Published in final edited form as:

Immunol Res. 2012 December ; 54(0): . doi:10.1007/s12026-012-8308-4.

Mucosal immunology of tolerance and allergy in the gastrointestinal tract

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Abstract

The mucosal immune system typically exists in a state of active tolerance to food antigens and commensal bacteria. Tolerance to food proteins is induced in part by dendritic cells residing in the intestinal mucosa and implemented by regulatory T cells. Food allergy occurs when immune tolerance is disrupted and a sensitizing immune response characterized by food-specific IgE production occurs instead. Experimental food allergy in mice requires use of adjuvant or exploitation of alternate routes of sensitization to induce allergic sensitization, and can aid in understanding the mechanisms of sensitization to food allergens and the pathophysiology of gastrointestinal manifestations of food allergy. Recent work in the understanding of mucosal immunology of tolerance and allergy in the gastrointestinal tract will be discussed.

Keywords

Food allergy; Oral tolerance; Epithelium; CD103+ dendritic cells; Th2 cells; Anaphylaxis

Introduction

The immune system of the gastrointestinal (GI) tract is continuously exposed to food antigens and endogenous flora. Most often, mucosal immunity functions in a state of tolerance, allowing food proteins to be absorbed and commensal bacteria to exist in the GI tract. However, in the case of food allergy, specific foods stimulate a robust adaptive immune response, involving generation of Th2 cytokine-producing T cells, allergen-specific IgE, and, ultimately, mast cell and basophil degranulation upon allergen re-exposure. The immunologic mechanisms behind the break in tolerance and subsequent sensitization to food allergen are incompletely understood. Since food allergy is a disease with increasing incidence in westernized countries [1] for which no curative treatment exists, further understanding of the immunologic mechanisms of tolerance and allergy in the GI tract will help to guide treatment strategies for food allergy in the future.

Oral tolerance: the normal response to food proteins

Oral tolerance to food protein was first described in 1911 by experiments that showed that anaphylaxis in guinea pigs could not be induced to proteins that were already present in their diet [2, 3]. Oral tolerance is defined as a systemic suppression of cellular or humoral immune responses to an antigen by prior administration of the antigen by the oral route [4]. It is a process that begins in infancy and is mediated by the GI mucosal immune system.

The role of the GI mucosa in establishing oral tolerance is complex. The GI tract sees approximately 70–100 g of protein daily, and the immune regulation in response to this antigen load relies on a number of factors: physical barriers of the epithelium and its secreted products, luminal digestion of antigens, and a suppressive immune milieu including the presence of regulatory T cells [5]. The barrier function of the GI tract includes a hydrophobic layer of mucin oligosaccharides that trap antigen and secretory IgA that prevents absorption of food proteins across the intestinal epithelium [6]. As food proteins pass through the stomach and duodenum, gastric acid and other digestive enzymes destroy their conformational and linear epitopes and break them down into di- and tripeptides, rendering them less immunogenic while simultaneously permitting absorption of peptides and amino acids as nutrients [7].

Three different types of cells in the intestinal mucosa can sample proteins that escape digestion. Specialized epithelial cells called microfold (M) cells are found in the dome epithelium overlying Peyer's patches. They can take up particulate antigen due to their limited glycocalyx, their sparse cytoplasm, and their high endocytic activity, allowing for efficient delivery to the underlying immune cells. Intestinal epithelial cells (IECs) can also transport soluble antigens by a transcellular mechanism, and dendritic cells (DCs) can directly sample antigen by extending dendrites into the intestinal lumen [8].

Once antigen has been sampled, it generates the production of regulatory T cells that suppress effector T-cell responses in an antigen-specific manner. Feeding of antigen induces regulatory CD4⁺ and CD8⁺ T cells that can transfer tolerance to a naïve animal [9, 10]. Weiner and colleagues described mouse and human Th3 cells, a CD4⁺ T cell subset expressing TGF- β , IL-4, and IL-10 and suppressing by a TGF- β -dependent mechanism [11, 12]. In depletion studies, it has been shown that antigen-specific induced Tregs (CD4⁺Foxp3⁺) are necessary for the development of oral tolerance and prevention of food allergy [13,14]. However, other cells such as CD8⁺ T cells have also been shown to mediate tolerance. Arnaboldi et al. [15] showed that CD8⁺ T cells could induce tolerance by feeding OVA epitopes that induced CD8⁺ Tregs. Subsequent immunization with whole OVA led to suppressed production of Th1 and Th17 cytokines and inhibited delayed-type hypersensitivity reactions. Transfer of CD8⁺ mesenteric lymph node (MLN) cells from peptide-fed mice was sufficient to induce suppression in naïve mice.

