

# Immunotherapy in the treatment of food allergy: focus on oral tolerance

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## Purpose of review

Food allergy is a serious and growing problem. Although the current standard of care for patients with food allergies is based on avoidance of the trigger, increased understanding of the mechanisms involved in oral tolerance has shifted focus of treatment and prevention toward inducing tolerance. Here we discuss the relationship of food allergy to oral tolerance and review recent oral tolerance studies, focusing on the mechanistic role of antigen presenting cells and the generation of regulatory T cells in mice and humans.

## Recent findings

Specialized intestinal antigen presenting cells are conditioned by spatial and soluble microenvironmental factors to promote tolerance to dietary antigen primarily via the induction of regulatory T cells. Retinoic acid, a vitamin A metabolite, has been recently identified as a key environmental factor in this process.

## Summary

Future clinical trials for food allergy immunotherapy will benefit from approaches, which target the oral tolerance pathways currently being elucidated.

## Keywords

dendritic cell, food allergy, immunotherapy, oral tolerance, retinoic acid, T regulatory cell

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## Introduction

Food allergy affects 6% of children younger than 3 years of age and approximately 4% of adults in the United States [1]. A recent report from the Centers for Disease Control confirmed these figures, and also identified an 18% increase in food allergies amongst the United States children over the past 10 years [2]. Food-induced anaphylaxis is the most common cause of anaphylaxis treated in hospital emergency departments [3]. At present, the standard of care for food allergy includes strict avoidance of food allergens and ready access to self-injectable epinephrine [4]. The difficulty in avoiding food allergens and the potential for sudden and life-threatening reactions can diminish health-related quality of life for patients and their families [5–7]. Thus, novel treatment strategies for food allergy are needed.

Although the impact of food allergies is substantial, their prevalence is remarkably low considering the complexities of the mucosal immune system. The gastrointestinal tract, which is the largest immunologic organ in the body [8], is normally tolerant of exposure to an enormous array of commensal bacteria and ingested proteins. A single epithelial layer divides the rich antigenic milieu of the

lumen from the lamina propria, a loose stroma of connective tissue populated by lymphocytes and other immune cells. In this context, dietary antigens interact with unique populations of antigen-presenting cells (APCs), which are believed to actively suppress T cell responses. Thus oral tolerance can be defined as the antigen-specific suppression of cellular or humoral immune responses following previous oral exposure to the antigen [8,9]. This hyporesponsiveness likely evolved as an analog of self-tolerance to prevent hypersensitivity reactions to food proteins and bacterial antigens present in the commensal flora. Food hypersensitivity can thus be considered a failure in establishing oral tolerance or a breakdown in existing tolerance.

For centuries, tolerance induction has been used as a strategy for preventing allergic reactions. In 1829 Dakin reported that certain populations of Native Americans ate poison ivy leaves to prevent contact hypersensitivity reaction to urushiol [10]. In a classic experiment, Wells later found that guinea pigs repeatedly fed hen's egg protein were protected from anaphylaxis when injected with the protein [11]. Experiments in the mid-twentieth century established that the inhibition of active immune

