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IL-9–producing cells in the development of IgE-mediated food allergy

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Abstract

Food allergy is a harmful immune reaction driven by uncontrolled type-2 immune responses. Considerable evidence demonstrates the key roles of mast cells, IgE, and TH2 cytokines in mediating food allergy. However, this evidence provides limited insight into why only some, rather than all, food allergic individuals are prone to develop life-threatening anaphylaxis. Clinical observations suggest that patients sensitized to food through the skin early in life may later develop severe food allergies. Aberrant epidermal thymic stromal lymphopoietin and interleukin (IL) 33 production and genetic predisposition can initiate an allergic immune response mediated by dendritic cells and CD4⁺TH2 cells in inflamed skin. After allergic sensitization, intestinal IL-25 and food ingestion enhance concerted interactions between type-2 innate lymphoid cells (ILC2s) and CD4⁺TH2 cells, which perpetuate allergic reactions from skin to the gut. IL-4 and crosslinking of antigen/IgE/FcεR complexes induce emigrated mast cell progenitors to develop into the multi-functional IL-9–producing mucosal mast cells, which produce prodigious amounts of IL-9 and mast cell mediators to drive intestinal mastocytosis in an autocrine loop. ILC2s and TH9 cells may also serve as alternative cellular sources of IL-9 to augment the amplification of intestinal mastocytosis, which is the key cellular checkpoint in developing systemic anaphylaxis. These findings provide a plausible view of how food allergy develops and progresses in a stepwise manner and that atopic signals, dietary allergen ingestion, and inflammatory cues are fundamental in promoting life-threatening anaphylaxis. This information will aid in improving diagnosis and developing more effective therapies for food allergy–triggered anaphylaxis.

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Introduction

IgE-mediated food allergy is an adverse immune response that occurs shortly after ingestion of food. For reasons unknown, the prevalence of food allergy has increased significantly over the past decade, affecting 3–4% of adults and 4–8% of children in the United States [1]. After exposure to the causal food allergens, the symptoms of food-induced anaphylactic reaction are variable, ranging from mild cutaneous swelling and abdominal discomfort to life-threatening anaphylaxis, characterized by vomiting, diarrhea, hypotension, and cardiovascular collapse [2]. Although less common, food allergy-induced life-threatening anaphylaxis is responsible for approximately 30,000 E.R. visits and 150 deaths per year in the U.S. For healthy individuals, ingesting food results in developing immunologic hyporesponsiveness or oral tolerance. It is unclear why oral tolerance toward innocuous foods fails to be established or breaks down in some individuals. Recent clinical studies demonstrate that food immunotherapy provides some protective effects or achieves short-term “sustained unresponsiveness” to food allergens for some subjects [3, 4]. However, the outcome of these food immunotherapy approaches appears ineffective in achieving complete de-sensitization or re-establishing long-term tolerance [4]. Notably, although some subjects may obtain some degree of protection after repeated exposure to food allergens, others often develop adverse gastrointestinal (GI) symptoms and increased risk of systemic adverse reactions [4]. Likewise, it has been a conundrum why some individuals with food allergy exhibit a mild discomfort characterized by pruritus of the lips or urticaria but others experience life-threatening anaphylactic reactions after consuming the same food allergen. These observations underscore that food immunotherapy for food allergy is not yet ready for clinical practice and that our knowledge of the immunologic mechanisms underpinning the development of allergic reactions in the GI tract remains to be established. Recent studies point to the involvement of IL-9 in regulating the pathogenesis of allergic disorders. This review focuses on summarizing recent advances in our understanding of IL-9 and the cellular sources of IL-9 that contribute to allergic disease progression and promote susceptibility to life-threatening, IgE-mediated food allergy.

