

The impact of bacterial infection on mast cell degranulation

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Abstract In developed countries, the prevalence of allergy is on the rise. Although the causes are unknown, it seems that (1) the disappearance of microbiota may play a role in the increase of allergies and (2) exposure to bacterial infections during childhood decreases the incidence of allergies. Although several cell types are involved in the development of allergy, mast cells play a major role in orchestrating inflammation. Upon activation, mast cell secretory granules fuse with the plasma membrane, resulting in the release of a number of inflammatory mediators. In addition to allergy, mast cells contribute to the innate immune response against a variety of bacteria. This is accomplished through the secretion of cytokines and other soluble mediators. Interestingly, there is growing evidence that mast cells exposed to bacteria down-regulate degranulation in response to IgE/Allergen stimulation. This inhibitory effect seems to require direct contact between bacteria and mast cells, but the intracellular mechanism by which bacterial contact suppresses allergic responses is unknown. Here, we review different aspects of mast cell physiology and discuss hypotheses as to how bacteria may influence mast cell degranulation.

Keywords Allergy · Mast cell · Infection · Bacteria · Degranulation · SNAREs · Phagocytosis · Membrane fusion

Introduction

During the last several decades, the incidence of allergy and asthma has risen dramatically [1, 2], spurring intense research into the molecular mechanisms of allergic reaction. In parallel, investigation of the impact of genetic and environmental factors on the development of atopy has increased [3, 4]. Epidemiological studies suggest that the most exceptional increase in allergic disorders has occurred in industrialized countries [4]. This rise has coincided with a sharp reduction in the prevalence of infectious diseases due to the advent of vaccines, antibiotics and increased

sanitation. In the mid 1980s, Strachan proposed the ‘hygiene hypothesis’, which suggested that the increase in atopy may be linked to a reduced exposure to parasitic infections as well as other pathogenic and non-pathogenic microorganisms [5]. Since then, there have been many revisions made to this theory. While numerous epidemiological and experimental data have shown an inverse relationship between infection and the development of atopy, a number of studies have also found no association between infection and allergy [6–8].

Mechanistic explanations for the ‘hygiene hypothesis’ have typically invoked the T-helper-type (T_H1/T_H2) model [9]. T-Helper (T_H) cells respond to infection and induce inflammation through the production of numerous cytokines. T_H cells are divided into several types based on their cytokine profile [10]. Among them, T_H1 cells produce proinflammatory mediators and contribute to cell-mediated immunity as observed during infection. T_H2 cells are important for the production of IgE and IgG₁ antibodies by B cells and are considered anti-inflammatory [10, 11].

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Allergic inflammation is primarily a T_H2 -mediated process that results in the production of systemic IgE. On the other hand, most bacterial infections induce a T_H1 -skewed response. It has been proposed that the decrease in pathogen exposure early in life, thus a lack of T_H1 stimuli, results in immune responses that are primarily T_H2 -biased. As such, infants would be more susceptible to developing allergic diseases and asthma. However, a T_H1/T_H2 imbalance cannot be the sole mechanism since helminth infections that induce a T_H2 response help protect against allergy [6, 9]. Therefore, the correlation between infection and allergy in the development of atopy remains a subject of debate. In order to understand such a complex interaction, it is necessary to examine the consequences that different infections may have on the molecular mechanisms of allergic inflammation.

In this review, we will explore how bacterial infections impact the function of mast cells, a key cellular player in the development of allergic reactions. Mast cells play an important role in both innate and adaptive immunity. Although mostly recognized for their role in allergies, mast cells also participate in the immune response to bacterial infection. Localized at the host-environment interface, mast cells are prevalent in both connective tissues (i.e., the skin) as well as mucosal tissues such as the gastrointestinal tract and lungs [12]. This localization allows mast cells to respond to both allergens and bacteria and makes them potentially able to coordinate the impact of bacterial infections on the development of allergies.

Mast cells in allergy

The allergic response is a complex process that involves both innate and adaptive branches of the immune system. The use of mast cell-deficient mice has allowed investigators to examine the *in vivo* role of mast cells in allergy and asthma. Mice lacking mast cells exhibited reduced airway reactivity in asthma models as well as reduced allergic symptoms and inflammation [13–15]. Moreover, reconstituting mast cell-deficient mice with mast cells from different knockout mice has established a role for mast cell-derived mediators such as $TNF-\alpha$ and MIP2 in the development of allergy [16–18].

Mast cells possess electron dense lysosome-like secretory granules filled with a number of preformed and *de novo* synthesized mediators [19]. These mediators can direct both innate and adaptive immunity. This is most exemplified by the role mast cells play in allergy. Initial exposure to an allergen results in the expansion of allergen-specific T cells and the production of IgE by activated B cells [3]. The high-affinity $Fc\epsilon$ receptor I ($Fc\epsilon RI$), present on the surface of mast cells, binds the newly synthesized

IgE. As a result, mast cells become sensitized. During subsequent exposures to the same allergen, the IgE bound on the mast cell surface in turn binds the multivalent allergen (Fig. 1, step 1) [6]. This results in the cross-linking of $Fc\epsilon RI$ on the plasma membrane and the activation of mast cells. The signaling cascade that follows receptor engagement (Fig. 1, step 2) ultimately leads to mast cell degranulation (Fig. 1, step 6), during which, mast cell secretory granules fuse with the plasma membrane and release their contents [3, 20].

