

Experimental Approaches to the Study of Food Allergy

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KEY CONCEPTS

- Animal models of IgE-mediated anaphylaxis in response to oral challenge with common food allergens have been developed in rodents, pigs, and dogs.
- Experimental approaches have been used to show that perturbation of intestinal barrier function, digestive capacity, or the composition of the gut flora can influence the development of allergic sensitization.
- Cutaneous exposure to peanut can lead to allergic sensitization in the absence of exogenous adjuvants.
- Several therapeutic approaches have been developed that use microbial products in combination with immunotherapy to induce safe desensitization to food allergens.

Current experimental animal models of food allergy

Most experimental animal models of food allergy have focused on IgE-mediated, immediate hypersensitivity reactions and anaphylaxis. In recent years, models have also been developed to address the pathophysiology of eosinophilic disorders of the gastrointestinal tract. Other food-allergic disorders such as food protein-induced enterocolitis and proctocolitis have not yet been modeled. As we gain more understanding of these disorders from the *in vitro* study of patient samples, we may in future be able to establish appropriate new animal models for the study of additional non-IgE-mediated food-allergic disorders.

Models of IgE-mediated food allergy

The majority of experimental models of food allergy use rats or mice, where the major advantages are cost, availability of immunological reagents, and availability of genetic targeting strategies that allow for mechanistic studies of the pathophysiology of food allergy. Experimental animal models of IgE-mediated food allergy generally fall into two broad categories: oral sensitization or systemic sensitization to food allergens. Typically the systemic sensitization models have been used to study the gastrointestinal response to allergen re-challenge, while in contrast the oral sensitization

models are used to address mechanisms of sensitization or testing of protein allergenicity. (See Table 43.1 for an overview of IgE-mediated models of food allergy).

Oral sensitization models

One approach to model human food allergy has been to find strains of rodents that are susceptible to the development of allergy to oral administration of food allergens in the absence of adjuvant. The Brown–Norway (BN) rat is a high IgE-responder strain that has been shown to develop IgG and IgE antibodies in response to prolonged daily oral exposure to ovalbumin (OVA) [1]. When orally challenged with OVA, the BN rat develops an increase in intestinal permeability, suggestive of a local anaphylaxis response in the gastrointestinal tract. This was not accompanied by a drop in blood pressure or respiratory rate, however, suggesting a lack of systemic anaphylaxis [2]. The B10A strain of mouse was also shown to respond to prolonged oral OVA exposure with the development of OVA-specific IgE and IgG and release of histamine after systemic OVA challenge [3].

Recently Hogan and colleagues have developed an adjuvant-free model of allergic food sensitization by transgenic expression of the bean α -amylase inhibitor (α AI) in peas [4]. Mice orally administered α AI-pea homogenate develop α AI-specific IgE, and develop early- and late-phase hypersensitivity responses in skin when the allergen is cutaneously administered [5]. Allergic inflammation in lung in response to intratracheal administration was also reported with this model [4]. Transgenic expression of α AI was necessary

for allergenicity, and was associated with expression of a slightly modified form of α AI that had enhanced immunogenicity. This finding highlights the usefulness of adjuvant-free systems for careful assessment of protein allergenicity, particularly in light of recent findings that many allergens appear to have intrinsic adjuvant-like activity [6–8].

Other models of oral sensitization have incorporated adjuvants such as cholera toxin (CT) to induce sensitization to orally delivered antigens. Snider *et al.* [9] showed that mice orally administered two feeds of hen's egg lysozyme (HEL) or OVA together with CT-generated antigen-specific IgE antibodies, and were primed for systemic anaphylaxis in response to systemic antigen challenge. In addition, immunization of mice with CT and HEL primed for HEL-specific secretory responses in the mouse intestine, as measured by Ussing chamber analysis of short-circuit current (Isc). Li *et al.* modified this model to establish models of oral sensitization to peanut [10] and whole milk [11] in C3H/HeJ mice with an outcome of systemic anaphylaxis to oral challenge. Variations of these models in C3H strains and Balb/c mice have been used to begin to address the immune mechanisms of allergic sensitization to common food allergens [12–16].

