

Mechanisms of allergic diseases

Series editors: Joshua A. Boyce, MD, Fred Finkelman, MD, William T. Shearer, MD, PhD, and Donata Vercelli, MD

Mechanisms of immune tolerance relevant to food allergy

Brian P. Vickery, MD,^a Amy M. Scurlock, MD,^b Stacie M. Jones, MD,^b and A. Wesley Burks, MD^a Durham, NC, and Little Rock, Ark

The intestine has an unenviable task: to identify and respond to a constant barrage of environmental stimuli that can be both dangerous and beneficial. The proper execution of this task is central to the homeostasis of the host, and as a result, the gastrointestinal tract contains more lymphocytes than any other tissue compartment in the body, as well as unique antigen-presenting cells with specialized functions. When antigen is initially encountered through the gut, this system generates a robust T cell-mediated hyporesponsiveness called oral tolerance. Although seminal observations of oral tolerance were made a century ago, the relevant mechanisms are only beginning to be unraveled with the use of modern investigational techniques. Food allergy is among the clinical disorders that occur from a failure of this system, and therapies that seek to re-establish tolerance are currently under investigation. (J Allergy Clin Immunol 2011;■■■:■■■-■■■.)

Key words: Oral tolerance, regulatory T cell, forkhead box protein 3, dendritic cell, food allergy, microbiota, vitamin A, TGF- β , CD103

Food allergy, which is hypothesized to result from a defect in oral tolerance, is a common, serious, and growing problem in

Abbreviations used

APC: Antigen-presenting cell
DC: Dendritic cell
Foxp3: Forkhead box protein 3
iTreg: Inducible regulatory T
MALT: Mucosa-associated lymphoid tissue
MLN: Mesenteric lymph node
RA: Retinoic acid
Treg: Regulatory T
TSLP: Thymic stromal lymphopoietin

developed countries. Patients with food allergy have pathological immune responses to ingested food antigens and can rapidly experience harmful adverse symptoms on re-exposure. Although a recent meta-analysis identified variation in prevalence rates,¹ recent survey data from the Centers for Disease Control and Prevention indicate that the current prevalence of food allergy in US children is approximately 4%, an increase of almost 20% in the last decade.² Increases in food allergy prevalence have also been observed in methodologically rigorous birth cohort studies, which use precise sampling techniques and well-defined outcome measures, suggesting that increasing prevalence is not simply caused by self-diagnosis or increased recognition of the disorder.³ Similar trends in the prevalence of asthma, allergic rhinitis, and atopic dermatitis support the general concept that atopic diseases are increasingly common.⁴

Interestingly, the likelihood that spontaneous clinical tolerance will develop in persons with food allergy varies depending on the allergen. In general, resolution of allergy to egg, milk, wheat, and soy can be expected, although sensitivity can persist into the second decade of life, which is longer than previously appreciated. In contrast, most patients allergic to peanut, tree nuts, and seafood will not outgrow their disease and must maintain strict elimination diets. The natural history of allergy to other important proteins, such as sesame and mustard, is largely unknown. In addition, current diagnostic testing for food allergy cannot predict a patient's risk for anaphylaxis or determine a subject's threshold dose to trigger symptoms. Therefore affected subjects and families must be constantly vigilant to avoid inadvertent exposure; however, accidental ingestions frequently occur even in the most cautious patients.⁵

The inability to completely eliminate the possibility of anaphylaxis and the associated limitations in everyday activities are great sources of uncertainty and stress on affected families. Over time, health-related quality of life can be adversely affected; in some families the degree of impairment is similar to that seen with other serious chronic diseases of childhood, such as type 1 diabetes or ventilator-dependent chronic respiratory failure.⁶ Although

From ^athe Division of Pediatric Allergy and Immunology, Duke University School of Medicine, Durham; and ^bthe Division of Pediatric Allergy and Immunology, the University of Arkansas for Medical Sciences, Little Rock.

Supported by the Food Allergy & Anaphylaxis Network; the Food Allergy Project; the Gerber Foundation; National Institutes of Health (NIH) grant 1 R01-AI06874-01A1, NIH T32 training grant, and NIH grant 1 UL1 RR024128-01 from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH); the NIH Roadmap for Medical Research (contents are solely the responsibility of the authors and do not necessarily represent the official view of NCRR or NIH); the Dorothy & Frank Robins Family; and the National Peanut Board.

Disclosure of potential conflict of interest: B. P. Vickery has received research support from the National Institutes of Health (NIH)/National Institute of Allergy and Infectious Diseases (NIAID), the Thrasher Research Fund, and Cephalon. A. M. Scurlock has received research support from the NIH. S. M. Jones has received research support from the National Peanut Board, NIH/NIAID, DYAX Corp, and the Food Allergy Initiative and is on the medical advisory board of the Food Allergy and Anaphylaxis Network (FAAN) and the steering committee of Sanofi-Aventis. A. W. Burks has consulted for ActoGenix NV, Intelliject, McNeil Nutritionals, Novartis, Pfizer, and Schering-Plough; is a minority stockholder in Allertein and MastCell, Inc; has served on an advisory board for Dannon Co Probiotics and an expert panel for Nutricia; has received research support from the NIH, FAAN, and the Wallace Research Foundation; has provided legal consultation or expert witness testimony on the topic of food allergy; and has served on the medical board of directors of FAAN.

Received for publication September 21, 2010; revised December 3, 2010; accepted for publication December 20, 2010.

Reprint requests: Brian P. Vickery, MD, DUMC Box 2644, Durham NC 27710. E-mail: brian.vickery@duke.edu.

0091-6749/\$36.00

© 2011 American Academy of Allergy, Asthma & Immunology

doi:10.1016/j.jaci.2010.12.1116

Terms in boldface and italics are defined in the glossary on page ■■■.

the effect 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, is constantly exposed to an enormous array of exogenous antigens, including commensal bacteria and ingested proteins.⁷ A single epithelial layer separates this antigenic load from the lymphocytes, **antigen-presenting cells** (APCs), stromal cells, and other immune cells in the lamina propria that together comprise the mucosa-associated lymphoid tissue (MALT). Within the MALT, unique populations of dendritic cells (DCs) interact with dietary antigens and determine the fate of the resulting adaptive response (ie, immunity vs tolerance).⁸ In this context immune tolerance is defined as the antigen-specific suppression of cellular or humoral immune responses. When the initial antigen exposure is mediated through the gastrointestinal tract, a robust T cell-mediated suppression develops called oral tolerance.⁹

This review will focus on the relationship between the component parts of the complex gastrointestinal mucosal immune system and IgE-mediated food allergy. Because most of the evidence with regard to immune tolerance to dietary antigens is derived from experimental animals, we will primarily discuss these model systems. Where possible, we will review the evidence for similar phenomena in human biology and the relevant applications for clinical medicine.

PHYSICAL FACTORS OF DIETARY ANTIGENS

After ingestion, dietary proteins undergo digestion by enzymes in the saliva and stomach, as well as by gastric acid. This

TABLE I. Biochemical factors that promote allergenicity

Molecular weight <70 kd
Glycosylation
Resistance to thermal or chemical denaturation
Abundance in food source
Linear epitopes
Solubility in water

processing results in reduced protein immunogenicity, likely through the destruction of conformational epitopes. However, specific biochemical properties common among different food groups confer resistance to this physical and chemical degradation, which collectively maintain the allergenicity of these proteins on reaching the small intestine (Table I). Additional factors that disrupt normal digestion, such as coadministration of antacids, have been shown in animal models to result in a breakdown in oral tolerance induction.¹⁰ Other features of mucosal defense include a hydrophobic layer of **mucin oligosaccharides**, which trap antigen, and both constitutive (ie, human β -defensin 1) and inducible (LL-37 and human β -defensin 2 and 3) antimicrobial peptides.

