

# Mast Cells as Regulators of Adaptive Immune Responses in Food Allergy

Yasmeen S. El Ansari<sup>a,b,1</sup>, Cynthia Kanagaratham<sup>a,c,1</sup>, and Hans C. Oettgen<sup>a,c,\*</sup>

<sup>a</sup>Boston Children's Hospital and Harvard Medical School, Boston, MA; <sup>b</sup>Institute of Laboratory Medicine, Philipps University Marburg, Marburg, Germany; <sup>c</sup>Department of Pediatrics, Harvard Medical School, Boston, MA

Mast cells are a critical first line of defense against endogenous and environmental threats. Their participation in innate immunity is well characterized; activation of toll like receptors as well as receptors for complement, adenosine, and a host of other ligands leads to mast cell release of preformed mediators contained within granules along with newly synthesized arachidonic acid metabolites, cytokines, and chemokines. These confer protective effects including the induction of mucus secretion, smooth muscle contraction, and activation of common itch and pain sensations, all of which act to promote expulsion of noxious agents. While their innate immune role as sentinel cells is well established, recent research has brought into focus their separate but also critical function in adaptive immunity particularly in the setting of IgE mediated food allergies. Crosslinking of FcεR1, the high affinity receptor for IgE, when bound to IgE and antigen, triggers the release of the same factors and elicits the same physiologic responses that occur after activation by innate stimuli. Though IgE-activated mast cells are best known for their role in acute allergic reactions, including the most severe manifestation, anaphylaxis, accumulating evidence has suggested an immunoregulatory effect in T cell-mediated immunity, modulating the balance between type 2 immunity and tolerance. In this review, we outline how mast cells act as adjuvants for food antigen driven Th2 cell responses, while curtailing Treg function.

## INTRODUCTION

Food allergy is a potentially deadly immunological disorder triggered by the ingestion of food protein antigens. While the specific cause of food tolerance breakdown that leads to food allergies is not well understood,

various environmental, genetic, and endogenous triggers have been implicated in their development [1,2]. Food allergy can manifest with a range of symptoms. The most common form, which is driven by the activation of mast cells by food-specific IgE antibodies, can present rapidly after food ingestion with abdominal pain, vomiting,

\*To whom all correspondence should be addressed: Hans Oettgen, Boston Children's Hospital and Harvard Medical School, Boston, MA; Email: [hans.oettgen@childrens.harvard.edu](mailto:hans.oettgen@childrens.harvard.edu).

Abbreviations: TSLP, thymic stromal lymphopoietin; DC, dendritic cell; PGD2, prostaglandin D2; LTC4, leukotriene C4; LTD4, leukotriene D4; LTE4, leukotriene E4; ILC2, innate lymphoid cells type 2; Treg, T regulatory cells; Tfh, T follicular helper cell; TRPV1, transient receptor potential cation channel subfamily V number 1; MCPT-1, mouse mast cell protease 1; NKT, natural killer T cell; SCF, stem cell factor; TCR, T cell receptor.

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<sup>1</sup>YSEA and CK contributed equally to this work.

hives, angioedema, difficulty breathing, and shock [2]. Given the lack of reliable correlation between IgE levels and symptoms, it has been difficult to accurately diagnose this form of food allergy in patients; instead, food challenge has become the gold standard for assessment. Despite diagnostic difficulties, the prevalence of food allergies is on the rise, affecting around 8% of children, and 4% of adults [3], and thus increasing the rate of fatal food anaphylaxis – the most serious manifestation of a reaction to food. Therefore, it remains vital to elucidate the mechanisms underlying the immunopathophysiology of food allergy in order to develop therapies that might improve quality of life and prevent near-death reactions.