DCs have also been shown to be involved in regulation of tolerance. Induced Foxp3⁺ Tregs are preferentially generated by the CD103⁺ subset of gastrointestinal DCs that migrate from the gastrointestinal lamina propria to the MLN [16, 17]. Resident CX₃CR1⁺ DC/macrophages also play a role in the expansion of regulatory T cells after antigen feeding; it has been shown that CD4⁺ Tregs first home to the lamina propria and are subsequently expanded by resident DCs/macrophages [13]. In a model of allergic contact dermatitis, liver plasmacytoid dendritic cells (pDCs) have been shown to trigger Tregs in secondary lymphoid organs that suppress antigen-specific CD8⁺ T cells upon re-exposure to allergen via the skin [18]. Similarly, systemic depletion of pDCs prevented tolerance induction by antigen feeding; pDCs appear to induce anergy or deletion of antigen-specific T cells in the liver via a CD4⁺-independent mechanism [19].

Allergic sensitization is commonly thought to be a result of a defect in immune tolerance, but this has not been definitively shown. For example, patients with inflammatory bowel disease have impaired tolerance responses to a fed neo-antigen [20], but similar studies are needed and have yet to be performed in patients with food allergy.

The adaptive immune response leading to sensitization to foods

Patients with IgE-mediated food allergy develop food-specific IgE antibodies that are thought to be downstream of an inappropriate Th2-skewed immune response. Studies of patients that have outgrown their peanut allergy, or atopic controls, have shown a weak but detectable food-specific T-cell response, characterized by low levels of TNF- and IFN- production. In contrast, patients with clinical food allergy have a greater number of allergen-specific CD4⁺ T cells and express a unique profile of cytokines, including IL-4, IL-5, and IL-13 [21–24]. Factors that play a role in the initiation of these Th2 responses to food allergens are therefore of significant interest and have been the focus of study in mouse models of food allergy.

Breaking tolerance with adjuvant: the role of dendritic cells in the GI tract

Because oral administration of antigen typically leads to tolerance rather than to sensitization, murine models of food allergy employ cholera toxin (CT) as an adjuvant to break tolerance and induce sensitization to milk proteins, peanut proteins, and OVA [25–28]. Exposure to allergen with CT results in increased antigen-specific IgE and Th2 cytokine production by spleen or MLN cells, with induction of anaphylaxis upon oral or systemic re-administration of the allergen. CT can therefore be used as a tool to probe mucosal mechanisms that are responsible for diverting a response of tolerance to one of allergic sensitization.

We examined the mechanisms by which gastrointestinal dendritic cells contribute to the process of allergic sensitization in mice [29]. We observed that CT altered the phenotype of the normally tolerogenic CD103⁺ DC subset. In vivo feeding of CT resulted in enhanced migration and maturation of the CD103⁺ DCs to the MLN. We also observed increased induction of Th2 cytokines when these DCs were purified and cocultured with CD4⁺ T cells. These effects of CT were limited to the MLN and did not affect DCs in peripheral lymph nodes. CT also specifically upregulated expression of genes that influence Th2 cell skewing in CD103⁺ DCs, including Jagged-2 and OX40L. Anti-OX40L neutralizing antibodies suppressed Th2 skewing without affecting IFN- or IL-17, indicating that OX40L on CD103⁺ DCs is capable of selectively inducing Th2 cytokines by responder T cells. We speculate that environmental factors that induce OX40L expression on gastrointestinal DCs, or genetic polymorphisms that lead to heightened activity of OX40-OX40L signaling, could play a role in food allergic sensitization in human disease. Similar to our findings with OX40L and CT, Yang and colleagues have shown that the adjuvant staphylococcal enterotoxin B (SEB) acts on gastrointestinal DCs to induce Th2 skewing through upregulation of the surface molecule TIM-4 [30, 31]. Figure 1 illustrates our current understanding of the role of gastrointestinal DCs in the development of allergic sensitization.