Systemic sensitization models

Models of systemic sensitization of rodents (most commonly OVA/alum sensitization of rats and mice) were developed to examine local responses to allergen challenge in the gastrointestinal tract [17–21]. Rats and mice systemically sensitized to OVA generate a local intestinal secretory response driven by active chloride secretion. One approach to study this secretory response is preparation of perfused intestinal loops of anesthetized rats where changes in water and ion content are measured in the perfusate [22]. Radioactive tracers have been used to address epithelial and vascular permeability in response to luminal allergen challenge [17,20,23]. Alternatively, Ussing chambers have been used to study active ion secretion in response to luminal or serosal allergen challenge [24–26], and also to measure gastrointestinal permeability changes in response to allergen challenge [25,27,28]. Systemically sensitized rats have also been used to study motility changes in response to allergen challenge [21]. These studies have shown that luminal allergen challenge results in an immediate hypersensitivity reaction in the gastrointestinal tract, characterized by mast cell degranulation, active ion secretion (and therefore water secretion), increased permeability to macromolecules, and disrupted intestinal motility. The pathophysiology of local hypersensitivity reactions in the gastrointestinal tract will be covered in greater detail later in the chapter.

Recent studies have extended the systemic sensitization model by administering multiple feeds of OVA to induce an *in vivo* allergic diarrhea response. Mice systemically sensitized to OVA with alum and then fed with multiple high-dose feeds of OVA (every second day) have severe acute

diarrhea in response to OVA challenge beginning after three feeds of OVA [29,30]. Studies by Brandt *et al.* demonstrated that the model is dependent on both mast cells and IgE [29]. Other investigators have used Freund's adjuvant to systemically sensitize mice to OVA, and repetitive feeding of OVA ($\times 10$) also induces a diarrheal response [31]. In contrast to the model using alum adjuvant, this model is localized to the large intestine, and is mediated by Th2 CD4+ T-cells that home to the large intestine and induce diarrhea in a STAT-6 dependent manner [31]. It is not clear if this model is also representative of an immediate allergic reaction in the intestinal mucosa.

Large animal models

Non-rodent models of food allergy have been developed that feature symptoms that may be more closely related to human food-allergic reactions than rodent models. Although these large animal models are unlikely to be used widely for mechanistic studies, they offer a unique opportunity to test potential therapeutics in a non-rodent model prior to human trials. Dogs, like humans, develop spontaneous allergic disease, most commonly atopic dermatitis. It was found that 32.7% of dogs with allergic skin disease seen at a veterinary dermatology practice had food hypersensitivity, indicating a similar association of atopic dermatitis and food hypersensitivity in dogs [32]. A colony of spontaneous food-allergic dogs (maltese/beagle cross) has been described by Jackson *et al.* at North Carolina State University [33,34]. These dogs have hypersensitivity to soy and corn, manifesting as pruritic skin disease, otitis, and colitis that resolves in response to a restricted diet and recurs upon food challenge.

Experimental food allergy in dogs has also been described. Dogs in an atopic spaniel/basenji dog colony maintained at the University of California, Davis, have been shown to develop allergy to peanut, tree nuts, soy, wheat, barley, and milk when immunized with allergen extracts in alum at birth [35,36]. The sensitized dogs were described as undergoing severe gastrointestinal and systemic symptoms after allergen challenge, and were treated with epinephrine, diphenhydramine, and intravenous fluids post-challenge. This dog model was used to show that immunotherapy of allergic dogs with peanut plus heat-killed *Listeria monocytogenes* (HKL) in incomplete Freund's adjuvant could improve symptom scores in peanut-allergic dogs [35].

A food allergy model using newborn pigs has also been described [37]. Piglets were sensitized by intraperitoneal injection of peanut extract plus CT as adjuvant. The majority of piglets responded to oral challenge on days 39 and 53 after sensitization with Grade 2 symptoms, described as including vomiting, lethargy/malaise, tremors, convulsions, reduced activity not activated by prodding, or major areas of edematous rashes. A minority of animals had severe respiratory distress requiring epinephrine. Gastrointestinal manifestations of food allergy included diarrhea in the majority

