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Molecular and cellular mechanisms of food allergy and food tolerance

R. Sharon Chinthrajah, MD,[a,b,e]* Joseph D. Hernandez, MD, PhD,[b,c,e]* Scott D. Boyd, MD, PhD,[c,e] Stephen J. Galli, MD,[c,d,e]‡ and Kari C. Nadeau, MD, PhD[a,b,e]‡ *Stanford, Calif*

Ingestion of innocuous antigens, including food proteins, normally results in local and systemic immune nonresponsiveness in a process termed oral tolerance. Oral tolerance to food proteins is likely to be intimately linked to mechanisms that are responsible for gastrointestinal tolerance to large numbers of commensal microbes. Here we review our current understanding of the immune mechanisms responsible for oral tolerance and how perturbations in these mechanisms might promote the loss of oral tolerance and development of food allergies. Roles for the commensal microbiome in promoting oral tolerance and the association of intestinal dysbiosis with food allergy are discussed. Growing evidence supports cutaneous sensitization to food antigens as one possible mechanism leading to the failure to develop or loss of oral tolerance. A goal of immunotherapy for food allergies is to induce sustained desensitization or even true long-term oral tolerance to food allergens through mechanisms that might in part overlap with those associated with the development of natural oral tolerance. (J Allergy Clin Immunol 2016;137:984-97.)

Key words: *Food allergy, microbiome, sensitization, desensitization, immunotherapy, tolerance, regulatory T cells, basophils, mast cells, dendritic cells*

Abbreviations used

APC:	Antigen-presenting cell
DC:	Dendritic cell
DNFB:	2,4-Dinitrofluorobenzene
EPIT:	Epicutaneous immunotherapy
Foxp3:	Forkhead box protein 3
GALT:	Gut-associated lymphoid tissue
GPR:	G protein–coupled receptor
M cell:	Microfold cell
MLN:	Mesenteric lymph node
OIT:	Oral immunotherapy
OVA:	Ovalbumin
SCFA:	Short-chain fatty acid
SLIT:	Sublingual immunotherapy
Treg:	Regulatory T

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To maintain immune tolerance, the immune system must not only be able to distinguish self from nonself antigens but also to discriminate between innocuous nonself and threatening nonself antigens. The gastrointestinal tract represents a unique challenge to the immune system in making these distinctions and in maintaining tolerance for several reasons. It is the largest interface between the body and the external environment, with the intestinal mucosa having a surface area of more than 300 m^2.[1] As such, it encounters a huge quantity and diversity of foreign antigens representing nonself antigens (ie, >30 kg of food proteins each year),[2] as well as the products of trillions of resident bacteria representing more than 1000 species.[3]

Maintaining tolerance requires complex interactions between nonimmune cells and cells making up the gut-associated lymphoid tissue (GALT), which contains 10^{12} lymphoid cells per meter of gut and more immunoglobulin-producing cells than the rest of the body.[4,5] These cells must act in concert to limit inflammatory responses to resident bacteria and food proteins that could lead to tissue injury, keep microbes confined to the gut, and recognize and respond to pathogens that can cause tissue injury or disease. Failure to achieve an appropriate balance in these roles can lead to a loss of tolerance, resulting in inflammatory diseases, such as inflammatory bowel disease, or responses to innocuous food antigens, such as those occurring in patients with celiac disease and ***IgE***-mediated food allergies.

From the Departments of [a]Medicine, [b]Pediatrics, [c]Pathology, and [d]Microbiology and Immunology and the [e]Sean N. Parker Center for Allergy & Asthma Research, Stanford University School of Medicine.

*These authors contributed equally to this work.

‡These authors contributed equally to this work as co-senior authors.

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Corresponding author: Kari C. Nadeau, MD, PhD, Division of Pulmonary and Critical Care Medicine, Department of Medicine, Sean N. Parker Center for Allergy and Asthma Research, Stanford University, Stanford University School of Medicine, 269 Campus Dr, CCSR 3215, MC 5366, Stanford, CA 94305-5101. E-mail: knadeau@stanford.edu.

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Terms in boldface and italics are defined in the glossary on page 985.

GLOSSARY

α4β7: An integrin expressed on lymphocytes that is shown to promote T-cell homing into gut-associated lymphoid tissues through its binding to mucosal addressin cell adhesion molecule, which is present on high endothelial venules of mucosal lymphoid organs.