Alternate routes of sensitization

It has been hypothesized that sensitization to food antigen may occur if the primary exposure to food allergens occurs through non-oral routes. This is largely due to the observation that some children have adverse reactions upon their first ingestion of allergens [32], suggesting that oral ingestion may not be the only means of sensitizing to allergen. One study found that children with a positive food challenge to peanut were more likely to have

used creams containing peanut oil [33], raising the possibility that skin exposure may be promoting sensitization. Indeed, mice have been shown to successfully sensitize to allergen in an adjuvant-independent manner through epicutaneous routes [34–36]. However, others have found that mechanical injury of the skin (through tape-stripping) is necessary for the induction of allergic sensitization through the epicutaneous route [37, 38]. Interestingly, one study by Dioszeghy et al. [39] demonstrated that epicutaneous immunotherapy downregulates the allergen-specific response in sensitized mice, indicating that desensitization can also be achieved through antigen exposure through the skin. It is not clear whether these conflicting results are due to differences in the type of antigen used, dose of exposure, or timing of exposure.

We examined whether the route of primary exposure to a food allergen could influence the nature of the immune response [40]. Mice were exposed to the milk allergen β -lactalbumin (ALA) via intragastric, sublingual, nasal, and cutaneous routes. None of the routes of exposure led to the induction of ALA-specific IgE in the absence of adjuvant. When CT adjuvant was present, all routes of exposure led to significant production of ALA-specific IgE, although cutaneous exposure resulted in the highest amount of specific IgE and a greater severity of anaphylaxis, as compared with oral sensitization. Similar to our findings with oral CT, we observed that CT drove DCs to the draining lymph node where they generated a Th2-skewed T-cell response. The DC delivering antigen from the skin to the lymph node in response to CT was the langerin^{neg} dermal DC. These results suggest that the skin is not inherently pro-allergenic, but, given the appropriate environmental triggers that can provide adjuvant activity, it can act as a physiologic site of allergic sensitization to food allergens. As with sensitization through the gut, further studies are needed to identify adjuvant-like environmental triggers that may play a role in human disease.

Systemic and gastrointestinal manifestations of food allergy

In order to trigger allergic reactions, ingested antigen must retain the ability to bind IgE antibodies. Of approximately 10,000 ingested proteins, only a small minority elicit allergy. A common characteristic of that minority is stability to heat, acid, and protease digestion [41], although not every protein with these characteristics acts as a food allergen. Similarly, heat treatment of food can either enhance or reduce protein immunogenicity [42–44]. We have shown that inherent properties of allergens and the ways in which those allergens are processed can significantly alter the way that allergens are handled in the gastrointestinal mucosa, thereby influencing the clinical response.

Antigen uptake: the importance of antigen getting systemically absorbed

We examined three of the main allergens from milk (β -lactalbumin [ALA], β -lactoglobulin [BLG], and casein) to determine how gastrointestinal antigen handling altered sensitization and clinical responsiveness to those allergens [45]. Soluble allergens ALA and BLG were readily transcytosed through enterocytes, and oral administration of soluble ALA and BLG to sensitized mice induced systemic anaphylaxis. In contrast, casein, which is relatively insoluble, was taken up instead through the Peyer's patches, and only systemic (intraperitoneal) administration of antigen induced anaphylaxis in mice sensitized to casein. Heat treatment of the soluble allergens ALA and BLG rendered them insoluble, and like casein, they trafficked into the Peyer's patches and were unable to trigger anaphylaxis by the oral route. This was also shown to be true for egg allergens, where heat treatment abolished clinical reactivity to the allergens through changing the gastrointestinal uptake rather than through decreasing the IgE binding of the allergens [46]. Despite their poor ability to elicit anaphylaxis by the oral route, insoluble allergens generated stronger T-cell and B-cell responses upon primary oral exposure [45]. These results suggest that clinical reactivity to a food allergen is dependent on two factors: the ability to trigger primary sensitization

followed by the ability to be absorbed and systemically disseminated upon re-exposure. This may explain why levels of IgE (or other immune parameters) are not always predictive of clinical reactivity to foods. Furthermore, this may explain why the majority of children with egg allergy or milk allergy are clinically tolerant to heated egg [47, 48] or heated milk [49]. Heated milk-tolerant patients had IgE-binding patterns similar to those who had outgrown their allergy and were more likely to progress to resolution of cow's milk allergy than heated-milk-reactive patients [50].

Other work has also emphasized the importance of systemic uptake of allergen in triggering anaphylaxis symptoms. Strait et al. [51] addressed this question by looking at the inhibitory effects of IgA and IgG on IgE-mediated anaphylaxis. They found that serum antigen-specific IgA and IgG suppressed mast cell degranulation and protected against anaphylaxis. IgA was protective even in mice lacking the pIgR receptor to transport it into the intestinal lumen, and application of IgA directly into the gastrointestinal tract did not protect against anaphylaxis induced by oral challenge. These results argue that food allergen triggers systemic anaphylaxis first by being disseminated systemically, rather than by triggering mast cells locally within the gastrointestinal tract that propagate symptoms systemically.