Table 43.1 Animal models of food allergy

Model	Sensitization	Challenge	Outcome
OVA: systemic (Various rat/mice)	OVA + alum systemic	Single, gastrointestinal	Immediate changes in GI physiology (secretion, motility, permeability); late-phase inflammation
Allergic diarrhea (1) (Balb/c mice)	OVA + alum systemic	Multiple, gastrointestinal	Acute diarrhea beginning at ~3rd feed, allergic inflammation in small intestine
Allergic diarrhea (2) (Balb/c mice)	OVA + CFA systemic	Multiple, gastrointestinal	Acute diarrhea beginning at ~10th feed, allergic inflammation in large intestine
CT adjuvant models	Allergen + CT oral	Single, gastrointestinal	Systemic anaphylaxis (susceptible strains) or "local anaphylaxis"
CT adjuvant models	Allergen + CT oral	Single, systemic	Systemic anaphylaxis
Brown–Norway rat	OVA (oral)	Systemic	Plasma histamine
Transgenic pea	α AI-pea (oral)	Respiratory or cutaneous	Allergic inflammation of lung or skin dependent on challenge site
Allergic dog	Allergens + alum subcutaneous	Graded food challenge	Vomiting, diarrhea, lethargy
Neonatal swine	Peanut + CT i.p.	Repeated food challenges	Diarrhea, respiratory distress, anaphylaxis

CFA, Complete Freund's Adjuvant.

of responders, and changes in the mucosal architecture of the small intestine including vascular engorgement, villous tip damage, and submucosal edema. Therefore, while separate rodent models are used to study anaphylaxis versus gastrointestinal manifestations of food allergy, the newborn pig model has the advantage of producing both types of immediate reactions to food allergens.

Models of eosinophilic-allergic disorders

The models outlined above are focused on IgE-mediated immediate hypersensitivity reactions to food allergens. Although gastrointestinal eosinophilia is a feature in some of these models, eosinophils do not appear to play a pathological role in the immediate hypersensitivity reactions. To address gastrointestinal eosinophilic disorders, Hogan *et al.* developed a murine model of eosinophilic gastroenteritis [38,39]. As with the IgE-mediated models of allergic diarrhea, the model was initiated by systemic sensitization of Balb/c mice with OVA in alum. To induce gastrointestinal eosinophilia, mice were administered encapsulated OVA followed by acidified water to release the OVA in the small intestine. Sensitized mice fed encapsulated OVA had an eotaxin-dependent infiltration of eosinophils in the jejunum and priming of OVA-specific IgE and IgG1 in the serum [39]. This was associated with weight loss, gastromegaly, and impaired gastric emptying. Weight loss and gastromegaly were eotaxin dependent [38]. How eosinophils induce gastrointestinal pathology, how they become activated, and interactions with other cell types such as

enteric nerves remain to be determined using this model. The reader is referred to Chapter 15 for an in-depth review of the pathophysiology of eosinophilic disorders of the gastrointestinal tract.

Pathophysiology of food allergy: progress with animal models

Mechanisms of sensitization

A working hypothesis in experimental food allergy research is that allergic sensitization to food proteins occurs via the gastrointestinal tract. It is also well accepted that the normal response to protein antigens delivered via the oral route is one of active tolerance mediated by the induction of regulatory cells (see Chapter 7 for a discussion of immunological tolerance). Therefore one area of research focus has been to determine the conditions that could skew a tolerogenic response to an allergenic response.

Gut barrier

It has been hypothesized that age of exposure to food allergens is a critical factor since infants have not yet developed a mature intestinal barrier. The barrier function is composed of many components, including tight junction formation between intestinal epithelial cells, secretion of IgA, and gastric and duodenal digestion of proteins. The role of these factors in allergic sensitization has been addressed in a number of studies. The role of gastric digestion was tested by interfering with gastric acid in mice prior to administration

of fish proteins by gavage [40]. Mice treated with ranitidine or omeprazole to suppress gastric acid had a significant enhancement of allergen-specific IgE to the fed allergens compared to mice administered the fish allergens without acid suppression. In contrast, there was no development of IgE reactivity to mouse chow. These results suggest that in the absence of pre-existing tolerance, interference with normal digestion can promote sensitization.

IgA is thought to be protective against allergy by interfering with uptake of antigens from the intestinal lumen. Fecal antigen-specific IgA has been shown to be upregulated in mice with tolerance, but not sensitization to the milk protein β -lactoglobulin (BLG) [15]. The role of IgA in experimental food allergy has not yet been directly tested through the use of mice deficient in IgA or the polymeric immunoglobulin receptor.