Secretory IgA has generally been considered to provide important tolerogenic function by binding to luminal antigens and preventing absorption (ie, “immune exclusion”), although its specific importance has been controversial.¹¹ A recent study showed that mice deficient in the receptor that secretes IgA and IgM into the intestinal lumen are hypersensitive to IgG-mediated anaphylaxis; nonetheless, they can be tolerized by an oral feed before systemic priming.¹² In this model tolerance

GLOSSARY

ANTIGEN-PRESENTING CELLS (APCS): Professional APCs are effective at endocytosing foreign antigens and displaying antigen to T cells in the context of MHC. Classically, macrophages, DCs, and B cells are professional APCs and express costimulatory molecules both constitutively and on stimulation. Nonprofessional APCs require stimulation before expression of MHC class II and include activated epithelial and endothelial cells.

MUCIN OLIGOSACCHARIDES: Intestinal mucins are primarily cell-surface mucins (MUC1, 3A, 3B, 4, 12, 13, and 17) that require glycosylation and sulfonation for proper assembly and function. Abnormalities in Muc1 and Muc2 are associated with gastritis, increased bacterial overgrowth, and colitis.

NATURAL KILLER T (NKT): NKT cells are specialized $\alpha\beta$ T cells that recognize glycolipid antigens that are CD1d restricted. The function of NKT cells in human allergic diseases is unclear.

OCCUDIN, CLAUDIN, AND JAM-ZO1: Tight junctions are important for organizing epithelial polarity and for epithelial integrity. Tight junction proteins include the transmembrane proteins occludins, claudins, junctional adhesion molecules (JAMs), and crumb. Some intestinal pathogens, including *Helicobacter pylori* and *Shigella* species, increase mucosal permeability by interfering with tight junction protein function and location.

PEYER PATCHES: One part of the gut-associated lymphoid tissue (GALT), Peyer patches are lymphoid follicles that are found primarily in the distal small intestine. Peyer patches are located in the intestinal wall directly underneath microfold cells, where they are positioned to directly encounter soluble antigens from the lumen. Other parts of the GALT include lamina propria T and B cells, intraepithelial lymphocytes, and the MLNs.

PLASMACYTOID DCs: DC subsets include myeloid and plasmacytoid DCs. Plasmacytoid DCs express Toll-like receptors 7 and 9 and express IFN- α .

RETINOIC ACID: Nuclear steroid receptors, peroxisome proliferator-activated receptor (PPAR) and retinoic acid receptors (RARs), both bind RA, which is derived from vitamin A. In the intestinal tract dietary retinoic acid is an important cofactor used by DCs to promote the development of Foxp3⁺ Treg cells.

REGULATORY T CELLS: Natural Treg helper cells are thymically derived CD4⁺ cells that generally express TGF- β and Foxp3 and provide specific immunosuppression primarily to self-antigens. Adaptive Treg cells, also known as T_H1 or T_H3 cells, are CD4⁺ T cells that acquire functionally suppressive properties after exposure to exogenous antigens in the periphery.

16s RIBOSOMAL RNA: Because 16s Ribosomal RNA is unique among species, sequencing of these subunits from bacteria is the molecular platform for analyzing the variability and influence of the microbiome on organ-specific disease states (eg, the intestinal tract, oropharynx, and respiratory tract).

THYMIC STROMAL LYMPHOPOEITIN (TSLP): TSLP is an IL-7-like cytokine expressed in activated epithelium, which promotes antigen presentation by DCs through the induction of second signal molecules, such as OX40, CD40, and CD80. These signals lead to the “inflammatory T_H2” response and the development of allergic inflammation.

$\gamma\delta$ T CELLS: $\gamma\delta$ T cells recognize nonpeptide antigens that can be upregulated on cells because of stress and that resemble pathogen-associated molecular patterns/danger-associated molecular patterns. $\gamma\delta$ T cells might be important in maintaining intestinal epithelial integrity because the lack of $\gamma\delta$ T cells decreases tight junction protein phosphorylation (claudin and occludin) and loss of epithelial integrity with increased pathogen translocation.

The Editors wish to acknowledge Seema Aceves, MD, PhD, for preparing this glossary.

was transferrable by CD4⁺CD25⁺ splenocytes, suggesting that cellular mechanisms can compensate for an impaired immune exclusion mechanism. However, a recent case-control study from a larger placebo-controlled trial examining probiotics for allergy prevention in high-risk infants showed that the risk of atopy was inversely correlated with fecal IgA levels.¹³ These data serve as one example of the complex and complementary forces that act to suppress immunity in the gut.

Gastrointestinal epithelial dysfunction has been thought to contribute to food hypersensitivity through both increased sensitization caused by a leaky barrier and the heightened T_H2 effector response that ensues. Epithelial junction complexes, also called adherens junctions, and tight junctions provide structural integrity to the gut barrier.¹⁴ However, this barrier is not completely formed at birth, and although not well understood, structural integrity might take time to fully develop.⁷ In mice the permeability of this barrier is further influenced by exposures to microbial pathogens, such as viruses, alcohol, nonsteroidal anti-inflammatory drugs, and other toxins, as well as cytokines (eg, IL-9), immune cells, and apoptotic pathways. These environmental exposures ultimately result in changes in gene expression and phosphorylation of tight junction proteins, such as *occludins*, *claudins*, and *JAM-ZOI* proteins, which in turn are associated with changes in intestinal mast cells and allergic sensitization.^{15,16}

Interestingly, intestinal permeability was assessed in infants with food allergy by calculating the urinary ratio after ingestion of freely diffusible lactulose and normally unabsorbed mannitol.¹⁷ Infants with food allergy were noted to have a lower ratio, indicating increased intestinal permeability, when compared with normal healthy young children. Investigators examined this ratio in children who had been on an allergen-free diet for at least 6 months and determined that intestinal permeability remained increased in children with food allergy, despite the absence of food allergen stimulation. Further evidence linking gastrointestinal epithelial barrier dysfunction and food allergy comes from studies in immunosuppressed patients after solid-organ transplantation who had food allergy while receiving systemic calcineurin inhibitors. Initially, investigators assumed this allergy was the result of transfer of sensitized donor lymphocytes. However, it is now theorized that medication-induced decreases in cellular ATP levels altered the integrity of junctional complexes, resulting in increased intestinal permeability.¹⁸

Mutations in the gene encoding filaggrin also lead to profound epidermal barrier dysfunction and are highly prevalent in patients with atopic dermatitis, which is in turn associated with an increased prevalence of food allergy. Acquired barrier defects associated with decreased filaggrin expression have been observed in the esophagi of patients with eosinophilic esophagitis and are thought to be secondary to IL-13.¹⁹ However, no studies to date have examined the mechanistic relationship of filaggrin mutations to IgE priming in the gut or clinical food allergy.²⁰

Increasing evidence suggests that the mucosal epithelium is likely to be more involved in tolerance than simply by acting as a physical barrier. Epithelial cells are known to express MHC class II molecules on their basolateral membranes and thus might act as nonprofessional APCs, which do not express conventional costimulatory molecules, favoring anergy.²¹ In addition, factors derived from the gut epithelium are generally believed to condition the DCs in the stroma, dampening immune responses and promoting gut homeostasis.²²

