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# Food-induced Anaphylaxis: Mast Cells as Modulators of Anaphylactic Severity1

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## Abstract

A food-induced anaphylactic reaction can occur within seconds to a few hours following exposure to the causal food allergen and often affects multiple organ systems including gastrointestinal (GI), cutaneous, respiratory and cardiovascular. A conundrum in the allergy field is that consumption of the same allergen can cause reactions of vastly different severity in separate individuals; one patient may experience a mild non-life-threatening reaction characterized by pruritis of lips or urticaria whereas another may experience a life-threatening reaction that involves respiratory and cardiovascular compromise leading to loss of consciousness and sometimes death. While there are tests available to determine the predictive risk value of a positive food challenge test or clinical reactivity, there is currently no reliable method to distinguish between individuals who are at risk of mild non-life-threatening versus life-threatening reaction. Recent research has significantly advanced our understanding of the involvement of immune pathways in the effector phase of food-induced anaphylaxis, a void remains regarding our understanding of the contribution of these pathways to severity of disease. In this review, we discuss mild, non-life-threatening versus life-threatening food-induced anaphylaxis and factors (co-morbidities and immune-activation) that predispose individuals to more severe disease. Furthermore, we summarize recent advancements in our understanding of the involvement of underlying immune pathways in systemic and food-induced anaphylaxis in mouse systems and discuss how these pathways may contribute to more severe disease phenotype.

## Introduction

A recent review of the literature estimated that food allergy affects greater than 1–2%, but less than 10%, of the population (1). A meta-analysis of epidemiological studies on food

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allergy estimated that 12–13% of individuals perceive that they have a food allergy; however, studies that utilized specific testing to confirm food allergy indicate a prevalence rate of 3–5% (2, 3). Evidence from pediatric studies indicates that food allergies may be are on the rise, with an 18% increase in pediatric food allergy over the last decade (4) and a 300% increase in the prevalence of self-reported peanut allergy in children over a decade (5). Multiple studies throughout the world (e.g. Australia, Canada, United Kingdom and USA) utilizing direct and indirect methodologies indicate that greater than 1% of school-aged children are now affected by peanut allergy (3, 6–9). Severe food allergy-related reactions, termed food-triggered anaphylaxis, are serious, life threatening and responsible for 30,000–120,000 emergency department visits, 2,000–3,000 hospitalizations and approximately 150 deaths per year in the USA (10, 11). Clinical studies indicate that food reactions account for 30–75% of anaphylactic cases in emergency departments in North America, Europe, Asia and Australia (10, 12–14). The prevalence of food-related anaphylaxis is unclear; however, clinical data from the USA and Australia indicate that it is on the increase. The American studies, which employed similar methodologies of similar geographical locations for cohorts a decade apart, revealed a 71–100% increase in food-induced anaphylaxis in the 1993–1997 cohort compared to the 1983–1987 cohort (15, 16). Review of anaphylaxis fatalities and hospital admissions in Australia from 1997 to 2005 revealed a 350% increase in food-induced anaphylaxis admissions over this period (17); furthermore, evaluation of the trends in hospitalizations for anaphylaxis in Australia from 1993–1994 to 2004–2005 revealed a continuous annual increase in rate of hospital admissions for anaphylaxis (8.8% per year). Notably, from 1994–1995 to 2004–2005, admissions for anaphylaxis caused by food had an average annual increase of 13.2%. The rate increased across all age groups; however, the most significant increase was within the 0–4 age group, which observed a 5.5-fold increase in rate of admissions over the same time period (18). Importantly, these increases were not necessarily attributable to increases in atopy as hospital admissions for asthma over the same time period (1993–1994 to 2003–2004) declined by 43% among children aged between 0–14 years (18, 19).

## Clinical Manifestations of Anaphylaxis

The onset of symptoms from food-induced anaphylaxis is variable, occurring within seconds to a few hours following exposure to the causal food allergen. Symptoms often affect multiple organ systems including gastrointestinal (GI), cutaneous, respiratory and cardiovascular (10, 20). Food-induced anaphylactic patients do not generally present with a consistent constellation of symptoms. Moreover, the kinetics of onset and the sequence and severity of symptoms often vary from individual to individual and may also even differ in the same individual between repeated episodes or in response to different foods (10). Cutaneous symptoms (e.g. erythema pruritus, urticaria and angioedema) are the most common type of symptom, occurring in more than 80% of cases (21–23). GI symptoms (i.e. nausea, abdominal pain, vomiting and diarrhea) (1, 24) are also relatively common, particularly in pediatric cases, and can be the primary manifestation of food-induced anaphylaxis with minimal involvement of other organs. Respiratory (i.e. deep repetitive cough, chest tightness, rhinorrhea and wheezing) symptoms are also relatively common (10, 25, 26), and asthma is thought to be the primary cause of death in food-allergic individuals (27–29). Cardiovascular symptoms in food-induced anaphylactic reactions are not common, particularly in infant and preschool children (30–32), occurring in 39% of food-allergic reactions and rarely in the absence of respiratory arrest (30). It is postulated that cardiovascular and respiratory collapse leads to the hypotensive state and the subsequent presentation of symptoms including nausea, vomiting, diaphoresis, dyspnea, hypoxia, dizziness, and possibly seizures and collapse. (33–35).