Factors that influence epithelial permeability have also been shown to be associated with sensitization to food proteins. In rats, psychological stress is commonly modeled by cold-restraint or water-avoidance protocols, which induce a corticotropin-releasing hormone (CRH)-mediated increase in epithelial permeability in the jejunum and colon [41,42]. Stress has recently been shown to enhance sensitization to orally delivered antigens [43]. Unstressed rats that were intragastrically administered the antigen horseradish peroxidase (HRP) in adjuvants (alum and pertussis toxin) did not develop sensitization to HRP (as measured by IgE or presence of local immediate hypersensitivity reactions upon re-challenge). However, if rats were subjected to water avoidance stress prior to administration of HRP in adjuvants, there was sensitization to HRP. *In vitro* antigen challenge of intestinal segments from sensitized rats showed characteristic increases in ion secretion and epithelial permeability, responses previously shown to be mast cell and IgE dependent. It should be noted that stress also has a number of effects on the immune system that may contribute to sensitization by other mechanisms. It was recently shown that mice colonized with *Candida albicans* develop a mast-cell-dependent defect in the gut barrier against macromolecules [44]. When infected mice were administered OVA, there was a significant rise in OVA-specific immunoglobulin production (IgE, IgG) compared to uninfected mice. Like stress, infection likely has many other effects beyond perturbation of barrier function, so these studies do not directly indicate that increasing delivery of an antigen across the epithelial barrier is in itself sufficient to induce sensitization. However, combined with the data on gastric acid suppression they suggest that normal gastrointestinal barrier function is required for tolerance to food proteins.

Immune mechanisms of allergic sensitization to food proteins

A number of groups have started to examine the host immune mechanisms, rather than external factors, by

which an allergenic immune response to an orally delivered protein is induced instead of the predicted response of tolerance. Signaling of T-cells through CTLA-4 rather than CD28 inhibits T-cell activation, and CTLA-4 pathways have been shown to be important in the generation of oral tolerance [45]. Blockade of CTLA-4 was tested in a murine model of peanut allergy, and was shown to significantly increase the IgE and clinical response to peanut when administered with CT, but not in the absence of CT [46]. Therefore CTLA-4 is an important suppressive factor preventing allergic sensitization, but absence of CTLA-4 signaling does not appear to convert a tolerogenic response to an allergenic response, and other factors (provided experimentally by adjuvant) are required for development of sensitization to peanut. Other groups have shown that T-cells isolated from liver [47], mesenteric lymph node, or spleen [16] can transfer the allergen-specific immunoglobulin response, indicating that allergen-specific T-cells are generated locally within the gastrointestinal tract. CT, which is used experimentally to generate an allergic response to co-administered antigens, has been shown to induce the migration and maturation of dendritic cells (DCs) to the mesenteric lymph node [48] and migration of DCs to T-cell areas in the Peyer's patch [49]. This maturation results in more robust T-cell activation, and migration is necessary for delivery of captured antigens to sites of efficient T-cell interaction. It is not yet understood how food allergens initiate the same response in the absence of experimental adjuvants such as CT, but the very interesting finding that peanut itself has intrinsic adjuvant activity [6] suggests that these additional migratory or maturation signals may be provided by the allergen or associated proteins.

Gut flora

The composition of the gut flora has been hypothesized to have a critical role in the development of allergic sensitization. One of the first indications that this could be true was the finding that the development of oral tolerance was impaired in germ-free rodents [50]. However, there are conflicting data on the role of gut flora in oral tolerance. Investigators using particulate antigens (sheep red blood cells) or haptens have described a lack of tolerance development in germ-free mice [50], while results with a soluble antigen such as OVA have been conflicting [51–53]. A confounding factor is the impact of the gut flora on the development of the mucosal immune system, such that impairment in oral tolerance may not be due to conditioning of the mucosal immune system by gut flora, but rather may be due to the underdeveloped gastrointestinal-associated lymphoid tissue. This may be of particular relevance to antigens trafficking through the Peyer's patches, as particulate antigens such as sheep red blood cells would be expected to do.

Bashir *et al.* examined the impact of TLR4 deficiency on oral sensitization to peanut in a mouse model of anaphylaxis

[54]. They found that TLR4 deficiency was associated with susceptibility to peanut sensitization and associated with enhanced peanut-specific IgE production, increased Th2 cytokine production and increased severity of peanut-induced anaphylaxis. In addition, treatment of TLR4+/+ mice with broad-spectrum antibiotics to suppress the gut flora resulted in enhanced sensitization to peanut. We also observed that TLR4 deficient mice had enhanced Th2 cytokine production and increased severity of peanut-induced anaphylaxis [55], but this was not observed in all strains of mice or to the milk allergen BLG [55] or OVA (unpublished observations). The difference in TLR4 influence on allergic responsiveness to peanut versus other allergens could potentially relate to the route of sensitization, for example peanut has been shown to traffic through the Peyer's patch [56]. Alternatively, the unique interaction between TLR4 and peanut sensitization may relate to the ability of peanut to bind DC-SIGN and have adjuvant-like activity on DCs as recently shown by Shreffler *et al.* [6].