ANTIGEN-PRESENTING CELLS (APCs): Cells that present antigens through MHCs on their surfaces to T-cell receptors on T cells.

Ara h 1, Ara h 2: Proteins found in peanuts that are known to be food antigens.

αVβ8: A member of the integrin family of transmembrane proteins that mediates cell-cell and cell–extracellular matrix adhesion.

B220: A CD45 isoform and a commonly used B-cell marker predominantly expressed on all mouse B lymphocytes.

BUTYRATE: A short-chain fatty acid and major microbial fermentation metabolite in the lumen of the colon that has been shown to be a critical mediator of the colonic inflammatory response. Without butyrates for energy, colon epithelial cells undergo autophagy and die.

CCR7: A chemokine receptor involved in the adhesion and migration of immune cells. Signals mediated by this receptor regulate T-cell homeostasis in lymph nodes and facilitate DC migration (eg, from the gut to the mesenteric lymph nodes).

CCR9: A chemokine receptor involved in the adhesion and migration of immune cells. CCR9 has also been shown to promote the migration of T lymphocytes (T cells) to the gastrointestinal tract.

CD11c: A cell-surface molecule expressed on many immune cells, with especially high abundance on many dendritic cells.

CD14: A coreceptor for bacterial LPS and other pathogen-associated molecular patterns, such as lipoteichoic acid, which is expressed on subsets of monocytes, dendritic cells, and other hematopoietic cells.

CD45: A receptor-linked protein tyrosine phosphatase that is expressed on all leukocytes.

CHOLERA TOXIN: A highly toxic protein secreted by the bacterium *Vibrio cholerae*, which causes severe gastric inflammation in animals. It is often used as an adjuvant to induce an immune response in biological experiments.

CpG SITES: Regions of DNA where a cytosine nucleotide occurs next to a guanine nucleotide separated by only 1 phosphate. Methylation of the cytosine within CpG sites of a gene can turn the gene off through epigenetic regulation.

CX3CR1: A chemokine receptor involved in the adhesion and migration of immune cells. It is expressed on a subset of phagocytic cells in the small intestine.

DENDRITIC CELLS (DCs): Professional antigen-presenting cells that link the innate and adaptive immune systems by capturing and then presenting antigens to T cells.

FOLLICULAR HELPER T (T_{FH}) CELLS: A specific subset of effector T cells that traffic to the B-cell areas of secondary lymphoid tissues, such as through interactions mediated by the chemokine receptor CXCR5 and its ligand, CXCL13. T_{FH} cells can regulate antigen-specific B-cell development and antibody production.

HAPTENS: Small molecules that elicit an immune response only when covalently bound to a large carrier, typically a protein antigen.

IgA: The main immunoglobulin found in mucous secretions. Secretory IgA is resistant to degradation by proteolytic enzymes in the gastrointestinal tract, where it provides protection against pathogens.

IgE: An antibody (immunoglobulin) associated with type 2 immunity, including allergic responses. Found only in mammals, IgE antibodies bind allergens and can help to enhance host resistance to parasites (eg, helminths and protozoans) and increase resistance to venoms in mice. When bound to allergens and FcεRI on basophils and mast cells, antigen- and IgE-induced aggregation of FcεRI can trigger release of histamine, proteases, prostaglandins, leukotrienes, chemokines, and cytokines.

IgG_4: A subtype of immunoglobulin IgG, IgG_4 can be produced in part to dampen inflammation by helping to curtail Fc receptor (FcR)–mediated processes.

IL-5: A major maturation and differentiation cytokine expressed by T_H2 cells and eosinophils in mice and human subjects. IL-5 has been shown to play an instrumental role in eosinophilic inflammation in patients with allergic diseases.

IL-6: A cytokine implicated in a wide variety of inflammation-associated disease states, it is involved in the maturation of B cells and has been shown to be an endogenous pyrogen capable of inducing fever in patients with autoimmune diseases or infections.

IL-10: A cytokine produced primarily by monocytes and, to a lesser extent, by lymphocytes (particularly Treg cells) and mast cells that has pleiotropic effects in immunoregulation and inflammation by limiting the immune response to pathogens and thereby limiting damage to the host.

