Published in final edited form as:

Clin Rev Allergy Immunol. 2018 October ; 55(2): 107–117. doi:10.1007/s12016-018-8680-5.

Mechanisms of oral tolerance

Leticia Tordesillas and M. Cecilia Berin

Jaffe Food Allergy Institute, Immunology Institute, Mindich Child Health Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Abstract

Oral tolerance is a state of systemic unresponsiveness that is the default response to food antigens in the gastrointestinal tract, although immune tolerance can also be induced by other routes, such as the skin or inhalation. Antigen can be acquired directly by intestinal phagocytes, or pass through enterocytes or goblet cell associated passages prior to capture by dendritic cells (DCs) in the lamina propria. Mucin from goblet cells acts on DCs to render them more tolerogenic. A subset of regulatory DCs expressing CD103 is responsible for delivery of antigen to the draining lymph node and induction of Tregs. These DCs also imprint gastrointestinal homing capacity, allowing the recently primed Tregs to home back to the lamina propria where they interact with macrophages that produce IL-10 and expand. Tregs induced by dietary antigen include Foxp3+ Tregs and Foxp3-Tregs. In addition to Tregs, T cell anergy can also contribute to oral tolerance. The microbiota plays a key role in the development of oral tolerance, through regulation of macrophages and innate lymphoid cells that contribute to the regulatory phenotype of gastrointestinal dendritic cells. Absence of microbiota is associated with a susceptibility to food allergy, while presence of Clostridia strains can suppress development of food allergy through enhancement of Tregs and intestinal barrier function. It is not clear if feeding of antigens can also induce true immune tolerance after a memory immune response has been generated, but mechanistic studies of oral immunotherapy trials demonstrate shared pathways in oral tolerance and oral immunotherapy, with a role for Tregs and anergy. An important role for IgA and IgG antibodies in development of immune tolerance is also supported by studies of oral tolerance in humans. The elucidation of key pathways in oral tolerance could identify new strategies to increase efficacy of immunotherapy treatments for food allergy.

Keywords

Oral tolerance; Regulatory T cells; Antigen-presenting cells; Immunotherapy; Food allergy; Microbiota

Introduction

Oral tolerance is defined as the active suppression of specific immune responses to antigens first encountered in the gastrointestinal tract. This process protects from inappropriate

Address Correspondence to: M. Cecilia Berin, PhD, Department of Pediatrics, Box 1198, One Gustave L. Levy Place, Icahn School of Medicine at Mount Sinai, New York, NY, USA 10029, cecilia.berin@mssm.edu, Tel: 1-212-824-8442.

Conflict of interest: L. Tordesillas and M.C. Berin declare that they have no conflict of interest.

immune responses to the plethora of foreign antigens derived from food or commensal organisms normally encountered in the gastrointestinal tract. When oral tolerance does not occur, immune reactivity to food or microbes can lead to food allergy or inflammatory bowel disease, respectively. In mouse models, oral tolerance is commonly induced by feeding antigen before immunization, with one single high dose (50-100 mg) or consecutive doses of 0.5-1 mg of antigen for 5 to 7 days. Oral tolerance is measured by decreased antigen-specific antibodies, reduced cytokine production by lymph nodes cells after antigen restimulation *in vitro*, or reduction of allergic symptoms or delayed hypersensitivity reaction after allergen challenge *in vivo*. In humans, induction of oral tolerance to a neoantigen has also been measured by decreased antibody production and T cell proliferation after immunization [1,2]. There is growing interest and support for the application of oral tolerance in the prevention and treatment of food allergy.

Gastrointestinal Antigen Uptake

Dietary antigens are subjected to digestion into peptides and amino acids. However, a small fraction can reach the immune system underlying the gut epithelium in an intact form, being able to interact with antigen presenting cells. Disruption of digestion of dietary proteins with antacids has been related with increased induction of IgE to antigens in the diet [3,4].

Antigen that reaches the intestinal epithelium can be transported by different routes dependent on its properties, such as size and solubility, which could lead to the induction of tolerance or immunity [5]. Microfold or M cells are flattened epithelial cells overlying the Peyer's patches, which are organized lymphoid structures distributed along the mucosal tissue. M cells are specialized in the uptake of particulate antigens such as of viruses and bacteria. Sampling through M cells has been associated with induction of IgA production [6], which contributes to immune exclusion by neutralizing antigens in the lumen.

Soluble antigen is absorbed by enterocytes and transported following two major routes, the transcellular route (in vesicles) and the paracellular route (between cells). Epithelial cells sample antigen from the lumen, and during transcytosis, it is degraded in lysosomal compartments, although some antigen can be released into the basolateral space. Partially degraded antigens within endocytic vesicles can be loaded onto MHCII and these exosomes are released from the basolateral membrane, to interact with dendritic cells (DCs) [7,8]. Under homeostatic conditions, tight junctions between enterocytes prevent the paracellular passing of antigens. An increased transport of intact antigen through epithelial cells has been related with its allergenic activity [9,10].

Goblet cells also have an important role in the transport of soluble antigen. Low molecular weight soluble antigen injected into the intestinal lumen *in vivo* preferentially filled a subset of epithelial cells corresponding to goblet cells, producing goblet-cell-associated antigen passages (GAPs)[11]. These GAPs delivered antigen exclusively to CD103⁺CX3CR1⁻ lamina propria DCs, a DC subset that has been associated with development of a tolerance response. Increased mucin secretion by goblet cells induced increased frequency of GAPs and higher amount of antigen delivery. Thus, antigen delivery pathways favoring interaction with CD103⁺ DCs or IgA production could be related with induction of oral tolerance (e.g.

through Peyer's patches, goblet cells, or exosomes) while a higher resistance to proteolytic digestion and increased paracellular transport would be related with sensitization, although the contribution of each of these pathways to tolerance and immunization still needs to be determined.

In addition to transport through the epithelium, intestinal phagocytes such as macrophages and DCs extend dendrites between enterocytes and sample antigen directly from the lumen without disrupting tight junctions between cells [12,13]. Soluble antigen is efficiently taken up from the intestinal lumen by CX3CR1⁺ macrophages. They send protrusions to sample antigen in a mechanism dependent on the expression of CX3CR1 [12,14]. By contrast, CD103⁺ DCs are less efficient in sampling soluble antigen [12,15], although they have been shown to capture bacteria by using intraepithelial dendrites [15]. CX3CR1^{high} macrophages do not migrate to the mesenteric lymph nodes (MLN) under steady state conditions, and a cooperation between CX3CR1^{high} cells and migratory CD103⁺ DCs was identified [12]. After antigen uptake, CX3CR1⁺ macrophages transfer peptide-MHCII complexes via gap junctions to CD103⁺ DCs, which migrate to the MLN and present antigen to naïve T cells [12]. In Fig. 1, different routes of antigen uptake are summarized.

Induction of tolerance in the intestine

Role of regulatory T cells

The role of T cells in mediating oral tolerance was shown by depleting CD4⁺ T cells [16] or transferring tolerance by adoptive transfer of CD4⁺ T cells [17]. Although thymic-derived regulatory T cells (Tregs) are not required for oral tolerance [18,19], peripherally induced antigen-specific CD4⁺CD25⁺ Foxp3⁺ Tregs are necessary for oral tolerance [18]. Mutations in the Foxp3 locus are associated with a loss of peripheral tolerance and development of severe food allergy in humans [20], and specific depletion of Foxp3⁺ cells abrogates oral tolerance [21]. Altogether, these results support a key role of induced Foxp3⁺ Tregs in oral tolerance.

Other Treg populations have been described in the context of oral tolerance, including Th3 and Tr1 cells. Th3 cells expressing TGFβ (detected by surface expression of latency-activated peptide or LAP) are induced after antigen feeding, or by oral anti-CD3 antibody administration [17,22,23]. The relationship between Foxp3⁺ and LAP/Th3 cells is not clear. Both suppress through a mechanism dependent on TGFβ, and Th3 cells can induce Foxp3⁺ cells by TGFβ production [24]. Furthermore, activated Foxp3⁺ Tregs can express LAP on their surface [25]. Thus, it is possible that there is some plasticity between these Treg subsets and that they present an overlapping function. Tr1 cells suppress immune responses through IL10. Tr1 cells play a key role in prevention of colitis [26,27], but their role in oral tolerance is not clear, with studies showing conflicting results [18,28,29]. Nature of antigen [29] or the experimental model used in these studies could determine the phenotype of Tregs involved in the oral tolerance response. The relationship between different Treg subsets is not clear as they all have the capacity to produce IL-10 and they present overlapping surface markers. Foxp3⁺ Tregs can condition DCs to induce IL-10-secreting Tr1 cells by a mechanism dependent on IL-27 [30], again showing the potential interaction between these Treg subsets.