Clinical features of food allergy

Food-induced allergic reactions are often caused by peanuts, tree nuts, cow’s milk, wheat, fish, and shellfish proteins in older children and adults [5, 6]. The primary organs targeted by food-induced allergic reactions are the skin, GI, and respiratory tract. After exposure to a causal food allergen, acute adverse reactions can occur within minutes to hours with clinical symptoms involving the skin (hives and angioedema), GI tract (oral allergy syndrome characterized by swelling of the lips, tongue, and throat; intestinal anaphylaxis characterized by abdominal pain, vomiting, and diarrhea), and respiratory tract (asthma and allergic rhinitis). Food-induced allergic reactions can also cause the exacerbation of chronic allergic diseases, such as atopic dermatitis, asthma, and GI disorders [7]. Current knowledge cannot explain why some individuals fail to establish and maintain immune tolerance to food throughout life. Furthermore, it is perplexing that some individuals with food allergy only experience a mild discomfort, whereas others develop symptoms of life-threatening anaphylaxis that involves GI, respiratory, and cardiovascular systems. The design of current therapeutic approaches for food allergy are based on the concept of allergen immunotherapy

using different mucosal routes, such as oral, sublingual, and epicutaneous [8, 9]. Food immunotherapy can induce some degree of desensitization and provide protection in a majority of children with egg and milk allergy by raising the reaction threshold [4]. However, the initial benefits of the protection sometimes wear off, and some individuals develop severe adverse responses to the therapy, particularly the GI symptoms [4, 10]. The goal of food immunotherapy in developing sustained unresponsiveness and eventually tolerance to causal foods has not been achieved for clinical practice. Currently, there is no reliable method to distinguish between individuals who are at risk of mild non-life-threatening versus life-threatening reaction. Identifying biomarkers that can determine the predictive risk value of a positive food challenge test or clinical reactivity to food immunotherapy will be beneficial to the clinical applications of food immunotherapy. Given the rapidly increasing prevalence of food allergy in the past decade, it is an urgent need to better understand the factors and mechanisms that underlie the development of IgE-mediated food allergy, which will lead to improved therapeutic approaches.

Oral tolerance and regulatory T cells

Food allergies usually develop at a very young age, within the first few years of birth, and rarely start at later stages in life. Clinical evidence suggest that the higher risk for neonates and young children to develop food allergy is because of the immature state of GI mucosa [11]. It has been hypothesized that exposure to food allergens in the early life may be effective in establishing oral tolerance before allergic sensitization occurred. While functioning to combat invading microbes and limit their persistence in the mucosa, the epithelial lumen and GI-associated lymphoid tissue (GALT) are continuously exposed to the influx of foods and develop active immune tolerance to food and commensal antigens. It has been established that FOXP3-expressing induced regulatory T cells (iTregs) play a key role of in maintaining intestinal tolerance to encountered antigens [12, 13]. The state of unresponsiveness to ingested antigens or oral tolerance can be established through tolerogenic CD103⁺ dendritic cells (DCs), which capture and present luminal food antigens to induce the generation of iTregs and/or IL-10- and IFN- γ -secreting Tr1 lymphocytes in the presence of TGF- β and retinoic acid [14, 15]. These antigen-specific iTregs express FOXP3, CTLA4, and CD25 and can dampen the induction of CD4⁺TH2 cells and control allergic inflammation at the mucosal sites [16, 17]. A role of Treg cells in mediating oral tolerance is further substantiated in a study using the ‘DEREG’ mice, which express the diphtheria toxin (DT) receptor under the control of the *Foxp3* gene [12]. Treatments with DT result in the depletion of Treg cells, which eventually leads to the loss of established oral tolerance in ‘DEREG’ mice [12]. Children who outgrow or become tolerant to their cow’s milk allergy have higher frequencies of circulating CD4⁺CD25⁺ Treg cells than those in the individuals who maintain clinically active allergy [18]. Though these observations demonstrate the pivotal role of iTreg in developing tolerance to ingested foods, the immunologic pathways that result in the failure to develop oral tolerance and the loss of established oral tolerance need to be better understood.