One such factor, constitutively expressed by the gut epithelium, is *thymic stromal lymphopoietin* (TSLP). TSLP is an IL-7–like cytokine that has been shown to activate expression of OX40 ligand on DCs and drive T_H2 differentiation. Thus TSLP is a critical mediator of allergic inflammation in the lung and skin. By contrast, in the gut TSLP appears to play a regulatory role, limiting deleterious T_H1 and T_H17 inflammation in models of helminth infection and colitis.²³ Although incompletely understood, this regulation can occur at the level of the DC, which expresses the TSLP receptor and has been shown to develop tolerogenic properties after TSLP exposure. Interestingly, regulatory responses to dietary allergens are evidently normal in TSLP receptor-deficient animals.²⁴ These findings suggest that although allergic sensitization in the gut might be mediated or regulated through TSLP, it is not required for oral tolerance. Little is known about the role of TSLP in food sensitization in human subjects. However, a recent study identified TSLP gene expression in the esophagi of patients with eosinophilic esophagitis and showed that genetic variants in TSLP and the TSLP receptor are associated with the disease.²⁵

IMPORTANCE OF ANTIGEN DOSE AND TIMING

Mucosal responses to soluble protein antigens early in life tend to be T_H2 biased, which has led to the general idea that this response occurs by default in both animals and human subjects.²⁶ Genetics plays a clear role in murine models, in which certain strains have exaggerated T_H2 bias, whereas others tend to be resistant to sensitization.²⁷ More recent evidence supports that impairment in *regulatory T cell* (Treg cell) induction, and innate immunity might also contribute to T_H2 polarization in early life.²⁸ Although these findings might be expected in atopic infants, prospective birth cohort studies have shown that IgE production to egg, milk, and peanut commonly occurs, even in healthy infants.²⁹ In nonallergic subjects this T_H2 bias appears to be transient, and IgE levels decrease, possibly through a counterbalancing induction of antigen-specific T_H1 responses (ie, IFN- γ); in contrast, these T_H2 responses consolidate and strengthen in allergic children, perhaps through induction of IL-4 signaling.³⁰

In murine models high-dose exposure to antigen in early life, even a single isolated dose, can produce lymphocyte anergy, whereas low-dose exposure, especially when repeated, induces Treg cell development.³¹ Interestingly, the differences in the actual dosing in these studies is quite small. Emerging evidence in human disease suggests that exposure to the proper dose of antigen during this critical period in early life is important for the shaping of the appropriate immune response to foods. Several epidemiologic studies have implicated delayed introduction in the increased prevalence of peanut allergy.^{32,33} Similarly, there is evidence that delayed introduction of cereals is associated with a higher risk of wheat allergy,³⁴ although methodological limitations in retrospective studies make definitive conclusions difficult. Recently, European and American guidelines for the introduction of potentially allergenic solid foods were revised to reflect the position that insufficient high-quality evidence exists to support delayed weaning as a preventative (ie, tolerogenic) strategy.^{35,36} However, early introduction is not automatically better because mature immune regulation might require time. In a set of classic experiments, Strobel and Ferguson³⁷ showed that immunologic priming is enhanced in neonatal

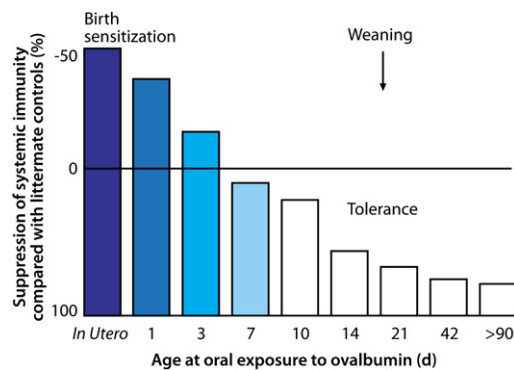


FIG 1. Immunity but not tolerance occurs after allergen exposure in early life. Neonatal mouse pups were exposed to ovalbumin through intra-amniotic injection 24 to 36 hours before birth or fed ovalbumin (1 mg/g body weight) or saline at days 1, 3, 7, 14, or 42. When rechallenged, animals exposed to ovalbumin before the seventh day of life did not have tolerance but instead robust humoral and cell-mediated immune responses, which persisted up to 14 weeks. Although it had long been known that tolerance was the default response to oral antigen administration in adult mice, these experiments demonstrated that oral exposure in early life could result in active immunologic priming rather than suppression.

mouse pups fed antigen in the first few days of life, whereas tolerance develops only after waiting 7 to 10 days to introduce antigen (Fig 1); if and how this “window” of priming versus tolerance translates to human subjects is unknown. Cow’s milk is typically the first potentially allergenic exposure, often occurring early in life, and yet cow’s milk is by far the most common food to which children are allergic.³¹ Although oral tolerance has been shown to occur across a range of doses, frequent or continuous exposure to relatively low doses typically results in robust oral tolerance induction. Defining the most appropriate time and dose for tolerance induction in human subjects is a great research need. Interventional studies are underway to investigate the importance of early-life oral exposure in tolerance development.

THE MICROBIOME AND ORAL TOLERANCE

Another critical influence on the gastrointestinal mucosal immune response is the microbial stimulation provided by the enteric flora, which by adulthood number approximately 100 trillion in the large intestine, providing essential nutritional and immunologic benefits.³⁸ Previous studies of germ-free mice demonstrated that colonization of the gastrointestinal tract is required for proper organization and development of both mucosal and secondary lymphoid tissues. These animals have impaired antibody responses and do not experience oral tolerance.³⁹ Thus the interaction beginning at birth between the intestinal microbiome and the MALT probably represents the primary stimulus for proper postnatal immune development. There is increasing evidence that signals from innate immune receptors, such as Toll-like receptors, play an important role in intestinal homeostasis,⁴⁰ in the genesis of Treg cells,⁴¹ and in the outcome of allergic disease.⁴² Investigators have identified specific microbial products (ie, polysaccharide A from *Bacteroides fragilis*) that interact with Toll-like receptors and promote downstream induction of Treg cells, which subsequently modulate intestinal inflammation in murine models of experimental colitis.⁴¹ However, determining the specific microbial signal or signals that are most critical

in the development of tolerogenic versus allergic outcomes has proved challenging, as evidenced by the overall disappointing results of probiotic trials on the prevention and treatment of allergic disease.⁴³ This difficulty might be largely technical because historically most studies have relied on culture-based methods to attempt to identify an incredibly diverse ecosystem of which many members are fastidious or simply unculturable. New deep-sequencing technologies, which focus on the unique 16s ribosomal subunit of bacterial RNA (*16s ribosomal RNA*), allow investigators to identify previously unknown organisms and promise to revolutionize our understanding of these critical microbes.⁴⁴ Coupling 16s rRNA sequencing techniques with high-throughput approaches allows profiling of entire bacterial communities, forming the basis of the Human Microbiome Project to characterize the entire flora (<http://nihroadmap.nih.gov/hmp/>). By using traditional methods, it has already been shown that allergic children are less frequently colonized with enterococci and bifidobacteria during the first year of life than healthy children.⁴⁵ The Human Microbiome Project will very likely shed important new light on this critical influence of mucosal immune responses.