Apart from the induction of Tregs, anergy and T cell depletion have also been shown as mechanisms of oral tolerance. Anergy refers to T-cell unresponsiveness to the antigen, while depletion is the apoptosis of antigen-specific T cells. High dose antigen exposure induces anergy or depletion while low dose antigen leads to induction of Tregs [31,32]. Although much of the focus in the oral tolerance field has focused on active suppression by Tregs, the role of T cell anergy and deletion in immune tolerance is supported by recent human studies which will be discussed later.

Induction of Tregs by DCs in the intestine

The site of oral tolerance induction has been identified as the MLN [33], while Peyer's patches are not necessary as oral tolerance could be established in the absence of these structures [34,35].

Oral tolerance is initiated by CD103⁺ DCs that capture antigen in the lamina propria and migrate to the MLN, where they induce differentiation of naïve T cells into Tregs, through a mechanism dependent on TGF β and retinoic acid [36–38]. CD103⁺ DCs express high levels of RALDH2, an enzyme that metabolizes retinal into retinoic acid. Retinoic acid is also required to program newly primed T cells to home to the intestine through expression of the chemokine receptor CCR9 and the integrin $\alpha_4\beta_7$ [39]. Stromal cells from MLN also express high levels of RALDH2 and they were found to be required *in vivo* to induce gut-homing T cells [40]. In addition, CD103⁺ DCs express high levels of indoleamine 2,3-dioxygenase (IDO), an enzyme involved in tryptophan catabolism, that is involved in induction of Foxp3⁺ Tregs [41]. After the induction of Tregs in the MLN, antigen-specific Tregs migrate to the lamina propria where they are expanded [21]. Migration of Tregs to the lamina propria is necessary for successful induction of oral tolerance, as mice deficient in β_7 integrin and MADCAM as well as CCR9-deficient mice, markers necessary for T cell migration to the lamina propria, have impaired oral tolerance [21,42]. CX3CR1⁺ macrophages expressing high levels of IL-10 are necessary for expansion of Tregs in the lamina propria, and failure of this tissue T cell expansion impairs development of oral tolerance. In Fig. 2 a schematic representation of the mechanism of oral tolerance is shown.

To determine the requirement of CD103⁺ DCs in oral tolerance, Esterhazy et al used a variety of mouse models to specifically ablate myeloid lineage-specific APC during oral antigen exposure [43]. Classical DCs were required for oral tolerance and peripheral Treg induction, while macrophages and monocyte-derived cells were dispensable. Migratory IRF8-dependent CD103⁺CD11b[−] DCs had the highest expression of Aldh1a2, Tgfb2 and Itgb8 (coding for RALDH2, TGF- β 2 and integrin beta-8, respectively) and other genes related with Treg generation and gut homing, and had the highest capacity to induce Foxp3⁺ Tregs. IRF8-dependent cDCs (containing migratory CD103⁺CD11b[−] and resident CD8 α ⁺CD11b^{low}) were required for peripheral Foxp3⁺ Treg polarization upon oral antigen *in vivo*, but depletion of these cells had no effect on oral tolerance. These findings suggest that although CD103⁺CD11b[−] may be uniquely involved in the polarization of Foxp3⁺ Tregs, there is redundancy in the contribution of DC subsets to the phenomenon of oral tolerance.

Other sites implicated in oral tolerance induction

A complementary role of liver and MLN has been identified. After high doses of antigen exposure, oral tolerance can be induced by deletion of antigen specific CD8⁺ and CD4⁺ T cells [44,45]. This mechanism is mediated by plasmacytoid DCs (pDCs) present in the liver and MLN, and occurs in the liver [45]. As an early site of exposure, pDCs in the liver are involved in a rapid deletion of antigen-specific T cells trafficking from the blood, followed by generation of Tregs in the MLN by CD103⁺ DCs leading to complete suppression of delayed-type contact hypersensitivity [45]. A role for tonsils, which are secondary lymphoid organs located in the oropharynx and nasopharynx, has also been proposed in the development of oral tolerance due to their privileged anatomical location [46]. Human tonsils are highly enriched in total CD4⁺ Foxp3⁺ Tregs as well as allergen-specific Tregs. Among the different DC subsets present in tonsils, pDCs induce Foxp3⁺ Tregs *in vitro* with suppressive capacity, and the number of tonsil pDCs was found to be decreased in atopic donors compared to non-atopic individuals [46]. DCs from the mouse oral mucosa and submucosa are also capable of generating suppressive Tregs expressing IFN γ and IL10 in the cervical lymph nodes [47]. These results suggest that DCs from tonsils and sublingual mucosa can also participate in the first step of oral tolerance induction to food and aeroallergens.

Factors involved in induction of oral tolerance

The local gut immune environment presents factors that promote the development of a tolerance response. As stated before, mucin secretion can favor the induction of Tregs by increasing antigen transport by goblet cells and uptake by tolerogenic CD103⁺ DCs [11]. In addition, hyperglycosylated mucin MUC2 conditions CD103⁺ DCs and intestinal epithelial cells towards a regulatory phenotype. MUC2 suppresses the expression of inflammatory cytokines and increases TGF- β , IL-10 and RALDH expression by inducing the transcription factor β -catenin via dectin-1 [48]. Another factor that affects the development of oral tolerance is the secretion of GM-CSF by intestinal ROR γ t⁺ innate lymphoid cells (ILC3) that promotes Treg homeostasis [49]. GM-CSF production is regulated by microbial signals, through a cross-talk between IL1 β -producing macrophages and ILC3 in the intestinal mucosa, and acts on gut DCs and macrophages, promoting their tolerogenic phenotype. In the infant acquiring nutrition through breastfeeding, differences in milk composition, such as levels of cytokines or presence of immunoglobulins could promote the development of tolerance to antigens present in breastmilk. In mice, presence of immunoglobulins in breastmilk enhanced uptake of antigen by the pups through the neonatal Fc Receptor, and enhanced the generation of Tregs [50]. TGF- β present in the milk also enhanced the generation of tolerance to antigens carried in the breastmilk [51].

Non-oral routes of tolerance induction

Induction of immune tolerance is not restricted to the oral and gastrointestinal mucosa, and can be induced by other routes such as airways or skin. Inhaled antigen can induce tolerance and generation of antigen-specific Tregs. DCs isolated from lung after respiratory exposure to OVA induce IL-10 producing Tr1 cells, and can transfer tolerance in adoptive transfer

experiments in an IL-10 dependent manner [52]. Nasal administration of anti-CD3 was shown to attenuate autoantibody production and lupus development by the induction of IL-10-producing LAP⁺ T cells, which had the phenotype of Th3 cells but suppressed both in a IL-10 and TGF β -dependent mechanism [53]. A role of Foxp3⁺ Tregs in the development of tolerance via respiratory route has also been identified [54]. Both LAP⁺ Foxp3- and Foxp3⁺ Tregs were found to be induced by inhalant antigen in lung-draining lymph nodes and were able to block subsequent antigen sensitization to OVA after priming, and lung inflammation after challenge [55]. In another study, Foxp3⁺ Tregs were induced in the spleen after inhaled OVA and suppression of subsequent sensitization was found to be dependent on TGF- β but not IL-10 [56]. In summary, different Treg subsets have been identified to play a role in airway tolerance. Different findings on the contribution of each subset may be due to different tolerance protocols or compartments analyzed, but clearly there is functional overlap between Treg subsets. Induction of tolerance to inhaled allergens is dependent on migratory DCs, which transport antigen from the airway mucosa to the thoracic lymph nodes [57] [58]. A role of pDCs in mediating protection against inhalant antigen has also been demonstrated, as depletion of pDCs increase antigen-specific Th2 responses and transfer of pDCs before sensitization prevented asthma [59,60].