Skin epithelial cytokines and allergic sensitization

Skin is the largest organ of the body and functions as the major barrier to protect against mechanical stress, environmental insults, and micro-organism invasion. Exposure to foreign proteins derived from allergens, bacteria, and viruses can provoke distinct immune reactions driven by the specialized immune components throughout the layers of skin. It has been suggested that these environmental triggers and genetic factors involved in skin barrier function can shape the immune responses against food antigens, leading to failure to establish oral tolerance. A recent clinical study showed that infants with atopic eczema were prone to be sensitized to egg as early as 4 months of age [19], suggesting that some patients with atopic dermatitis (AD) in early life may have a higher risk of developing food allergy [20, 21]. Patients with loss-of-function mutations in the filaggrin gene, which functions to maintain skin barrier integrity, were found to have a higher risk for food sensitization [22]. Indeed, eczema and filaggrin gene loss-of-function mutations were identified as risk factors for food sensitization in a population-based study of pediatric food allergy [22]. Evidence from murine studies further demonstrate that the skin may be a critical route for allergic sensitization to food antigens prior to establishing immune tolerance, leading to the susceptibility to developing experimental food allergy [23–26]. Thus, the findings from clinical and murine studies support the hypothesis that sensitization occurring through the skin barrier in early life may promote the development of allergic reactions to food antigens and counteract the establishment of oral tolerance.

Skin exposures to allergens or exogenous molecules, such as lectins, proteases, or chitins, which can act as the mucosal TH2 adjuvants, often trigger the production of the epithelial-derived TH2-promoting cytokines [27–29, 26]. One of the epithelial-derived cytokines is thymic stromal lymphopoietin (TSLP), which has been shown to predispose the skin to allergic sensitization and induce type-2 inflammation [30]. Originally, TSLP, a distant paralog of IL-7, was identified as a factor secreted by a thymic stromal cell line and that supports B cell development [31]. In human studies, strong TSLP expression can be detected in the epithelia of Hassall's corpuscles in the thymic medulla [32], airway of patients with asthma [33], and skin from AD lesions [34]. Several factors are capable of inducing strong TSLP production by epidermal keratinocytes, including: vitamin D3 analogs (the ligand for vitamin D receptor), skin injury (skin picking or excoriation), and activated toll-like receptors by viral, bacterial, and fungal ligands [35–37]. Mechanistically, TSLP activates dendritic cells (DCs) to create a type-2 permissive microenvironment by producing large amounts of chemokines to recruit inflammatory cells [30]. TSLP-activated DCs can induce CD4⁺TH2 cell differentiation and maintain functional attributes of CD4⁺TH2 memory/effector cells, thereby driving an antigen-specific DC-T cell-mediated allergic immune response [34, 38, 39]. A recent study showed that TSLP can also function in driving IL-3-dependent basophil hematopoiesis, which further potentiates the DC-T cell-mediated allergic immune response [40, 23]. Repeated topical application of ovalbumin (OVA) plus vitamin D3 analog induces skin allergic inflammation in mice, which later develop AD. These AD-like mice become susceptible to ingested OVA and eventually develop symptoms of experimental food allergy [25, 23, 24]. In contrast, the DC-specific disruption of TSLP receptor resulted in a failure to develop antigen-specific IgE after epicutaneous sensitization

and resistance to developing experimental food allergy [41]. It appears that prior to establishing oral tolerance, aberrant epidermal TSLP production may provoke allergic sensitization to foods through the interactions between DCs, basophils, and CD4⁺TH2 immune cells, resulting in increased risk for developing food allergy.

In addition to *TSLP*, the epithelial-derived cytokine *IL33* and *ST2/IL1RL1* (IL-33 receptor) genes were identified by recent genome-wide association studies of asthma as major susceptibility gene loci, implicating their involvement in the development of allergic diseases [42, 43]. Although the mechanisms underlying the induction and release of epithelial-derived IL-33 remain elusive, perturbing the skin barrier after injury result in liberating intracellular IL-33 [44]. The bioactive form of released IL-33 can bind to the IL-33 receptor (IL-33R), which consists of ST2 (IL-1R4) and IL-1RAcP (IL-1R3), and is primarily expressed on type-2 inflammatory cell types, such as mast cells (MCs), type-2 innate lymphoid cells (ILC2s), eosinophils, and CD4⁺TH2 cells, in the context of type-2 inflammation [45]. The signals derived from the IL-33/IL-33R complex can activate the MyD88 and IRAK-4 pathway, which enhances the production of TH2 cytokines and thereby results in the development of allergic inflammation [44]. Indeed, the injury after repeated skin stripping can induce an increase of circulating IL-33, which enhances IgE-mediated mucosal mast cell (MMC) degranulation in the gut, resulting in the development of an anaphylactic response to ingested antigens [46]. Thus, the skin epithelium-derived cytokines TSLP and IL-33 can function to induce allergic sensitization to food antigens after skin injury has occurred.