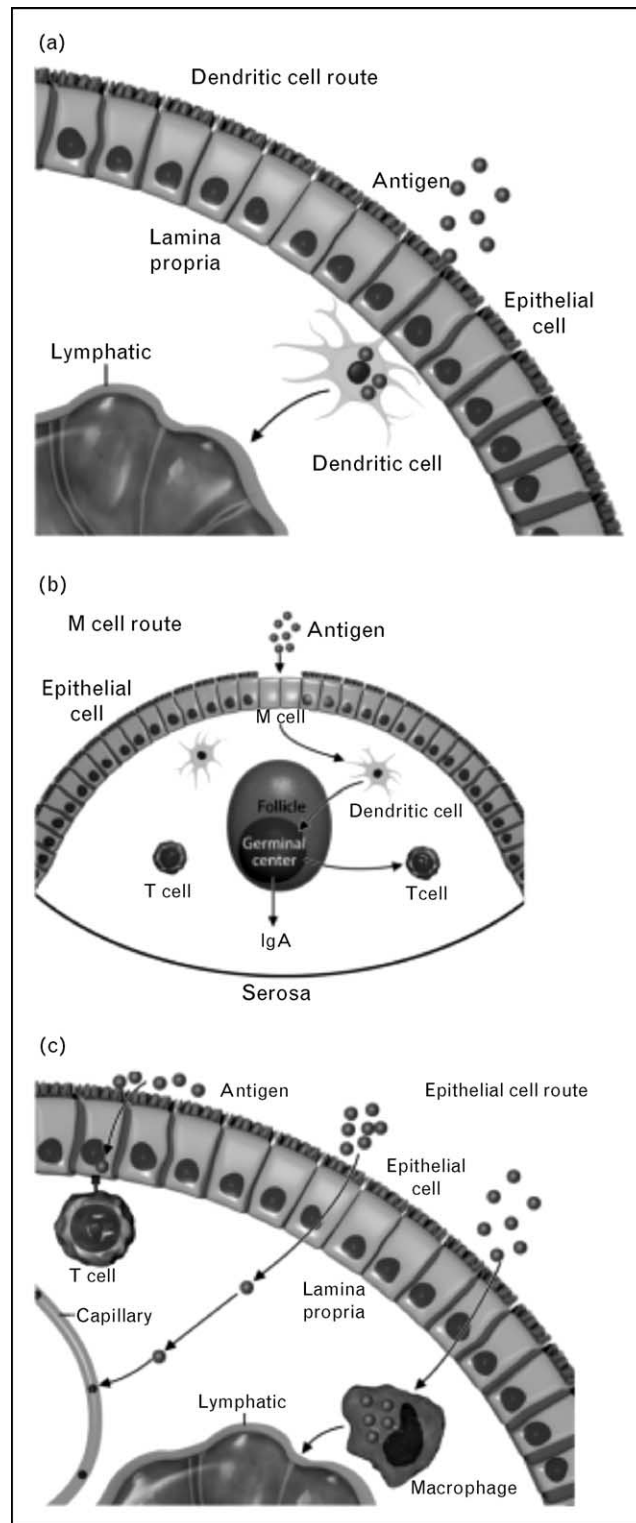
responses following oral feeding of an antigen is T cell-mediated [12]. Age, genetics, route of initial antigen exposure, and the physical properties of allergens have also been shown to impact the development of tolerance. More recently, understanding oral tolerance has been recognized as a key component in developing strategies for preventing and treating food allergies. Data from early-phase clinical trials suggest both sublingual and oral immunotherapy are effective in reducing sensitivity to allergens, but more study is needed to understand if and how tolerance is generated. In this article we discuss the relationship of food allergy and oral tolerance, review the mechanisms of oral tolerance, and discuss important new observations on the roles of antigen-presenting cells in tolerance. The reader is referred to a recent review in this journal and others for information on novel clinical prevention and treatment paradigms for food allergy [13,14\*,15].

### Initial responses to dietary proteins

Exposure to foreign antigens in the gut leads to protective local and systemic immunologic responses. Secretory IgA, a noninflammatory antibody isotype which functions to sequester antigen in the lumen, is initially locally produced at mucosal surfaces [16]. The systemic immune system may also be primed, inducing humoral and cell-mediated immune memory. For most dietary proteins and commensal bacteria, these local and systemic factors encourage immunologic tolerance. These initial tolerogenic interactions are also critical in stimulating proper maturation of the immune system. Mice that are raised in germ-free conditions or reared on a balanced but protein-free diet have poorly developed gut-associated lymphoid tissue that resembles suckling mice [17,18]. Such mice also have low numbers of lymphocytes and levels of circulating IgG and IgA, as well as cytokine production consistent with  $T_H2$  responses.