Clinical studies have revealed differences in symptoms of anaphylaxis between children and adults (20, 36). Allergic reactions are more common in children; however, adults experience anaphylaxis more often (13). When an anaphylactic reaction does occur in pediatric cases, it is often triggered by foods such as tree nuts or peanuts, whereas in adults, anaphylaxis is often induced by insect venom or drugs (20). An anaphylactic reaction in adults often involves cutaneous symptoms (90% of the time) and frequently involves cardiovascular compromise; while cutaneous symptoms are also common (~ 80%) in pediatric cases of anaphylaxis, children experience respiratory symptoms more often than adults (36).

## Severity of Anaphylaxis

There has been a significant effort to develop a grading system by which to gauge and categorize anaphylaxis severity and to determine factors that may influence reaction severity (37–41). However, this has proven to be difficult as food-induced anaphylactic patients do not generally present with a consistent constellation of symptoms. Consequently, determination of food-induced anaphylaxis severity cannot necessarily be predicted based on clinical history (21, 42). While there is significant debate, it is generally accepted that any cardiovascular or respiratory involvement indicates a severe anaphylactic reaction (22, 37). Based upon a study in 2004 that performed a retrospective chart analyses of clinical features of acute generalized hypersensitivity reactions in 1,149 patients, a simple grading system based upon a minimum set of predictors of hypotension and hypoxia was developed. The author demonstrated that features including incontinence, collapse and confusion strongly correlated with hypotension and hypoxia (37). Other features, including dyspnea, stridor and wheeze, also significantly correlated with hypotension and hypoxia but were less closely associated. Common skin features, including urticaria, erythema and angioedema, did not correlate with hypoxia and hypotension (37). On the basis of predictors of hypotension and hypoxia as determined by a stepwise logistic regression analysis, the author developed a system whereby severe reactions were defined by cyanosis or SpO2% at any stage and hypoxia, confusion, collapse, loss of consciousness or incontinence; moderate grade reactions were defined by features suggesting respiratory, cardiovascular or GI involvement including dyspnea, stridor, wheeze, nausea, vomiting, dizziness, diaphoresis, chest or throat tightness and abdominal pain, and mild reactions were defined by symptoms that involved skin and subcutaneous tissues only. Notably, this grading system correlated well with epinephrine usage (37). While there were some limitations of these analyses, the study revealed a correlation between GI manifestations and severity (hypotension) of anaphylaxis. This is consistent with recent clinical reports showing that a clinical history of chronic/relapsing GI symptoms and the degree of GI permeability positively correlate with the severity of anaphylaxis in humans (43–47). Several factors have been identified as increasing the risk of severe food-induced anaphylaxis including atopic history, a pre-existing food allergy and co-existence of asthma (48–50). While there have been a number of foods identified as triggers for food-induced anaphylaxis, the most common foods to induce severe reactions or death are peanuts and tree nuts (22, 27, 51).

## Effector Mechanisms in Anaphylaxis

Early clinical evidence suggested that anaphylaxis was classically mediated by antigen cross-linking of antigen-specific IgE bound to FcεRI on mast cells stimulating mast cell degranulation and the rapid release of secondary mediators (e.g. histamine, proteoglycans, platelet-activating factor [PAF], serotonin, tryptase and chymase) and lipid-derived mediators (i.e. $PGD_2$ and $LTC_4$, $LTD_4$ and $LTE_4$) (52–56). These mediators are thought to act on target cells to promote the pathophysiological features of disease including urticaria, diarrhea, bronchoconstriction, respiratory and cardiovascular collapse, the latter of which reflects a decrease in intravascular volume resulting in decreased vital organ perfusion and

shock (35, 53, 54, 57–61). Indeed, increased levels of several mast cell mediators including PAF, lipoxygenase products (LTB4, LTC4 and LTD4), cyclooxygenase products (PGD2), trypase, chymase, histamine and cytokines (IL-6, IL-10 and soluble tumor necrosis factor [TNF] receptor I) have been observed in blood of human patients with anaphylaxis.