Perpetuations of allergic reactions from the skin to gut

The mucosal immunity of the GI tract is complex and contains a variety of immune and nonimmune structural cells to mount protective immune response against pathogens while maintaining the state of unresponsiveness toward both foods and commensal bacteria. Though allergic sensitization occurring at the skin barrier is thought to be the first step in the path to developing food allergy, the immunologic pathway that propagates allergic reactions to foods from skin to the GI tract remains elusive. Additionally, after food sensitization, the factors at the mucosal site of the GI tract that break oral tolerance and confer the susceptibility to developing food allergy later in life remain to be elucidated.

IL-25 (IL-17E), a distinct IL-17 cytokine member, was first identified by a sequence homology search of a genomic DNA database [47]. Unlike other IL-17 cytokine members that exert potent pro-inflammatory responses, an early study suggests that IL-25 exerts potent function to promote a type 2 immune response [47]. Administering IL-25 or forced expression of an IL-25 transgene induces strong TH2 immune responses, characterized by eosinophilia; upregulated expression of the TH2 cytokines IL-4, IL-5, IL-13 in several tissues; and increased serum IgE and IgG1 [48, 49]. A recent study further elucidated that tuft cells, one of the five intestinal epithelial cell lineages, can produce IL-25 constitutively, functioning in maintaining intestinal ILC2 homeostasis and limiting the TH1- and TH17-mediated inflammation induced by commensal flora [50–52]. After helminth infection, the intestinal epithelium-derived IL-25 mounts protective type-2 immunity against parasitic infection (e.g., helminth), possibly by activating ILC2s to secrete IL-13 [53, 52]. These

findings suggest that intestinal epithelium-derived IL-25 may play a role in inducing allergic reactions to dietary proteins in the GI tract after allergic sensitization has occurred. Indeed, allergic sensitization induces an increased level of intestinal epithelium *IL25* expression, which can be further upregulated after intragastric food challenge prior to the onset of anaphylaxis [54]. Compared to their wild-type controls, the genetically modified murine strains with overexpression or deficiency of intestinal IL-25 signals are shown to be more susceptible or resistant, respectively, to developing symptomatic features of experimental food allergy, including allergic diarrhea, hypothermia, intestinal mastocytosis, increased serum OVA-specific IgE, and mast cell protease 1 (MCPT-1) [54]. Both intestinal ILC2s and ingested antigen-induced CD4⁺TH2 cells express high levels of surface IL-17RB, a component of the IL-25 receptor complex, and are capable of producing TH2 cytokines in response to IL-25 stimulation to promote the development of experimental food allergy. Notably, ILC2s alone are insufficient to mount anaphylactic reactions in these naïve or sensitized mice [54]. After allergic sensitization has occurred, repeated ingested antigen challenge triggered the expansion and activation of CD4⁺TH2 cells, which secrete IL-2 to potentiate the function of ILC2s in producing large amounts of IL-5 and IL-13 [55, 54]. Subsequently, IL-13 produced by activated ILC2s can enhance allergic reactions to ingested antigen, possibly by inducing goblet cell hyperplasia, increasing intestinal permeability, and modulating gut barrier function. These studies indicate that the intestinal epithelium-derived IL-25 plays an important role in promoting allergic reactions to ingested antigens via enhancing the concerted interactions between ILC2s and antigen-induced CD4⁺TH2 cells after allergic sensitization has occurred. The findings support the view that IL-25 may bridge the cross-talk between skin and gut to propagate allergic reactions, which are amplified by intestinal ILC2s and CD4⁺TH2 cells at the effector phase of IgE-mediated food allergy.