Following oral ingestion, gastric acidity and luminal digestive enzymes degrade dietary proteins, which often results in the destruction of immunogenic epitopes. Interrupting this process in animal models disrupts tolerance and leads to hypersensitivity [19]. Immunogenic proteins, which escape luminal digestion, can then interact with the mucosal immune system in at least three ways, as depicted in Fig. 1. First, lamina propria dendritic cells have been shown to sample exogenous antigens by extending processes through the paracellular epithelial space and into the lumen [20]. Additionally, specialized intestinal epithelial cells called microfold or M cells, which overlie Peyer's patches (PP) can transport particulate antigens and deliver them to subepithelial dendritic cells [21]. Finally, intestinal epithelial cells, which constitutively express MHC class II molecules on their basolateral membranes [22,23], may take up dietary

**Figure 1** Intestinal antigen presentation



(a) Dendritic cells extend processes through the epithelium and into the lumen. (b) M cells overlying Peyer's patches take up particulate antigens and deliver them to subepithelial dendritic cells. (c) Soluble antigens possibly cross the epithelium through transcellular or paracellular routes to encounter T cells or macrophages in the lamina propria. Adapted with permission from [14\*].

antigens and act as nonprofessional APCs. Some soluble antigens may also passively traffic through paracellular channels in damaged gut epithelium. There are several unique subpopulations of mucosal dendritic cells, which process and present dietary antigens to T cells in local lymphoid tissue; the functional and phenotypic attributes of these dendritic cell subsets are critically important, still being investigated, and beyond the scope of this review [24<sup>\*\*</sup>]. Dietary antigen presentation occurs either in the PP or the mesenteric lymph node (MLN) but CCR7-dependent chemotaxis to the MLN, not PP, has been shown to be critical for oral tolerance induction [25]. Tolerogenic local factors in the gut microenvironment are thought to influence the dendritic cell-T cell interaction and result in suppression of immune responses.

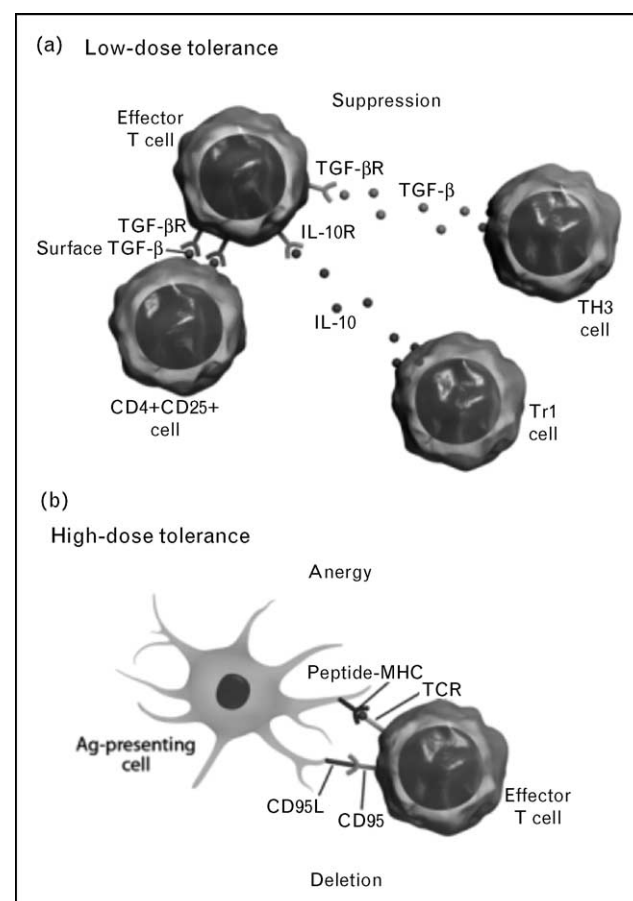
### Mechanisms of oral tolerance: T cells

Antigen dose also influences oral tolerance mechanisms, represented in Fig. 2: low-dose exposures favor active suppression by regulatory T cells whereas high doses result in clonal anergy/deletion [26]. We will first consider regulatory T cells, which express either CD4 cell [27–29] or CD8 cell [30], and suppress immune responses through soluble or cell-bound regulatory cytokines, such as IL-10 and TGF- $\beta$  [8]. Antigen-specific regulatory cells migrate to lymphoid organs, where they inhibit the generation of effector cells, and to target organs, where they release cytokines, which are capable of nonspecific suppression [9].