Induction of tolerance to food antigens can also be achieved through intact skin. Although skin antigen exposure has been associated with sensitization (reviewed in [61]), exposure of the skin to food allergens such as milk can generate tolerance [62]. Factors that break immune tolerance through the skin include damage to the skin (tape stripping), microbial adjuvants such as staphylococcus enterotoxin B, or endogenous adjuvant activity within foods such as peanut [63,64]. All of these factors induce an innate inflammatory response in skin that contributes to sensitization. However, the presence of adjuvant activity within foods does not preclude tolerance induction through skin. Immune tolerance can be generated by epicutaneous peanut or OVA [65,66]. Thus, many natural routes of exposure to allergens have the capacity to generate immune tolerance in the absence of adjuvant factors or inflammation.

Microbiota and diet

Several studies in mice have demonstrated an association between altered intestinal microbiota and impaired oral tolerance, shown by the development of food allergy. Mice treated with antibiotics, and germ-free mice or mice with low-diversity microbiota have increased basal levels of IgE and enhanced sensitization to food allergens [67,68]. Transfer of microbiota to germ-free mice early in life protects against subsequent development of food allergy [67]. Transgenic mice carrying a gain-of-function mutation resulting in susceptibility to food allergy by oral exposure (*Il4raF709* mice) have a different gut microbiota signature compared to wild type mice [69] and transfer of gut microbiota from *Il4raF709* mice to germ-free mice can transfer food allergy susceptibility. Moreover, in a study where responders and non-responders to food allergy development were found among genetically identical mice using a mouse model of OVA sensitization under gastric-acid suppression, those that were protected from food allergy presented distinct microbiota composition from susceptible mice [70]. Although it is unknown if a particular microbiota species promotes food allergy, some species such as Clostridia, Bifidobacterium and

Bacteroides, have been related with suppression of food allergy in mouse studies [68,71–73]. Most studies have focused on the role of gut microbiota, but skin is also rich in microbiota that modulate skin immunity [74]. Due to the increasing evidence that points to the skin as the main site of sensitization to food antigens [61], it is probable that skin microbiota regulates susceptibility to food allergy.

Tregs play a prominent role in the regulation of oral tolerance by the microbiota. Tregs are induced by Clostridia species and other bacterial strains [68,71,72,75,76]. A subset of ROR γ t-expressing Tregs is induced by microbiota, and is required to suppress Th2 responses [76,77]. Vitamin A from the diet is required for the generation of ROR γ t+ Tregs [76]. Microbial generation of Tregs has been shown to occur by both MyD88-dependent and independent mechanisms [78], indicating pathways in addition to TLR recognition may be required. Microbial suppression of IgE production is also dependent on MyD88 signaling in B cells [79]. Non-TLR pathways include the generation by microbiota of short-chain fatty acids, in particular acetate and butyrate, induced by fermentation of dietary fiber [80–82]. High fiber diet in mice can protect from food allergy through the production of short-chain fatty acids and vitamin A metabolism [83]. Thus, there is a close association between diet and microbiota. Dietary factors can shape gut microbiota [84,85], and thereby Tregs and development of oral tolerance. Tregs in the intestine are dependent not only on stimulation from the microbiota, but antigens derived from the diet also are necessary for maintenance of intestinal Tregs [86].

Another mechanism of microbiota-induced tolerance is through maintenance of the integrity of intestinal epithelial barrier. Colonization of germ-free mice with Clostridia species upregulates IL-22 production by ROR γ t⁺ innate lymphoid cells and T cells in the lamina propria of the intestine, which contributes to a reduced permeability to oral antigens [68]. Antibody isotype class-switching to IgA is also induced by Clostridia colonization [68,71,72] which can further suppress antigen uptake.

Therapeutic application of immune tolerance

Induced tolerance, whether oral or by other routes, can effectively prevent immune responses including food allergy. There is a great deal of interest in determining whether the process of immune tolerance can suppress an existing immune response, which is critical for immune tolerance to be used therapeutically. Low dose allergen immunotherapy has been used for many years for venom and aeroallergens, and is actively being tested for the treatment of IgE-mediated food allergies using oral, sublingual, epicutaneous, and subcutaneous routes. A key question is whether allergen immunotherapy induces desensitization, a transient state of reduced clinical reactivity, or true immune tolerance. Clinical trials test for the presence of sustained unresponsiveness, defined as clinical protection after a period of discontinuation of treatment. There is disagreement about the length of treatment avoidance required to determine if true tolerance has occurred. We will review the evidence that immune pathways known to contribute to oral tolerance contribute to immunotherapy-induced clinical improvement in the context of food allergy.

Induction of Tregs versus anergy and deletion

While Tregs are required for oral tolerance, generation of Tregs during immunotherapy for food allergy is not clear. Reduction of allergen-specific Th2 cytokine production is observed after oral immunotherapy [87–89]. However, this has been mainly attributed to T cell anergy [90]. Using tetramers to identify antigen-specific CD4⁺ T cells and single-cell gene-expression profiling, Ryan and colleagues showed that in peanut-allergic individuals showing sustained tolerance after OIT, there was an expansion of anergic memory (low expression of CD28, Ki-67, CD69 and CD45RA) and non-allergic (high expression of CD27 and low expression of IL-4 and IL-13) antigen-specific CD4⁺ T cells [91], but no induction of antigen-specific Tregs. Other studies using proliferation-based approaches or examining bulk Tregs (identified as Foxp3⁺) have shown hypomethylation of Foxp3 leading to enhanced Foxp3 expression [92], and increased frequency of Foxp3⁺ T cells [88], in peanut-allergic patients in OIT-induced tolerance. Differences between studies in the finding of Treg induction could be related with the use of different strategies to identify antigen-specific T cells in patients. For example, tetramers may identify cells with high-affinity TCR interaction while proliferation-based assays may allow for a wider range of TCR affinities, as well as identification of bystander-activated T cells. A limitation of human studies is lack of access to tissue sites such as gastrointestinal mucosa, and studies in blood may not fully reflect immunotherapy-induced immune changes.

Preclinical models of food allergy and immunotherapy have identified several novel regulatory mechanisms contributing to therapeutic immune tolerance. Allergic mice have been shown to have impaired Treg generation in response to OIT [23,93]. One contributing mechanism of Treg suppression is IgE-mediated activation and IL-4 production by mast cells, and blockade of IgE during OIT can enhance generation of Tregs and clinical tolerance [93]. Susceptible *Il4raF709* mice have Th2 reprogrammed Tregs that produce IL-4 and contribute to disease, and transfer of healthy Tregs can suppress disease [94]. These results support the hypothesis that oral tolerance pathways are impaired in allergic patients receiving OIT, and suggest that additional immunomodulation is required for effective tolerance induction.

Induction of therapeutic tolerance to foods by epicutaneous or sublingual routes is also being explored. In mouse models, epicutaneous immunotherapy (EPIT) using Viaskin[®] patches is effective in inducing protection against allergic gastrointestinal inflammation and anaphylaxis [23,95], and EPIT can generate a population of gut-homing LAP⁺ Tregs that suppress mast cell activation and food-induced anaphylaxis by a TGF- β -dependent mechanism [23]. Alternative routes of immunotherapy that bypass the intestine may therefore be an effective strategy for generation of Tregs and induction of immune tolerance.

Role of antibodies in tolerance

The role of antibodies in immune tolerance is poorly understood. Mouse models of oral tolerance use the suppression of immunization-induced antibody and T cell responses as a read-out of tolerance. However, it is known that antigen feeding can induce the production of IgA antibodies that contribute to tolerance at mucosal sites through immune exclusion. Healthy human subjects often have detectable antigen-specific IgG to food allergens in the

absence of clinical symptoms [96]. Early introduction of peanut in children resulting in protection from peanut allergy was associated with a substantial increase of peanut-specific IgG4 [97], suggesting that IgGs could have an important role in development of oral tolerance. In response to allergen immunotherapy, there is also an elevation of allergen-specific IgA and IgG responses. Patients receiving peanut OIT have a polyclonal expansion and increased somatic mutation of IgG4 antibodies [98,99] indicating that an increased affinity of IgG antibodies could effectively block IgE binding. In addition to blocking mechanisms, IgG antibodies induced during OIT can actively suppress mast cell and basophil activation by acting on Fc γ R2 [100]. IgG antibodies can also suppress IgE-mediated IL-4 production by mast cells, thus releasing the brake on Treg generation [101].