A distinct role of IL-9 in type-2 immunity

The pleiotropic cytokine IL-9, a member of the common γ -chain/ γ -receptor cytokine family, was originally identified as a T cell and MC growth factor [56]. Because IL-9 is often produced concomitantly with other TH2 cytokines, IL-9 has been categorized as one of the type-2 cytokines secreted by CD4⁺TH2 cells. Numerous lines of evidence from human and murine studies support the view that IL-9 functions as a TH2 cytokine to promote type-2 immune responses. Studies in patients with asthma indicate that IL-9 production is associated with the increase of mucus genes, as evidenced by mucus hyperplasia [57]. In a murine model of allergic lung disease, transgenic mice with constitutive IL-9 overexpression in the lung display pronounced infiltration of eosinophils and lymphocytes, mucus production, and sub-epithelial collagen deposition in the airway and the resulting phenotype of increased airway hyperresponsiveness (AHR) [58, 59]. Conversely, treatments with anti-IL-9 antibody significantly reduced pulmonary eosinophilia, serum IgE levels, goblet cell hyperplasia, airway epithelial damage, and AHR in sensitized mice challenged with antigen intranasally [60]. However, deficiency of IL-9 in sensitized mice does not prevent the development of allergic lung diseases [61]. It appears that other TH2 cytokines or factors can exert functions or mechanisms that are shared with those of IL-9 in enhancing the pathogenesis of airway allergic inflammation.

A distinctive role of IL-9 was later demonstrated in studies of murine models for parasitic intestinal infection. Transgenic mice overexpressing IL-9 mounted stronger protective immune responses against *Trichinella spiralis* infection by producing increased IgG1 antibodies and developing more pronounced intestinal mastocytosis, resulting in a rapid parasitic expulsion in the gut [62]. Correspondingly, IL-9-deficient mice were ineffective in rapidly expelling *T. spiralis* during the acute phase, despite there being no difference in worm burden between IL-9-deficient and wild-type mice at the chronic phase of parasitic infection [63]. It is possible that intestinal IL-9 production was induced rapidly to provide the MC-dependent protection but declined several days post infection, when CD4⁺TH2 cell-mediated, antigen-specific immunity began to develop [63]. Indeed, in a pulmonary granuloma model, IL-9 was essential to induce a rapid and robust generation of pulmonary mastocytosis and goblet cell hyperplasia but not the infiltration of eosinophils and development of a CD4⁺TH2 cell-mediated immune response [64]. Furthermore, recent studies point to a pivotal role for IL-9 in promoting intestinal mastocytosis and driving experimental food allergy [65]. Repeated intragastric OVA challenge failed to increase intestinal mastocytosis, serum MCPt-1, the incidence of allergic diarrhea, or hypothermia in sensitized IL-9- or IL-9R-deficient mice, even though they produced OVA-specific IgE and IgG normally [66]. These findings demonstrate that IL-9 is an atypical TH2 cytokine that functions primarily in promoting MC-mediated immune responses and may be a key molecular factor in promoting the intestinal anaphylactic response to ingested food.

TH9 cells in type-2 immunity

Detailed re-analyses of the molecular program of CD4⁺T cell differentiation induced by TGF- β have led to the identification of an IL-9-producing CD4⁺ cell subset, termed TH9 cells [67, 68]. Unlike classical CD4⁺TH2 cells that express GATA3 transcription factor, TH9 cells are found to express primarily the transcription factors PU.1 and IRF4 and a low level of GATA3, thereby producing primarily IL-9 and some TH2 cytokines (IL-4, IL-5, and IL-13) [69, 70]. Because CD4⁺TH2 cells can also be induced into IL-9-producing T cells by TGF- β in the presence of IL-4, it has been suggested that CD4⁺TH2 or TH9 cells share the T cell developmental program. Indeed, parallel to the studies showing the effects of epithelial-derived cytokines TSLP and IL-25 on CD4⁺TH2 cell differentiation and function [71, 34, 72], recent studies demonstrate that both cytokines can also promote TH9 cell generation and function [73, 74]. In a murine model of allergic lung disease, TH9 cells were detected in the lung of mice challenged intranasally with house dust mite allergen [75], and the presence of *Il9* transcript expression was dependent on transcription factor PU.1 [69]. Transfer of OVA-specific TH9 cells is sufficient to induce the infiltration of inflammatory cells, mucus secretion, and MC accumulation in the airway in this murine model of allergic lung disease [70, 76]. IL-9 has also been tightly correlated with host-protection against parasites. Using IL-9 reporter mice, TH9 cells were shown to be involved in mounting a protective immune response against *Nippostrongylus brasiliensis* infection [77]. Indeed, mice transferred with parasitic antigen-specific TH9 cells were sufficient to expel worm infection [63]. Notably, IL-9-secreting T cells can be detected in the peripheral blood of patients with asthma or inflamed skin [75, 78]. Interestingly, levels of both TH2- and TH9-associated genes were increased in children with peanut allergy, and *IL9* expression level