Cutaneous sensitization

Although the natural site of exposure to food proteins in humans is through the gastrointestinal tract, several studies have also indicated that sensitization may occur through skin. The skin is a common site of symptoms in food-allergic patients, and patients often have food-related skin symptoms (urticaria or atopic dermatitis) in the absence of any gastrointestinal symptoms. One potential explanation for this common observation is that sensitization occurs cutaneously and when cells are re-exposed they will home back to the skin using skin-specific homing markers (cutaneous lymphocyte-associated antigen (CLA)) and chemokines (e.g. CCL17, CCL27) [57]. A number of studies have shown that mice can be sensitized epicutaneously as indicated by antigen-specific IgE responses [58–60]. Hsieh *et al.* have shown that Balb/c mice sensitized to OVA by application of OVA to the skin respond to oral challenge with systemic anaphylaxis (measured by symptom score and plasma histamine) and histological changes in intestine and lung [61]. In the same study, the authors show that this sensitization process is IL-4 dependent. In addition, Strid *et al.* have shown that Balb/c mice exposed to peanut proteins via the skin develop peanut-specific IgE, IgG1, and a Th2-dominated T-cell cytokine response [62]. Epicutaneous exposure to peanut was shown to not only abrogate oral tolerance responses, but also prime for Th2-skewed immunity. During oral challenge, the mice epicutaneously primed with peanut developed symptoms of anaphylaxis. The latter study [62] required mild abrasion of the skin (by stripping with tape) for sensitization, but exposure via the skin results in an allergenic immune response in the absence of exogenous adjuvants. It is not clear what factors in the skin predispose to this allergenic response, or if endotoxin in OVA preparations or endogenous adjuvant activity in peanut are critical in the development of sensitization.

The only study to examine the role of TLR4 in epicutaneous sensitization to OVA used CT as adjuvant, and did not show an influence of TLR4 on allergic sensitization to OVA [63]. These studies have not yet been done in the absence of adjuvant.

These studies clearly show that allergic sensitization resulting in food-allergic-type symptoms can be generated by cutaneous sensitization. The link between oral challenge and cutaneous symptoms as seen in patients with food allergy has not yet been clearly modeled. It has been shown that mice orally sensitized to a model food allergen (transgenic expression of α -AI) will develop immediate (mast-cell mediated) and delayed (T-cell mediated) cutaneous hypersensitivity reactions in response to intradermal injection in the footpads [5]. In addition, several murine models of food antigen-induced anaphylaxis report transient cutaneous symptoms, including itching and swelling around the eyes, ears, and mouth, in response to oral challenge [9,10]. Delayed cutaneous reactions in response to oral challenge have not yet been convincingly modeled, although it has been reported that mice sensitized to milk developed atopic dermatitis-like lesions in response to mouse chow contaminated with milk proteins [64].

Pathophysiology of immediate hypersensitivity reactions in the gut

Experimental models of food allergy have been used to elucidate the consequences of allergen challenge in the gastrointestinal tract. As outlined above, rats or mice systemically sensitized to OVA develop increased ion secretion, epithelial permeability, and intestinal motility in response to allergen challenge. Ussing chambers have been particularly instrumental in uncovering mechanisms of epithelial dysfunction. Intestinal segments are mounted in Ussing chambers that immobilize the tissue between oxygenated chambers bathing the luminal or serosal sides of the tissue. Tissues are voltage clamped so that the Short circuit current (I_{sc}) is equal and opposite to the current generated by the tissue by active transport. Therefore, I_{sc} is a measure of active transport of the tissue. The compartmentalization of buffers bathing the serosal and luminal sides of the tissue also allow for measurement of permeability to small and large molecular weight tracers. Tissues can also be "challenged" with allergen on the luminal or serosal side of the intestinal segment. As outlined in the section on models of systemic sensitization to food proteins, luminal allergen challenge of sensitized mice and rats produce a local immediate hypersensitivity response, mediated by mast cells [28,65], and characterized by increased chloride ion secretion and permeability to macromolecules. There is bi-directional communication between mast cells and enteric nerves within the gastrointestinal tract [66,67], and mast cell–nerve interactions are critical to the generation of secretory and motility changes in response to allergen challenge [65,68]. Different mast cell mediators