The capacity for mast cells to quickly secrete large quantities of mediators is also due to compound exocytosis (Fig. 1, step 5). This process relies on the fusion of granules with the plasma membrane as well as the fusion of granules with themselves [20, 21]. The mediators that are rapidly released in response to $Fc\epsilon RI$ -mediated activation include bioactive amines (histamine and serotonin in rats and mice, only histamine in humans), heparin, proteases (β -hexosaminidase), and pre-stored growth factors and cytokines, such as $TNF-\alpha$. *De novo* production of lipid mediators (leukotrienes and prostaglandins), cytokines, chemokines, and growth factors also occurs in response to $Fc\epsilon RI$ -dependent activation [3, 19, 22]. The local release of preformed mediators is responsible for the symptoms of the early-phase reaction in allergy. Acute allergic symptoms include vasodilation, smooth muscle contraction, increased mucus production, and increased vascular permeability [3]. The gradual release of mast cell-derived *de novo* synthesized cytokines and chemokines contributes to the activation and recruitment of other immune cells to the site of inflammation during late-phase allergic reactions [3, 22]. In particular, mast cell-derived IL-3, IL-5, and GM-CSF activate eosinophils and basophils, while IL-8 and $TNF-\alpha$ activate neutrophils. IL-3 and IL-9 also reciprocally enhance mast cell differentiation. Mast cell-produced IL-13 drives B-cell growth and differentiation, thus functioning as a positive-feedback loop [3, 22]. The impact of mast cell mediators on the immune response and in the development of pathology associated with allergy establishes them as pivotal players in allergic inflammation.

Mast cell degranulation at the molecular level

Mast cell secretory granules

A hallmark feature of mast cells is the large number of granules densely packed in the cytoplasm. Histochemical and subcellular fractionation techniques have revealed heterogeneity among the cytoplasmic granules. As such, they can be divided into different populations based on their luminal and membrane content [23–25]. In Interferon- γ stimulated bone marrow-derived mast cells (BMMCs),

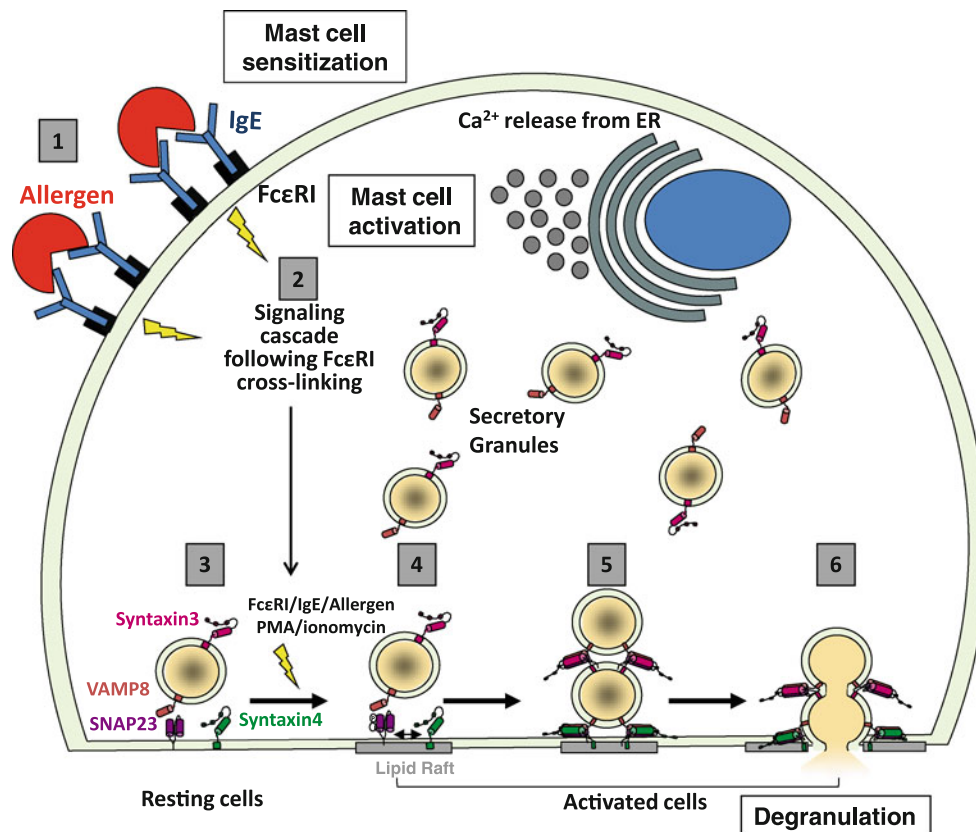


Fig. 1 Mast cell activation and degranulation. (1) Allergen-specific IgE is produced during the first exposure to an allergen and binds the FcεRI on the surface of mast cells. During subsequent exposures to the same allergen, the allergen binds to IgE bound on the mast cell. This results in the cross-linking of the FcεRI and mast cell activation. (2) A signaling cascade is initiated in response to FcεRI cross-linking. Ca²⁺ release from the ER is one downstream consequence of FcεRI cross-linking. (3) In resting cells, the t-SNAREs SNAP23 and Syntrophin4 are located on the plasma membrane, while the v-SNARE VAMP8 and the t-SNARE Syntrophin3 are located on the granules. (4) Upon FcεRI cross-linking, the t-SNARE SNAP23 is phosphorylated.