Mast cells play a critical role in the pathogenesis of food allergies. These first-line defenders are stationed at the interface between our bodies and the environment and serve as effectors of anaphylactic reactions. Their numbers increase several-fold in patients with IgE-dependent hypersensitivity reactions [4]. FcεR1, the high affinity IgE receptor on mast cells, when crosslinked following the binding of the antigen to the IgE, activates a signaling cascade that leads to mediator release and cytokine production. Vasoactive mediators released by mast cells such as histamine and serine proteases quickly induce immediate reactions such as smooth muscle constriction, vascular leak with tissue edema, vasodilation, and mucus production [5]. Such host responses confer a vital adaptive advantage in the expulsion of helminths and detoxification of venoms but are pathogenic when triggered in response to harmless food antigens. IgE-FcεR1 aggregation on mast cells also leads to the production of a plethora of cytokines that exert both proinflammatory and immunomodulatory effects. In recent years it has become clear that in the setting of food allergy, mast cells not only function as effector cells of hypersensitivity reactions, but also as inducers of emerging immune responses, such as Th2 expansion and suppression of the development and function of regulatory T cells (Treg) [6]. This review will cover the roles of mast cells as regulators of the T cell mediated immunopathophysiology of food allergies.

## THE ROLE OF MAST CELLS IN SENSITIZATION AND ANAPHYLAXIS

### Sensitization

Allergic sensitization is defined as the process by which the exposure to food antigens results in the production of IgE antibodies. A number of steps are involved in the generation of immune responses to food antigens and several key factors are known to specifically drive Th2 responses and suppress Treg (Figure 1). Ingested allergens are first encountered by intestinal epithelial cells. Under some conditions these epithelial cells produce IL-25,

IL-33, and thymic stromal lymphopoietin (TSLP). After crossing the epithelium by active transcellular transport or passive intracellular paths, allergens are encountered by mucosal dendritic cells (DC). Under the influence of epithelium-derived IL-25 and IL-33, these DC acquire a Th2-promoting phenotype. Antigen presenting cells that have picked up and processed food antigen migrate to draining mesenteric lymph nodes, to prime naïve CD4+ T cells. These CD4+ T cells take on a Th2 phenotype, expressing the transcription factor GATA3 and producing pro-allergic cytokines such as IL-4 and IL-13 [7].

### IgE Production

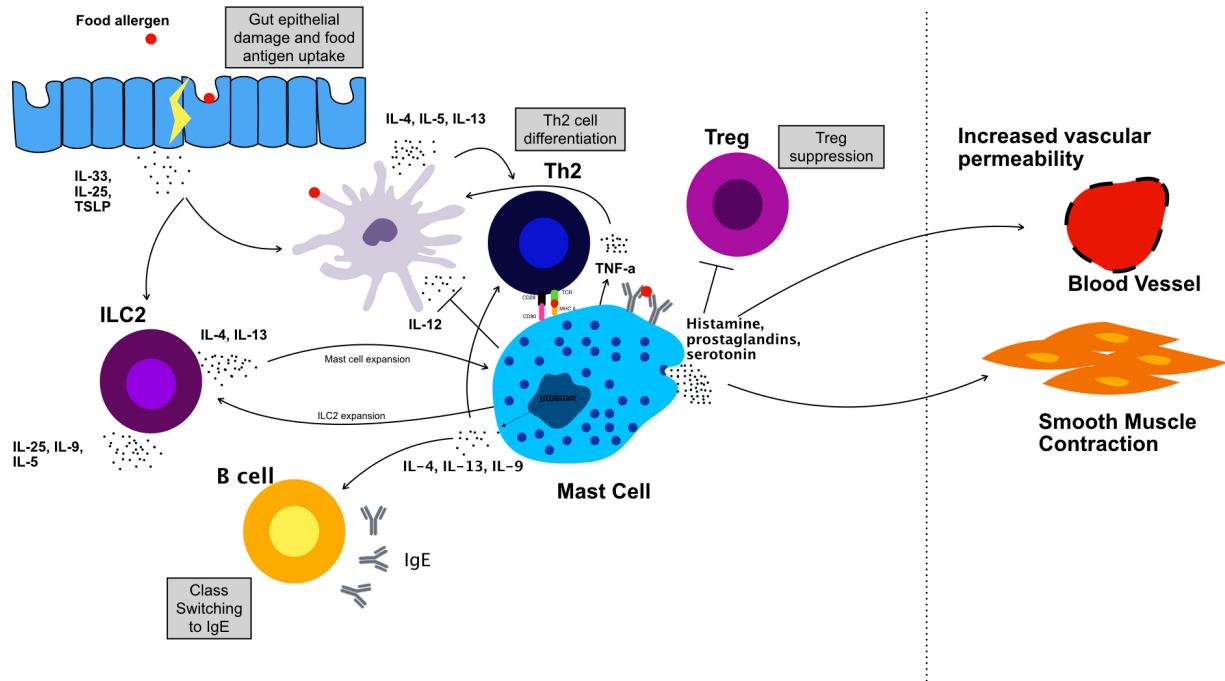
Ultimately, innate and adaptive immune cells prime the immune response to drive IgE production. IgE production driven by IL-4, along with IL-13, is a key event in sensitization. An issue of great interest in food allergy has been to identify the key early sources of IL-4 that might prime Th2 responses. A number of innate immune cells, including basophils, mast cells, and natural killer T cells (NKT) cells are known to produce IL-4. In the setting of food allergy, mast-cell-derived IL-4 appears critical to drive early Th2 responses *in vivo* [8]. IL-4 is critical in inducing Th2 responses and acts directly on B cells to induce germline transcription and IgE class switching (Figure 1). However, optimal IgE production may also require IL-13 as shown by Gowthaman *et al.* [9]. Their study of T follicular helper cells (Tfh) identified a novel subset of IL-4- and IL-13-producing Tfh that are needed to produce IgE with a high affinity to the antigen [10,11]. Following class switching, IgE antibodies become long lived when bound to their high affinity IgE receptor FcεR1 found on allergic cell types such as eosinophils, basophils, and mast cells.

