



Food allergy: a glimpse into the inner workings of gut immunology

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Purpose of review

How food protein becomes recognized as an allergen remains a fundamental question. Previous studies indicated that the pathophysiology of food allergy is because of a skewed Th2 response to specific food glycoproteins. The focus has now shifted to understanding how a failure of regulatory mechanisms results in food allergy. This review summarizes the recent findings elucidating the small intestine's role in the pathophysiology of food allergy and the immune mechanisms of oral tolerance.

Recent findings

Gut homeostasis and immunity occur via a complex interplay of innate and adaptive immune responses. Immune exclusion is performed mainly by secretory IgA, although there are back-up mechanisms in place to induce oral tolerance when secretory IgA is lacking. Oral tolerance cannot occur in murine models lacking T regulatory cells, for which Foxp3⁺ is a key marker. Migration of Foxp3⁺ T cells from the mesenteric lymph nodes (MLNs) to the lamina propria occurs via gut-homing signals. Also in the MLNs are CD103⁺ dendritic cells, which drive the differentiation of Foxp3⁺ T cells in the presence of TGF- β and retinoic acid produced from dietary vitamin A. Lastly, microenvironmental signals from the microbiome can serve to enhance these interactions.

Summary

We have focused primarily on local immunologic variables that may affect the induction of oral tolerance in the gut and the mechanisms elucidated in animal models. However, many other variables such as genetics, commensal microbiota, and diet are likely to be important factors.

Keywords

CD103⁺ dendritic cell, forkhead box protein-3, oral tolerance, regulatory T cell, secretory IgA

INTRODUCTION

Food allergy is a growing problem among developed countries. Recent estimates approximate prevalence to be 4–8% in the United States [1–4]. Similar trends have been observed in other atopic disorders, such as asthma and atopic dermatitis. Interestingly, relatively few foods are responsible for more than 90% of food allergy. These include milk, egg, soy, and wheat (which are frequently outgrown), as well as peanut, tree nuts, fish, and shellfish (which tend to be life-long). Allergy to seeds, such as sesame and mustard, is also not uncommon.

Foods are introduced first to the gastrointestinal tract mucosa, which contains more lymphoid cells and produces more antibodies than any other site in the body. The gut has the critical task of determining friend from foe. Tolerance is the normal response to food antigens and is characterized by the secretion of IL-10 and TGF- β by T lymphocytes. IL-10 is a regulatory cytokine that favors T-cell

anergy and also activates antigen-specific secretory IgA antibody production [5]. TGF- β has an immunosuppressive effect on B and T cells, maintaining immune nonresponsiveness to commensal bacterial and food antigens. It is also a switch factor for secretory IgA production [6].

Normally, immunologic responses at the epithelium lead to a progression of events aimed at inactivating and removing offending antigens or

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KEY POINTS

- Immune exclusion is performed mainly by secretory IgA, although there are back-up mechanisms in place to induce oral tolerance when secretory IgA is lacking. Systemic IgA may play more of a role in anaphylaxis.
- Oral tolerance cannot occur in murine models lacking T regulatory cells, for which Foxp3⁺ is a key marker.
- Migration of Foxp3⁺ T cells from the mesenteric lymph nodes to the lamina propria appears to be a key step in oral tolerance development. Migration occurs via gut-homing signals, including integrin $\alpha 4\beta 7$ and chemokine receptor nine (CCR9).
- CD103⁺ dendritic cells in the mesenteric lymph nodes drive the differentiation of Foxp3⁺ T cells in the presence of TGF- β and retinoic acid produced from dietary vitamin A.

microbes in addition to developing a tolerogenic response to nonpathogenic proteins, such as foods. Factors involved in oral tolerance induction include antigen dose (high dose and low dose) and form (soluble), as well as host characteristics such as microbiome content, genetics, age, and gastric acid suppression.