can serve as an indicator to distinguish patients with peanut allergy from those with peanut sensitization only, suggesting that TH9 cells may be involved in peanut-specific responses [79]. Together, these studies suggest that CD4⁺TH9 cells are a specialized T cell subset with functions distinct from that of classical CD4⁺TH2 cells in promoting effective type-2 immunity.

IL-9–producing mucosal mast cells amplify intestinal mastocytosis

Food-induced anaphylaxis is an immediate allergic reaction, which depends on the presence of food-specific IgE and MCs expressing the high-affinity IgE receptor, FcεR. After allergic sensitization has occurred, secretion of IL-4 and IL-13 by antigen-induced CD4⁺TH2 cells causes isotype switching in B cells and them to produce IgE, which binds the FcεRI to form the IgE/FcεRI complexes on the surface of MCs. Mechanistically, an allergen exposure results in the binding of allergen to the IgE/FcεRI complexes, which in turn crosslinks the IgE/FcεRI complexes, leading to the activation of downstream signaling cascade in MCs. Subsequently, the activated MCs rapidly release the vasoactive and preformed pro-inflammatory mediators, including histamine, tryptase, carboxypeptidase A, leukotrienes, and platelet-activating factor (PAF), which drive the acute phase of the allergic response and physiologic alterations, thereby causing diarrhea, nausea and vomiting, abdominal cramping, and shock (anaphylaxis) [80]. An early study using mice deficient of MCs shows that MCs may play an important role in regulating gut barrier function and thereby the development of intestinal anaphylactic responses [81]. Though these studies highlight a role for IgE and MCs in driving the clinical presentations of IgE-mediated food allergy, it is not known what causes the differences in the symptoms among individuals with food allergy. Specifically, why only some, rather than all, individuals who are competent to generate MCs and have high levels of dietary allergen–specific IgE develop life-threatening anaphylaxis. Some clues are provided by studies using rodent models. Despite all developing a MC pool and acquiring antigen-specific IgE normally after allergic sensitization, murine strains are distinctively different in their susceptibility to systemic anaphylaxis [25]. This enigma hints that other molecular and cellular factors are involved in the development of life-threatening anaphylaxis.

It has been well documented that MCs show marked heterogeneity and can be divided into two major subclasses, connective tissue mast cells (CTMCs) and mucosal mast cells (MMC9s). CTMCs are derived from fetal liver progenitors and are primarily located in stromal tissues, whereas MMC9s originate from bone marrow progenitors and reside in the gut and lung. Of particular interest are the GI MMC9s, which appear to have unique cellular and molecular properties, including the insensitivity to certain degranulating agents and drugs. Since IL-9 is the key cytokine to drive mastocytosis [65, 66], identifying the cellular source of IL-9 may provide clues in understanding mechanisms underlying the susceptibility to food-induced anaphylaxis. It was originally hypothesized that TH9 cells are the primary IL-9 producers that drive intestinal mastocytosis. Unexpectedly, comparative analyses of cellular components among murine strains with different susceptibility to food allergy have uncovered that IL-9–producing mucosal mast cells (MMC9s) are the principal intestinal IL-9 producers [25]. MMC9s are a novel type of multi-functional mucosal MCs and exhibit the following characteristics: i) having a phenotype of the MMC lineage (Lin[−]c-