FUNDAMENTAL IMPORTANCE OF APCs

A complex interplay of the aforementioned physical factors; antigen character, dose, and timing; and adjuvant effects of innate immune stimulation (eg, microbiota and microbial toxins) ultimately shapes the development of oral tolerance through the same final common pathway by directly or indirectly influencing the APCs. Much recent research has begun to demonstrate that mucosal DCs are probably the most critical determinant of allergic sensitization versus tolerance in naive subjects. These DCs can encounter ingested antigen in one of 3 ways: by extending dendrites through the paracellular space between epithelial cells to sample luminal contents, by directly interacting with the epithelial cells, and by taking up antigen in the *Peyer patch*, specialized lymphoid tissue that is immediately adjacent to microfold cells.⁴⁶ The properties of the antigen itself (ie, particulate vs soluble) to a certain extent determine the route of exposure, and it is likely that each route will involve distinct DC populations. The relative contributions of each of these DC populations to oral tolerance is incompletely understood but is beginning to be unraveled, and the CCR7-dependent migration of CD103⁺CD11c⁺ DCs to the mesenteric lymph nodes (MLNs) appears to be a critical step.⁴⁷

Although multiple mechanisms of tolerance are likely and might include anergy or deletion of T cells, substantial evidence links oral tolerance to the ability of mucosal DCs to induce forkhead box protein 3 (Foxp3)-positive Treg cells in MLNs.⁴⁸ The integrin CD103, as well as *retinoic acid* (RA), indoleamine-2,3-dioxygenase, B7 family costimulatory molecules, and TGF- β , all appear to act on the DCs to positively influence this conversion.⁴⁹⁻⁵³ By contrast, lamina propria DCs that do not express CD103 are strongly proinflammatory. These data suggest that tolerogenic DCs might receive site-specific signals from the intestinal epithelium through interactions with E-cadherin, a ligand of CD103; it is likely that spatial conditioning by the mucosal epithelium provides a unique microenvironment that orchestrates antigen presentation toward appropriate inflammatory versus tolerogenic outcomes in the MLNs (Fig 2).⁵⁴ Exposure of mucosal DCs to RA also has been shown to induce

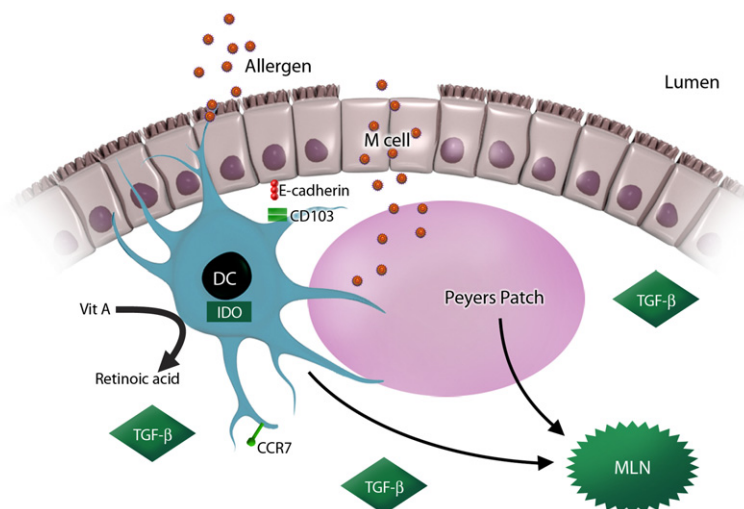


FIG 2. Microenvironmental signals initiate tolerogenic DC responses. Oral tolerance depends on CD103⁺ mucosal DCs encountering luminal antigens and transporting them to the MLNs. This process requires CCR7 and is positively influenced by local factors, such as RA, TGF- β , and indoleamine-2,3-dioxygenase (*IDO*). CD103⁺ DCs conditioned by these stimuli then interact with cognate T cells to actively suppress immune reactivity. *Vit A*, Vitamin A.

expression of the $\alpha 4\beta 7$ integrin and CCR9 on lymphocytes, which provide signals to recirculate back to the MALT.⁵⁵⁻⁵⁷ Importantly, studies of human subjects have observed similar RA-associated phenotypic and functional features of CD103⁺ MLN DCs, implicating them as potential therapeutic targets for the regulation of intestinal immune responses.⁵⁸ In addition to its role in peripheral conversion of Foxp3⁺ Treg cells, RA can also condition DCs to induce T cell-independent IgA production^{57,59} in the gut of mice and human subjects, which might be a complementary mechanism in hyporesponsiveness to dietary proteins. Taken together, these data confirm that RA, acting in concert with TGF- β , powerfully modulates tolerogenic intestinal immune responses by participating in the peripheral conversion of inducible Foxp3⁺ Treg cells in the MLNs, imprinting lymphocytes with gut-homing signatures, and stimulating IgA production.⁶⁰⁻⁶² Interestingly, dietary vitamin A is the sole source of RA because it cannot be synthesized by human subjects or animals; thus the effect of RA on the intestinal immune response represents a key adaptation to local environmental factors.

APCs other than conventional DCs might also participate in oral tolerance induction. For example, intestinal macrophages can also efficiently induce Foxp3⁺ Treg cells in an IL-10-, RA-, and TGF- β -dependent fashion.⁶³ **Plasmacytoid DCs**, a specialized DC subset known for their ability to produce vast quantities of type I interferons, can also activate inducible Foxp3⁺, as well as IL-10⁺, T_H1 Treg cells in both mice and human subjects under various conditions.⁶⁴⁻⁶⁷ Plasmacytoid DCs in the murine liver, which account for almost 30% of the CD11c⁺ DC population there, can induce oral tolerance to fed protein and hapten by inducing anergy or deletion of 70% to 80% of antigen-specific CD4⁺ and CD8⁺ T cells in addition to generating CD4⁺CD25⁺ Treg cells.^{68,69} This suggests that oral tolerance might be operative through multiple mechanisms in multiple tissue compartments. Hepatic tolerance was studied recently in human subjects by isolating liver DCs from surgical specimens. Compared with peripheral blood-derived DCs, liver DCs produced vastly greater amounts of IL-10, greater suppression of T-cell responses in mixed lymphocyte

culture, and the exclusive IL-10-dependent generation of CD4⁺CD25⁺Foxp3⁺ Treg cells from naive precursors.⁷⁰

T CELLS: PRIMARY EFFECTORS OF ORAL TOLERANCE

Integration of environmental information by DCs results in specific activation and differentiation of T-cell subsets, including the Foxp3⁺ Treg cells, which we will consider first, as the primary effector of oral tolerance. However, other mechanisms that affect T-cell activity also influence tolerance, such as anergy and deletion. In addition, although most work has focused on T cells expressing the $\alpha\beta$ T-cell receptor and CD4, there appears to be at least some role for other types of T cells in tolerance models, such as CD8⁺ T cells, $\gamma\delta$ T cells, and perhaps **natural killer T** (NKT) cells.^{9,71}

Repeated exposures to low doses of antigen are thought to be the optimal stimulus for the development of Treg cells, which suppress immune responses through soluble or cell-bound regulatory cytokines, such as IL-10 and TGF- β .⁷ These antigen-specific regulatory cells migrate to lymphoid organs, where they inhibit the generation of effector cells, and to target organs, where they release cytokines that are capable of nonspecific suppression.⁹ Natural CD4⁺CD25⁺ Treg cells develop in the thymus and express the specific transcription factor Foxp3, which confers regulatory function to these cells to block both T_H1 and T_H2 responses. Natural CD4⁺CD25⁺ Treg cells mediate suppression through cell surface-bound TGF- β , although suppression can possibly occur independently of TGF- β .⁷²

Inducible regulatory T (iTreg) cells are CD4⁺ cells that can differentiate from naive precursors, acquiring regulatory properties in the periphery after exposure to antigen. In many cases these cells acquire the expression of Foxp3, and they exist in at least 2 forms distinguished by the anti-inflammatory cytokines produced: IL-10 (T_R1 cells) and TGF- β (T_H3 cells). Whereas natural CD4⁺CD25⁺ Treg cells are thought to primarily govern peripheral tolerance to self-antigens, iTreg cells are more likely