appear to mediate effects on epithelial ion secretion and barrier function. Ion secretion is driven by mast-cell-derived histamine, serotonin, and prostaglandins [24]. In contrast, mast cell proteases, including tryptase acting on protease-activated-receptor 2 (PAR2), appear to be major mediators of epithelial permeability changes in the gastrointestinal tract [69,70].

Studies have demonstrated that in the gastrointestinal tract there is a mast-cell-dependent “late-phase” response generated after oral allergen challenge [71–73]. In the stomach this is mediated by mast-cell-derived TNF- α [72]. The late-phase response in the small intestine is characterized by a mononuclear cell infiltrate, disrupted epithelial barrier function, and ion secretion [73]. Immediate effects of mast cell degranulation on epithelial cells combined with a late-phase response are likely underlying mechanisms responsible for allergen-induced diarrhea *in vivo*, which has been confirmed to be mast cell dependent [29]. The central role of mast cells in allergen-induced pathophysiology of the gastrointestinal tract is shown schematically in Fig. 43.1.

Early studies using the Ussing chamber system to examine epithelial responses to allergen demonstrated that addition of OVA to the luminal or serosal side of intestinal segments from systemically sensitized rats resulted in a rapid ion secretion response (15 seconds when added serosally, 3 minutes when added lumenally) [24]. The difference in the response time reflects the time required for antigen to cross the epithelial cell layer prior to triggering lamina propria mast cells that degranulate and cause epithelial ion secretion. Within the last 10 years, it has been shown that this rapid transepithelial antigen transport across intestinal epithelial cells is a feature of sensitized intestine [27,28,74,75]. Rapid transepithelial transport is antigen specific [27], and is mediated by IgE and CD23 on the epithelial cell surface [74,75]. This enhanced uptake is mast cell independent [28], and epithelial CD23 expression is regulated by IL-4 and IgE [74]. Recently these findings have been extended to human systems [76–80], and we have recently shown that CD23 and food-specific IgE are present within stool of food-allergic patients (post-food challenge), but not in controls [76]. It has not yet been shown if interference with this system can prevent anaphylaxis in murine models of food-induced anaphylaxis, although blocking antibodies against CD23 prevent the development of local hypersensitivity reactions in the gut [75]. Figure 43.2 illustrates the role of CD23 in the transepithelial transport of IgE. Improving barrier function through the use of glucagon-like peptide 2 (GLP-2) has also been shown to prevent local hypersensitivity reactions in the gut through a CD23-independent mechanism [81]. Clearly factors that prevent uptake of allergen from the gastrointestinal lumen are effective in prevention of allergen-induced gastrointestinal dysfunction. Probiotics have also been shown to enhance intestinal epithelial barrier function [82], which may be one mechanism by which they may have therapeutic effect.

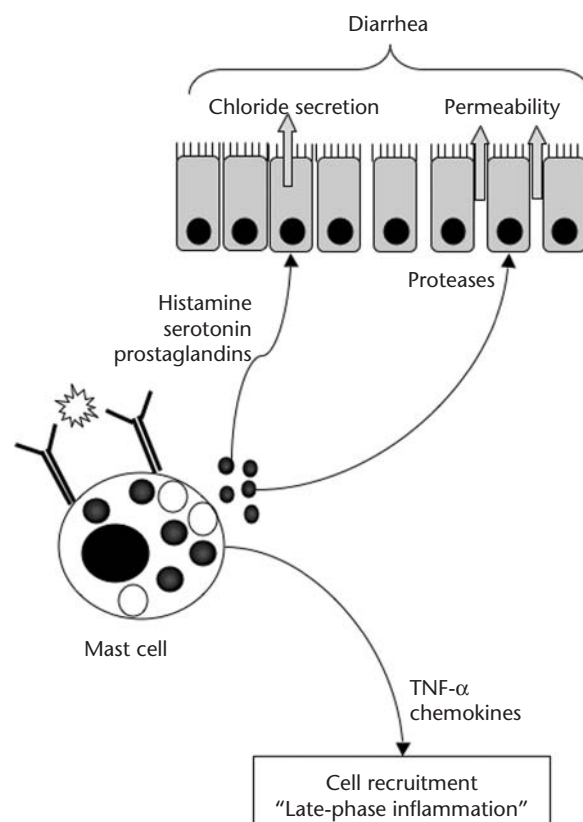


Figure 43.1 Mast cell mediators and the pathophysiology of gastrointestinal hypersensitivity reactions.