The failure to develop or the breakdown of oral tolerance leads to food allergy [7,8], which may be IgE mediated, non-IgE mediated, or both. How food protein becomes recognized as an allergen in a naïve individual remains a fundamental question. Traditionally, the development of food allergy was primarily attributed to a skewed Th2 allergic response. However, it is now recognized that the failure of regulatory mechanisms may play a critical role in this Th2 skewing [9].

This review will address the recent findings that have contributed to our understanding of the small intestine's role in the pathophysiology of food allergy and mechanisms of oral tolerance.

LUMINAL BARRIERS

The gut immune system is the most common site of primary allergen sensitization in both IgE-mediated and non-IgE-mediated food allergy in children. The surface area of the intestinal epithelium represents a large landscape of perhaps 3200 square feet (300 m²) when villi, crypts, and folds are considered [10[¶]]. Intact proteins and allergenic epitopes are taken up by specialized cells in the gastrointestinal tract and transported to antigen-presenting cells (APCs). Antigens may be taken up from the gut lumen by three cell types [7,8]: intestinal epithelial cells (IECs), dendritic cells, and Microfold cells (M cells, which overlay Peyer's patches). The site of entry may

dictate the nature of the immune response to the antigen.

Peyer's patches consist of germinal centers comprised of B lymphocytes, which produce IgA. Notably, at least 80% of the body's immunoglobulin-producing cells are situated in the gut mucosa. Secretory IgA binds and neutralizes food proteins that have retained their immunogenic epitopes following digestion. The importance of secretory IgA in the exclusion of food allergens has been controversial. Mucosal IgA antibodies are hypothesized to inhibit food allergen-induced anaphylaxis by binding antigen prior to systemic absorption. For example, one study showed that mice deficient in the receptor that secretes IgA and IgM into the intestinal lumen (polymeric immunoglobulin receptor) were hypersensitive to IgG-mediated anaphylaxis [11]. However, these mice showed compensatory tolerance after being fed food antigen (ovalbumin), at least in part mediated by CD4⁺CD25⁺ T regulatory cells. This model may mimic the 'leaky gut' environment in early infancy, a time when oral tolerance is normally achieved.

In addition, Strait *et al.* [12[¶]] showed that systemic IgA or IgG rather than enteric IgA antibodies protected against IgE-mediated food-induced anaphylaxis, implying that ingested food antigens must be absorbed systemically to induce an allergic reaction. Allergen activation of the intestinal mucosal mast cells alone, in this model, did not appear to be adequate to provoke a systemic allergic response [12[¶]]. This may be at least in part due to the much lower ratio of specific antibody to antigen present in the gut lumen compared to the systemic circulation. The study also demonstrated that ingested antigen can be absorbed systemically at a speed consistent with the clinical manifestations of anaphylaxis. The authors also assert that the severity of anaphylaxis appeared to be related more closely to the initial or peak plasma concentration of antigen rather than the total time the antigen concentration remained elevated.

T CELLS

A variety of T cells are involved in the pathogenesis of food allergy and oral tolerance induction. They are found in several locations including the gut epithelium, lamina propria, Peyer's patches, and peripheral blood, and have differing effector functions depending on their cytokine profiles.

A key transcription factor, Foxp3, is thought to suppress Th1 and Th2 polarization and is expressed by CD4⁺CD25⁺ regulatory T cells or Tregs. Tregs also include Th3 and Tr1 cells. These play a primary role in controlling the adaptive immune response and

maintenance of tolerance. Functional milk-specific suppressive $\text{Foxp3}^+\text{CD4}^+\text{CD25}^+$ Tregs, for example, were found in higher numbers in children with milk allergy who became tolerant to heated milk compared with children allergic to all forms of milk or nonallergic controls [13]. Furthermore, in normal mice it has been shown that upon stimulation (notably without $\text{TGF-}\beta$), up to 70% of neonatal $\text{CD4}^+\text{Foxp3}^-$ T cells differentiated into Treg cells ($\text{CD4}^+\text{Foxp3}^+$) compared to less than 10% in adult mice [14], highlighting the role of age on gut immune responses.