Facilitated antigen uptake through IgE/CD23

The uptake of food allergens across the mucosal barrier is influenced not only by properties of the allergen, but also by the immune status of the individual. We have shown that allergic sensitization enhances the transcellular uptake of antigens across the intestinal epithelium [52]. Enhanced uptake is allergen-specific but mast cell-independent [53], suggesting a mechanism of recognition at the epithelial cell surface. This recognition is due to a facilitated uptake mechanism composed of allergen-IgE complexes acting on the low-affinity IgE receptor CD23 expressed on the intestinal epithelium [54]. We and others have shown that this mechanism is also present in human intestinal epithelium [55, 56]. This mechanism provides for the selective delivery of intact allergen across the intestinal epithelium and may explain the clinical reactivity to minute quantities of food allergens in sensitized individuals.

Mechanism of systemic anaphylaxis

The “classical pathway” of systemic anaphylaxis (IgE-mediated anaphylaxis) in rodents has been shown to involve cross-linking of IgE-bound Fc RI on mast cells, leading to degranulation and release of platelet-activating factor (PAF) and histamine [57]. This pathway is inhibited when mice are deficient in mast cells, functional IgE, Fc RI, and IL-4 [58]. Meanwhile, the “alternative pathway” of IgG-mediated anaphylaxis involves IgG, Fc RIII, macrophages, basophils, and PAF; blockade of any of these components has been shown to inhibit this pathway [57,58].

These pathways have been examined in the context of experimental peanut allergy by Jordana et al. In a series of papers, they have systematically addressed the role of peanut-specific IgE and IgG, Fc receptors, mast cells, basophils, macrophages, and inflammatory mediators [59–61]. They found that the majority of clinical responsiveness to peanut was mediated by the classical pathway, involving peanut-specific IgE bound to FcεRI on mast cells [59]. However, they also showed concurrent involvement of the alternative pathway, demonstrating that peanut-specific IgG1 acting on Fc RIII macrophages also contributed to anaphylaxis symptoms and that blockade of both pathways was essential for complete abrogation of peanut-induced anaphylaxis in mice [60]. Similarly, blockade of both histamine and PAF was required for abrogation of symptoms [61]. Their data indicate that peanut-induced anaphylaxis is the result of several immune pathways that act in concert to

elicit a full immune response, and highlight the difficulty of therapeutically targeting a single immune pathway for the treatment of food allergy.

Gastrointestinal manifestations of food allergy

In a subset of food allergic individuals, symptoms are primarily localized to the gastrointestinal tract. This is particularly true for non-IgE-mediated food allergies, where T cells may play a more direct role in pathophysiology. Lin et al. [62] studied local gastrointestinal immune events by performing biopsies on individuals with gastrointestinal manifestations of food allergy. Healthy volunteers served as controls. They found that allergen ingestion was associated with a significant allergic inflammatory histopathology in the duodenum, characterized by increased numbers of mast cells, eosinophils, and T lymphocytes expressing IL-4. This was true for subjects with or without detectable food-specific IgE. Gastrointestinal manifestations of food allergy are modeled experimentally in a murine model of IgE-mediated allergic diarrhea. Repeated feeding of antigen to systemically primed mice results in acute self-limited diarrhea and a Th2-biased local inflammation [63]. We have shown that CD4⁺ T cells producing Th2 cytokines are sufficient to transfer disease to naïve recipients [64].

IL-4 and IL-13 are highly expressed in the intestine of mice with allergic diarrhea [63, 64]. There are conflicting data regarding their role in gastrointestinal manifestations of food allergy, specifically regarding whether they are involved in the effector phase of allergic diarrhea or whether they function primarily by promoting IgE class switching. These cytokines may be involved in the effector phase of allergic diarrhea by increasing the sensitivity of intestinal smooth muscle, epithelium, and vasculature to mediators released by mast cells [65]. IL-13 has been shown to have direct effects on epithelial architecture and ion secretion, the driving force for diarrhea [66]. However, Brandt et al. [67] demonstrated that although IL-4 and IL-13 were essential for the development of allergic diarrhea and allergic inflammation in the intestine, blockade of IL-4 or IL-13 did not prevent acute episodes of diarrhea once inflammation had already been established. The cytokine IL-9 has also been shown to be necessary for the development of gastrointestinal manifestations of food allergy, but not systemic anaphylaxis, by promoting intestinal mastocytosis [68, 69].