As a result, its cognate t-SNARE, Syntrophin4 is recruited into lipid rafts where they interact to form a t-SNARE complex. (5) VAMP8 present on the secretory granule interacts with the t-SNARE complex Syntrophin4/SNAP23 on the plasma membrane to form a stable four-helix bundle. (6) This interaction brings the opposing membranes into close proximity, leading to fusion. Fusion of the secretory granule with the plasma membrane results in the release of inflammatory mediators. Compound exocytosis also occurs. Upon activation, SNAP23 also relocates to the granules. It is speculated that t-SNAREs Syntrophin3/SNAP23 on the granule fuse to another granule containing the VAMP8. This results in the rapid release of mediators

three different types of granules have been identified—Type I, II, and III [24]. Type I granules (amine-free) contain conventional lysosomal markers such as mannose 6-phosphate receptor (MPR). These granules are located along the endocytic pathway as they can be labeled with fluid phase markers [24]. Type II and III granules are amine-containing secretory granules. Type II granules contain only serotonin and MPR and stain positive for fluid phase markers, while type III granules contain serotonin and do not label with fluid phase markers [24]. The granule luminal content also varies between mast cell phenotypes (connective versus mucosal mast cells). In contrast to BMMCs, the RBL-2H3 cell line, a widely used rat mast cell line, only contains type I and type III granules [23, 26]. In addition to differentiating granules by luminal content, Puri and Roche have further distinguished granule populations based on their dependence upon different

SNARE protein isoforms which are components of the membrane fusion machinery [25].

SNARE proteins

Mast cell degranulation has long been implicated in the pathology associated with allergy and has been well characterized at the molecular level. The fusion of secretory granules with the plasma membrane is a crucial step in degranulation. SNARE proteins are part of the core fusion machinery involved in mast cell exocytosis [27]. SNAREs form a highly conserved family of proteins that mediate fusion of intracellular membranes in all eukaryotic cells. These proteins are present on almost all intracellular compartments and form a stable four-helix bundle that brings opposing membranes into close proximity to drive membrane fusion [28, 29] (Fig. 1 steps 3–6). SNARE

proteins are divided into two categories based on their relative location—target- (t-) and vesicle- (v-) SNAREs [30]. While the v-SNARE is always a single protein, the t-SNARE consists of three subunits—one heavy chain and two light chains [31]—that pre-assemble to form the t-SNARE complex. The t-SNARE light chains can be composed of two individual proteins or one protein that is typically lipid-anchored to the membrane [28, 29, 31]. The specificity of fusion is encoded in the association of a particular t-SNARE complex with its cognate v-SNARE [32–35]. Thus, fusion events within the cell are regulated by different SNARE complexes.

The SNARE complex involved in IgE-dependent mast cell degranulation has been investigated in depth and is composed of the t-SNARE Syntaxin4 (heavy chain)/SNAP23 (light chains), both located at the plasma membrane and the cognate v-SNARE VAMP8, associated with granules [36] (Fig. 1, step 3). In addition, VAMP7, a v-SNARE localized on granules, also participates in mast cell degranulation through the interactions with Syntaxin4/SNAP23 in primary human mast cells [37].

SNARE-mediated fusion of secretory granules with the plasma membrane is a multi-step process (Fig. 1, steps 3–6). Following mast cell activation (Fig. 1, step 1–2), the lipid-raft associated SNAP23 is phosphorylated by the host kinase IKK β [38, 39] (Fig. 1, steps 3–4). Phosphorylation of SNAP23 results in the recruitment of Syntaxin4 into the lipid rafts to form a t-SNARE complex [39, 40] (Fig. 1, step 4). Once the t-SNARE complex has formed, the v-SNARE VAMP8 assembles with the t-SNARE complex Syntaxin4/SNAP23 and brings the granule membrane in close apposition to the plasma membrane (Fig. 1, step 5). As the SNARE complex zippers, the space between both membranes is reduced to a minimum and fusion occurs, leading to the release of the granule content [36] (Fig. 1, step 6). The inhibition or knockout of the t-SNAREs Syntaxin4 and SNAP23, or of the v-SNARE VAMP-8, severely inhibits mast cell degranulation [25, 36, 41]. Allergen sensitization of mast cells increases the expression of the exocytic SNAREs SNAP23 and Syntaxin4 [42], which supports the critical role of these SNARE proteins in IgE-mediated mast cell degranulation.