In addition, IgE antibodies can regulate mast cell survival, and proliferation in settings of allergy [12]. Studies have shown that, in the absence of survival cytokines, binding of IgE to FcεR1 can provide a survival signal to mast cells [13]. Furthermore, in the presence of increased levels of IgE and IL-4, FcεR1 density on mast cells is upregulated [4]. Parasitized mice that have IgE also have enhanced mast cell responses compared with those that do not, and injecting IgE-secreting hybridomas in the IgE-lacking mice results in similar responses [9,14].

## GENERAL MECHANISMS OF MAST CELL EFFECTOR FUNCTION IN ALLERGIC DISEASE

### Mast Cell Degranulation

Activation of IgE-FcεR1 on mast cells and basophils is central to the pathogenesis of food allergy. Antigen recognized by the IgE bound to mast cell or basophils leads



**Figure 1. IgE dependent activation of mast cells in the gut leads to a Th2 cell-mediated immune response.** During sensitization, damage to the epithelial barrier in the gastrointestinal tract (due to trauma or toxins) leads to increased antigen entry and secretion of epithelium derived IL-33, IL-25, and thymic stromal lymphopietin (TSLP). Upon antigen recognition and processing by antigen presenting cells, IL-33, IL-25, and TSLP promote dendritic cell differentiation into a Th2 promoting cell. Mature APCs migrate to the mesenteric lymph nodes (not shown) to prime naïve T cells into Th2 cells. This response consequentially leads to ILC2 expansion and B cell class switching to IgE [7]. IgE binds to its high affinity receptor FcεR1a on mast cells, and upon subsequent antigen-receptor cross linking cause them to degranulate [12]. Products of degranulation such as histamine cause increased vascular permeability and smooth muscle contraction. Mast cell produced cytokines such as IL-4 and IL-13 further amplifies the Th2 response by acting on B cells to produce more IgE, and further promote expansion of ILC2 [2]. In a bidirectional manner, ILC2 can also drive mast cell expansion via IL-4, and IL-13. IL-9 derived mast cell also acts with a positive feedback loop to promote mast cell proliferation/accumulation [29]. Mast cells facilitate crosstalk with T cells by presenting antigen via MHC [41]. While mast cells support Th2 responses, their activation also suppresses Treg function [42].

to receptor aggregation, activation of signaling pathways, degranulation, and activation of the cell. Degranulation results in the release of preformed mediators (like histamine, tryptase, and other proteases), rapidly synthesized lipid mediators (PGD<sub>2</sub>, LTC<sub>4</sub>, LTD<sub>4</sub>, and LTE<sub>4</sub>), and the transcription of cytokine genes (IL-4, IL-6, and IL-13) [12].