Levels of histamine and tryptase have been associated with anaphylactic reaction severity (62–68). Notably, serum tryptase in patients with insect sting-induced anaphylaxis correlated with the degree of hypotension (69). However, elevated total tryptase levels are typically not elevated in individuals with food-induced anaphylaxis or in those with a positive food challenge test in which anaphylaxis symptoms are observed (49, 70). Recently, Vadas and colleagues reported elevated levels of PAF in medication-, insect sting- and food-induced anaphylaxis (63). Notably, the levels of PAF positively correlated with severity of the anaphylactic response (63). In a retrospective study, Vadas and colleagues also assessed PAF acetylhydrolyase activity in nine pediatric and adult cases of fatal anaphylaxis. PAF acetylhydrolase is a phospholipase A2 that hydrolyzes the sn-2 acetyl residue of PAF causing loss of function (71). Vadas and colleagues showed that PAF acetylhydrolase activity was significantly lower in patients with fatal anaphylaxis than in controls, suggesting that a reduced ability of PAF acetylhydrolase to inactivate PAF may contribute to anaphylaxis severity (72). While these studies suggest an important contribution for PAF and PAF acetylhydrolase in anaphylaxis, alternate explanations, such as severe hypoxia and subsequent systemic necrosis reducing PAF acetylhydrolase activity in patients with fatal anaphylaxis, have yet to be ruled out (73).

## Mechanisms of Anaphylaxis in Mice

Experimental studies in rodent models of anaphylaxis have provided corroborative evidence demonstrating that IgE-mediated anaphylaxis is critically dependent on FcεRI/mast cells (53). Moreover, mice deficient in IL-4/IL-4 receptor alpha (IL-4Rα), mast cells, FcεRI or IgE are protected against IgE-mediated anaphylaxis (53). Experimental studies employing various drug antagonists (histamine receptor 1, histamine receptor 2 and PAF) demonstrated a role for histamine, and to a lesser extent PAF, in active IgE-mediated systemic anaphylaxis (53). However the demonstration of systemic anaphylaxis in mice that lacked mast cells (c-kit–deficient W/Wv mice), FcεRI, or IgE provided evidence for the existence of an IgE/FcεRI/mast cell-independent pathway in anaphylaxis (74–76). These findings have led to intense focus on defining alternative pathways in the development of anaphylaxis and the identification of an alternative anaphylaxis-associated pathway that is mediated by IgG antibodies, the activating low-affinity IgG receptor FcγRIII and macrophages (53, 77). The FcγRIII/macrophage-dependent pathway of anaphylaxis closely resembles IgE/FcεRI/mast cell–mediated shock; however, there are some important differences. For example, PAF, rather than histamine, is primarily responsible for the development of shock in the FcγRIII/macrophage-dependent system (53). Tachycardia is more prominent when anaphylaxis is induced in mast cell–sufficient than in mast cell–deficient mice, suggesting that it is likely dependent on IgE and histamine (53, 76). Although IgG antibody-dependent complement activation can produce anaphylatoxins, including C3a and C5a, these do not appear to be important in the IgG-mediated alternative pathway. This may reflect the predominant involvement of IgG1, which does not activate complement, in most experiments. Recent investigations have also revealed a role for basophils in IgG-mediated and not IgE-mediated anaphylaxis in mice (78). These studies indicated that basophils were dispensable for IgE-mediated anaphylaxis but that depletion of basophils, but not macrophages or neutrophils or natural killer “NK” cells, ameliorated IgG-mediated anaphylaxis (78). The IgG/basophil-mediated response involved FcγRIII receptors and was dependent on PAF (78). Assessment of active systemic anaphylaxis in mice has also recently illuminated the involvement of IgG antibodies, FcγRIIIA and FcγRIV, PAF and neutrophils (79). Analysis of mice that are

deficient in all IgE and IgG receptors (FcγRI/ FcγRIIB/FcγRIIIA/FcεRI/FcεRII) other than FcγRIV revealed a role for this receptor in systemic anaphylaxis (79). Neutrophil depletion by anti-Gr1 monoclonal antibody inhibited active and passive systemic anaphylaxis, and the anaphylaxis phenotype could be recapitulated by reconstitution of mice with either human or murine neutrophils (79). Collectively, these studies indicate the existence of a classical pathway of anaphylaxis involving IgE-mast cells and an alternative pathway of anaphylaxis involving IgG FcγRIII, FcγRIV, macrophages, basophils and neutrophils in mice. However, because deletion of FcεRIα results in a gain of function for FcγRIII, it remains possible that deletion of all other stimulatory FcRs increases the importance of FcγRIV in anaphylaxis (75).