In mast cells, degranulation occurs not only through the fusion of individual granules with the plasma membrane but also through granule–granule fusion, a process called compound exocytosis (Fig. 1, steps 5–6) [20, 21]. Guo and colleagues have demonstrated that SNAP23 is involved in this process both at the plasma membrane and in granule–granule fusion, where it relocates following mast cell activation [43]. Contrary to SNAP23, Syntaxin4 does not appear to relocate on the granule membrane. Therefore, it is unlikely that Syntaxin4 participates to granule–granule fusion [43]. Interestingly, Syntaxin3, another heavy chain,

is located on the granules and has been shown to interact with SNAP23 [44]. Although the function of Syntaxin3 in mast cells is still unclear, it is speculated that it participates in compound exocytosis by forming a functional t-SNARE complex with SNAP23 on secretory granules [45] (Fig. 1, step 5). The role SNARE proteins play in mast cell degranulation and subsequent pathology emphasizes the importance of SNARE proteins in the development of disease.

Regulatory proteins of SNARE-mediated fusion in mast cells

Although SNARE proteins form the core machinery for membrane fusion, additional levels of regulation are needed to ensure that fusion does not spontaneously occur. Numerous regulatory proteins of SNARE function have been identified. In this section, we only examine the role of three classes of regulatory proteins important in mast cell degranulation—synaptotagmins, complexins, and Munc18 proteins.

Synaptotagmins constitute a family of Ca²⁺ sensors that play a major role in Ca²⁺-dependent exocytosis. This function has been demonstrated using the neuronal system in which synaptotagmin1 (Syt1) is expressed [46]. In neurons, Syt1 binds to a number of effectors including SNAREs and anionic phospholipids such as phosphatidyl serine (PS). Mechanistically, it is thought that Syt1 binds SNARE proteins at a low affinity in the absence of Ca²⁺. When mast cells are stimulated and Ca²⁺ levels increase, the affinity of Syt1 for SNARE proteins and PS increases [46]. Interactions with Syt1 and phospholipids such as PS have been shown to induce membrane curvature leading to membrane destabilization thus favoring membrane fusion [46].

Mast cells express different synaptotagmin isoforms, including Syt 1, 2, 3, 5, and 9 [47, 48]. Overexpression of Syt1 and Syt2 in RBL-2H3 mast cells results in increased β -hexosaminidase release when compared with the control [47, 49], suggesting that Syt1 and Syt2 are negative regulators of degranulation. However, bone marrow-derived mast cells (BMMC) from Syt2 knockout mice showed 70% decrease in the secretion of histamine and β -hexosaminidase [50], suggesting that Syt2 functions as a positive regulator of degranulation. The difference of granule composition between RBL-2H3 and BMBCs might explain such inconsistencies. While BMBCs contain three granule populations, RBL-2H3 mast cells only possess two distinct granule populations [26]. Additionally, RBL-2H3 mast cells are mucosal-like while the BMBCs from the Syt2 knock-out mice are connective tissue-type mast cells [50, 51]. Interestingly, Syt2 in RBL-2H3 mast cells appears to be localized on the lysosomes rather than on the

secretory granules. This suggests that Syt2 is likely involved in lysosomal secretion [47]. In summary, while it is clear that synaptotagmins play a role in Ca^{2+} -dependent exocytosis in mast cells, the mechanism by which they operate remains unclear.

Complexins (CPX) form another important family of regulatory proteins that are expressed in both neuronal and non-neuronal tissues. There are four complexin isoforms, namely CPX1–4 [52]. Complexin1 (CPX1), mostly expressed in neurons, interacts with assembled t-SNARE complexes and clamps partially zippered t-/v-SNARE complexes in a primed state, inhibiting complete fusion [53, 54]. The clamping by complexin1 is Ca^{2+} insensitive [53–56]. However, addition of synaptotagmin1 and Ca^{2+} to the SNARE-CPX1 complex induces a rapid release of CPX1. It appears that the conformational change in synaptotagmin1 due to Ca^{2+} binding displaces CPX1 from the SNARE complex thus releasing the clamp and allowing fusion to occur [55, 57].

Mast cells have been shown to express complexin2 (CPX2). Knockdown of CPX2 inhibits mast cell degranulation by reducing the Ca^{2+} sensitivity of the fusion step. This suggests that CPX2 is a positive regulator of mast cell degranulation and enhances calcium sensitivity of fusion [58]. Interestingly, recombinant CPX2 has been shown to interact with Syntaxin3, but not Syntaxin4. Furthermore, the affinity of Syntaxin3 for CPX2 was increased with the addition of SNAP23 and VAMP8 [59]. These data support a role for Syntaxin3 in mast cell degranulation. Previous studies using protein overexpression of Syntaxin4 and Syntaxin3 only demonstrated a role for Syntaxin4 in degranulation [36]. Syntaxin3 interacts with Munc18-2, another regulatory protein, in addition to CPX2. Syntaxin4, on the other hand, has only been shown to interact with Munc18-3 [60]. Therefore, it might be more difficult to experimentally tease out the function of Syntaxin3 due to the fact that Syntaxin3 appears more tightly regulated [59].