Although depletion of Foxp3^+ cells eradicates oral tolerance, generation of Treg cells alone may not be sufficient to establish oral tolerance in the gut. Hadis *et al.* [15²²] utilized several mouse models to demonstrate that gut homing and expansion of Tregs in the intestinal lamina propria hold a previously unappreciated role establishing oral tolerance. Lamina propria lymphocytes (LPLs) are thought to be memory T cells. They are exposed to soluble antigens crossing the epithelial barrier of the gut. Once activated, they migrate to gut-associated lymphoid structures, such as gut-draining mesenteric lymph nodes (MLNs). T cells are activated in the MLN, expressing integrin $\alpha 4\beta 7$ and chemokine receptor 9 (CCR9), which are homing factors that guide them to the small intestine. Hadis *et al.* [15²²] reported that Foxp3^+ Treg cells, after activation in the MLN, require gut-homing receptors to first migrate and then proliferate and accumulate in the intestinal lamina propria. In mice having gut-homing defects, oral tolerance could not be established despite retaining their ability to generate Foxp3^+ cells in the lymph nodes. Thus, this model proposes a step-wise generation of Foxp3^+ Treg cells to establish gut tolerance. First, dendritic cells capture antigen and transport it to MLNs where antigen-specific naïve T cells are activated to generate a 'founder pool' of Foxp3^+ Treg cells. Second, activated T cells home from the MLN to the intestinal mucosa, specifically the lamina propria, where local expansion can occur.

The role of Tregs was further explored in humans by Savilahti *et al.* [16] who found that Foxp3 mRNA expression was highest in children with persistent cow's milk allergy after β -lactoglobulin stimulation. A weak Foxp3 response was found in nonatopic milk-tolerant individuals. This group suggested that anergy rather than upregulation of Foxp3 in Treg activation is the primary mechanism of oral tolerance. However, the study utilized circulating T cells that were frozen and thawed, a process known to adversely affect APCs and Tregs.

Intestinal $\gamma\delta$ T cells, although less common than $\alpha\beta$ T cells, are found in abundance in the gut mucosa

where they make up the majority of intestinal epithelial lymphocytes (IELs). IELs lie between IECs and LPLs. Previously, IELs have been found to be mostly anergic to antigenic stimulation, suggesting a tolerogenic role, although they are not well characterized. Bol-Schoenmakers *et al.* [17] investigated the potential functional role of $\gamma\delta$ T cells in peanut sensitization in a mouse model of peanut allergy. Induction of peanut allergy resulted in a significant decrease in the percentage of $\gamma\delta$ T cells in intestinal tissues and Peyer's patches. In addition, $\gamma\delta$ TCR blockade prior to the induction of sensitization resulted in higher peanut-specific IgE and greater mast cell degranulation. These findings suggest a regulatory role for $\gamma\delta$ T cells in the development of allergic sensitization, although mechanistically their involvement is not clear.

DENDRITIC CELLS

Antigen presentation is mostly achieved by professional APCs, mainly dendritic cells [18], which then present antigenic peptides (via MHC) to T cell receptors (TCRs) on CD4^+ T cells, leading to T-helper cell differentiation. Activated dendritic cells hold a crucial role in the establishment of allergy. It appears that the type of dendritic cell that is activated may provide a clue as to whether allergy or tolerance will develop. Origin, in addition to type, may also play a role in the tolerogenic capacity of dendritic cells.

Murine intestinal dendritic cells were originally defined as CD11c^+ class II MHC cells. Two subsequent subsets have been defined in the murine gut based on two markers that are essentially mutually exclusive: α_E integrin CD103 and fractalkine receptor CX3CR1 . The CD103^+ dendritic cell subset, whose development depends on the dendritic cell-specific growth factor Flt3 ligand, originates in the lamina propria and migrates to the MLNs to present antigen to naïve CD4^+ T cells, although it is not entirely clear as to how they obtain antigen. The ability to extend transepithelial dendrites into the gut lumen to sample antigens appears to be a function of CX3CR1^+ dendritic cells, which represent a nonmigratory gut-resident population [19] having both dendritic cell and macrophage-like characteristics, but without the ability to prime naïve T cells [20]. In contrast, CD103^+ lamina propria dendritic cells migrate to the MLNs in a CCR7 -dependent manner [21] after acquisition of antigen. In the MLN, these dendritic cells drive the differentiation of gut-homing Foxp3^+ regulatory T cells in the presence of $\text{TGF-}\beta$ and retinoic acid produced from dietary vitamin A [22,23]. Vitamin A is an important dietary factor supporting secretory immunity by