#### *Effects of Mast Cell-Derived IL-4 and IL-9 on Mast Cell Homeostasis and Functions*

In addition to initiating immediate hypersensitivity, cytokines produced by mast cell activation support the recruitment of pro-allergic cells that contribute to a secondary late phase reaction. Mast cells favor the development of Th2 responses for their survival, most notably by synthesizing the Th2-inducing cytokine IL-4. IL-4 was the first cytokine discovered to be synthesized by mast cells upon activation and, similarly to IgE, also induces mast

cell proliferation and survival, and enhances FcεR1 expression in humans [15,16]. Many research groups have demonstrated that injecting exogenous IL-4 into mice promotes mast cell expansion [8,17,18]. It also promotes the expansion and activation of many cell types, such as eosinophils and B cells, respectively. Furthermore, along with IL-13 and TNF-α, IL-4 acts on B and T cells to promote a positive feedback loop amplifying the production of mast cells and IgE [19].

Among the cytokines produced by mast cells, IL-9 has recently become of great interest to scientists in the field of mast cell biology and allergy. IL-9 is an important cytokine involved in mast cell expansion, acting synergistically with stem cell factor (SCF) to promote the proliferation of mast cells and their progenitors [20]. Mucosal mast cells have been shown to produce IL-9. These IL-9-producing mast cells increase in number with allergen challenge and are needed for food-allergy-asso-

ciated phenotypes such as incidence of diarrhea, expansion of intestinal mast cell number, and increase in serum concentration of mouse mast cell protease 1 (MCPT-1) following antigen challenge (as shown in mice ablated of IL-9-producing mast cells) [21]. However, the serum concentration of antigen-specific IgE in these mice remains the same as those with normal levels of IL-9 [22,23]. This indicates that IL-9 plays a role in the anaphylaxis phase following challenge but not in the sensitization phase. Overexpression of IL-9 promotes the expansion of intestinal mast cell numbers [23]. Overlapping small intestine transcriptome profile signatures can be observed between mice overexpressing IL-9 and those that have undergone anaphylaxis [24]. An increase in expression of allergy-associated genes such as CPA3a, FcεR1a, Sprr2a, Mcpt1, and Mcpt2 can be observed in both groups. IL-9 has also been shown to promote increased intestinal permeability that is suspected to be mediated by the increased number of mast cells in the small intestine [24].

### ILC2 Expansion

Mast cell cytokines not only promote mast cell homeostasis, but also orchestrate the expansion of various cells types that interact with one another and lead to the overall phenotype of food allergy. Innate cells predominantly found at mucosal barriers called type 2 innate lymphoid cells (ILC2) are a unique subset of hematopoietic cells that can interact with mast cells. ILC2 act like Th2 cells, but without T cell receptors (TCR), and release large amounts of IL-5, IL-13, IL-4, IL-25, and IL-9, which along with Th2-secreted cytokines orchestrates allergic immunity [25]. Mast cells can regulate the expansion of ILC2. ILC2 promote allergic inflammation and hinder the production of allergen-specific Treg. IgE-activated mast cells can drive the intestinal expansion of ILC2 that produce IL4, IL-5, IL-9, and IL-13 and contribute to allergy phenotypes [26-28]. In the absence of IgE and mast cells, ILC2 expansion is lost [28]. Recent studies have also shown that the relationship between ILC2 and mast cells is bidirectional. In a model of epicutaneous allergen sensitization, Levy-Castillo *et al.* show that ILC2-derived IL-4 and IL-13 drive mast cell numbers in the intestine (Figure 1) [29]. Collectively, activation of the mast cell compartment can modulate its own maintenance as well expand different cell types that interact in a complex interplay to sensitize the host against a food antigen.