The last family of SNARE regulatory proteins that we will discuss here is the Munc18 family. Three isoforms of Munc18 have been identified—Munc18-1, Munc18-2 and Munc18-3 [61]—and each of them has been shown to interact with specific Syntaxins [62]. Using a variety of cellular systems, it has been demonstrated that this family of proteins is essential for exocytosis [61–63]. Mast cells express Munc18-2 and Munc18-3, but not Munc18-1 [60, 64]. Initial studies conducted by Martin-Verdeaux and colleagues have shown that Munc18-2 overexpression inhibits Fc ϵ RI-mediated degranulation. This finding was later confirmed by knocking down Munc18-2 in RBL-2H3. In these cells, mast cell degranulation was also inhibited [65]. Using immunoprecipitation, it has been observed that Munc18-2 mainly interacts with Syntaxin3 and less so with Syntaxin2. No interaction was observed between

Munc18-2 and Syntaxin4. However, Syntaxin4 was shown to interact with Munc18-3 [60]. Together these data suggest that Munc18-2 is involved in the regulation of mast cell degranulation through its interaction with Syntaxin3. Interestingly, co-overexpression of Munc18-2 and Syntaxin3 induced a relocalization of Munc18-2 to Syntaxin3 containing compartments, supporting a tight relationship between Munc18-2 and Syntaxin3. Syntaxin4 overexpression did not impact Munc18-2 distribution [65]. The exact mechanism of how Munc18-2 and Munc18-3 function in mast cell degranulation remains to be clarified.

The mechanism by which mast cell degranulation occurs is a complex process. Diverse populations of granules fuse with the plasma membrane upon IgE/allergen cross-linking. This fusion process is mediated in part by SNARE proteins. In order to ensure that granule fusion does not spontaneously occur, a number of Ca^{2+} -dependent and Ca^{2+} -independent proteins regulate SNARE-mediated fusion.

Mast cells in bacterial infection

Role of mast cells in infection: in vivo studies

In addition to contributing to allergic inflammation, it has become evident that mast cells are important for the innate immune response to bacterial infections [12]. The generation of mast cell-deficient mice has led to the finding that mast cells are important for the clearance of a number of bacteria. *Klebsiella pneumonia*, a common causative agent of peritonitis and urinary tract infections, is one model organism that has been used to examine mast cell involvement in bacterial infections. Mast cell-deficient mice are unable to survive *Klebsiella pneumonia* challenge. However, upon specific reconstitution of mast cells to the peritoneal cavity, the mast cell-deficient mice were able to survive challenge similar to wild-type mice [66]. It has also been demonstrated that mast cells are important for the protection from *Mycoplasma pneumonia* [67], a common respiratory pathogen, as well as *Listeria monocytogenes* [68].

Mast cell secretion plays a role in the host response to bacterial infection. Mast cells secrete a number of mediators, including proinflammatory cytokines, in response to bacterial infection. More specifically, histamine, IL-6, and TNF- α have been shown to be important mast cell-derived mediators during bacterial infection [69–71]. Malaviya and colleagues demonstrated that mast cell-derived TNF- α was a key cytokine for the recruitment of neutrophils during *E. coli* infection [70]. Additionally, mast cell-derived TNF- α was shown to increase expression of E-selectin in the endothelium during *E. coli* infection, which enhanced the

recruitment of dendritic cells to the site of infection [72]. Using *Staphylococcus aureus*-derived peptidoglycan, a cell wall component of the bacterium, Marshall, and colleagues found that mast cells themselves as well as the mediators histamine and IL-6 regulate the recruitment of dendritic cells into the lymph nodes [69]. In addition to contributing to the recruitment of inflammatory cells to the site of infection, mast cells have also been shown to have direct antimicrobial activity. Co-incubation of mast cells with *pneumococci* resulted in bacterial death. This antimicrobial activity is activated by the pneumococcal toxin pneumolysin. Pneumolysin stimulated the release of the cathelicidin LL-37, an antimicrobial peptide, which was responsible for pneumococcal death [73].

It is clear that mast cells provide protection against a variety of bacterial infections. This is partly accomplished through the secretion of a number of preformed and de novo synthesized mediators, which recruit other immune cells to the site of infection. In order to define the relationship between mast cells and bacteria in more depth, many have examined the interactions between mast cells and bacteria in vitro.

In vitro interactions between mast cells and bacteria

In vitro analysis of mast cell interactions with bacteria have yielded many interesting results. Typically, bacteria are cleared from circulation by professional phagocytes—namely macrophages, dendritic cells, and neutrophils. Mast cells were added to this class of phagocytic cells in 1968 [74, 75]. Co-incubation of mast cells with bacteria results in internalization of the bacteria via phagocytosis [76]. Briefly, phagocytosis begins at the cell surface where bacteria bind different receptors on the plasma membrane. Next, the cell engulfs the pathogen resulting in the formation of a nascent phagosome derived from the plasma membrane. This membrane bound compartment harboring the bacteria is trafficked into the endocytic pathway where it fuses with the early endosomes, late endosomes, and subsequently, lysosomes. It is within the phagolysosomal compartment that the bacteria are digested [77, 78].

Similar to mast cell degranulation, SNARE proteins mediate all fusion events in phagocytosis [79]. Knowing the different SNARE partners involved in this process is critical to understand how efficient the mast cell phagocytic pathway is, and how important this pathway may be for clearing bacterial infections. However, the SNARE machinery involved in this process has not been characterized. Our laboratory is actively investigating this machinery and is in the process of identifying which SNAREs control the different steps of mast cell phagocytosis.