Conclusion

Immune mechanisms including peripherally induced antigen-specific Tregs of various phenotypes, T cell anergy or deletion, and production of IgA and IgG antibodies contribute to the state of immune non-responsiveness known as oral tolerance. It is becoming evident that these same immune pathways can be induced by allergen immunotherapy, although the induction of tolerance is less effective when applied as a treatment than as prevention. New strategies focused on maximizing the regulatory environment in which oral tolerance is induced may improve the efficacy of immunotherapy treatments. Modulation of the microbiota to promote strains associated with gastrointestinal Tregs, production of short-chain fatty acids and increased barrier integrity could potentially generate a milieu prone to develop a tolerance response during immunotherapy. Alternative routes to oral exposure, such as the skin, may also be effective in bypassing the altered gut immune environment to induce a regulatory response in allergic patients.

References

1. Kraus TA, Toy L, Chan L, Childs J, Mayer L. Failure to induce oral tolerance to a soluble protein in patients with inflammatory bowel disease. *Gastroenterology*. 2004; 126(7):1771–1778. [PubMed: 15188172]
2. Husby S, Mestecky J, Moldoveanu Z, Holland S, Elson CO. Oral tolerance in humans. T cell but not B cell tolerance after antigen feeding. *J Immunol*. 1994; 152(9):4663–4670. [PubMed: 8157979]
3. Untersmayr E, Bakos N, Scholl I, Kundi M, Roth-Walter F, Szalai K, Riemer AB, Ankersmit HJ, Scheiner O, Boltz-Nitulescu G, Jensen-Jarolim E. Anti-ulcer drugs promote IgE formation toward dietary antigens in adult patients. *Faseb J*. 2005; 19(6):656–658. doi:04-3170fje [pii] 10.1096/fj.04-3170fje. [PubMed: 15671152]
4. Untersmayr E, Scholl I, Swoboda I, Beil WJ, Forster-Waldl E, Walter F, Riemer A, Kraml G, Kinaciyan T, Spitzauer S, Boltz-Nitulescu G, Scheiner O, Jensen-Jarolim E. Antacid medication inhibits digestion of dietary proteins and causes food allergy: a fish allergy model in BALB/c mice. *J Allergy Clin Immunol*. 2003; 112(3):616–623. [PubMed: 13679824]
5. Knoop KA, Miller MJ, Newberry RD. Transepithelial antigen delivery in the small intestine: different paths, different outcomes. *Curr Opin Gastroenterol*. 2013; 29(2):112–118. DOI: 10.1097/MOG.0b013e32835cf1cd [PubMed: 23380572]
6. Rios D, Wood MB, Li J, Chassaing B, Gewirtz AT, Williams IR. Antigen sampling by intestinal M cells is the principal pathway initiating mucosal IgA production to commensal enteric bacteria. *Mucosal Immunol*. 2016; 9(4):907–916. DOI: 10.1038/mi.2015.121 [PubMed: 26601902]
7. Mallegol J, Van Niel G, Lebreton C, Lepelletier Y, Candalf C, Dugave C, Heath JK, Raposo G, Cerf-Bensussan N, Heyman M. T84-intestinal epithelial exosomes bear MHC class II/peptide

- complexes potentiating antigen presentation by dendritic cells. *Gastroenterology*. 2007; 132(5): 1866–1876. DOI: 10.1053/j.gastro.2007.02.043 [PubMed: 17484880]
8. Menard S, Cerf-Bensussan N, Heyman M. Multiple facets of intestinal permeability and epithelial handling of dietary antigens. *Mucosal Immunol*. 2010; 3(3):247–259. DOI: 10.1038/mi.2010.5 [PubMed: 20404811]
 9. Perrier C, Corthesy B. Gut permeability and food allergies. *Clin Exp Allergy*. 2011; 41(1):20–28. DOI: 10.1111/j.1365-2222.2010.03639.x [PubMed: 21070397]
 10. Tordesillas L, Gomez-Casado C, Garrido-Arandia M, Murua-Garcia A, Palacin A, Varela J, Konieczna P, Cuesta-Herranz J, Akdis CA, O'Mahony L, Diaz-Perales A. Transport of Pru p 3 across gastrointestinal epithelium - an essential step towards the induction of food allergy? *Clin Exp Allergy*. 2013; 43(12):1374–1383. DOI: 10.1111/cea.12202 [PubMed: 24261947]
 11. McDole JR, Wheeler LW, McDonald KG, Wang B, Konjufca V, Knoop KA, Newberry RD, Miller MJ. Goblet cells deliver luminal antigen to CD103+ dendritic cells in the small intestine. *Nature*. 2012; 483(7389):345–349. doi:nature10863 [pii] 10.1038/nature10863. [PubMed: 22422267]
 12. Mazzini E, Massimiliano L, Penna G, Rescigno M. Oral tolerance can be established via gap junction transfer of fed antigens from CX3CR1(+) macrophages to CD103(+) dendritic cells. *Immunity*. 2014; 40(2):248–261. DOI: 10.1016/j.immuni.2013.12.012 [PubMed: 24462723]
 13. Rescigno M, Urbano M, Valzasina B, Francolini M, Rotta G, Bonasio R, Granucci F, Kraehenbuhl JP, Ricciardi-Castagnoli P. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nat Immunol*. 2001; 2(4):361–367. [PubMed: 11276208]
 14. Niess JH, Brand S, Gu X, Landsman L, Jung S, McCormick BA, Vyas JM, Boes M, Ploegh HL, Fox JG, Littman DR, Reinecker HC. CX3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance. *Science*. 2005; 307(5707):254–258. [PubMed: 15653504]
 15. Farache J, Koren I, Milo I, Gurevich I, Kim KW, Zigmund E, Furtado GC, Lira SA, Shakhar G. Luminal bacteria recruit CD103+ dendritic cells into the intestinal epithelium to sample bacterial antigens for presentation. *Immunity*. 2013; 38(3):581–595. DOI: 10.1016/j.immuni.2013.01.009 [PubMed: 23395676]
 16. Garside P, Steel M, Liew FY, Mowat AM. CD4+ but not CD8+ T cells are required for the induction of oral tolerance. *Int Immunol*. 1995; 7(3):501–504. [PubMed: 7794826]
 17. Chen Y, Kuchroo VK, Inobe J, Hafler DA, Weiner HL. Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. *Science*. 1994; 265(5176):1237–1240. [PubMed: 7520605]
 18. Mucida D, Kutchukhidze N, Erazo A, Russo M, Lafaille JJ, Curotto de Lafaille MA. Oral tolerance in the absence of naturally occurring Tregs. *J Clin Invest*. 2005; 115(7):1923–1933. [PubMed: 15937545]
 19. Curotto de Lafaille MA, Kutchukhidze N, Shen S, Ding Y, Yee H, Lafaille JJ. Adaptive Foxp3+ regulatory T cell-dependent and -independent control of allergic inflammation. *Immunity*. 2008; 29(1):114–126. [PubMed: 18617425]
 20. Torgerson TR, Linane A, Moes N, Anover S, Mateo V, Rieux-Laucat F, Hermine O, Vijay S, Gambineri E, Cerf-Bensussan N, Fischer A, Ochs HD, Goulet O, Ruemmele FM. Severe food allergy as a variant of IPEX syndrome caused by a deletion in a noncoding region of the FOXP3 gene. *Gastroenterology*. 2007; 132(5):1705–1717. doi:S0016-5085(07)00403-9 [pii] 10.1053/j.gastro.2007.02.044. [PubMed: 17484868]
 21. Hadis U, Wahl B, Schulz O, Hardtke-Wolenski M, Schippers A, Wagner N, Muller W, Sparwasser T, Forster R, Pabst O. Intestinal tolerance requires gut homing and expansion of FoxP3+ regulatory T cells in the lamina propria. *Immunity*. 2011; 34(2):237–246. DOI: 10.1016/j.immuni.2011.01.016 [PubMed: 21333554]
 22. Ochi H, Abraham M, Ishikawa H, Frenkel D, Yang K, Basso AS, Wu H, Chen ML, Gandhi R, Miller A, Maron R, Weiner HL. Oral CD3-specific antibody suppresses autoimmune encephalomyelitis by inducing CD4+ CD25– LAP+ T cells. *Nat Med*. 2006; 12(6):627–635. DOI: 10.1038/nm1408 [PubMed: 16715091]
 23. Tordesillas L, Mondoulet L, Blazquez AB, Benhamou PH, Sampson HA, Berin MC. Epicutaneous immunotherapy induces gastrointestinal LAP+ regulatory T cells and prevents food-induced anaphylaxis. *J Allergy Clin Immunol*. 2016; doi: 10.1016/j.jaci.2016.03.057