Recently, we sought to identify markers that distinguish IgE- from IgG-mediated anaphylaxis (80). Employing passive models of systemic anaphylaxis, we demonstrated that both IgG- and IgE-mediated anaphylaxis were characterized by a decrease in the number of peripheral blood basophils and monocyte percentages and an increase in the number of neutrophils (80). IgE-mediated, but not IgG-mediated, anaphylaxis was associated with increased levels of serum IL-4 (1000-fold), sIL-4Rα (60–70%) and IL-4Rα expression on $CD4^+$ T-cells (40–70%). IgG-mediated anaphylaxis was shown to differentiate from IgE-mediated anaphylaxis by neutrophil FcγRIII expression. Moreover, passive IgG-mediated anaphylaxis was associated with a 28–60% reduction in FcγRIII on neutrophils. Importantly, using *in vitro* and *in vivo* systems, we provided data suggesting that a similar effect could occur in humans (80). These studies indicate that a number of immune component parameters can distinguish between IgE- and IgG-mediated anaphylaxis.

## Experimental Models of Food-induced Anaphylaxis

Over the last decade, researchers have developed several mouse models of food-induced allergy and anaphylaxis in order to begin to delineate the important immune pathways involved in augmentation of the sensitized state and the key effector pathways in the induction of the myriad manifestations of food-induced anaphylaxis (81–83). Recently, a number of excellent reviews have been published that describe the current state of knowledge of the immune pathways involved in $CD4^+$ T-cell Th2-development, antigen-specific IgE production and GI mastocytosis (83, 84). Therefore, in this section, we will focus on the involvement of these immune mechanisms in the modulation of severity of food-induced anaphylactic reactions.

There are a number of described experimental mouse models of oral antigen-induced anaphylaxis, whereby mice are sensitized to antigen (Ag) [peanut (PN), ovalbumin (OVA), cow milk [CM] or cashew [CSH] or hazelnut [HZ]) via an oral, peritoneal or transdermal route in the presence of an adjuvant (alum, cholera toxin [CT], staphylococcus aureus [SEB]) and are subsequently challenged orally with antigen that induces a shock syndrome characterized as systemic anaphylaxis but which also commonly induces GI symptoms, such as diarrhea (85–88). In the transdermal CSH or HZ models oral gavage of antigen primarily induced systemic shock (88, 89). In the Oral Ag-CT, Oral Ag-SEB models, both shock and diarrhea are induced whereas the initial description of the intraperitoneal (i.p.) sensitization model, involving two i.p. immunizations with chicken OVA and the adjuvant alum (aluminum hydroxide) and subsequent oral OVA challenge, only induced diarrhea (87). Recent refinement of this model involving one i.p. immunization and multiple oral challenges have demonstrated induction of both GI symptoms (diarrhea) and systemic symptoms (bronchoconstriction and shock) (I.P. Alum-OVA model) (90, 91).

As multiple, independent pathways have been identified as mediating anaphylaxis in mice, it is important for the increased understanding of food-induced anaphylaxis to clarify which of

these pathways contribute to the manifestations of oral antigen-induced anaphylaxis. Studies using the Oral Ag-CT model indicate that it is primarily an IgE-mediated response: the oral antigen-induced anaphylaxis in this model is associated with elevated levels of IgE and histamine production, and passive cutaneous anaphylactic (PCA) reactions induced by immune sera from cow milk-sensitized mice were eliminated by heat inactivation (85). The Oral Ag-SEB model is also associated with increased histamine and IgE (92), and the diarrhea and shock response can be abolished by anti-histamine treatment (personal communication Dr. Paul Bryce). The I.P. Alum-OVA model stimulates increased IgE and GI mastocytosis (90, 93). Notably, blockade of mast cell activity with mast cell-stabilizing agents and mast cell-neutralizing antibodies or inhibition of IgE activation (*FceRI*$^{-/-}$ mice and mice treated with anti-IgE antibodies) abolishes systemic and GI symptoms of oral antigen-induced anaphylaxis in the I.P. Alum OVA model (90, 91, 93). Furthermore, *Igg1*$^{-/-}$ mice develop diarrhea and shock response at levels comparable to that of WT mice, indicating that the IgG1 alternative pathway does not have a role in oral antigen-induced anaphylaxis induced by i.p. antigen/alum (90). Thus, in contrast to parenteral-induced anaphylaxis, oral antigen-induced anaphylaxis in mice is dependent on the classical mast cell/IgE-dependent pathway, and there is no involvement of the alternative IgG/macrophage/basophil pathway to the effector phase of disease.

