intracellular Ca\(^{2+}\), and it could provide a mechanism by which Ca\(^{2+}\)/calmodulin-mediated processes...
are regulated (McLaughlin and Murray, 2005). One of these well-studied processes is the rapid inactivation of STIM1-mediated SOCE, which has recently been shown to depend on the binding of Ca\(^{2+}\)/calmodulin to a specific sequence in STIM1 (Mullins et al., 2009). Inhibition of this interaction by MARCKS association with Ca\(^{2+}\)/calmodulin would provide a plausible mechanism for the enhancing effects of PKC-phosphorylated MARCKS on SOCE and consequent enhancement of granule exocytosis.

**CELL MOTILITY AND CHEMOTAXIS**

Cell migration is critical for various biological functions, such as embryogenesis, wound healing, and immune responses, and it can also contribute to the pathogenesis of diseases including cancer and transplant rejection. Understanding basic mechanisms of cell motility has been a goal of scientific investigations since the emergence of optical microscopy. Cell motility requires the actin cytoskeleton, asymmetric morphology of the cell, and polarized intracellular signaling (Mitchison and Cramer, 1996; Petrie et al., 2009). Polarization and development of leading and trailing edges of the cell mediate cell locomotion by dynamic extension and retraction of cellular protrusions, including pseudopods, filopodia, and lamellipodia (Gupton and Gertler, 2007).

Cells often respond to a gradient of external factors or asymmetric environmental cues by means of a compass or steering mechanism coupled to basal motility machinery, resulting in directed migration. Similarly, chemotaxis occurs in response to soluble cues (Bourne and Weiner, 2002; Arrieumerlou and Meyer, 2005). Mast cells accumulate at sites of inflammation in response to parasitic and bacterial infections (Madden et al., 1991; Echtenacher et al., 1996). Differentiated mucosal mast cells are known to redistribute from the submucosa or crypt area to the lamina propria and intraepithelial regions of jejunal villi during the course of an immune response to certain parasitic infections (Friend et al., 1996). Mast cell chemotaxis toward IgE-specific antigen was first described for RBL cells (Orida et al., 1983) and has also been characterized in bone marrow-derived mouse mast cells (Kitaura et al., 2005; Olivera et al., 2006). Recently, we observed with real-time imaging that Ca\(^{2+}\) influx via Orai1 plays an important role in regulating both spontaneous, random motility, as well as chemotaxis toward antigen for both RBL mast cells and bone marrow-derived rat mast cells (J. Lee et al., submitted). Inhibition of Ca\(^{2+}\) influx, or knockdown of the Ca\(^{2+}\) entry channel protein, Orai1, by shRNA in RBL cells reduces both of these processes. RBL cells expressing the Ca\(^{2+}\) sensor, GCaMP3, exhibit spontaneous Ca\(^{2+}\) transients that depend on Ca\(^{2+}\) influx, and their appearance correlates with cell motility. Our results thus identify a novel Ca\(^{2+}\) influx-mediated, Orai1-dependent mechanism for mast cell migration.

**OTHER Ca\(^{2+}\)-DEPENDENT PROCESSES**

In addition to degranulation and chemotaxic responses to antigen-mediated crosslinking of IgE receptor complexes, rat mast cells undergo additional exocytotic processes that depend on Ca\(^{2+}\) mobilization. One of these is the stimulated outward trafficking of recycling endosomes that are labeled with FITC–cholera toxin B (CTB) bound to ganglioside GM1, which we previously characterized (Naal et al., 2003; Smith et al., 2010). We found that this process depends on Ca\(^{2+}\) mobilization and normal cholesterol levels, but not on PKC activity. In the absence of extracellular Ca\(^{2+}\), this process is transient, indicating that Ca\(^{2+}\) influx is necessary for a sustained response. This stimulated trafficking
targets delivery of CTB/GM₁ to spatially restricted sites where \( \text{IgE} \) receptors are clustered and activated (Wu et al., 2007), and this is reminiscent of the cholesterol-dependent delivery of TNF\( \alpha \) to phagocytic cups in murine macrophages (Murray et al., 2005; Kay et al., 2006). The macrophage studies provided evidence that recycling endosomes participate in cytokine secretion in those cells. TNF\( \alpha \), as well as other cytokines, are synthesized \textit{de novo} in rodent mast cells in response to activation via IgE receptors (Baumgartner et al., 1994). Stimulated secretion of TNF\( \alpha \) does not involve trafficking to secretory lysosomes as it does for pre-stored TNF\( \alpha \) in human mast cells (Gordon and Galli, 1991). Both the initial phase of stimulated transcriptional activation and the secretion of TNF\( \alpha \) require \( \text{Ca}^{2+} \) mobilization (Baumgartner et al., 1994). Activation of NFAT transcriptional factors via