Mast cells are required for allergen-induced increases in epithelial chloride secretion and permeability, two driving forces for onset of diarrhea. Mast cells produce a vast array of bioactive mediators, and these have differing effects on pathophysiology. Rapidly released mediators such as histamine, serotonin, and prostaglandins have immediate (within minutes) effects on epithelial chloride secretion. Proteases impair epithelial barrier function within 30–60 minutes, whereas mast-cell-derived cytokines and chemokines can participate in late-phase inflammatory reactions occurring within hours to days after allergen challenge.

Harnessing host–microbial interactions for therapy

The ability of microbial products to downregulate allergic sensitization to food proteins has been of significant interest in the development of potential therapeutics. The use of CpG oligodeoxynucleotides (ODN), bacterial vectors and adjuvants, and probiotics have been tested in animal models for their ability to prevent or treat experimental food allergy. Bashir *et al.* have shown that administration of CpG ODN together with peanut and CT results in impairment of sensitization to peanut (reduced IgE, reduced IL-13, enhanced IFN- γ) and abolishment of peanut-induced anaphylaxis [54]. The same group has also shown that helminth infection inhibits development of allergic sensitization and anaphylaxis in response to peanut challenge,

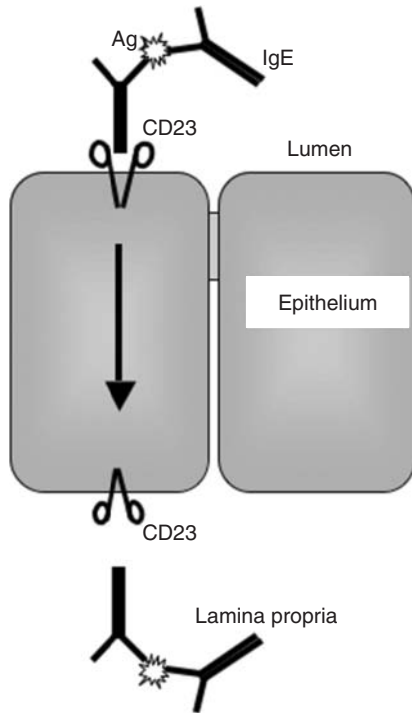


Figure 43.2 CD23 facilitates the entry of antigen from the gut lumen. CD23 is constitutively expressed in human intestinal epithelium, and is induced in rodent models of food allergy. CD23 facilitates the apical-to-basolateral transport of IgE–antigen complexes and delivers these complexes to immune cells in the lamina propria.

via an IL-10-dependent pathway [83], which supports the hypothesis that exposure to microbial products can promote the development of regulatory cells that suppress allergic sensitization. This is also supported by studies showing that helminth infections are protective against intestinal inflammation in murine colitis models through IL-10-dependent [84] and -independent [85,86] pathways. Although a helminth-based therapy has been developed for ulcerative colitis [87], this approach has not been studied as a therapeutic option in food-allergic disorders.

Of major interest in the development of therapeutics is a means of inhibiting pre-existing sensitization. Two studies have been published using HKL as an adjuvant to inhibit sensitization and reactivity to peanut. Li *et al.* showed that administration of HKL to peanut-sensitized mice inhibited peanut-specific IgE, peanut-induced anaphylaxis, and modified the peanut-specific Th2/Th1 cytokine balance [88]. Frick *et al.* used peanut- or milk-sensitized dogs to show that HKL plus specific antigen could significantly inhibit antigen-induced allergic responsiveness and skin test reactivity [35]. Other studies testing the effect of HKL on OVA-induced airway hyperresponsiveness have shown that HKL induces IFN- γ -producing “Th1 regulatory” cells that express the regulatory transcription factor Foxp3 and suppress allergic inflammation in an IL-10-dependent manner [89]. These Th1-like Tregs are generated by CD8⁺ DCs, and by

an IL-10- and IL-12-dependent mechanism. It is likely that the same mechanism is responsible for the suppression of peanut-specific IgE and peanut-induced anaphylaxis, but this has not been directly tested.