## MAST-CELL-MEDIATED REGULATION OF T CELL RESPONSES

We and others have been interested in the question of whether mast cells, as prolific producers of cytokines, including pro-Th2 IL-4, regulate adaptive immune responses in food allergy. Support for this idea is provided

by evidence for mast cells' roles in the induction of other adaptive immune responses including asthma and contact hypersensitivity. As extensively studied and reviewed by Galli, FcεR1-dependent mast cell activation can enhance the development and intensity of T cell responses. Even though not required for the induction of OVA-specific T cells in the sensitization phase of mouse airway inflammation, mast cells and mast-cell-derived TNF enhance recruitment of lymphocytes and Th2 cytokine production in the challenge phase [30]. Studies using IgE- or mast-cell-deficient mice show that IgE primes mast cells in a way that promotes sensitization and exacerbates pathology. Mast-cell-deficient Kit<sup>W-sh/W-sh</sup> mice can be used to make reconstituted mice whereby the biological response to the Kit mutation is corrected by the adoptive transfer of mast cells. Reber *et al.*, show that mast-cell-deficient mice reconstituted with sensitized mast cells have increased airway responsiveness and tissue remodeling as a result of mast-cell-derived mediators [30]. Similarly, mast cells are key players in mouse models of cutaneous contact hypersensitivity. Mice lacking mast cells or IgE antibodies exhibit defective emigration of dermal dendritic cells and priming of T cells during the sensitization phase of this classic immune response, consistent with a powerful adjuvant role for mast cells in immune sensitization [31,32]. These findings taken together suggest a role for mast cells as effectors of adaptive immunity.

Several murine models of food allergy have been used to query the role of mast cells in regulating adaptive immune responses in food allergy. Brandt and colleagues developed a model of experimental oral allergen-induced diarrhea to study the effect of recurrent allergen ingestion on mast cells. In this model wildtype mice are injected intraperitoneally twice with OVA/alum, 2 weeks apart, and then challenged intragastrically with up to 50 mg of OVA 2 weeks after the last sensitization. Mice are repeatedly challenged every other day, for up to 10 challenges. They showed that incremental increases in the number of enteral allergen challenges is directly correlated with the number of mast cells in the jejunum, as well as the occurrence of diarrhea. They show that diarrhea is dependent on IgE, as administering anti-IgE attenuates the onset of diarrhea and the expansion of mast cell number in the small intestine. Additionally, repeated intragastric OVA challenge further amplifies the Th2 response, measured by increasing total and specific IgE after multiple challenges, and the expression of Th2 cytokines in the jejunum [33]. This work of Brandt and colleagues highlights a role for IgE-mediated mast cell activation in allergic diarrhea and hints at a role for mast cells in driving Th2 responses but does not solidify the connection.

Our own studies of mast cells as innate inducers of Th2 and IgE responses to food allergens in mice took advantage of a different model. We used F709 mice

which harbor a disinhibited form of IL-4R $\alpha$ , increasing sensitivity of the receptor to IL-4 by enhancing ligand-induced STAT6 phosphorylation, thus enhancing Th2 cell responses and IgE production. These animals are inherently atopic, demonstrating high levels of specific IgE production following ingestion or inhalation of allergens even in the absence of any adjuvant. Using these animals, we found that repeated feeding of either OVA or peanut is sufficient to induce Th2 responses like IgE production, mast cell expansion, and anaphylaxis after challenge. Using F709 mice that lacked mast cells alongside animals reconstituted with normal or IL-4-deficient mast cells, we observed that IL-4-producing mast cells are needed to induce peanut-specific Th2 cells and IgE. Intact Fc $\epsilon$ R1 signaling is necessary for this Th2 adjuvant effect of IgE and mast cells as demonstrated using mice with a mast cell lineage targeted deletion of SYK, the proximal kinase in signal transduction by Fc $\epsilon$ R1. Atopic F709 mice displayed strong Th2 responses yet failed to generate stable tolerogenic Treg responses. Treg produced in F709 mice are skewed towards a Th2 profile by expressing GATA3. Whereas the OVA/alum model utilized by Brandt *et al.* had hinted at amplification of the Th2 response by enteral allergen, via a Th2 cytokine RNA readout, we clearly show that IgE receptor signaling via mast cells promotes sensitization to enteral allergens [6]. Because food allergy results from a break in oral tolerance, and tolerance is maintained by Treg, we measured the effect of *ex vivo* peanut stimulation on Foxp3<sup>+</sup> dividing cells in WT and F709 mice. WT mice that received the weekly dose of peanut butter developed oral tolerance, measured by an expansion of peanut activated Foxp3<sup>+</sup> cells. In contrast to F709 mice, which exhibited strong anaphylactic reactions upon ingestion challenge, the WT animals showed no signs of reaction. In a separate protocol designed to mimic oral immunotherapy, administration of a small molecule SYK inhibitor in the course of allergen exposure, meant to paralyze mast cell activation by IgE via Fc $\epsilon$ R1, facilitated the reemergence of tolerance [6]. IL-4, can also destabilize Foxp3 expression in Treg, while activating Th2 and Th9 pathways [34].