Kit⁺ST2⁺β7integrin^{lo}); ii) secreting prodigious amounts of IL-9 (~2.0 pg/mL per cell) and other TH2 cytokines, including IL-4 and IL-13, in lesser amounts; iii) exhibiting a small innate helper cell–like morphology with few metachromatic granules in their scanty cytoplasm; iv) secreting MC proteases and histamine. MMC9s are scarce in the small intestines of immunologically naïve mice and expand considerably after repeated ingested antigen exposure [25]. IL-4, provided by induced CD4⁺TH2 cells, and antigen crosslinking of IgE/FcεRIα complexes are both essential for the induction and expansion of MMC9s after allergic sensitization [25]. Although IL-9 is not required for the development of MMC9s, IL-9/IL-9R signals are essential for effective MMC9 expansion in an autocrine manner [25]. Notably, intestinal epithelium–derived IL-33 can enhance MMC9 function by inducing robust IL-9 production, which further amplifies intestinal mastocytosis, resulting in the development of an anaphylactic response to ingested antigens [25]. Given their anatomic location, characteristics, and function, MMC9s may serve as a key player that bridges the crosstalk between skin and gut by perpetuating allergic reactions and amplifying anaphylactic responses to dietary proteins. Indeed, an increased frequency of duodenal MMC9-like cells and expression levels of *Il9* and MC-specific transcripts were associated with atopic cases that developed comorbid allergic diseases, such as eczema and food allergy [25]. These findings suggest that the induction of the MMC9 developmental pathway may represent a pivotal checkpoint in acquiring the susceptibility to life-threatening anaphylaxis and provide a conceptual paradigm by linking atopic status (IL-4), dietary antigen and IgE/FcεR complex interactions, and inflammatory cues (exemplified by IL-33) with food allergy. Additionally, it will be intriguing to test whether food ingestions can activate antigen specific CD4⁺TH9 cells to produce IL-9 to amplify MMC9 induction, thereby augmenting the anaphylactic response to food allergens in the gut.

Conclusions

Over the past decades, research findings have led to the identification of food allergens and improvements of food allergy diagnosis [82]. However, our knowledge of the immunologic pathways that underlie the susceptibility to life-threatening anaphylaxis remain limited. Currently, there are no reliable biomarkers to predict or immunotherapeutic approaches to prevent the development of food allergy. Considerable clinical and experimental evidence indicate that multiple molecular and cellular factors and stepwise mechanisms are involved in the development of food allergy. (i) *Allergic sensitization phase*: routes of antigen exposure other than the GI tract are considered to be responsible for the initial induction of TH2 immune responses. Environmental insults triggered by lectins, proteases, or chitins and/or a genetic predisposing factor (filaggrin gene loss-of-function mutation) may initiate inflammatory reactions to induce the epidermal TSLP and/or IL-33 production that triggers allergic sensitization. TSLP endows DCs to promote a TH2-permissive microenvironment, which induces CD4⁺TH2 cell–mediated immune response and the generation of antigen-specific IgE. (ii) *Allergy propagation phase*: after allergic sensitization established through a parenteral route, food allergen ingestion activates emigrated antigen-specific CD4⁺TH2 memory/effector cells, resulting in the loss of GI-associated tolerogenic mechanisms. In the presence of CD4⁺TH2 cells and IL-25 stimulation, gut resident ILC2s produce large amounts of IL-13, which promote goblet cell hyperplasia and intestinal permeability, and