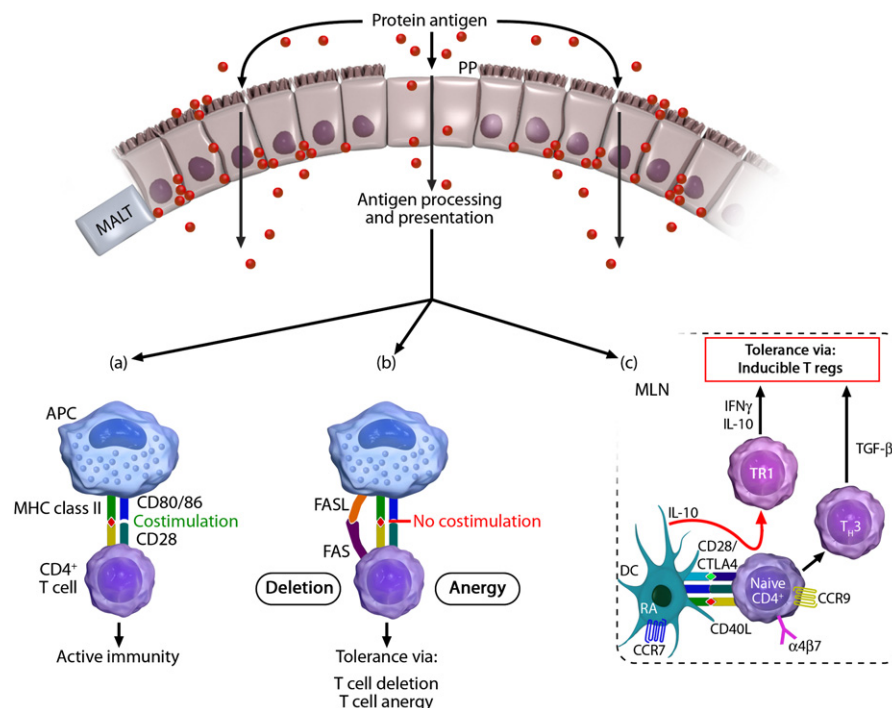


FIG 3. T lymphocytes are the ultimate effectors of oral tolerance. Once antigens pass through the gut epithelium and are taken up, processed, and presented by APCs on MHC class II, one of several fates is possible, depending on the conditions present. (a), An active immune response ensues when the T cell receives input from its costimulatory molecule CD28, which binds CD80/86 on the APC. (b), Reactive clones are rendered anergic or deleted if the TCR-peptide-MHC interaction occurs without costimulation or in the presence of FAS–FAS ligand (FASL) interaction, respectively. (c), Inducible or adaptive Treg cells, which express the gut-homing receptors CCR9 and $\alpha 4\beta 7$, are generated in the MLNs through interactions with tolerogenic DCs. T_H1 cells are formed in the presence of the suppressive cytokine IL-10 and go on to themselves produce IL-10, whereas T_H3 cells produce TGF- β . CTLA4, Cytotoxic T lymphocyte–associated antigen 4; PP, Peyer patch.

responsible for tolerance to exogenous substances, such as allergens. A series of elegant experiments confirmed that Foxp3⁺ iTreg cells are required to establish mucosal tolerance to inhaled allergen in mice.⁷³

Induction of iTreg cells was also recently shown to convey tolerance in a food allergy model. In this system the lectin SIGNR1 expressed by lamina propria DCs recognized glycosylated residues on fed antigen, leading to IL-10 production and the generation of functional IL-10⁺ T_H1 cells and oral tolerance.⁷⁴ In human subjects T_H1 cells are known to develop after frequent exposure to allergen and are associated with the development of clinical tolerance.⁷⁵

It is likely that defects in Treg cell activity contribute to the development of food allergy. Whereas in mice substantial data link Treg cells to mucosal allergen tolerance,⁷² in human subjects the evidence is limited and largely indirect. The importance of Treg cells in human intestinal function is primarily demonstrated in patients with immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX), in whom severe enteropathy and food allergy are part of a fatal immunoregulatory disorder caused by *FOXP3* mutations.⁷⁶ A deletion in the noncoding region of *FOXP3* that impairs mRNA splicing has been associated with a milder IPEX immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome variant that consists of severe enteropathy, atopic dermatitis, and food allergies.⁷⁷ Probably the most widely cited study of patients

with more typical food allergy examined children with non-IgE-mediated cow's milk intolerance, in which clinical tolerance usually develops within a year or 2 of diagnosis.⁷⁸ Compared with children who maintain clinically active intolerance, the development of tolerance is associated with higher numbers of circulating CD4⁺CD25⁺ Treg cells and decreased *in vitro* proliferative responses to bovine β -lactoglobulin in PBMCs; depletion of CD25⁺ cells from the PBMCs of tolerant children led to a 5-fold increase in proliferation against β -lactoglobulin *in vitro*. However, the relevance of these findings to IgE-mediated cow's milk allergy is not known. In another *in vitro* study of children with milk allergy, lymphocytes were cultured from the duodenal mucosa in the presence of milk. These cells displayed a typical T_H2 cytokine profile when restimulated but only very low amounts of TGF- β and IL-10, suggesting a defect in iTreg cell function.⁷⁹ In a seminal study Shreffler et al⁸⁰ provided substantial evidence that Treg cells are involved in clinical tolerance to food allergens by examining the cells from children with milk allergy, heated milk-tolerant (ie, intermediate phenotype) children with former milk allergy, and healthy control children, all of whom had normal total percentages of polyclonal Treg cells. However, whereas control subjects had low numbers of milk-specific Treg cells, there was a dose-dependent increase in the numbers of milk-specific Treg cells from allergic to heated milk-tolerant to fully tolerant children. Furthermore, these cells, which had a phenotype of CD4⁺CD25^{hi}CD45RO⁺CD27⁺

cytotoxic T lymphocyte-associated antigen 4 (CTLA-4)-positive CD127⁺, were indirectly shown to have functional suppressive capacity.⁸⁰

In contrast to the low-dose exposures that induce Treg cells, high-dose tolerance is mediated by lymphocyte anergy or clonal deletion (Fig 3).³¹ 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 counterreceptors on APCs (CD80, CD86, and cytotoxic T lymphocyte-associated antigen 4).⁸¹ Clonal deletion in oral tolerance occurs by means of FAS-mediated apoptosis.⁸² Low- and high-dose tolerance are likely not mutually exclusive and might have overlapping functionality.⁹ TGF- β is secreted during clonal anergy and deletion, and in the absence of cofactors, such as IL-6, that with TGF- β drive T_H17 responses, likely contributes to an immunosuppressive environment in the gut. Although there is evidence that it is liberated during apoptosis, the primary source of TGF- β remains one of the most significant unresolved questions in tolerance; other contributors of TGF- β in this environment include the gut epithelium and immature mucosal DCs. Ultimately, no matter the source, TGF- β is required for the differentiation of inducible Foxp3⁺ Treg cells in murine models of oral tolerance.⁴⁸