We hypothesize that the adjuvant and Th2-polarizing effects of mast cells in food allergy may be mediated by actions of their mediators on DC. In support of this concept is the observation by Kitawaki and colleagues showing that mast cell activation via IgE can suppress DC-derived IL-12 in co-culture experiments [35]. Furthermore, small molecule mast cell activators such as compound 48/80 can enhance migration of DC to draining lymph nodes by upregulating the lymph node homing receptor CCR7. This is thought to be mediated by mast cell derived TNF [36,37]. DC are crucial in priming T cell responses for their subsequent role in antibody responses.

Other studies suggest roles for mast-cell-derived

histamine and mast cell OX40L/IL-6 in suppressing Treg function *in vitro* [38,39]. Recently Tamaka *et al.* extended this observation by showing that histamine suppresses Treg *in vivo* in a murine model of chronic allergic contact dermatitis. In this model, you observe mast cell expansion and an increase in IL-4, while a decrease in Treg remains, consistent with our F709 food allergy model. Utilizing histamine-deficient mice, Tamaka *et al.* show that a lack of histamine results in an increased infiltration of Treg, and therefore fewer mast cells and less IL-4. Even though unknown, it is probable that mast cells are the major source of histamine in this model [40].

Lastly, it has been proposed that mast cells might influence T cells by presenting antigen via MHC I or II [41]. T cells also happen to be stationed in close proximity to mast cells in allergic mucosal tissues, facilitating crosstalk between the two cell types (Figure 1) [42].

### Basophil IL-4 Promotes Th2 Responses

While our main focus in this review has been on the regulatory role of mast cells in innate and adaptive Type 2 responses, it is important to remember that, similarly, IgE aggregation of Fc $\epsilon$ R1 on basophils causes release of mediators and contributes to the allergic phenotype as well. Whereas mast cells are long-lived differentiated tissue-resident sentinel cells, basophils are transient and circulate in the blood. They are recruited in response to immunological stimuli. Basophils are not abundant in the intestinal mucosa at baseline and are not therefore usually considered as instigators of immune sensitization or effectors of acute reactions in food allergy. However, recent studies have revealed an important role for basophils as priming sources of IL-4 in models of IgE-mediated food allergy arising from cutaneous exposure to antigen. In a model of epicutaneous sensitization, followed by intragastric antigen administration, Hussain *et al.* showed a role for basophil-derived IL-4 in promoting Th2 polarization *in vitro*. The same study also demonstrated that the absence of basophil IL-4 results in reduced allergic responses *in vivo* [43]. Noti and colleagues have shown that keratinocyte-derived TSLP promotes basophil responses in the skin [44]. Kawakami *et al.* studied the contributions of basophils in the effector phase of food allergy using an OVA/alum model of sensitization, followed by intragastric OVA challenge, and found that the depletion of basophils before challenge resulted in reduced diarrhea and clinical symptoms [45]. Mast cell numbers, and serum levels of MCPT-1 were also reduced in the basophil-depleted mice, again suggesting that IL-4 produced by basophils promotes Th2 differentiation that leads to IgE production. Reber and colleagues reported that selective ablation of mast cells or basophils reduced peanut-induced anaphylaxis in a mouse model of peanut allergy [46]. Thus appreciation for the contributions of

basophils to both sensitization and allergic responses is coming into sharper focus.