Mouse model research investigating the role of mast cell mediators in the induction of the systemic and GI manifestations of anaphylaxis indicates that various mast cell mediators contribute to discrete components of disease in various tissues (81). For instance, the hypothermic component of shock response is mediated by histamine as it can be blocked by histamine H1 and H2 receptor antagonism whereas the secretory diarrhea response is dependent on PAF and serotonin (87). Interestingly, employing the I.P. Alum-OVA model, we recently observed that while the diarrhea and shock response were attenuated in *FceRI*$^{-/-}$ mice, there were no differences in airway hyperresponsiveness (AHR) between OVA-challenged wild-type and *FceRI*$^{-/-}$ mice, suggesting that the FcεRI/IgE pathway is important in GI and cardiovascular symptoms but is dispensable for the AHR response associated with oral antigen-triggered anaphylaxis (91). Notably, treatment of mice with CD4$^{+}$ T-cell-depleting antibody (GK1.5) abrogated the AHR response in oral OVA-gavaged *FceRI*$^{-/-}$ mice, indicating that the OVA challenge-induced AHR response in *FceRI*$^{-/-}$ mice is CD4$^{+}$ T-cell dependent. This finding is consistent with the previous demonstration of CD4$^{+}$ T-cell involvement in the development and maintenance of AHR in mouse models of allergic airway disease (94). There are considerable data indicating an important contribution of CD4$^{+}$ T-cell-derived IL-13, and to a lesser extent IL-4, in the onset of AHR associated with the asthmatic phenotype (95–97). However, the CD4$^{+}$ T-cell-mediated AHR response in food-induced anaphylaxis does not appear to involve IL-4 or IL-13, as we observed no effect of oral OVA gavage on pulmonary IL-4 or IL-13 levels or on IL-4- or IL-13-driven processes (e.g. mucus cell hyperplasia) between vehicle- and OVA-gavaged wild-type mice (91). Previous clinical studies describing double-blinded, placebo-controlled food challenge of food allergic patients have reported the development of airway reactivity changes in the absence of bronchopulmonary obstruction (98, 99). Interestingly, asthma and atopy are considered a risk factor for severe anaphylaxis (20); therefore, determining the contribution of a pre-existing $Th_2$ phenotype to the IgE-independent AHR response in oral antigen-triggered anaphylaxis will be important for delineation of immune pathways involved in the induction of AHR.

Why oral antigen-induced anaphylaxis is IgE-dependent while parenteral antigen-induced anaphylaxis is mediated by both IgG and IgE pathways is unclear. The involvement of the IgE or IgG pathways in anaphylaxis is dependent on the concentration of antigen that enters the systemic circulation following oral or parenteral antigen challenge and the rate with which it accesses IgG-bearing macrophages/basophils or IgE-bearing mast cells. In the

absence of blocking antibody, ~100-fold less antigen is required to induce IgE-mediated than IgG-mediated anaphylaxis (100). Ingested antigen is digested and/or excreted, and thus relatively small quantities are absorbed. Furthermore, the ingested antigen is absorbed over several minutes, unlike parenteral antigen administration, which happens rapidly. Thus, it is likely that oral antigen ingestion only provides sufficient antigen to induce the IgE-dependent pathway. Consistent with this idea, blockade of the IgE but not the IgG pathway abrogated both systemic and GI anaphylaxis induced by oral antigen (90).

## Immune Regulation of Oral Antigen-induced Anaphylaxis Severity in Mice

Organ system involvement varies among species and directs the clinical course of anaphylaxis (101). In humans, compromise of either the cardiovascular or respiratory system defines a severe reaction (21, 102). Indeed, anaphylactic symptoms including confusion, collapse, unconsciousness and shock strongly correlate with hypotension and hypoxia (47). Oral antigen challenge is thought to stimulate vasodilatation and sequestering of blood in the capillary bed, likely due in part by pulmonary venous vasodilatation and fluid extravasation, which causes respiratory and cardiovascular collapse leading to hypotension and hypoxia (103). However, the demonstration of obstructing edema of the upper airways and of the acute pulmonary emphysema observed in anaphylaxis suggests extravasation of fluid in the third space in humans during an anaphylactic reaction (104). Consistent with this possibility, Black and Kemp demonstrated an increase in the specific gravity of blood in an individual experiencing a ragweed-induced anaphylactic reaction, suggesting increased hemoconcentration and increased capillary permeability (105). Similarly, 5 of 26 patients with idiopathic anaphylaxis had elevated erythrocyte sedimentation rates, indicating increased extravasation in human anaphylaxis (106). Fluid extravasation and vasodilatation is thought to lead to a decrease in circulating blood volume of up to 35% within 10 minutes of allergen exposure (33).