Interestingly, maternal breast milk might be another important source of TGF- β relevant to mucosal tolerance. Elegant animal models of neonatal weaning have begun to dissect the respective contributions of breast milk TGF- β ⁸³ to allergic asthma and breast milk allergen⁸⁴ to food allergy. All mammalian milks contain TGF- β , and receptors for TGF- β have been identified in the rodent small intestine,⁸⁵ where they mediate uptake of exogenous TGF- β , which acts as a negative regulator of inflammation⁸⁶; oral administration of exogenous TGF- β has been shown to enhance oral tolerance to coadministered ovalbumin and β -lactoglobulin.^{87,88} Further studies of neonatal mice indicate that allergen-IgG immune complexes are formed in breast milk, which might act on an intestinal Fc γ receptor in a TGF- β -independent way to induce mucosal tolerance and protection from allergic asthma through Treg cell induction.⁸⁹

Although breast-feeding has been repeatedly demonstrated to have a positive effect on the prevention or delay of atopic dermatitis in human subjects, it is admittedly a weak effect and does not translate to the prevention of food allergy or asthma. However, clinical studies of allergy prevention are beset by methodologic flaws that weaken their associations, and few have performed in-depth mechanistic analysis. Certainly food allergens are known to pass into human milk, which also contains TGF- β , of primarily the TGF- β 2 isotype⁸⁷; however, mothers of allergic children might themselves be on an elimination diet. Therefore exposure during breast-feeding is complex, and most studies of breast-feeding on clinical outcomes did not analyze the content of the milk. Breast milk concentrations of TGF- β have been shown to fluctuate between women and throughout the day,⁹⁰ and mothers of allergic children have been shown to have lower breast milk TGF- β levels.⁸⁵ Likewise, children with food allergy have fewer TGF- β ⁺ cells in the small intestine when compared with healthy control subjects and children with celiac disease.⁹¹ With roles in epithelial growth, IgA production, DC maturation, and Treg cell differentiation, TGF- β clearly provides key signals for oral tolerance induction. Further study is needed to unravel the complex and crucial activities of this pleiotropic molecule.

BRINGING ORAL TOLERANCE INDUCTION TO THE CLINIC

There is a great clinical need for a disease-modifying therapy for patients with food allergy. Both allergen-specific and nonspecific approaches to tolerance induction are currently being studied, and promising pilot data in human subjects exist.⁹²⁻⁹⁵ Although clinical desensitization and immune modulation have been demonstrated, the strength of the current evidence from early clinical trial designs is insufficient to change practice. Findings from several current trials with randomized, double-blind, placebo-controlled designs are eagerly anticipated. Although the induction of *bona fide* immune tolerance in patients with food allergy is a long-term goal, it is not clear whether this is possible in any of the approaches under study. If so, the mechanisms of an interventional therapy might not resemble the kind of mucosal tolerance that occurs naturally.

CONCLUSIONS

The gastrointestinal mucosal immune system must constantly analyze antigenic information and respond appropriately to pathogens, commensals, and food antigens. These responses require a complex immunoregulatory network, of which the hallmark is the induction of oral tolerance. Both host- and antigen-specific properties, as well as dietary and other environmental factors, are important in determining the proper adaptive immune response. Multiple immunologic determinants, including APCs/DCs, T cells, cytokines, and antibodies, play essential roles in shaping the tolerogenic response, and specific characteristics of each of these key players are being identified. Advances in our understanding of the complexities of the gastrointestinal mucosal immune response and the mechanisms of oral tolerance induction will inform the development of future therapies and improve the care and quality of life of patients with food allergy.

REFERENCES

- Chafen JJS, Newberry SJ, Riedl MA, Bravata DM, Maglione M, Suttorp MJ, et al. Diagnosing and managing common food allergies: a systematic review. *JAMA* 2010;303:1848-56.
- Branum AM, Lukacs SL. Food allergy among children in the United States. *Pediatrics* 2009;124:1549-55.
- Venter C, Arshad SH, Grundy J, Pereira B, Clayton CB, Voigt K, et al. Time trends in the prevalence of peanut allergy: three cohorts of children from the same geographical location in the UK. *Allergy* 2010;65:103-8.
- Gupta R, Sheikh A, Strachan DP, Anderson HR. Time trends in allergic disorders in the UK. *Thorax* 2007;62:91-6.
- Sicherer SH, Sampson HA. Food allergy. *J Allergy Clin Immunol* 2010;125 (suppl 2):S116-25.
- Cummings AJ, Knibb RC, King RM, Lucas JS. The psychosocial impact of food allergy and food hypersensitivity in children, adolescents and their families: a review. *Allergy* 2010;65:933-45.
- Chehade M, Mayer L. Oral tolerance and its relation to food hypersensitivities. *J Allergy Clin Immunol* 2005;115:3-12.
- Coombes JL, Powrie F. Dendritic cells in intestinal immune regulation. *Nat Rev Immunol* 2008;8:435-46.
- Faria AMC, Weiner HL. Oral tolerance. *Immunol Rev* 2005;206:232-59.
- Untersmayr E, Jensen-Jarolim E. The role of protein digestibility and antacids on food allergy outcomes. *J Allergy Clin Immunol* 2008;121:1301-8.
- Brandtzaeg P. Update on mucosal immunoglobulin A in gastrointestinal disease. *Curr Opin Gastroenterol* 2010;26:554-63.
- Karlsson MR, Johansen F, Kahu H, Macpherson A, Brandtzaeg P. Hypersensitivity and oral tolerance in the absence of a secretory immune system. *Allergy* 2010;65: 561-70.
- Kukkonen K, Kuitunen M, Haahtela T, Korpela R, Poussa T, Savilahti E. High intestinal IgA associates with reduced risk of IgE-associated allergic diseases. *Pediatr Allergy Immunol* 2010;21:67-73.