Another experimental approach to immunotherapy has been to administer modified (non-IgE binding) recombinant peanut allergens. These were administered to peanut-sensitized mice by intrarectal administration within a heat-killed *E. coli* vector [90]. Despite the heavy microbial load normally present within the large intestine, administration of the *E. coli* vector itself had some transient immunomodulatory effects, but maximal prolonged suppression of IgE- and peanut-induced anaphylaxis was observed with *E. coli* carrying modified peanut proteins. The mechanism by which this preparation has immunomodulatory effects, or if this suppression of anaphylaxis is mediated by regulatory cells that suppress peanut-specific T- and B-cells, is currently under investigation.

Probiotics have been shown to have clinical efficacy in patients with atopic dermatitis or food allergen-exacerbated eczema [91–95]; however, relatively little has been investigated in experimental animal models. Adel-Patient *et al.* generated *Lactococcus lactis* strains expressing the milk protein BLG and administered them orally to mice [96]. After administration, mice were sensitized to BLG by intraperitoneal injection in incomplete Freund’s adjuvant. Only one of the test strains inhibited BLG-specific IgE in serum, but several of the strains induced a significant increase in BLG-specific IgG2a. Surprisingly both Th1 and Th2 cytokines were enhanced by administration of BLG-expressing *L. lactis* strains. Although a BLG-expressing *Lactobacillus casei* has also been developed [97], the effect of these strains on clinical reactivity to BLG in mice has not been reported to date. Kim *et al.* reported that administration of *Bifidobacterium bifidum*, *Lactobacillus casei*, or *E. coli* to mice in the mouse chow significantly reduced OVA-specific IgE and IgG1 in a murine model of oral sensitization to OVA [98]. Anaphylaxis symptoms were not assessed. Oral administration of probiotics (VSL-3) to mice with experimental colitis has been shown to inhibit inflammation (in both Th1-mediated and innate-immunity-mediated models of colitis) in a TLR9-dependent manner [99]. Given the fact that CpG has been shown to inhibit peanut-induced anaphylaxis in a murine model of food allergy [54], the use of oral probiotics may also potentially activate TLR9 and downregulate allergic sensitization in food allergy; however, this remains speculative at this point.

Reductionist models for food-allergy research

Animal models are not the only experimental approach to food allergy, and reductionist models have been used to examine specific aspects of the allergic response to food allergens. The use of Ussing chambers has been introduced in a

previous section of the chapter. Ussing chambers can be used not only for the *in vitro* measurement of local hypersensitivity reactions of gastrointestinal tissue from animal models, but modified chambers have been developed for use with patient biopsies [100,101]. This technique has been reported as a potential diagnostic tool for food-allergic disorders [100], and has been used to study transepithelial antigen trafficking in inflammatory bowel disease [101]. In cases where endoscopy and biopsy are called for, this technique may be valuable for studying local responses to food allergens in humans.

Modeling of the human gastrointestinal epithelium can also be done using cell lines that polarize when grown on filter supports. T84 cells, Caco-2 cells, HT-29 subclones, and HCA-7 cells all polarize when grown on transwell filters. These cell lines form tight junctions and have well-differentiated apical and basolateral domains. Human intestinal cell monolayers have been used to address issues related to transepithelial allergen transport [102,103], antibody-mediated antigen uptake [76,77,79], and when co-cultured with immune cells can model epithelial pathophysiology in response to local immune activation [104].

Mast cells, like human epithelial cells, have also been modeled using cell line systems. The most common is the rat basophil leukemia cell line, or RBL. This cell line has been transfected with the human high-affinity IgE receptor, and through this method can be used to assess the reactivity of human serum samples to different allergens [105,106]. Bischoff and colleagues have also published protocols for the isolation of human intestinal mast cells [107], which allows for the study of tissue-specific characteristics of human mast cells.

Reductionist approaches may be most useful for modeling the interaction of cells and allergens within the gastrointestinal mucosa, as access to intestinal tissues from food-allergic patients is limited due to the invasive measures needed to acquire them. The development of co-culture systems will then begin to address cell-cell interactions in a well-controlled experimental environment, and can begin to move findings using rodent systems into a human tissue-based experimental system.

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