In the mouse, the shock organ is the capillary bed, and anaphylaxis causes capillary bed dilatation and extravasation leading to severe hypovolemia and fatal tissue hypoxia (107, 108). Consistent with this, anaphylaxis in mice is associated with a significant increase in hemoconcentration (108). Using an experimental approach in order to identify modulators of severity of food-triggered anaphylaxis, we recently evaluated symptom parameters of mouse food-induced anaphylaxis to determine whether they correlate with severity and immune parameters that are required for anaphylaxis (91). A consequence of the hypovelmia-induced shock in mice is hypothermia (81, 109, 110); thus, we specifically choose the systemic symptom of hypothermia as an indicator for severe anaphylaxis in mice. Experimental studies suggest that the only two immune requirements for oral antigen-induced anaphylaxis are antigen-specific IgE and GI mastocytosis (90, 93). Absence of these two features in mice prevents the development of oral antigen-induced anaphylaxis characterized by GI and systemic symptoms (90, 93, 111). Thus, we looked for a relationship between systemic symptoms of oral antigen-induced anaphylaxis (I.P. Alum-OVA model) and immune parameters including antigen-specific IgE and GI mast cells.

A total of seven oral gavage challenges administered on alternate days to OVA-sensitized BALB/c mice triggers an oral-antigen-induced anaphylactic reaction that has both GI and systemic (hypothermia) manifestations. Surprisingly, we observed no relationship between IgE levels and hypothermia (maximum temperature change) following the seventh oral challenge, suggesting that IgE levels do not modulate the severity of disease. Similarly, while allergen-specific IgE levels in humans have a 95% predictive risk value of a positive food challenge test or clinical reactivity, IgE levels do not predict the type or severity of any reaction that may occur. Interestingly, the mouse GI mast cell levels positively correlated with maximum temperature loss (hypothermia), suggesting a relationship between GI mast

cell levels and the systemic manifestations of oral antigen-induced anaphylaxis (91). We next assessed symptoms of anaphylaxis following the fourth oral gavage challenge in the I.P. Alum-OVA model. This experiment was prompted by our observation of an ~50% penetrance of oral antigen-induced anaphylaxis in wild-type mice following this challenge and of the anaphylactic reaction ranging from mild to moderate severity (hypothermia) in those mice that do develop anaphylaxis. These analyses revealed a strong correlation between GI mast cell density and the shock (systemic) response (91), corroborating or earlier finding. Importantly, the serum levels of the mast cell activation marker MCPT-1 positively correlated with change in body temperature, indicating a strong relationship not only between the GI mast cell density and the severity of oral antigen-induced anaphylaxis but also the GI mast cell activation and the severity of oral antigen-induced anaphylaxis (91). To begin to delineate the molecular basis of intestinal mast cell regulation of severity of oral antigen-induced anaphylaxis we hypothesized that intestinal mast cells induce intestinal capillary bed dilatation and extravasation leading to severe hypervolemia and increased severity of food-induced anaphylaxis. Notably, we have observed significant mesenteric vascular leak as evidenced by increased Evan's blue extravasation and associated hypothermia in OVA-primed WT mice following oral gavage (91).

The demonstration that GI mast cell density correlated with increasing severity of systemic symptoms of anaphylaxis led us to speculate that mice with increased GI mast cell levels would experience more severe GI and systemic symptoms of oral antigen-induced anaphylaxis. To directly test this hypothesis, we examined severity of IgE-mediated anaphylaxis in BALB/c wild-type and iIL-9Tg mice. iIL-9Tg mice have increased numbers of mast cells in their intestines but not in other tissues (93). Furthermore, we have not observed any differences in the level of total IgE or in $CD4^+$ T-cell and B-cell functions in iIL-9Tg mice compared to wild-type mice. Consistent with our hypothesis, the level of hypothermia during anaphylaxis induced by either anti-IgE treatment, the I.P. OVA-alum model or a passive oral antigen anaphylaxis model was significantly elevated (i.e. greater negative change in temperature) in mice that possessed a GI-specific increase in numbers of mast cells (93) compared to wild-type mice (91). Furthermore, other systemic features, including cutaneous and mesenteric vascular leak and AHR, were also significantly elevated in iIL-9Tg mice compared to wild-type mice (91). These data indicate that increased GI mast cell numbers increase the severity of passive IgE-mediated systemic anaphylaxis.