14. Dahan S, Roth-Walter F, Arnaboldi P, Agarwal S, Mayer L. Epithelia: lymphocyte interactions in the gut. *Immunol Rev* 2007;215:243-53.
15. Forbes EE, Groschwitz K, Abonia JP, Brandt EB, Cohen E, Blanchard C, et al. IL-9- and mast cell-mediated intestinal permeability predisposes to oral antigen hypersensitivity. *J Exp Med* 2008;205:897-913.
16. Groschwitz KR, Hogan SP. Intestinal barrier function: molecular regulation and disease pathogenesis. *J Allergy Clin Immunol* 2009;124:3-20.
17. Ventura M, Polimeno L, Amoruso A, Gatti F, Annoscia E, Marinaro M, et al. Intestinal permeability in patients with adverse reactions to food. *Dig Liver Dis* 2006;38:732-6.
18. Levy Y, Davidovits M, Cleper R, Shapiro R. New-onset post-transplantation food allergy in children—is it attributable only to the immunosuppressive protocol? *Pediatr Transplant* 2009;13:63-9.
19. Blanchard C, Stucke EM, Burwinkel K, Caldwell JM, Collins MH, Ahrens A, et al. Coordinate interaction between IL-13 and epithelial differentiation cluster genes in eosinophilic esophagitis. *J Immunol* 2010;184:4033-41.
20. van den Oord R, Sheikh A. Filaggrin gene defects and risk of developing allergic sensitisation and allergic disorders: systematic review and meta-analysis. *BMJ* 2009;339:b2243.
21. Hershberg RM, Cho DH, Youakim A, Bradley MB, Lee JS, Framson PE, et al. Highly polarized HLA class II antigen processing and presentation by human intestinal epithelial cells. *J Clin Invest* 1998;102:792-803.
22. Iliev ID, Matteoli G, Rescigno M. The yin and yang of intestinal epithelial cells in controlling dendritic cell function. *J Exp Med* 2007;204:2253-7.
23. Ziegler SF, Artis D. Sensing the outside world: TSLP regulates barrier immunity. *Nat Immunol* 2010;11:289-93.
24. Blazquez AB, Berin MC. Gastrointestinal dendritic cells promote Th2 skewing via OX40L. *J Immunol* 2008;180:4441-50.
25. Sherrill JD, Gao P, Stucke EM, Blanchard C, Collins MH, Putnam PE, et al. Variants of thymic stromal lymphopoietin and its receptor associate with eosinophilic esophagitis. *J Allergy Clin Immunol* 2010;126:160-5, e3.
26. Weiner HL, Friedman A, Miller A, Khoury SJ, Al-Sabbagh A, Santos L, et al. Oral tolerance: immunologic mechanisms and treatment of animal and human organ-specific autoimmune diseases by oral administration of autoantigens. *Ann Rev Immunol* 1994;12:809-37.
27. Berin MC, Zheng Y, Domaradzki M, Li XM, Sampson HA. Role of TLR4 in allergic sensitization to food proteins in mice. *Allergy* 2006;61:64-71.
28. Prescott SL. Allergic disease: understanding how in utero events set the scene. *Proc Nutr Soc* 2010;69:366-72.
29. Sigurs N, Hattevig G, Kjellman B, Kjellman NM, Nilsson L, Björkstén B. Appearance of atopic disease in relation to serum IgE antibodies in children followed up from birth for 4 to 15 years. *J Allergy Clin Immunol* 1994;94:757-63.
30. Holt PG. Prenatal versus postnatal priming of allergen specific immunologic memory: the debate continues. *J Allergy Clin Immunol* 2008;122:717-8.
31. Burks AW, Laubach S, Jones SM. Oral tolerance, food allergy, and immunotherapy: Implications for future treatment. *J Allergy Clin Immunol* 2008;121:1344-50.
32. Du Toit G, Katz Y, Sasieni P, Mesher D, Maleki SJ, Fisher HR, et al. Early consumption of peanuts in infancy is associated with a low prevalence of peanut allergy. *J Allergy Clin Immunol* 2008;122:984-91.
33. Fox AT, Sasieni P, du Toit G, Syed H, Lack G. Household peanut consumption as a risk factor for the development of peanut allergy. *J Allergy Clin Immunol* 2009;123:417-23.
34. Poole JA, Barriga K, Leung DY, Hoffman M, Eisenbarth GS, Rewers M, et al. Timing of initial exposure to cereal grains and the risk of wheat allergy. *Pediatrics* 2006;117:2175-82.
35. Greer FR, Sicherer SH, Burks A. Effects of Early nutritional interventions on the development of atopic disease in infants and children: the role of maternal dietary restriction, breastfeeding, timing of introduction of complementary foods, and hydrolyzed formulas. *Pediatrics* 2008;121:183-91.
36. Høst A, Halken S, Muraro A, Dreborg S, Niggemann B, Aalberse R, et al. Dietary prevention of allergic diseases in infants and small children. *Pediatr Allergy Immunol* 2008;19:1-4.
37. Strobel S, Ferguson A. Immune responses to fed protein antigens in mice. 3. Systemic tolerance or priming is related to age at which antigen is first encountered. *Pediatr Res* 1984;18:588-94.
38. Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. *Nat Rev Immunol* 2009;9:313-23.
39. Mowat A, Parker L, Beacock-Sharp H, Millington OR, Chirdo F. Oral tolerance: overview and historical perspectives. *Ann N Y Acad Sci* 2004;1029:1-8.
40. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R. Recognition of commensal microflora by Toll-like receptors is required for intestinal homeostasis. *Cell* 2004;118:229-41.
41. Round JL, Mazmanian SK. Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. *Proc Nat Acad Sci U S A* 2010;107:12204-9.
42. Holt PG, Strickland DH. Soothing signals: transplacental transmission of resistance to asthma and allergy. *J Exp Med* 2009;206:2861-4.
43. Yao T, Chang C, Hsu Y, Huang J. Review article: probiotics for allergic diseases: realities and myths. *Pediatr Allergy Immunol* 2010;21:900-19.
44. Medini D, Serruto D, Parkhill J, Relman DA, Donati C, Moxon R, et al. Microbiology in the post-genomic era. *Nat Rev Micro* 2008;6:419-30.
45. Björkstén B, Sepp E, Julge K, Voor T, Mikelsaar M. Allergy development and the intestinal microflora during the first year of life. *J Allergy Clin Immunol* 2001;108:516-20.
46. Mowat AM. Anatomical basis of tolerance and immunity to intestinal antigens. *Nat Rev Immunol* 2003;3:331-41.
47. Worbs T, Bode U, Yan S, Hoffmann MW, Hintzen G, Bernhardt G, et al. Oral tolerance originates in the intestinal immune system and relies on antigen carriage by dendritic cells. *J Exp Med* 2006;203:519-27.
48. Curotto de Lafaille MA, Lafaille JJ. Natural and adaptive Foxp3+ regulatory T cells: more of the same or a division of labor? *Immunity* 2009;30:626-35.
49. Mucida D, Park Y, Kim G, Turovskaya O, Scott I, Kronenberg M, et al. Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. *Science* 2007;317:256-60.
50. Coombes JL, Siddiqui KR, Arancibia-Carcamo CV, Hall J, Sun C, Belkaid Y, et al. A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF- β and retinoic acid dependent mechanism. *J Exp Med* 2007;204:1757-64.
51. Sun C, Hall JA, Blank RB, Bouladoux N, Oukka M, Mora JR, et al. Small intestine lamina propria dendritic cells promote de novo generation of Foxp3 T reg cells via retinoic acid. *J Exp Med* 2007;204:1775-85.
52. Matteoli G, Mazzini E, Iliev ID, Mileti E, Fallarino F, Puccetti P, et al. Gut CD103+ dendritic cells express indoleamine 2,3-dioxygenase which influences T regulatory/T effector cell balance and oral tolerance induction. *Gut* 2010;59:595-604.
53. Fukaya T, Takagi H, Sato Y, Sato K, Eizumi K, Taya H, et al. Crucial roles of B7-H1 and B7-DC expressed on mesenteric lymph node dendritic cells in the generation of antigen-specific CD4+ Foxp3+ regulatory T cells in the establishment of oral tolerance. *Blood* 2010;116:2266-76.
54. Belkaid Y, Oldenhove G. Tuning microenvironments: induction of regulatory T cells by dendritic cells. *Immunity* 2008;29:362-71.
55. 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;204:1765-74.
56. Kang SG, Lim HW, Andrisani OM, Broxmeyer HE, Kim CH. Vitamin A metabolites induce gut-homing FoxP3+ regulatory T cells. *J Immunol* 2007;179:3724-33.
57. Mora JR, Iwata M, Eksteen B, Song S, Junt T, Senman B, et al. Generation of gut-homing IgA-secreting B cells by intestinal dendritic cells. *Science* 2006;314:1157-60.
58. Jaensson E, Uronen-Hansson H, Pabst O, Eksteen B, Tian J, Coombes JL, et al. Small intestinal CD103+ dendritic cells display unique functional properties that are conserved between mice and humans. *J Exp Med* 2008;205:2139-49.
59. Uematsu S, Fujimoto K, Jang MH, Yang B, Jung Y, Nishiyama M, et al. Regulation of humoral and cellular gut immunity by lamina propria dendritic cells expressing Toll-like receptor 5. *Nat Immunol* 2008;9:769-76.
60. Iwata M. Retinoic acid production by intestinal dendritic cells and its role in T-cell trafficking. *Semin Immunol* 2009;21:8-13.
61. Mucida D, Park Y, Cheroutre H. From the diet to the nucleus: vitamin A and TGF- β join efforts at the mucosal interface of the intestine. *Semin Immunol* 2009;21:14-21.
62. Strober W. Vitamin A rewrites the ABCs of oral tolerance. *Mucosal Immunol* 2008;1:92-5.
63. Denning TL, Wang Y, Patel SR, Williams IR, Pulendran B. Lamina propria macrophages and dendritic cells differentially induce regulatory and interleukin 17-producing T cell responses. *Nat Immunol* 2007;8:1086-94.
64. Chen W, Liang X, Peterson AJ, Munn DH, Blazar BR. The indoleamine 2, 3-dioxygenase pathway is essential for human plasmacytoid dendritic cell-induced adaptive T regulatory cell generation. *J Immunol* 2008;181:5396-404.
65. Sharma MD, Baban B, Chandler P, Hou D, Singh N, Yagita H, et al. Plasmacytoid dendritic cells from mouse tumor-draining lymph nodes directly activate mature Tregs via indoleamine 2, 3-dioxygenase. *J Clin Invest* 2007;117:2570-82.
66. Ito T, Yang M, Wang YH, Lande R, Gregorio J, Perng OA, et al. Plasmacytoid dendritic cells prime IL-10-producing T regulatory cells by inducible costimulator ligand. *J Exp Med* 2007;204:105-15.
67. Moseman EA, Liang X, Dawson AJ, Panoskaltis-Mortari A, Krieg AM, Liu YJ, et al. Human plasmacytoid dendritic cells activated by CpG oligodeoxynucleotides induce the generation of CD4+ CD25+ regulatory T cells. *J Immunol* 2004;173:4433-42.