Defects in regulatory T cell activity likely contribute to the development of food allergy. Natural CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells develop in the thymus and express the specific transcription factor forkhead box P3 (FOXP3) [31,32], which confers regulatory function to these cells to block both T<sub>H</sub>1 and T<sub>H</sub>2 responses [33]. Natural regulatory T cells mediate suppression through cell surface-bound TGF- $\beta$  [34], although suppression can possibly occur independently of TGF- $\beta$  [35]. Human FOXP3 mutations result in a fatal disorder characterized by immune dysregulation, polyendocrinopathy, and enteropathy (IPEX) [36]. A deletion in the noncoding region of FOXP3 that impairs mRNA splicing has been associated with an IPEX variant that includes severe enteropathy, atopic dermatitis, and food allergies [37].

Inducible regulatory T cells are CD4<sup>+</sup> cells which can acquire regulatory properties in the periphery, associated with the production of the anti-inflammatory cytokines IL-10 and TGF- $\beta$ . A series of elegant recent experiments confirmed that inducible Foxp3<sup>+</sup> T regs were required for the establishment of mucosal tolerance in mice [38<sup>\*</sup>]. Duodenal mucosal lymphocytes from children with milk-induced gastrointestinal diseases were cultured *in vitro* in the presence of milk. Upon restimulation with milk

**Figure 2 Mechanisms of oral tolerance**



(a) Low doses of antigen favor tolerance driven by regulatory T cells, which suppress immune responses through soluble or cell surface-associated toleragenic cytokines such as IL-10 and TGF- $\beta$ . (b) High-dose tolerance is mediated by lymphocyte anergy or clonal deletion. Anergy can occur through T-cell receptor ligation in the absence of costimulatory signals. Clonal deletion occurs by means of FAS-mediated apoptosis (CD95). TCR, T-cell receptor; Ag, antigen; TGF, transforming growth factor. Adapted with permission from [14<sup>\*</sup>].

protein, these cells released the T<sub>H</sub>2-associated cytokines IL-5 and IL-13, but only very low amounts of TGF- $\beta$  and IL-10, suggesting a defect in inducible regulatory T cell function [39].

Finally, among children with non-IgE mediated allergy to cow's milk, which is frequently outgrown, the development of tolerance is associated with the appearance of CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells. Children who outgrow their allergy have higher numbers of circulating CD4<sup>+</sup>CD25<sup>+</sup> T cells and decreased *in-vitro* proliferative responses to bovine  $\beta$ -lactoglobulin in peripheral blood mononuclear cells (PBMCs) than children who maintain clinically active allergy [40]. Depletion of CD25<sup>+</sup> cells from PBMCs of tolerant children leads to a five-fold increase in proliferation against  $\beta$ -lactoglobulin *in vitro*.

High-dose tolerance is mediated by lymphocyte anergy or clonal deletion. Anergy can occur through T cell receptor ligation in the absence of costimulatory signals provided by soluble cytokines, such as IL-2, or by interactions between receptors on T cells (CD28) and counter-receptors on APCs (CD80 and CD86) [41]. Clonal deletion occurs by means of FAS-mediated apoptosis [42], which can be blocked by the proinflammatory cytokine IL-12 [43].

Low-dose and high-dose tolerance may not be mutually exclusive and may have overlapping functionality [9]. Apoptotic T cells release TGF- $\beta$  in both latent and bioactive forms, and macrophages produce TGF- $\beta$  upon ingesting apoptotic cells [44,45]. Secretion of TGF- $\beta$  can be induced by treating T cells with anticytotoxic T lymphocyte antigen (CTLA)-4 antibodies, despite the evidence that CTLA-4 also appears to be involved in the induction of clonal anergy *in vivo* [34,46]. The secretion of TGF- $\beta$  through the various mechanisms of clonal anergy and deletion may contribute to an immunosuppressive environment in the gut. Indeed, the primary source of TGF- $\beta$  remains one of the most significant unresolved questions in tolerance; other contributors of TGF- $\beta$  in this environment include the gut epithelium and immature mucosal dendritic cells.