There are a number of potential explanations of how increased GI mast cells modulate severity of oral antigen-induced anaphylaxis (Figure 1). Firstly, elevated GI mast cell load may amplify the severity of oral antigen-triggered anaphylaxis via modulation of oral antigen absorption. We have previously demonstrated that iIL-9Tg mice have increased GI paracellular permeability (93). Altered GI permeability could promote increased systemic antigen absorption and dissemination, which may lead to increased IgE/FcεRI mast cell degranulation and increased severity of anaphylaxis (Figure 1). In support of this hypothesis, we have previously demonstrated a positive correlation between systemic antigen dose and severity of systemic anaphylaxis [12]. Interestingly, wheat allergen challenge tests on patients with wheat-dependent, exercise-induced anaphylaxis have revealed a positive correlation between blood gliadin levels and clinical symptoms of exercise- and aspirin-induced anaphylaxis (112). Furthermore, physical exercise or intake of aspirin promotes increases in GI permeability (113, 114), suggesting that altered GI permeability may contribute to the increased severity of food-induced anaphylaxis.

Alternatively, the increased severity of anaphylaxis in the iIL-9Tg mice may not necessarily pertain to mast cell-dependent physiological effects but could simply be due to the increased GI mast cell load. iIL-9Tg mice have an increased total number of GI mast cells: thus, following IgE-mediated activation, they release a greater level of mast cell-derived

mediators that could disseminate and induce increased systemic anaphylactic symptoms. Indeed, we observe evidence of increased mast cell activation in anaphylactic iIL-9Tg mice compared with wild-type mice (91).

So what evidence is there that intestinal mast cells are elevated in food allergy or anaphylaxis and that they contribute to disease severity? Clinical studies have demonstrated elevated levels of IgE-positive cells in duodenal biopsy specimens from food allergic patients compared to controls (115, 116). In one study, the level of IgE-positive mast cells in duodenal biopsy specimens from patients with positive SPTs was 2-fold higher than that observed in patients with negative SPTs (115). In the Caffarelli study the distribution of the number of IgE-positive cells in the biopsy specimens from the food-allergic children was significantly elevated compared with that of controls. Notably, the prevalence of elevated numbers of IgE-containing cells in food-allergic children was 92%. Importantly, the authors demonstrated that the IgE_positive cells were plasma cells and mast cells (2.7%) in the duodenal biopsies from children with food allergy. Mast cells were virtually absent and the infiltrate poor in the control duodenal biopsy samples (116). Lilllestol and colleague demonstrated increased levels of IgE-positive cells in duodenal biopsies from patients with self-reported food hypersensitivity. However, the IgE-bearing cell numbers did not correlate with increased levels of tryptase-positive mast cells (117). Importantly, studies have identified the existence of a tryptase$^{-}$ chymase $^{+}$ MC population in the mucosa and submucosa of the intestine in humans (118) and it is currently unclear whether the IgE-bearing positive cells were of a tryptase$^{-}$ chymase $^{+}$ MC phenotype. Other investigators have demonstrated increased intestinal MC activity in food allergic individuals. Moreover, activation of intestinal mast cells obtained from enzymatically dispersed duodenal biopsies by anti-IgE activation released more histamine in comparison to cells from non-allergic individuals ((119).

Systemic mastocytosis is a proliferative disorder of the hematopoietic mast cell progenitor lineage resulting in excessive numbers of mast cells in tissues that include the intestine (120). Notably, 25–50% of patients with systemic mastocytosis develop gastrointestinal symptoms, including abdominal pain, diarrhea, nausea and vomiting (121). Notably, treatment of patients with the mast cell stabilizing agent disodium cromoglycate is very effective in controlling the GI symptoms in patients with mastocytosis (122, 123). Indeed, two recent large studies consisting of a total of 320 cases of mastocytosis demonstrated an increased risk for development of anaphylaxis (124, 125); anaphylactic reactions have been reported in 22–49% of adult and 6–9% of pediatric patients with mastocytosis, whereas the lifetime prevalence in the general population is ~1% (126).

## Conclusions

A conundrum in the allergy field is that food-induced anaphylactic patients do not generally present with a consistent constellation of symptoms; thus, determination of food-induced anaphylaxis susceptibility and severity cannot necessarily be predicted based on clinical history. Experimental studies and corroborative clinical evidence indicate an important contribution for IgE-FcεRI-mast cells in the induction of the clinical manifestations of anaphylaxis. Recent experimental studies indicate that intestinal mast cell levels contribute to the severity of a food-induced anaphylactic reaction. The mechanism of action remains unclear, however intestinal mast cells may increase antigen absorption and induce increased systemic mast cell activation. Alternatively increased intestinal mast cell numbers may lead to increased intestinal mast cell activation and release of mast cell-derived mediators following oral allergen challenge, which amplifies the systemic symptoms of disease. While there is some circumstantial clinical evidence to support increased intestinal mast cells as a contributor to more severe disease further clinical and experimental analyses are required.