Mast cells internalize a variety of bacteria such as *E. coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*,

Pseudomonas aeruginosa, and *Mycobacteria tuberculosis* [76, 80–82]. Phagocytosis of bacteria in mast cells has been shown to occur through complement receptors, IgG receptors and through direct interactions with the bacterial adhesin, FimH [83]. Similar to other phagocytic cells, mast cells possess bactericidal activity, which relies on reactive oxygen species and acidification of the phagosome [76], suggesting that the lysosomal compartment is involved. Lysosomes contain a number of enzymes and toxic radicals that are able to digest internalized bacteria. In mast cells, one main function of the lysosome is secretory (see previous section in this review). Different populations of secretory granules have been shown to be located in both the endocytic and exocytic pathways as determined by the uptake of fluid phase markers [26]. This would position the secretory granules to fuse with the phagosomes as they are trafficked through the endocytic pathway. It remains to be determined whether mast cell secretory granules contribute to the bactericidal activity of mast cells.

Mast cell exposure to certain bacterial strains leads to mast cell activation. Incubation of mast cells with FimH⁺ *E. coli*, *Streptococcus pneumoniae*, *Staphylococcus pneumoniae*, *Pseudomonas aeruginosa*, *Mycoplasma pneumoniae*, and *Mycobacterium tuberculosis* results in the release of preformed and de novo synthesized mediators [71, 81, 84–88]. Studies with *E. coli* also revealed that the adhesin protein FimH interacts with CD48 on mast cells and is essential for inducing mast cell degranulation [71]. In order to induce significant degranulation, *Mycoplasma pneumoniae* and *Streptococcus pneumoniae* required up to 4 h of co-incubation with mast cells [85, 88]. This suggests a low, but constant level of degranulation rather than a rapid release as seen with IgE-mediated degranulation. It is important to note that not all bacteria induce mast cell degranulation. Co-incubation with commensal bacteria such as non-pathogenic *E. coli*, *Lactobacillus*, and *Bifidobacteria* strains does not induce mast cell degranulation [89–92]. Thus, the capacity of bacteria to stimulate mast cells appears to be specific.

In summary, in vitro experiments have shown that a variety of bacteria induce mast cell degranulation and cytokine production, which are important for microbial immunity. However, these experiments do not address the impact that bacterial exposure has on mast cell responses to IgE/Allergen-dependent activation—a potential link between bacterial infection and allergy.

Bacterial exposure down-regulates mast cell degranulation

In an effort to understand whether bacterial infection impacts IgE-dependent degranulation in mast cells, several in vivo and in vitro studies have been carried out. The

impact of bacterial exposure on allergic inflammation has primarily been examined with commensal or probiotic bacteria. In human trials, perinatal administration of *Lactobacillus rhamnosus* lessened the frequency of atopic dermatitis in at-risk children [93, 94]. This was also observed in mouse systems of allergic inflammation [95]. Administration of probiotics to adults has led to inconsistent data that seem to be dependent on the study design, probiotic strains and allergen tested [8, 96]. Therefore, it appears that the protective effects provided by *L. rhamnosus* may be highly dependent on the timing of the application as well as the choice of probiotic. In mice, the protective effect of the probiotics *L. rhamnosus* and *Bifidobacterium lactis* is associated with an increase in the regulatory T-cell compartment [97], which could explain the immuno-regulatory effects observed with *L. rhamnosus* administration. The above data suggest a model in which probiotics suppress the development of allergy by influencing the maturation of the developing immune system. However, one recent study suggests that probiotics can also impact the effector phase of allergic inflammation in sensitized mice. Using IgE-induced passive systemic anaphylaxis as their model system, Daëron and colleagues showed that *Lactobacillus casei* is able to induce protection against anaphylaxis. Additionally, they showed that *L. casei* inhibited mouse BMDC degranulation and that the inhibition was dependent on mast cell-bacteria contact [90]. Thus, probiotic administration impacts the development of the immune response in humans and recent data points to mast cell-bacteria interactions as another possible mechanism to explain mast cell hypo-responsiveness.

In an effort to directly examine the relationship between mast cells and bacteria, in vitro analysis of mast cell degranulation after bacterial exposure has been employed.

Metcalf and colleagues used a microarray approach to examine the impact that FimH⁺ *E. coli* exposure had on mast cell gene expression. Using a human mast cell line and primary human mast cells, they found a number of genes that were modulated in response to co-incubation with *E. coli*. Among the upregulated genes were proinflammatory cytokines and chemokines that are important for innate immunity. Of significance to IgE-dependent degranulation was the observed down-regulation of FcεRI surface expression. Therefore, one consequence of bacterial exposure may be a decrease in IgE-mediated responses [98]. The down-regulation of FcεRI on the surface obtained with *E. coli* was confirmed using *Lactobacillus rhamnosus* GG and *Bifidobacterium animalis* [99]. Likewise, a number of studies reported that exposure to different bacterial strains including probiotics and FimH⁺ *E. coli* led to a substantial decrease in mast cell degranulation [89–92, 98] (Table 1). Although a limited number of bacteria have been studied, the data suggest that bacterial exposure results in a reduced responsiveness to IgE-mediated degranulation. However, surface expression of FcεRI may not be a conclusive readout of mast cell function, as only a small number of receptors need to be cross-linked in order to induce degranulation [100]. Furthermore, a suppression of degranulation was still observed despite the recovery of FcεRI after 6 h of exposure to *Lactobacillus* [91]. This suggests that down-regulation of FcεRI may not be the only reason for the observed decrease in degranulation.