### Mechanisms of oral tolerance: antigen-presenting cells

Although much is known about the role of human and animal T cells in tolerance, relatively little is known about the upstream events which lead to their development. Several recent studies have focused on specialized mucosal dendritic cell populations and contributed important new insights in the generation of immunity and tolerance to oral antigens. New clues are emerging which begin to characterize how important factors in the intestinal microenvironment shape dendritic cell responses, which dendritic cells become activated, and how these conditioned dendritic cells then participate in tolerance induction.

Because tolerance normally develops after oral protein administration in mice, mucosal adjuvants are required for an immune response to dietary proteins. Important insights about tolerance are gained by studying its disruption by adjuvants, a concept explored in an excellent recent review [47<sup>••</sup>]. A mucosal adjuvant commonly used in food allergy models is cholera toxin, which stimulates T<sub>H</sub>2 responses and the production of allergen-specific IgE. A recent study used mice with an ovalbumin-specific transgenic T cell receptor to investigate the mechanism of action of cholera toxin in mucosal allergic sensitization [48<sup>•</sup>]. Ovalbumin sensitization in the presence of cholera toxin caused a subset of lamina propria dendritic cells to

expand, undergo maturation, and upregulate Jagged-2 and cell-surface OX40L. These mature dendritic cells then trafficked to the MLN, where they stimulated naïve T cells, skewing them towards a T<sub>H</sub>2 phenotype in an OX40L-dependent manner. OX40L is a critical downstream effector of the 'inflammatory T<sub>H</sub>2' response mediated by the epithelial cytokine thymic stromal lymphopoietin (TSLP) in human atopic dermatitis and asthma [49<sup>••</sup>]. TSLP is also produced by the human intestine, where it may participate in regulation of immune homeostasis [50]. Although adjuvants are not required for human food allergy, the clear role of OX40L in T<sub>H</sub>2 sensitization to food proteins, and its possible link to epithelial TSLP, deserves further study.

Substantial evidence links oral tolerance to the ability of CD11c+ mucosal dendritic cells to convert naïve T cells to regulatory T cells [23]. A series of recent studies has demonstrated that this ability can be conferred by the vitamin A metabolite retinoic acid, in the presence of TGF- $\beta$ . Dietary vitamin A is the sole source of retinoic acid, as it cannot be synthesized by humans or animals; thus the effect of retinoic acid on the intestinal immune response represents a key adaptation to local environmental factors. An initial report demonstrated that TGF- $\beta$  produced by mucosal dendritic cells induced Foxp3+ regulatory T cells, but only in the presence of dendritic cell-derived retinoic acid [51<sup>••</sup>]. These observations were subsequently confirmed by another group who further demonstrated that the retinoic acid-producing tolerogenic dendritic cell subset was found in the MLN and expressed CD103 as well as *aldh1a2*, a retinal dehydrogenase which converts retinal to retinoic acid [52<sup>••</sup>]. By contrast, CD103- dendritic cells were strongly proinflammatory, suggesting that tolerogenic MLN dendritic cells may be spatially conditioned by intestinal epithelium through interactions with E-cadherin, a ligand of CD103. Utilizing a green fluorescent protein-Foxp3 reporter system, another group adoptively transferred OVA-specific CD4+Foxp3- T cells. They observed de-novo peripheral conversion of Foxp3+ T regs in MLN and PP after oral antigen administration, which again required CD103+ lamina propria dendritic cells and retinoic acid [53<sup>••</sup>]. Retinoic acid also has been shown to induce lymphocyte expression of the  $\alpha$ 4 $\beta$ 7 integrin and CCR9, both of which confer gut-homing specificity [54,55,56<sup>•</sup>]. Importantly, these retinoic acid-associated phenotypic and functional features of CD103+ MLN dendritic cells have been recently observed in humans as well, implicating them as potential therapeutic targets for the regulation of intestinal immune responses [57<sup>••</sup>]. In addition to its role in peripheral conversion of Foxp3+ T regs, retinoic acid can also condition dendritic cells to induce T-cell independent B-cell IgA production [56<sup>•</sup>,58] in the gut of mice and humans, which may be a complementary mechanism in