24. Carrier Y, Yuan J, Kuchroo VK, Weiner HL. Th3 cells in peripheral tolerance. I. Induction of Foxp3-positive regulatory T cells by Th3 cells derived from TGF-beta T cell-transgenic mice. *J Immunol.* 2007; 178(1):179–185. doi:178/1/179[pii]. [PubMed: 17182553]
25. Tran DQ, Andersson J, Wang R, Ramsey H, Unutmaz D, Shevach EM. GARP (LRRC32) is essential for the surface expression of latent TGF-beta on platelets and activated FOXP3+ regulatory T cells. *Proc Natl Acad Sci U S A.* 2009; 106(32):13445–13450. DOI: 10.1073/pnas.0901944106 [PubMed: 19651619]
26. Asseman C, Mauze S, Leach MW, Coffman RL, Powrie F. An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. *J Exp Med.* 1999; 190(7): 995–1004. [PubMed: 10510089]
27. Kuhn R, Lohler J, Rennick D, Rajewsky K, Muller W. Interleukin-10-deficient mice develop chronic enterocolitis. *Cell.* 1993; 75(2):263–274. [PubMed: 8402911]
28. Gonnella PA, Chen YH, Waldner H, Weiner HL. Induction of oral tolerization in CD86 deficient mice: a role for CD86 and B cells in the up-regulation of TGF-beta. *J Autoimmun.* 2006; 26(2): 73–81. doi:S0896-8411(05)00138-1 [pii] 10.1016/j.jaut.2005.10.003. [PubMed: 16439314]
29. Zhou Y, Kawasaki H, Hsu SC, Lee RT, Yao X, Plunkett B, Fu J, Yang K, Lee YC, Huang SK. Oral tolerance to food-induced systemic anaphylaxis mediated by the C-type lectin SIGNR1. *Nat Med.* 2010; 16(10):1128–1133. doi:nm.2201 [pii] 10.1038/nm.2201. [PubMed: 20835248]
30. Awasthi A, Carrier Y, Peron JP, Bettelli E, Kamanaka M, Flavell RA, Kuchroo VK, Oukka M, Weiner HL. A dominant function for interleukin 27 in generating interleukin 10-producing anti-inflammatory T cells. *Nat Immunol.* 2007; 8(12):1380–1389. DOI: 10.1038/ni1541 [PubMed: 17994022]
31. Chen Y, Inobe J, Kuchroo VK, Baron JL, Janeway CA Jr, Weiner HL. Oral tolerance in myelin basic protein T-cell receptor transgenic mice: suppression of autoimmune encephalomyelitis and dose-dependent induction of regulatory cells. *Proc Natl Acad Sci U S A.* 1996; 93(1):388–391. [PubMed: 8552644]
32. Chen Y, Inobe J, Marks R, Gonnella P, Kuchroo VK, Weiner HL. Peripheral deletion of antigen-reactive T cells in oral tolerance. *Nature.* 1995; 376(6536):177–180. [PubMed: 7603570]
33. Worbs T, Bode U, Yan S, Hoffmann MW, Hintzen G, Bernhardt G, Forster R, Pabst O. Oral tolerance originates in the intestinal immune system and relies on antigen carriage by dendritic cells. *J Exp Med.* 2006; 203(3):519–527. [PubMed: 16533884]
34. Kraus TA, Brimnes J, Muong C, Liu JH, Moran TM, Tappenden KA, Boros P, Mayer L. Induction of mucosal tolerance in Peyer's patch-deficient, ligated small bowel loops. *J Clin Invest.* 2005; 115(8):2234–2243. DOI: 10.1172/JCI19102 [PubMed: 16041410]
35. Spahn TW, Fontana A, Faria AM, Slavin AJ, Eugster HP, Zhang X, Koni PA, Ruddle NH, Flavell RA, Rennert PD, Weiner HL. Induction of oral tolerance to cellular immune responses in the absence of Peyer's patches. *Eur J Immunol.* 2001; 31(4):1278–1287. [PubMed: 11298355]
36. Benson MJ, Pino-Lagos K, Roseblatt M, Noelle RJ. All-trans retinoic acid mediates enhanced T reg cell growth, differentiation, and gut homing in the face of high levels of co-stimulation. *J Exp Med.* 2007; 204(8):1765–1774. DOI: 10.1084/jem.20070719 [PubMed: 17620363]
37. Coombes JL, Siddiqui KR, Arancibia-Carcamo CV, Hall J, Sun CM, Belkaid Y, Powrie F. A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. *J Exp Med.* 2007; 204(8):1757–1764. doi:jem.20070590 [pii] 10.1084/jem.20070590. [PubMed: 17620361]
38. Sun CM, Hall JA, Blank RB, Bouladoux N, Oukka M, Mora JR, Belkaid Y. Small intestine lamina propria dendritic cells promote de novo generation of Foxp3 T reg cells via retinoic acid. *J Exp Med.* 2007; 204(8):1775–1785. DOI: 10.1084/jem.20070602 [PubMed: 17620362]
39. Iwata M, Hirakiyama A, Eshima Y, Kagechika H, Kato C, Song SY. Retinoic acid imprints gut-homing specificity on T cells. *Immunity.* 2004; 21(4):527–538. doi:S107476130400247X [pii] 10.1016/j.immuni.2004.08.011. [PubMed: 15485630]
40. Hammerschmidt SI, Ahrendt M, Bode U, Wahl B, Kremmer E, Forster R, Pabst O. Stromal mesenteric lymph node cells are essential for the generation of gut-homing T cells in vivo. *J Exp Med.* 2008; 205(11):2483–2490. doi:jem.20080039 [pii] 10.1084/jem.20080039. [PubMed: 18852290]