To determine whether FcεRI was the sole mechanism by which bacteria inhibit mast cell degranulation, investigators have bypassed FcεRI activation by directly stimulating degranulation with Ca²⁺ ionophores. Surprisingly, mast cells co-incubated with bacteria and stimulated with Ca²⁺ ionophores also exhibited reduced degranulation (Table 1).

Table 1 Bacterial strains that reduce mast cell degranulation in vitro

Type of bacteria	Type of mast cell (MC)	Bacteria viability	Number of bacteria	Secretion assay	Inhibition	Stimulus	Ref.
<i>E. coli</i> (Symbioflor1)	C1.MC/C57.1 (mouse)	Alive	2 × 10 ⁷ cfu/ml 1 × 10 ⁹ cfu/ml 2 × 10 ⁹ cfu/ml	Serotonin	~ 70%	IgE/antigen Ca ²⁺ ionophore	[89]
<i>E. coli</i> (Symbioflor1)	Peritoneal MC (mouse)	Alive	Minimum of 1 × 10 ⁹ cfu/ml	Serotonin	~ 60%	IgE/antigen Ca ²⁺ ionophore	[89]
<i>E. coli</i> (Symbioflor1)	Peritoneal MC from Symbioflor1 infected mouse	Alive	Minimum of 10 ⁷ cfu	Serotonin	~ 50%	IgE/antigen Ca ²⁺ ionophore	[89]
<i>Bifidobacteria</i>	RBL-2H3 (rat)	Heat killed	10 µg/ml	β-hexosaminidase	~ 2–58%	IgE/antigen	[92]
<i>Lactobacillus</i>	Bone marrow-derived MC (mouse)	Heat killed	100 µg/ml	β-hexosaminidase	~ 14–72%	IgE/antigen	[91]
<i>E. coli</i> K12 (FimH ⁺)	Human MC	Alive	1 × 10 ⁷ cfu/ml	β-hexosaminidase	~ 50%	IgE/antigen	[98]

Even though Ca^{2+} ionophores induce a more potent secretory response, significant reductions in serotonin and β -hexosaminidase release were still observed [89]. This effect was not limited to cell lines as primary mouse mast cells exhibited a similar response [89]. Therefore, the hypo-responsiveness of mast cells observed after bacterial exposure is not solely attributable to decreased Fc ϵ RI expression.

A few conclusions can be drawn from these data. (1) Bacterial exposures affect events downstream of Fc ϵ RI engagement. This is supported by the results obtained using Ca^{2+} ionophores, which bypass Fc ϵ RI. A downstream process that may be affected is the fusion of secretory granules with the plasma membrane. Since intracellular β -hexosaminidase levels are not affected by bacterial exposure [90], the hypo-responsiveness of mast cells may be due to a fusion defect of the granules with the plasma membrane. One possibility is that bacterial exposure impacts the phosphorylation or localization of SNAP23 and the subsequent formation of a fusion-competent t-SNARE complex. As a result, one would predict that β -hexosaminidase secretion would be reduced without affecting intracellular stores of β -hexosaminidase. (2) Bacterial exposure may affect the function of SNARE regulatory proteins, which as a result would impact the fusion of secretory granules with the plasma membrane. (3) Alternatively, bacterial exposure may impact other pathways, such as toll-like receptor stimulation, which may regulate degranulation.

Role of toll-like receptors in mast cell degranulation

Toll-like receptors (TLR) are a family of pattern-recognition receptors that play a major role in innate immunity. Bacterial, viral, parasitic, fungal, and yeast ligands are recognized by TLRs. These receptors are present on a wide variety of cell types in the innate immune system including mast cells [101]. Recognition of specific ligands by toll-like receptors triggers cellular activation and the recruitment of other immune cells to the site of infection. TLRs are characterized based on their relative location. TLR1, 2, 4, 5, 6, 10, and 11 are expressed on the cell surface, while TLR3, 7, 8, and 9 are localized in intracellular compartments [102]. Given the relationship between infection and allergy as well as the role that TLRs play in infection and inflammation, one can speculate that TLR signaling might impact the development of allergic inflammation.

Several groups have investigated the role of TLRs in the development of allergic inflammation. The most notable response is seen with TLR2 and TLR4. TLR2 and TLR4 are both localized to the cell surface and recognize surface components of a wide range of pathogens. Specifically, TLR2 recognizes bacterial triacylated lipopeptides such as

peptidoglycan while TLR4 recognizes lipopolysaccharide (LPS), a major component of the outer membrane of Gram-negative bacteria [102]. Using OVA-immunized mice, Rodriguez and colleagues showed that administration of bacterial lipopolysaccharide was able to suppress IgE-mediated pulmonary inflammation, airway eosinophilia, and hyperactivity [103]. Moreover, the suppressive effect of LPS was dependent on TLR4 and nitric oxide synthase 2 activity [103]. Another study examined the role of TLR2 and TLR4 in the progression of OVA-induced allergic inflammation. When TLR4 or TLR2 agonists were administered, a significant decrease in eosinophilia was observed. If the agonists were given before sensitization, there was a decrease in additional inflammatory components such as a decrease in total serum IgE, airway hyper-responsiveness, and the number of CD4 $^{+}$ T cells in the lung [104]. Using Timothy Grass Pollen, a different allergy model, Fuchs and colleagues demonstrated that TLR2 agonists reduced eosinophilia during chronic respiratory sensitization [105]. Altogether, these data suggest that TLR2 and TLR4 play a role in the development of allergic inflammation.