modulate gut barrier function. (iii) *Amplification of mastocytosis phase*: pronounced intestinal mastocytosis is a prerequisite for the development of food-induced anaphylaxis. It is possible that food allergen ingestion induces the increase of intestinal CD4⁺TH2 cells, which provide the IL-4 signaling to induce MC progenitors to develop into MMC9s. Additionally, crosslinking of the MMC9 surface IgE/FcεR complex with ingested antigens greatly enhances the expansion of MMC9s. IL-33, a potent inflammatory cytokine, induce MMC9s to secrete a large amount of IL-9, which further promotes MMC9 expansion and maturation in an autocrine loop, thereby driving the amplification of intestinal mastocytosis. Other cellular components also have the potential to enhance the amplification of intestinal mastocytosis. In response to IL-25 and/or IL-33, ILC2s also secrete IL-9 transiently, which facilitated IL-5 and IL-13 production in an autocrine manner [83]. By employing IL-4-eGFP (4GET) mice, a subset of T effector cells distinguished by their active *Il4* gene transcriptional activity was found to be capable of producing IL-9 [25], suggesting that TH9 cells may be involved in the induction and maturation of MMC9s during the development of experimental food allergy. These evidence support a plausible scenario that in addition to CD4⁺TH2 cells, ILC2s and TH9 cells may participate in the early induction and local accumulation of IgE-bearing MMC9s, which function to amplify intestinal mastocytosis and enable the potent anaphylactic reactivity to ingested antigens.

Currently, avoiding allergenic foods is the best form of management of food allergies. A number of therapeutic strategies have been used towards the treatment and prevention of food allergy, with limited to moderate success. While oral immunotherapy for infants at risk for peanut allergy (LEAP or Learning Early About Peanut allergy)[9], children with egg allergy [8], or with cow milk allergy [84] indeed induce some unresponsiveness in some individuals, the protection to casual food appears wear off, and some individuals develop severe adverse GI responses to the therapy. It would be interesting to test whether IL-9 producing cells, including innate MMC9s and ILC2s, and adaptive CD4⁺TH2 and TH9 cells, are involved in the loss of protection or the development of adverse GI symptoms during the regimen of oral immunotherapy. The ultimate goal of food allergy research is not only to reduce the allergic symptoms, but also to be able to potentiate a long-lasting state of unresponsiveness or “tolerance” to foods. Future, in-depth studies of human MMC9s, ILC2s, TH9 cells, and their associated molecules involved in the clinical reactivity of food allergy will provide the basis to translate the mechanistic findings from murine studies to further our understanding of human food allergy and the clinical application of this knowledge.

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Abbreviations

AD	atopic dermatitis
DCs	dendritic cells

GI	gastrointestinal
IL	interleukin
IgE	immunoglobulin E
ILC2s	type-2 innate lymphoid cells
LP	lamina propria
MCs	mast cells
MMC9s	IL-9–producing mucosal mast cells
OVA	ovalbumin
PAF	platelet-activating factor
TH2	T helper type 2
TH9	IL-9–producing T helper cells
TSLP	thymic stromal lymphopoietin

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Figure 1. Potential involvements of intestinal IL-9-producing cells in the development of immunoglobulin E (IgE)-mediated food allergy

In the allergic sensitization phase, environmental or mechanical triggers (or both) may induce skin keratinocytes to produce thymic stromal lymphopoietin (TSLP), which recruits and activates dendritic cells (DCs). TSLP-activated DCs migrate to draining lymph nodes to induce naïve CD4⁺T cells to differentiate into CD4⁺TH2 cells and maintain CD4⁺TH2 effector/memory pools. TSLP may also promote the generation of TH9 cells after sensitization occurs. In the allergy propagation phase, these CD4⁺TH2 cells migrate to the intestine and interact with resident type-2 innate lymphoid cells (ILC2s) to produce large amounts of IL-13 in response to intestinal IL-25 stimulation. In the amplification-of-mastocytosis phase, IL-4 signals provided by CD4⁺TH2 cells induce emigrated mast cell progenitors (MCPs) to become the multi-functional IL-9-producing mucosal mast cells (MMC9s), which then expand greatly after ingested antigens cross-link with MMC9 surface IgE/FcεR complex. The inflammatory cytokine IL-33 enhances IL-9 production by MMC9s, resulting in MMC9 maturation and the amplification of intestinal mastocytosis in an autocrine loop. In addition, IL-25 may directly act on TH9 cells to promote IL-9 production, resulting in the enhanced intestinal anaphylaxis. Thus, MMC9 induction may serve as a key cellular checkpoint to amplify and propagate allergic inflammation, resulting in the development of IgE-mediated food allergy. MMC, mucosal mast cell; STAT6, signal transducer and activator of transcription 6; TH2, T helper type 2 cell.