enhancing the IgA switch and intestinal homing of mucosal B cells. Retinol-metabolizing activity in murine small intestinal CD103⁺ dendritic cells has been shown to be imprinted locally in the small intestine by bile retinoids, thereby enhancing the ability to generate gut-homing effector T cells and to induce Foxp3⁺ T cell differentiation [24[¶]].

However, experiments in some murine models have suggested that when retinoic acid is present in a proinflammatory environment, it may paradoxically downregulate tolerance and promote inflammatory responses [25].

Other dendritic cells in the lamina propria include the plasmacytoid dendritic cells, which are a specialized subset known for their ability to produce high levels of type I interferons, but they, like CD103⁺ lamina propria dendritic cells, can also activate inducible Foxp3⁺ as well as IL10⁺ Tr1 Treg cells.

Smit *et al.* [26] demonstrated that expansion of the dendritic cell population prior to peanut exposure inhibited peanut-specific IgE production, mast cell degranulation, and Th2 cytokine production. This protective effect appears to be dependent on the expansion of plasmacytoid dendritic cells. In addition, peanut sensitization induced a skew towards fewer CD103⁺ dendritic cells. Specifically, CD103⁺ dendritic cells decreased in the lamina propria but absolute numbers increased in the MLN, confirming prior findings [22,23].

MICROENVIRONMENTAL SIGNALS

Activation of dendritic cells represents the initiation point of immunity. Dendritic cells via Toll-like receptors (TLRs) and C-type lectin receptors (CLRs) generate innate immune responses and can influence the adaptive response.

Zhou *et al.* [27[¶]] proposed that SIGNR-1 (CD209b), a CLR, helps condition lamina propria dendritic cells for induction of oral tolerance in a murine model of food allergy. Bovine serum albumin bound to 51 molecules of mannoside (Man51-BSA) delivered orally reduced the BSA-induced anaphylactic response. Man51-BSA targeted lamina propria dendritic cells expressing SIGNR1, inducing IL-10 expression. This in turn promoted the generation of CD4⁺ Tr1-like cells, but not of CD4⁺CD25⁺Foxp3 T cells. Similarly in human dendritic cells, the cell surface receptor, CD209 DC-SIGN (dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin) was found to mediate recognition of a lectin-containing peanut allergen, Ara h 1, *in vitro* [28].

Postulated adjuvants also include glycans. The glycan-binding protein, Galectin-9 (Gal-9), has been

shown previously to activate dendritic cells and promote dendritic cell maturation [29,30]. In a study by Chen *et al.* [31], Gal-9 expression by IECs, which is inducible by mast cell-derived tryptase, was shown to be increased in the duodenal mucosa of adult patients with food allergy compared to normal intestinal epithelia. Then using a mouse model, markers of allergic hypersensitivity (sIgE, IL-4, mast cells, and eosinophils) were shown to be suppressed in OVA-sensitized mice after treatment with anti-Gal-9 antibody. Thus, the authors concluded that IEC-derived Gal-9 contributes to sustaining the allergic status in the intestine. This may help explain the mechanism behind sustaining allergic hypersensitivity.

CONCLUSION

We have focused primarily on local immunologic variables that may affect the induction of oral tolerance in the gut and the mechanisms elucidated primarily in animal models. However, many other variables such as genetics, commensal microbiota, and diet are likely to be important factors. Understanding these processes is crucial for the development of novel therapeutic strategies to prevent or reverse hypersensitivity reactions to foods.

Acknowledgements

None.

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 177–178).

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