hyporesponsiveness to dietary proteins. Taken together, these important new data confirm that retinoic acid, acting in concert with TGF- $\beta$ , powerfully modulates tolerogenic intestinal immune responses by participating in the peripheral conversion of inducible Foxp3+ regulatory T cells, imprinting lymphocytes with gut-homing signatures, and stimulating IgA production. The impact of these significant findings has sparked several excellent recent reviews [59,60,61<sup>••</sup>].

Another vitamin, the steroid hormone vitamin D, has also been implicated in the modulation of allergic responses. Acquired either through skin exposure to sunlight or by consumption of fortified foods, vitamin D is central to calcium metabolism and bone health. However, the vitamin D receptor is also expressed on T cells, activated B cells, monocytes, dendritic cells, and epithelial cells, and its activation results in regulation of both innate and adaptive immune responses [62]. In general, the effect on T cells is to shift towards the Th2 phenotype by inhibiting Th1 development, although this may depend on the timing of vitamin D exposure. In addition, vitamin D has also been shown to promote the induction of regulatory T cells [63]. The immunologic effects of vitamin D exposure are thus complex, and interestingly, investigators have postulated that both deficiency [64] and excess [65] of this pleiotropic molecule could contribute to the loss of tolerance and increased prevalence of allergic diseases such as rhinitis, asthma, and anaphylaxis. There are epidemiologic data, which support both positions [66,67]. Clearly, well designed randomized clinical trials are needed to better evaluate the role of vitamin D in the human allergic response.

APCs other than conventional dendritic cells may also participate in oral tolerance induction. For example, intestinal macrophages can also efficiently induce Foxp3+ T regs in an IL-10, retinoic acid, and TGF- $\beta$ -dependent fashion [68<sup>•</sup>]. Plasmacytoid dendritic cells (pDC), an atypical dendritic cell subset known for the ability to produce vast quantities of type I interferons, can also activate inducible Foxp3+ as well as IL-10+ Tr1 regulatory T cells in both mice and humans under various conditions [69–72]. pDCs were also recently shown to induce oral tolerance to fed protein and hapten by inducing anergy or deletion of 70–80% of antigen-specific CD4+ and CD8+ T cells. Interestingly, these tolerogenic pDCs were found in the liver, where they make up almost 30% of the CD11c+ dendritic cell population [73<sup>•</sup>]. This suggests that oral tolerance may be operative via multiple mechanisms in multiple tissue compartments, with antigen-loaded mucosal dendritic cells trafficking via lymph to the MLN and soluble antigen encountered within the liver or portal circulation. Hepatic tolerance was studied recently in humans by isolating liver dendritic cells from surgical specimens. Compared

with peripheral blood-derived dendritic cells, liver dendritic cells produced vastly greater amounts of IL-10, greater suppression of T-cell responses in mixed lymphocyte culture, and the exclusive IL-10 dependent generations of CD4+CD25+FoxP3+ regulatory T cells from naïve precursors [74<sup>•</sup>]. Two excellent recent summaries review in greater detail the roles of APCs and the impact of their microenvironments on the development of tolerance [24<sup>••</sup>,75<sup>••</sup>].

## Conclusion

The development of treatments for patients who have, or are at risk for, food allergy critically depends on understanding oral tolerance induction. Ongoing food allergy clinical trials are currently targeting oral and mucosal forms of tolerance using immunotherapy with aqueous, powdered, and heat-denatured proteins. Most of these trials include immunologic as well as clinical analyses, in an effort to better understand the mechanism of human oral tolerance. Intestinal APCs and T regs are likely to be central to this mechanism, although many unanswered questions remain. Key factors such as retinoic acid are emerging as critical functional regulators, which represent important potential targets for future immunotherapeutic approaches to tolerance induction in food allergy.

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- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

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