Mast cells express a number of toll-like receptors on their surface. The role that mast cells play in (1) bacterial infection through the secretion of T $_H$ 1-stimulating mediators and (2) allergy through the secretion of T $_H$ 2-stimulating mediators have led many to investigate the impact of TLRs and TLR agonists on mast cell function. Studies using human mast cell lines showed that stimulation with the TLR4 agonist LPS increased the expression of TLR4 [106]. Additionally, exposure to TLR2 agonists, but not TLR4, resulted in decreased Fc ϵ RI expression and hypo-responsiveness to IgE-mediated activation [107]. In the rat mast cell line, RBL-2H3, Kasakura and colleagues demonstrated that TLR2 agonists suppressed IgE-mediated mast cell responses [108]. This is consistent with the results obtained in human cell lines. However, treatment of BMMC with TLR2 agonists did not reduce Fc ϵ RI expression or the binding of IgE. Moreover, it has been observed that TLR2 agonists reduced Ca^{2+} mobilization and ERK phosphorylation [108]. This suggests that TLR2 engagement impacts the signaling cascade downstream of Fc ϵ RI without impacting surface expression.

The role of TLR signaling in the development of allergy is still not well understood. It is clear that TLR engagement can influence the severity of allergic inflammation as well as impact the IgE-mediated response in mast cells. The study by Kasakura and colleagues hints at a possible link between IgE-mediated degranulation and TLR stimulation. While research into the relationship between mast cells, allergy and TLRs is rather new, it supports a model in which bacterial exposure negatively impacts allergic inflammation.

Concluding remarks

Mast cells play an important role in both innate and adaptive immunity. In addition, it is well established that mast cells are a critical component in the development of allergy. This is evidenced by the IgE-dependent release of secretory granules containing histamine, serotonin, and other chemical mediators that contribute to the immediate allergic response. Furthermore, mast cells release de novo produced cytokines that contribute to the development of late-phase allergic reactions. The striking increase in allergy over the past 40 years has stimulated research addressing the factors that influence the development of atopy, including the impact of bacterial infections.

Recent data demonstrates that mast cells participate in the innate immune response to several bacterial infections through the secretion of granule mediators and cytokines. Interestingly, mast cells not only produce mediators that direct the innate and adaptive immune response during bacterial infection, but also exhibit properties of the professional phagocytes. Mast cells have the capacity to internalize bacteria via phagocytosis and traffic the phagosomes to the lysosomal compartments where degradation occurs. Given that mast cell secretory granules are lysosome-like, it will be interesting to know whether these granules contribute to the digestion of bacteria. Additionally, it would be exciting to identify whether the SNAREs that mediate degranulation also participate in phagocytosis. Understanding the intimate interactions between mast cells and bacteria will help uncover to which extent bacterial infections can influence the development of allergy.

When exposed to a variety of commensal bacteria as well as *E. coli*, mast cells down-regulate FcεRI and become hypo-responsive to IgE-mediated activation. This effect is not solely due to a reduced surface expression of FcεRI since Ca^{2+} ionophores also induce a similar response. Altogether, this suggests an additional mechanism that regulates mast cell hypo-responsiveness. Toll-like receptor stimulation induces a similar effect in mast cells by down-regulating specific effector proteins in the FcεRI signaling cascade. However, the direct link between mast cells, TLRs, and infection is still missing. A vital experiment using mast cell-deficient mice reconstituted with mast cells from TLR and other knockout mice needs to be conducted. We cannot exclude other possible consequences of bacterial exposure. This includes the impact that bacteria have on intracellular trafficking events in mast cells.

Intracellular trafficking of vesicles is a highly regulated process, and altering one aspect of trafficking may have profound effects. That mast cells internalize and digest bacteria suggests that lysosomal secretory granules may participate in their anti-bacterial function. If these granules do contribute to phagocytosis, this may alter the secretory

granule populations and in turn affect degranulation. Additionally, given that direct TLR stimulation impacts the FcεRI signaling cascade, it is possible that bacterial exposure may impact other signaling events. One potential signaling event that would have detrimental effects on mast cell secretion is the phosphorylation of the t-SNARE SNAP23. Interestingly, the TLR2 signaling pathway involves IKK β , which is known to phosphorylate SNAP23 in vivo [39, 109]. Further signaling elements such as Ca^{2+} release have the potential to impact the regulation of SNARE fusion through regulatory proteins. Nevertheless, the impact bacterial exposure has on intracellular trafficking and SNARE-mediated function remains unknown.

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