Central role of the intestinal epithelium in gastrointestinal manifestations of food allergy

The intestinal epithelium is a target of allergic effector cells, and changes in epithelial barrier function and ion secretion are responsible for the diarrhea observed after allergen challenge [70]. Epithelial cells are also active participants in the immune response to food allergens. They are a rich source of chemokines that recruit effector cells to the gastrointestinal mucosa. One mechanism by which the epithelium can sense the presence of allergens is through IgE-allergen complexes binding to CD23. We hypothesized that in addition to functioning as a transport mechanism, CD23 could activate immune pathways in the intestinal epithelium. We observed that allergen-IgE complexes induce the release of the chemokine CCL20 from human IECs in a CD23-dependent manner [71]. We also found that epithelial supernatants could chemoattract naïve dendritic cells in a CCL20-dependent manner, and we hypothesized that this epithelial release of chemokines could contribute to inflammatory gastrointestinal manifestations of food allergy.

To test the *in vivo* role of CCL20 in gastrointestinal manifestations of food allergy, we used mice deficient in CCR6, the receptor for CCL20 [72]. CCR6 is expressed on memory T cells, B lymphocytes, and dendritic cells. We found that CCR6-deficient mice were resistant to the development of allergic diarrhea; this was associated with a lack of allergen-induced IL-4 and IL-13 expression in the intestinal mucosa. Through adoptive transfer experiments,

we showed that CCR6 on MLN lymphocytes was necessary for the homing of pathogenic Th2 cells to the intestinal lamina propria and development of allergen-triggered symptoms.

In addition to chemokines, epithelial cells are a source of cytokines that can promote the development of Th2-skewed inflammatory responses, including thymic stromal lymphopoietin (TSLP), IL-25, and IL-33 [73]. TSLP is a cytokine produced by epithelial cells that acts on dendritic cells, mast cells, and T and B lymphocytes [74], and it is a potent inducer of pro-allergic Th2 responses [75]. TSLP has also been shown to have a regulatory role by suppressing inflammatory Th1 and Th17 responses, particularly in the GI tract [76, 77]. Using mice deficient in the TSLP receptor, we found that TSLP was critical for the development of gastrointestinal manifestations of food allergy, but played no role in the development of systemic anaphylaxis or oral tolerance [78]. Symptoms could be restored to TSLP R^{-/-} mice by transfer of wild-type MLN lymphocytes producing Th2 cytokines. Antigen presentation assays showed that T cells, rather than gastrointestinal DCs, were the main targets of TSLP and suggested that the role of TSLP was in amplifying Th2 responses locally rather than in inducing a primary Th2 response. The central role of the epithelial cell in orchestrating gastrointestinal responses to food allergens is shown in Fig. 2.

Conclusion

The normal response of the mucosal immune system is one of active immune tolerance to food antigen. This tolerance is perturbed in food allergy, which can be modeled experimentally through the use of oral adjuvant such as CT or SEB, or through alternate routes of sensitization such as the skin. Using these models, we have begun to identify immune mechanisms underlying sensitization to food allergens, as well as mechanisms of systemic anaphylaxis and gastrointestinal manifestations of food allergy. The challenge currently facing us is to translate these findings back to human disease, and ultimately to find a means for re-establishing immune tolerance as a cure for food allergy.

Acknowledgments

Lauren Steele is supported by the Doris Duke Clinical Research Fellowship program. The work described from the Berin and Mayer labs was funded in part by NIH grant AI044236.

Biography

M. Cecilia Berin

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Fig. 1.

Adjuvant alters the phenotype of the normally tolerogenic intestinal DC. The pro-allergenic adjuvant CT enhances migration and maturation of CD103+ DCs to the draining MLN, where they induce the differentiation of Th2 effector cells. This Th2 induction is mediated by OX40L expression on DCs, which also upregulate Jagged 2 in response to CT

Fig. 2.

Epithelial cells orchestrate gastrointestinal manifestations of food allergy. Epithelial cells sense the presence of allergen–IgE complexes through the expression of CD23, leading to the release of chemokines including CCL20. The receptor for CCL20, CCR6, is found on B cells, DCs, and T cells. CCR6 on T cells is required for the development of gastrointestinal manifestations of food allergy in mice. The epithelial-derived cytokine TSLP acts directly on CD4⁺ T cells to amplify Th2 responses in the intestine. The Th2 cytokines IL-9, IL-4, and IL-13 participate in gastrointestinal manifestations of food allergy by promoting mastocytosis and having direct effects on the intestinal epithelium