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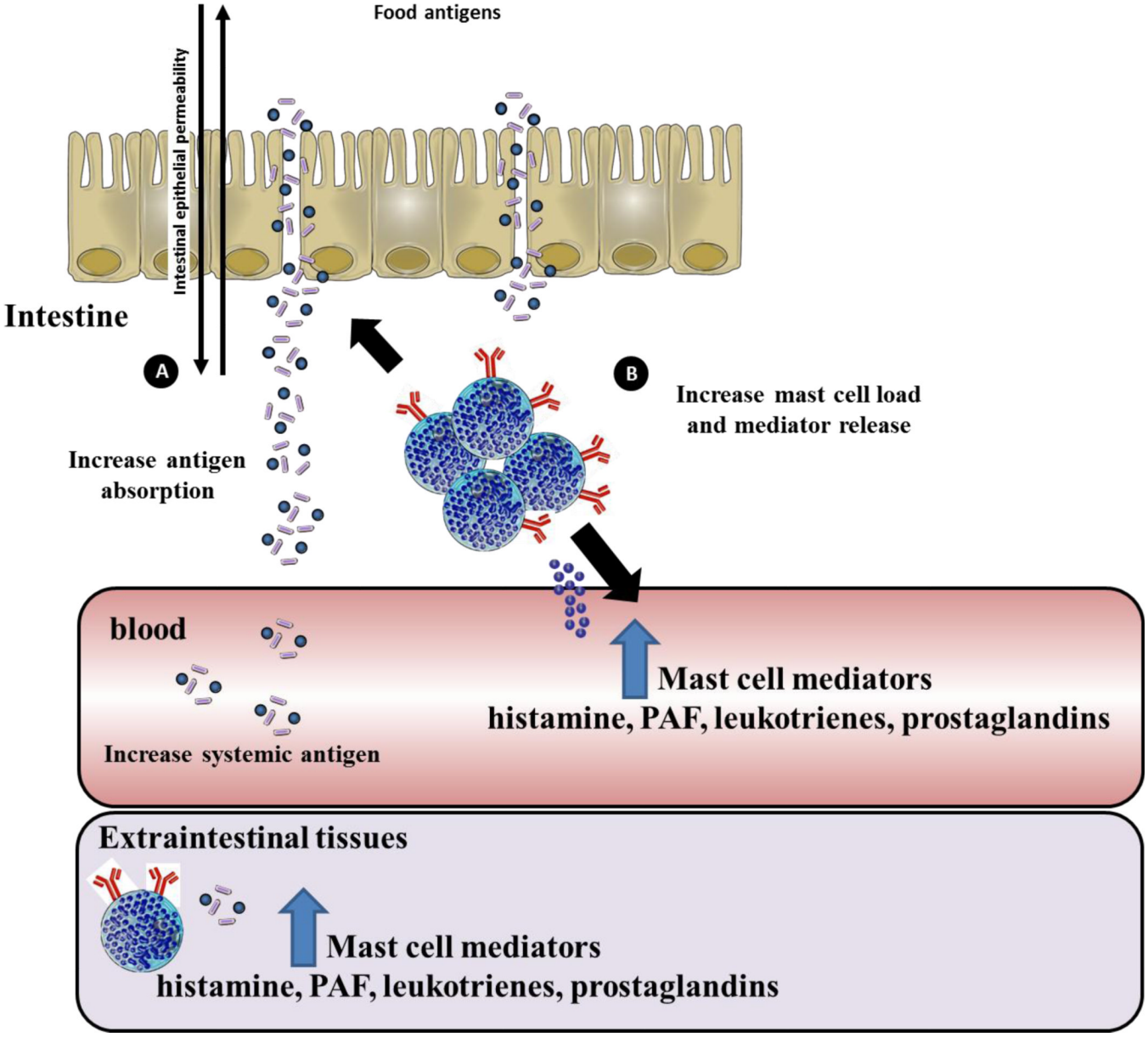


**Figure 1. Intestinal mast cells and severity of food-induced anaphylaxis**

Intestinal mast cells can regulate intestinal epithelial permeability (127). Increased mast cell levels could increase intestinal permeability and increase systemic antigen absorption, which would enable systemic mast cell activation and stimulation of respiratory and cardiovascular collapse. Alternatively, oral antigen absorption in the present high intestinal mast cell numbers can lead to increase mast cell activation and degranulation leading to increased systemic release of mast cell mediators, stimulation of respiratory and cardiovascular collapse and onset of severe food-induced anaphylactic reactions.