41. Matteoli G, Mazzini E, Iliev ID, Mileti E, Fallarino F, Puccetti P, Chieppa M, Rescigno M. Gut CD103+ dendritic cells express indoleamine 2,3-dioxygenase which influences T regulatory/T effector cell balance and oral tolerance induction. *Gut*. 2010; 59(5):595–604. doi:59/5/595 [pii] 10.1136/gut.2009.185108. [PubMed: 20427394]
42. Cassani B, Villablanca EJ, Quintana FJ, Love PE, Lacy-Hulbert A, Blaner WS, Sparwasser T, Snapper SB, Weiner HL, Mora JR. Gut-Tropic T Cells That Express Integrin $\alpha 4\beta 7$ and CCR9 Are Required for Induction of Oral Immune Tolerance in Mice. *Gastroenterology*. 2011; 141(6):2109–2118. doi:S0016-5085(11)01249-2 [pii] 10.1053/j.gastro.2011.09.015. [PubMed: 21925467]
43. Esterhazy D, Loschko J, London M, Jove V, Oliveira TY, Mucida D. Classical dendritic cells are required for dietary antigen-mediated induction of peripheral T(reg) cells and tolerance. *Nat Immunol*. 2016; 17(5):545–555. DOI: 10.1038/ni.3408 [PubMed: 27019226]
44. Dubois B, Joubert G, Gomez de Agüero M, Gouanvic M, Goubier A, Kaiserlian D. Sequential role of plasmacytoid dendritic cells and regulatory T cells in oral tolerance. *Gastroenterology*. 2009; 137(3):1019–1028. doi:S0016-5085(09)00519-8 [pii] 10.1053/j.gastro.2009.03.055. [PubMed: 19345221]
45. Goubier A, Dubois B, Gheith H, Joubert G, Villard-Truc F, Asselin-Paturel C, Trinchieri G, Kaiserlian D. Plasmacytoid dendritic cells mediate oral tolerance. *Immunity*. 2008; 29(3):464–475. [PubMed: 18789731]
46. Palomares O, Ruckert B, Jartti T, Kucuksezzer UC, Puhakka T, Gomez E, Fahrner HB, Speiser A, Jung A, Kwok WW, Kalogjera L, Akdis M, Akdis CA. Induction and maintenance of allergen-specific FOXP3+ Treg cells in human tonsils as potential first-line organs of oral tolerance. *J Allergy Clin Immunol*. 2012; 129(2):510–520. 520 e511–519. DOI: 10.1016/j.jaci.2011.09.031 [PubMed: 22051696]
47. Mascarell L, Lombardi V, Louise A, Saint-Lu N, Chabre H, Moussu H, Betbeder D, Balazuc AM, Van Overtvelt L, Moingeon P. Oral dendritic cells mediate antigen-specific tolerance by stimulating TH1 and regulatory CD4+ T cells. *J Allergy Clin Immunol*. 2008; 122(3):603–609 e605. doi:S0091-6749(08)01185-8 [pii] 10.1016/j.jaci.2008.06.034. [PubMed: 18774396]
48. Shan M, Gentile M, Yeiser JR, Walland AC, Bornstein VU, Chen K, He B, Cassis L, Bigas A, Cols M, Comerma L, Huang B, Blander JM, Xiong H, Mayer L, Berin C, Augenlicht LH, Velcich A, Cerutti A. Mucus enhances gut homeostasis and oral tolerance by delivering immunoregulatory signals. *Science*. 2013; 342(6157):447–453. DOI: 10.1126/science.1237910 [PubMed: 24072822]
49. Mortha A, Chudnovskiy A, Hashimoto D, Bogunovic M, Spencer SP, Belkaid Y, Merad M. Microbiota-dependent crosstalk between macrophages and ILC3 promotes intestinal homeostasis. *Science*. 2014; 343(6178):1249288. doi: 10.1126/science.1249288 [PubMed: 24625929]
50. Mosconi E, Rekima A, Seitz-Polski B, Kanda A, Fleury S, Tissandie E, Monteiro R, Dombrowicz DD, Julia V, Glaichenhaus N, Verhasselt V. Breast milk immune complexes are potent inducers of oral tolerance in neonates and prevent asthma development. *Mucosal Immunol*. 2010; 3(5):461–474. doi:mi201023 [pii] 10.1038/mi.2010.23. [PubMed: 20485331]
51. Rekima A, Macchiaverni P, Turfkruyer M, Holvoet S, Dupuis L, Baiz N, Annesi-Maesano I, Mercenier A, Nutten S, Verhasselt V. Long-term reduction in food allergy susceptibility in mice by combining breastfeeding-induced tolerance and TGF-beta-enriched formula after weaning. *Clin Exp Allergy*. 2017; 47(4):565–576. DOI: 10.1111/cea.12864 [PubMed: 27883236]
52. Akbari O, DeKruyff RH, Umetsu DT. Pulmonary dendritic cells producing IL-10 mediate tolerance induced by respiratory exposure to antigen. *Nat Immunol*. 2001; 2(8):725–731. [PubMed: 11477409]
53. Wu HY, Quintana FJ, Weiner HL. Nasal anti-CD3 antibody ameliorates lupus by inducing an IL-10-secreting CD4+ CD25– LAP+ regulatory T cell and is associated with down-regulation of IL-17+ CD4+ ICOS+ CXCR5+ follicular helper T cells. *J Immunol*. 2008; 181(9):6038–6050. [PubMed: 18941193]
54. Duan W, So T, Croft M. Antagonism of airway tolerance by endotoxin/lipopolysaccharide through promoting OX40L and suppressing antigen-specific Foxp3+ T regulatory cells. *J Immunol*. 2008; 181(12):8650–8659. [PubMed: 19050285]

55. Duan W, So T, Mehta AK, Choi H, Croft M. Inducible CD4+LAP+Foxp3- regulatory T cells suppress allergic inflammation. *J Immunol.* 2011; 187(12):6499–6507. DOI: 10.4049/jimmunol.1101398 [PubMed: 22079987]
56. Ostroukhova M, Seguin-Devaux C, Oriss TB, Dixon-McCarthy B, Yang L, Ameredes BT, Corcoran TE, Ray A. Tolerance induced by inhaled antigen involves CD4(+) T cells expressing membrane-bound TGF-beta and FOXP3. *J Clin Invest.* 2004; 114(1):28–38. DOI: 10.1172/JCI20509 [PubMed: 15232609]
57. Vermaelen KY, Carro-Muino I, Lambrecht BN, Pauwels RA. Specific migratory dendritic cells rapidly transport antigen from the airways to the thoracic lymph nodes. *J Exp Med.* 2001; 193(1): 51–60. [PubMed: 11136820]
58. Hintzen G, Ohl L, del Rio ML, Rodriguez-Barbosa JI, Pabst O, Kocks JR, Kregge J, Hardtke S, Forster R. Induction of tolerance to innocuous inhaled antigen relies on a CCR7-dependent dendritic cell-mediated antigen transport to the bronchial lymph node. *J Immunol.* 2006; 177(10): 7346–7354. doi:177/10/7346[pii]. [PubMed: 17082654]
59. de Heer HJ, Hammad H, Soullie T, Hijdra D, Vos N, Willart MA, Hoogsteden HC, Lambrecht BN. Essential role of lung plasmacytoid dendritic cells in preventing asthmatic reactions to harmless inhaled antigen. *J Exp Med.* 2004; 200(1):89–98. [PubMed: 15238608]
60. Xanthou G, Alissafi T, Semitekolou M, Simoes DC, Economidou E, Gaga M, Lambrecht BN, Lloyd CM, Panoutsakopoulou V. Osteopontin has a crucial role in allergic airway disease through regulation of dendritic cell subsets. *Nat Med.* 2007; 13(5):570–578. DOI: 10.1038/nm1580 [PubMed: 17435770]
61. Tordesillas L, Berin MC, Sampson HA. Immunology of Food Allergy. *Immunity.* 2017; 47(1):32–50. DOI: 10.1016/j.immuni.2017.07.004 [PubMed: 28723552]
62. Dunkin D, Berin MC, Mayer L. Allergic sensitization can be induced via multiple physiologic routes in an adjuvant-dependent manner. *J Allergy Clin Immunol.* 2011; 128(6):1251–1258 e1252. DOI: 10.1016/j.jaci.2011.06.007 [PubMed: 21762973]
63. Strid J, Hourihane J, Kimber I, Callard R, Strobel S. Epicutaneous exposure to peanut protein prevents oral tolerance and enhances allergic sensitization. *Clin Exp Allergy.* 2005; 35(6):757–766. [PubMed: 15969667]
64. Tordesillas L, Goswami R, Benede S, Grishina G, Dunkin D, Jarvinen KM, Maleki SJ, Sampson HA, Berin MC. Skin exposure promotes a Th2-dependent sensitization to peanut allergens. *J Clin Invest.* 2014; 124(11):4965–4975. DOI: 10.1172/JCI75660 [PubMed: 25295541]
65. Li W, Zhang Z, Saxon A, Zhang K. Prevention of oral food allergy sensitization via skin application of food allergen in a mouse model. *Allergy.* 2012; 67(5):622–629. DOI: 10.1111/j.1398-9995.2012.02798.x [PubMed: 22339388]
66. Dunkin D, Berin MC, Mondoulet L, Yeretsian G, Tordesillas L, Iuga A, Larcher T, Guillespie V, Benhamou PH, Colombel JF, Sampson HA. Epicutaneous Tolerance Induction to a Bystander Antigen Abrogates Colitis and Ileitis in Mice. *Inflammatory Bowel Diseases.* 2017 In Press.
67. Cahenzli J, Koller Y, Wyss M, Geuking MB, McCoy KD. Intestinal microbial diversity during early-life colonization shapes long-term IgE levels. *Cell Host Microbe.* 2013; 14(5):559–570. DOI: 10.1016/j.chom.2013.10.004 [PubMed: 24237701]
68. Stefka AT, Feehley T, Tripathi P, Qiu J, McCoy K, Mazmanian SK, Tjota MY, Seo GY, Cao S, Theriault BR, Antonopoulos DA, Zhou L, Chang EB, Fu YX, Nagler CR. Commensal bacteria protect against food allergen sensitization. *Proc Natl Acad Sci U S A.* 2014; 111(36):13145–13150. DOI: 10.1073/pnas.1412008111 [PubMed: 25157157]
69. Noval Rivas M, Burton OT, Wise P, Zhang YQ, Hobson SA, Garcia Lloret M, Chehoud C, Kuczynski J, DeSantis T, Warrington J, Hyde ER, Petrosino JF, Gerber GK, Bry L, Oettgen HC, Mazmanian SK, Chatila TA. A microbiota signature associated with experimental food allergy promotes allergic sensitization and anaphylaxis. *J Allergy Clin Immunol.* 2013; 131(1):201–212. DOI: 10.1016/j.jaci.2012.10.026 [PubMed: 23201093]
70. Diesner SC, Bergmayr C, Pfitzner B, Assmann V, Krishnamurthy D, Starkl P, Endesfelder D, Rothballer M, Welzl G, Rattei T, Eiwegger T, Szeplausi Z, Fehrenbach H, Jensen-Jarolim E, Hartmann A, Pali-Scholl I, Untersmayr E. A distinct microbiota composition is associated with protection from food allergy in an oral mouse immunization model. *Clin Immunol.* 2016; 173:10–18. DOI: 10.1016/j.clim.2016.10.009 [PubMed: 27789346]