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**FIGURE 6** | Effects of siRNA knockdown of endogenous MARCKS protein on \( \text{Ca}^{2+} \) (A) and degranulation (B) responses in RBL cells. (A) RBL cells transfected with irrelevant control siRNA or two different siRNA sequences specific for rat MARCKS (Gadi, 2012) were loaded with the \([\text{Ca}^{2+}]\) indicator indo-1 before stimulating with antigen in the absence of extracellular \( \text{Ca}^{2+} \). Then millimolar \( \text{Ca}^{2+} \) was added to enable SOCE. (B) RBL cells, transfected as in (A), and loaded with FITC–dextran (Gadi et al., 2011), were treated with 1 mM cytochalasin D and then stimulated with antigen. FITC fluorescence increases due to stimulated degranulation were monitored by steady state fluorimetry.
Ca²⁺/calcineurin-mediated dephosphorylation of these proteins is an important part of this process (Andrade et al., 2011). In contrast, constitutive synthesis and trafficking of another cytokine, TGF-β, does not require elevated cytoplasmic Ca²⁺ (Baumgartner et al., 1996). Thus, the requirement for elevated cytoplasmic Ca²⁺ for stimulated cytokine production suggests a different mechanism for their exocytic release than for constitutive biosynthetic protein trafficking. An interesting question to be pursued from these results is whether antigen-stimulated cytokine secretion in mast cells utilizes trafficking through recycling endosomes en route to the plasma membrane.

Stimulated outward trafficking of recycling endosomes labeled with FITC–CTB is inhibited by basic sphingosine derivatives that spontaneously flip to the inner leaflet of the plasma membrane. Sphingosine derivatives that do not readily flip, including N,N,N′,N″-trimethylsphingosine and glycosylated sphingosine (psychosine), do not inhibit this process (Smith et al., 2010). Using the ser → ala mutated MARCKS-ED construct, we further showed that inhibition by these long chain bases correlates with electrostatic neutralization of negatively charged PIPs at the cytoplasmic face of the plasma membrane. Similarly, the sphingosine derivatives that readily flip across the membrane inhibit antigen-stimulated Ca²⁺ mobilization and granule exocytosis, providing evidence for a general mechanism of phosphoinositide sequestration by these compounds (Smith et al., 2010). This consistent set of results lends new insight into the interactions by which sphingosines inhibit downstream signaling in many different published studies.

An additional Ca²⁺-dependent process that generates important mediators in stimulated mast cell responses is the activation of phospholipase A2 to produce arachidonic acid, which is the precursor for leukotrienes and prostaglandins, two different families of eicosanoids that act as ligands for specific G protein-coupled receptors (Beaven, 2009). Cytoplasmic phospholipase A2 is activated by Ca²⁺ binding together with Erk-dependent phosphorylation, which in turn is activated by the MAP kinase cascade (Hirasawa et al., 1995). These eicosanoid mediators can act in an autocrine fashion to synergize with antigen stimulation of mast cells, as well as to activate other cells in processes such as anaphylaxis (Beaven, 2009).

OUTSTANDING QUESTIONS

Mast cells are receiving increasing attention as mediators of innate immune responses in addition to their better-known role in the adaptive allergic response (Metz et al., 2007; Brown et al., 2008). Although mast cells are recruited to the gut where they undergo dramatic proliferation in response to certain parasitic infections such as with *Trichinella spiralis* (Friend et al., 1996), a recent study has shown that they do not contribute significantly to parasite clearance during the secondary immune response (Blum et al., 2009). This suggests that mast cells participate in the initiation of the Th2 immune response, rather than in the effector phase. Our studies summarized above suggest that antigen-specific recruitment of mast cells to small intestinal villi during such immune responses may be driven by Ca²⁺ mobilization in a process that utilizes STIM1-mediated activation of CRAC channels that are formed by Orai1. Understanding the mechanism by which these proteins and the Ca²⁺ response they elicit provide a cell polarization cue in response to antigen will be a challenging goal for future studies.

Mast cells also respond to other chemotactic cues, such as the lipophilic ligand for G protein-coupled receptors, sphingosine-1-phosphate (Jolly et al., 2004; Olivera et al., 2007). We found that RBL cells chemotax toward this ligand with a robust response, but in this case, chemotaxis is not dependent on extracellular Ca²⁺ (J. Lee et al., submitted). The mechanism of signaling involved in this process remains to be determined.

RBL mast cells undergo robust phagocytosis of beads coated with ligands for IgE receptors, and, under these conditions, granule exocytosis is severely restricted (Pierini et al., 1996). The mechanism for this differential regulation is unclear, and recent experiments indicate that Ca²⁺ mobilization and PKC recruitment to the plasma membrane are not limiting under these conditions (K. Corwith, unpublished results). In macrophages, trafficking of recycling endosomes to the forming phagosome has been shown to contribute to this process (Cox et al., 2000), and we are interested in the potential role of this trafficking in mast cell phagocytosis and in the signaling consequences. Recent studies have revealed a role for recycling endosomal trafficking in the maintenance of the parasitic vacuole that is formed by invading *Toxoplasma gondii* in mast cells, as well as disruption of normal FcεRI signaling by this process (N. Smith et al., in preparation). Together, these studies reveal dynamic alterations of IgE receptor signaling by phagocytosis or parasite invasion, but the molecular mechanisms of these alterations remain to be characterized.

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