68. Goubier A, Dubois B, Gheit H, Joubert G, Villard-Truc F, Asselin-Paturel C, et al. Plasmacytoid dendritic cells mediate oral tolerance. *Immunity* 2008;29:464-75.
69. Dubois B, Joubert G, Gomez de Aguero M, Gouanvic M, Goubier A, Kaiserlian D. Sequential role of plasmacytoid dendritic cells and regulatory T cells in oral tolerance. *Gastroenterology* 2009;137:1019-28.
70. Bamboat ZM, Stableford JA, Plitas G, Burt BM, Nguyen HM, Welles AP, et al. Human liver dendritic cells promote T cell hyporesponsiveness. *J Immunol* 2009;182:1901-11.
71. Middendorp S, Nieuwenhuis EES. NKT cells in mucosal immunity. *Mucosal Immunol* 2009;2:393-402.
72. Tang Q, Bluestone JA. The Foxp3+ regulatory T cell: a jack of all trades, master of regulation. *Nat Immunol* 2008;9:239-44.
73. 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;29:114-26.
74. Zhou Y, Kawasaki H, Hsu S, Lee RT, Yao X, Plunkett B, et al. Oral tolerance to food-induced systemic anaphylaxis mediated by the C-type lectin SIGNR1. *Nat Med* 2010;16:1128-33.
75. Akdis M, Verhagen J, Taylor A, Karamloo F, Karagiannidis C, Crameri R, et al. Immune responses in healthy and allergic individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. *J Exp Med* 2004;199:1567-75.
76. Bennett CL, Christie J, Ramsdell F, Brunkow ME, Ferguson PJ, Whitesell L, et al. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat Gen* 2001;27:20.
77. Torgerson TR, Linane A, Moes N, Anover S, Mateo V, Rieux-Laucat F, et al. Severe food allergy as a variant of IPEX syndrome caused by a deletion in a noncoding region of the FOXP3 gene. *Gastroenterology* 2007;132:1705-17.
78. Karlsson MR, Rugtveit J, Brandtzaeg P. Allergen-responsive CD4+CD25+ regulatory T cells in children who have outgrown cow's milk allergy. *J Exp Med* 2004;199:1679-88.
79. Beyer K, Castro R, Birnbaum A, Benkov K, Pittman N, Sampson HA. Human milk-specific mucosal lymphocytes of the gastrointestinal tract display a TH2 cytokine profile. *J Allergy Clin Immunol* 2002;109:707-13.
80. Shreffler WG, Wanich N, Moloney M, Nowak-Wegrzyn A, Sampson HA. Association of allergen-specific regulatory T cells with the onset of clinical tolerance to milk protein. *J Allergy Clin Immunol* 2009;123:43-52, e7.
81. Appleman LJ, Boussiotis VA. T cell anergy and costimulation. *Immunol Rev* 2003;192:161-80.
82. Chen Y, Inobe J, Marks R, Gonnella P, Kuchroo VK, Weiner HL. Peripheral deletion of antigen-reactive T cells in oral tolerance. *Nature* 1995;376:177-80.
83. Verhasselt V, Milcent V, Cazareth J, Kanda A, Fleury S, Dombrowicz D, et al. Breast milk-mediated transfer of an antigen induces tolerance and protection from allergic asthma. *Nat Med* 2008;14:170-5.
84. Lopez-Exposito I, Song Y, Jarvinen KM, Srivastava K, Li X. Maternal peanut exposure during pregnancy and lactation reduces peanut allergy risk in offspring. *J Allergy Clin Immunol* 2009;124:1039-46.
85. Penttila IA. Milk-derived transforming growth factor- β and the infant immune response. *J Pediatr* 2010;156(suppl 1):S21-5.
86. Li MO, Flavell RA. Contextual regulation of inflammation: a duet by transforming growth factor- β and interleukin-10. *Immunity* 2008;28:468-76.
87. Ando T, Hatsushika K, Wako M, Ohba T, Koyama K, Ohnuma Y, et al. Orally administered TGF- β is biologically active in the intestinal mucosa and enhances oral tolerance. *J Allergy Clin Immunol* 2007;120:916-23.
88. Penttila I. Effects of transforming growth factor-beta and formula feeding on systemic immune responses to dietary β -lactoglobulin in allergy-prone rats. *Pediatr Res* 2006;59:650-5.
89. Mosconi E, Rekima A, Seitz-Polski B, Kanda A, Fleury S, Tissandier E, et al. Breast milk immune complexes are potent inducers of oral tolerance in neonates and prevent asthma development. *Mucosal Immunol* 2010;3:461-74.
90. Hawkes JS, Bryan D, James MJ, Gibson RA. Cytokines (IL-1 β , IL-6, TNF- α , TGF- β 1, and TGF- β 2) and prostaglandin E2 in human milk during the first three months postpartum. *Pediatr Res* 1999;46:194-9.
91. Pérez-Machado M, Ashwood P, Thomson M, Latcham F, Sim R, Walker-Smith J, et al. Reduced transforming growth factor- β 1-producing T cells in the duodenal mucosa of children with food allergy. *Eur J Immunol* 2003;33:2307-15.
92. Jones SM, Pons L, Roberts JL, Scurlock AM, Perry TT, Kulis M, et al. Clinical efficacy and immune regulation with peanut oral immunotherapy. *J Allergy Clin Immunol* 2009;124:292-300.
93. Blumchen K, Ulbricht H, Staden U, Dobberstein K, Beschoner J, de Oliveira LCL, et al. Oral peanut immunotherapy in children with peanut anaphylaxis. *J Allergy Clin Immunol* 2010;126:83-91, e1.
94. Skripak JM, Nash SD, Rowley H, Brereton NH, Oh S, Hamilton RG, et al. A randomized, double-blind, placebo-controlled study of milk oral immunotherapy for cow's milk allergy. *J Allergy Clin Immunol* 2008;122:1154-60.
95. Clark AT, Islam S, King Y, Deighton J, Anagnostou K, Ewan PW. Successful oral tolerance induction in severe peanut allergy. *Allergy* 2009;64:1218-20.