71. Atarashi K, Tanoue T, Oshima K, Suda W, Nagano Y, Nishikawa H, Fukuda S, Saito T, Narushima S, Hase K, Kim S, Fritz JV, Wilmes P, Ueha S, Matsushima K, Ohno H, Olle B, Sakaguchi S, Taniguchi T, Morita H, Hattori M, Honda K. Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. *Nature*. 2013; 500(7461):232–236. doi:nature12331 [pii] 10.1038/nature12331. [PubMed: 23842501]
72. Atarashi K, Tanoue T, Shima T, Imaoka A, Kuwahara T, Momose Y, Cheng G, Yamasaki S, Saito T, Ohba Y, Taniguchi T, Takeda K, Hori S, Ivanov II, Umesaki Y, Itoh K, Honda K. Induction of colonic regulatory T cells by indigenous Clostridium species. *Science*. 2011; 331(6015):337–341. doi:science.1198469 [pii] 10.1126/science.1198469. [PubMed: 21205640]
73. Rodriguez B, Prioult G, Bibiloni R, Nicolis I, Mercenier A, Butel MJ, Waligora-Dupriet AJ. Germ-free status and altered caecal subdominant microbiota are associated with a high susceptibility to cow's milk allergy in mice. *FEMS Microbiol Ecol*. 2011; 76(1):133–144. DOI: 10.1111/j.1574-6941.2010.01035.x [PubMed: 21223329]
74. Naik S, Bouladoux N, Linehan JL, Han SJ, Harrison OJ, Wilhelm C, Conlan S, Himmelfarb S, Byrd AL, Deming C, Quinones M, Brechley JM, Kong HH, Tussiwand R, Murphy KM, Merad M, Segre JA, Belkaid Y. Commensal-dendritic-cell interaction specifies a unique protective skin immune signature. *Nature*. 2015; 520(7545):104–108. DOI: 10.1038/nature14052 [PubMed: 25539086]
75. Faith JJ, Ahern PP, Ridaura VK, Cheng J, Gordon JL. Identifying gut microbe-host phenotype relationships using combinatorial communities in gnotobiotic mice. *Sci Transl Med*. 2014; 6(220):220ra211. doi: 10.1126/scitranslmed.3008051
76. Ohnmacht C, Park JH, Cording S, Wing JB, Atarashi K, Obata Y, Gaboriau-Routhiau V, Marques R, Dulauroy S, Fedoseeva M, Busslinger M, Cerf-Bensussan N, Boneca IG, Voehringer D, Hase K, Honda K, Sakaguchi S, Eberl G. MUCOSAL IMMUNOLOGY. The microbiota regulates type 2 immunity through RORgamma(+) T cells. *Science*. 2015; 349(6251):989–993. DOI: 10.1126/science.aac4263 [PubMed: 26160380]
77. Sefik E, Geva-Zatorsky N, Oh S, Konnikova L, Zemmour D, McGuire AM, Burzyn D, Ortiz-Lopez A, Lobera M, Yang J, Ghosh S, Earl A, Snapper SB, Jupp R, Kasper D, Mathis D, Benoist C. MUCOSAL IMMUNOLOGY. Individual intestinal symbionts induce a distinct population of RORgamma(+) regulatory T cells. *Science*. 2015; 349(6251):993–997. DOI: 10.1126/science.aaa9420 [PubMed: 26272906]
78. Wang S, Charbonnier LM, Noval Rivas M, Georgiev P, Li N, Gerber G, Bry L, Chatila TA. MyD88 Adaptor-Dependent Microbial Sensing by Regulatory T Cells Promotes Mucosal Tolerance and Enforces Commensalism. *Immunity*. 2015; 43(2):289–303. DOI: 10.1016/j.immuni.2015.06.014 [PubMed: 26231118]
79. Hill DA, Siracusa MC, Abt MC, Kim BS, Kobuley D, Kubo M, Kambayashi T, Larosa DF, Renner ED, Orange JS, Bushman FD, Artis D. Commensal bacteria-derived signals regulate basophil hematopoiesis and allergic inflammation. *Nat Med*. 2012; 18(4):538–546. doi:nm.2657 [pii] 10.1038/nm.2657. [PubMed: 22447074]
80. Arpaia N, Campbell C, Fan X, Dikiy S, van der Veeken J, deRoos P, Liu H, Cross JR, Pfeffer K, Coffey PJ, Rudensky AY. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature*. 2013; 504(7480):451–455. DOI: 10.1038/nature12726 [PubMed: 24226773]
81. Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, Nakanishi Y, Uetake C, Kato K, Kato T, Takahashi M, Fukuda NN, Murakami S, Miyauchi E, Hino S, Atarashi K, Onawa S, Fujimura Y, Lockett T, Clarke JM, Topping DL, Tomita M, Hori S, Ohara O, Morita T, Koseki H, Kikuchi J, Honda K, Hase K, Ohno H. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature*. 2013; 504(7480):446–450. DOI: 10.1038/nature12721 [PubMed: 24226770]
82. Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly YM, Glickman JN, Garrett WS. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science*. 2013; 341(6145):569–573. DOI: 10.1126/science.1241165 [PubMed: 23828891]
83. Tan J, McKenzie C, Vuillermin PJ, Govere G, Vinuesa CG, Mebius RE, Macia L, Mackay CR. Dietary Fiber and Bacterial SCFA Enhance Oral Tolerance and Protect against Food Allergy