## THE ROLE OF ALTERNATIVE RECEPTORS AND THEIR LIGANDS IN MAST CELL ACTIVATION AND IMMUNOLOGICAL SENSITIZATION

The studies discussed in this review strongly point to a role for IgE in amplifying nascent allergic responses in the setting of recurrent food ingestion and accruing IgE production. While mast cells can promote sensitization in this setting, it remains still unclear if they can initiate responses in the pre-IgE-sensitized, immunologically naïve state. It is possible that other mast cell activation pathways are involved in the initial innate phase of food allergen responses. Mast cells express a range of activating receptors that recognize ligands such as N-formyl-met-leu-phe, adenosine and substance P. The newly appreciated Mas-related G protein-coupled receptor (Mrgprx2) expressed on mast cells (Mrgpra2 in mice) recognizes a broad range of small molecules involved including drugs, antimicrobial peptides, and neuromediators including substance P. Activation via this receptor causes mast cell degranulation independent of IgE [47]. It has been implicated in wound healing, pain, and itch sensations [48]. However, the role of this receptor in food allergy remains unclear. One could speculate that it is possible that foods contain ligands that can be recognized by Mrgprx2, which provide initial triggers for mast cell activation. One could speculate that neuron:mast cell interactions triggered by aversive responses or olfactory reflexes during early food introduction could activate mast cells by this pathway.

Others have hypothesized that foods are initially encountered in the context of microbial or other danger signals, that might promote sensitization, or via anatomic sites of exposure that favor induction of Type 2 responses. The latter possibility is suggested by the observation that most peanut-allergic patients experience clinical symptoms after their first known ingestion. This implies that initial sensitization has occurred through a non-enteral route; many peanut-allergic children have concurrent atopic dermatitis, a condition strongly conducive to epicutaneous antigen sensitization. A recent study by Moran and colleagues aimed to study the effect of indoor dust on airway sensitization to peanut in mice. They show that inhalational exposure to peanut in combination with indoor dust drives the production of peanut-specific IgE and subsequent effector responses [49]. This aligns nicely with previous studies that have looked at the effect of house dust mite (HDM) on mast cell function. Yu and Chen show that the major species *Dermatophagoides farinae* (Der f) in HDM is capable of activating mast cells *in vivo* in a non-IgE mediated process. Once activated, mast cells

produce an array of cytokines and chemokines, representing an early mast cell driven response in the sensitization process [50]. Emerging evidence has suggested an adjuvant role for the cysteine protease activity of the HDM allergen Der p 1 in activating initial innate pathways, and is known in promoting house dust mite induced airway inflammation [51]. Furthermore, a recent study by Serhan, Basso, *et al.* show that HDM with cysteine protease activity can activate peptidergic nociceptors expressing transient receptor potential cation channel subfamily V number 1 (TRPV1) and Tac1 in the skin. Substance P is released which then drives the degranulation of mast cells via Mrgprb2 [52]. This provides an example of how mast cells can initiate immune responses in the context of allergic skin inflammation.

## CONCLUSION

The separate but equally important roles of mast cells as both effectors of hypersensitivity reactions and inducers of Th2 responses has become increasingly clear. While targeting mast-cell-derived mediators (antihistamines, etc.) has been traditionally used as treatment, they are only partially effective, suggesting a larger contribution of mast-cell-derived cytokines in pathogenesis. Newer therapeutics such as omalizumab (anti-IgE), mepolizumab (anti-IL-5), and dupilumab (anti-IL-4/IL-13) have shown promise in atopic dermatitis, and chronic rhinosinusitis and are now in clinical trials for food allergy. Taken together, this suggests that mast cells immunomodulatory effects stretch beyond the magnitude of the hypersensitivity reactions but also for the subsequent development of T effector cell responses. Elucidating how mast cells amplify and thus control T effector function will help provide insight on new therapeutics in food allergy.

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