- through Diverse Cellular Pathways. *Cell Rep.* 2016; 15(12):2809–2824. DOI: 10.1016/j.celrep.2016.05.047 [PubMed: 27332875]
84. De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, Collini S, Pieraccini G, Lionetti P. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc Natl Acad Sci U S A.* 2010; 107(33):14691–14696. DOI: 10.1073/pnas.1005963107 [PubMed: 20679230]
 85. Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA, Bewtra M, Knights D, Walters WA, Knight R, Sinha R, Gilroy E, Gupta K, Baldassano R, Nessel L, Li H, Bushman FD, Lewis JD. Linking long-term dietary patterns with gut microbial enterotypes. *Science.* 2011; 334(6052):105–108. DOI: 10.1126/science.1208344 [PubMed: 21885731]
 86. Kim KS, Hong SW, Han D, Yi J, Jung J, Yang BG, Lee JY, Lee M, Surh CD. Dietary antigens limit mucosal immunity by inducing regulatory T cells in the small intestine. *Science.* 2016; 351(6275):858–863. DOI: 10.1126/science.aac5560 [PubMed: 26822607]
 87. Gorelik M, Narisety SD, Guerrero AL, Chichester KL, Keet CA, Bieneman AP, Hamilton RG, Wood RA, Schroeder JT, Frischmeyer-Guerrero PA. Suppression of the immunologic response to peanut during immunotherapy is often transient. *J Allergy Clin Immunol.* 2015; 135(5):1283–1292. DOI: 10.1016/j.jaci.2014.11.010 [PubMed: 25542883]
 88. Varshney P, Jones SM, Scurlock AM, Perry TT, Kemper A, Steele P, Hiegel A, Kamilaris J, Carlisle S, Yue X, Kulis M, Pons L, Vickery B, Burks AW. A randomized controlled study of peanut oral immunotherapy: clinical desensitization and modulation of the allergic response. *J Allergy Clin Immunol.* 2011; 127(3):654–660. doi:S0091-6749(11)00050-9 [pii] 10.1016/j.jaci.2010.12.1111. [PubMed: 21377034]
 89. Perezabad L, Reche M, Valbuena T, Lopez-Fandino R, Molina E, Lopez-Exposito I. Oral Food Desensitization in Children With IgE-Mediated Cow's Milk Allergy: Immunological Changes Underlying Desensitization. *Allergy Asthma Immunol Res.* 2017; 9(1):35–42. DOI: 10.4168/aair.2017.9.1.35 [PubMed: 27826960]
 90. Bedoret D, Singh AK, Shaw V, Hoyte EG, Hamilton R, DeKruyff RH, Schneider LC, Nadeau KC, Umetsu DT. Changes in antigen-specific T-cell number and function during oral desensitization in cow's milk allergy enabled with omalizumab. *Mucosal Immunol.* 2012; 5(3):267–276. DOI: 10.1038/mi.2012.5 [PubMed: 22318492]
 91. Ryan JF, Hovde R, Glanville J, Lyu SC, Ji X, Gupta S, Tibshirani RJ, Jay DC, Boyd SD, Chinthrajah RS, Davis MM, Galli SJ, Maecker HT, Nadeau KC. Successful immunotherapy induces previously unidentified allergen-specific CD4+ T-cell subsets. *Proc Natl Acad Sci U S A.* 2016; 113(9):E1286–1295. DOI: 10.1073/pnas.1520180113 [PubMed: 26811452]
 92. Syed A, Garcia MA, Lyu SC, Bucayu R, Kohli A, Ishida S, Berglund JP, Tsai M, Maecker H, O'Riordan G, Galli SJ, Nadeau KC. Peanut oral immunotherapy results in increased antigen-induced regulatory T-cell function and hypomethylation of forkhead box protein 3 (FOXP3). *J Allergy Clin Immunol.* 2014; 133(2):500–510 e511. DOI: 10.1016/j.jaci.2013.12.1037 [PubMed: 24636474]
 93. Burton OT, Noval Rivas M, Zhou JS, Logsdon SL, Darling AR, Koleoglou KJ, Roers A, Houshyar H, Crackower MA, Chatila TA, Oettgen HC. Immunoglobulin E signal inhibition during allergen ingestion leads to reversal of established food allergy and induction of regulatory T cells. *Immunity.* 2014; 41(1):141–151. DOI: 10.1016/j.immuni.2014.05.017 [PubMed: 25017467]
 94. Noval Rivas M, Burton OT, Wise P, Charbonnier LM, Georgiev P, Oettgen HC, Rachid R, Chatila TA. Regulatory T Cell Reprogramming toward a Th2-Cell-like Lineage Impairs Oral Tolerance and Promotes Food Allergy. *Immunity.* 2015; 42(3):512–523. DOI: 10.1016/j.immuni.2015.02.004 [PubMed: 25769611]
 95. Mondoulet L, Dioszeghy V, Puteaux E, Ligouis M, Dhelft V, Plaquet C, Dupont C, Benhamou PH. Specific epicutaneous immunotherapy prevents sensitization to new allergens in a murine model. *J Allergy Clin Immunol.* 2015; 135(6):1546–1557 e1544. DOI: 10.1016/j.jaci.2014.11.028 [PubMed: 25583102]
 96. Husby S, Oxelius VA, Teisner B, Jensenius JC, Svehag SE. Humoral immunity to dietary antigens in healthy adults. Occurrence, isotype and IgG subclass distribution of serum antibodies to protein antigens. *Int Arch Allergy Appl Immunol.* 1985; 77(4):416–422. [PubMed: 4018884]

97. Du Toit G, Roberts G, Sayre PH, Bahnson HT, Radulovic S, Santos AF, Brough HA, Phippard D, Basting M, Feeney M, Turcanu V, Sever ML, Gomez Lorenzo M, Plaut M, Lack G, Team LS. Randomized trial of peanut consumption in infants at risk for peanut allergy. *N Engl J Med*. 2015; 372(9):803–813. DOI: 10.1056/NEJMoa1414850 [PubMed: 25705822]
98. Hoh RA, Joshi SA, Liu Y, Wang C, Roskin KM, Lee JY, Pham T, Looney TJ, Jackson KJL, Dixit VP, King J, Lyu SC, Jenks J, Hamilton RG, Nadeau KC, Boyd SD. Single B-cell deconvolution of peanut-specific antibody responses in allergic patients. *J Allergy Clin Immunol*. 2016; 137(1):157–167. DOI: 10.1016/j.jaci.2015.05.029 [PubMed: 26152318]
99. Vickery BP, Lin J, Kulis M, Fu Z, Steele PH, Jones SM, Scurlock AM, Gimenez G, Bardina L, Sampson HA, Burks AW. Peanut oral immunotherapy modifies IgE and IgG4 responses to major peanut allergens. *J Allergy Clin Immunol*. 2013; 131(1):128–134. e121–123. doi:S0091-6749(12)01772-1 [pii] 10.1016/j.jaci.2012.10.048. [PubMed: 23199605]
100. Burton OT, Logsdon SL, Zhou JS, Medina-Tamayo J, Abdel-Gadir A, Noval Rivas M, Koleoglou KJ, Chatila TA, Schneider LC, Rachid R, Umetsu DT, Oettgen HC. Oral immunotherapy induces IgG antibodies that act through FcγRIIb to suppress IgE-mediated hypersensitivity. *J Allergy Clin Immunol*. 2014; 134(6):1310–1317 e1316. DOI: 10.1016/j.jaci.2014.05.042 [PubMed: 25042981]
101. Burton OT, Tamayo JM, Stranks AJ, Koleoglou KJ, Oettgen HC. Allergen-specific IgG antibody signaling through FcγRIIb promotes food tolerance. *J Allergy Clin Immunol*. 2017; doi: 10.1016/j.jaci.2017.03.045

Figure 1. Transport of dietary antigen through the intestinal epithelium

Soluble antigen that reaches the small intestine is absorbed by enterocytes (transcellular route). Antigen is usually degraded into small peptides inside vesicles. Peptides can be loaded into MHC-II in endosomes and be released to be taken up by CD103⁺ DCs. Antigens with resistance to proteolysis can reach the basolateral membrane in an intact form. Inflammation increases permeability and paracellular transport of soluble antigen. Goblet cells absorb antigens through goblet cell-associated antigen passages for delivery to CD103⁺ DCs. CXCR1⁺ macrophages extend their dendrites through the intestinal epithelium to sample antigen from the lumen, and transfer antigen to CD103⁺ for transport to the MLN. Particulate antigen, such as bacteria or virus, is taken up through M cells that overlie Peyer's patches.

Figure 2. Mechanism of oral tolerance

Dietary antigen is transported to the MLN by CD103+ DCs, that express high levels of RALDH, IDO, and TGF- β , facilitating naïve T cells to differentiate to Tregs. Tregs express gut-homing markers CCR9 and $\alpha_4\beta_7$, and migrate back to the lamina propria. There, expansion of Tregs is induced by high levels of IL-10 produced by macrophages. GM-CSF produced by ILC3 also contributes to homeostasis of Tregs, by acting on DCs and macrophages. GM-CSF production is dependent on microbiota signals, in a mechanism mediated by IL1- β production by macrophages. Absorbed antigen also reaches the liver through the portal vein, where it is presented by plasmacytoid DCs, that induce deletion of antigen-specific T cells. Deletion and anergy are also induced in the MLN in response to high dose of antigen.