IL-22: A cytokine that has important functions in host defense both at mucosal surfaces and in tissue repair. It appears to be unique in that it is produced by immune cells, including T-helper cell subsets and innate lymphocytes, but acts mostly on nonhematopoietic stromal cells, in particular epithelial cells, keratinocytes, and hepatocytes.

IL-25: A cytokine known to be involved in mucosal immunity. It induces production of the type 2 cytokines IL-4, IL-5, and IL-13.

IL-33: Belonging to the IL-1 family of cytokines, IL-33 potently drives production of type 2 cytokines. It is a ligand for IL-33 receptor (IL1RL1), an IL-1 family receptor that is selectively expressed on T_H2 cells and mast cells.

INHIBITORY FCγ RECEPTORS: Receptors that downregulate the immune complex–mediated inflammatory responses on phagocytes and IgE- and antigen-induced activation of mast cells and basophils when cross-linked with stimulatory Fcγ receptors (FcγRs).

INNATE LYMPHOID CELLS (ILCs): Innate immune cells that belong to the lymphoid lineage but cannot respond in an antigen-specific manner because they lack a B- or T-cell receptor. ILCs are a recently described group of cells with physiologic functions analogous in some ways to helper T cells and cytotoxic natural killer cells. They have a role in protective immunity and the regulation of homeostasis and inflammation. Their dysregulation has been shown to contribute to immune pathology and diseases, such as allergy and autoimmune disease.

MHC CLASS II: A complex that presents antigen derived from extracellular proteins to $CD4^+$ T cells.

MHC TETRAMERS: Fluorescently labeled tetrameric MHC-peptide complexes that enable the direct detection, quantification, and phenotypic characterization of antigen-specific T cells by using flow cytometry.

MICROFOLD CELLS (M CELLS): Specialized epithelial cells of the gastrointestinal tract that sample antigens.

OVALBUMIN: The most abundant protein found in egg white, ovalbumin is a well-characterized allergen used in immunologic studies in mice.

OX40–OX40 LIGAND: Members of the TNF superfamily expressed on a variety of cells, including activated $CD4^+$ and $CD8^+$ T cells. The OX40-OX40 ligand (OX40L) complex has been shown to regulate cytokine production from T cells (including differentiation to T_H2 cells), antigen-presenting cells, natural killer cells, and natural killer T cells and also modulate cytokine receptor signaling. In mice Treg cells can directly inhibit the FcεRI-dependent degranulation of mast cells through cell-cell contact involving OX40-OX40L interactions between Treg cells and mast cells, respectively. The OX40-OX40L complex plays a central role in the development of multiple inflammatory and autoimmune diseases.

PROPIONATE: A short-chain fatty acid and a major microbial fermentation metabolite in the human gut with putative health effects that extend beyond the gut epithelium.

RETINOIC ACID: A metabolite derived from retinol (vitamin A) that plays important roles in cell growth and differentiation, including differentiation of Treg cells.

STAPHYLOCOCCAL ENTEROTOXIN B: A superantigen produced by the bacterium *Staphylococcus aureus* that elicits cytokine release.

Staphylococcal enterotoxin B–induced inflammation can promote allergic inflammation.

TGF-β: A cytokine secreted by many cell types, including macrophages and mast cells, which controls proliferation, cellular differentiation, and other functions in most cells. It also promotes differentiation of Treg cells and IgA-secreting B cells.

THYMIC STROMAL LYMPHOPOIETIN (TSLP): A cytokine produced mainly by nonhematopoietic cells (eg, epithelial cells), which stimulates the maturation of T cells through activation of antigen-presenting cells, such as dendritic cells and macrophages.

TYPE 1 REGULATORY (TR1) CELLS: A subset of regulatory T cells that are Foxp3$^-$ and induced by chronic activation of CD4+ T cells by antigen in the presence of IL-10 and that mediate their suppressive effects through secretion of IL-10.

TYPE 2 INNATE LYMPHOID CELLS (ILC2s): ILC2s can produce the T_H2 cytokines IL-4, IL-5, IL-9, and IL-13 in response to helminth infections. They also have been implicated in the development of allergic inflammation. They require IL-7 for their development, which activates 2 transcription factors, RORα and GATA3.

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This review will focus on the complex mechanisms underlying the development of natural tolerance to food antigens and how these might break down in subjects who have IgE-mediated food allergies and anaphylaxis. Also, we will discuss how experimental immunotherapeutic approaches, some of them currently in clinical trials, have the potential to restore food tolerance.

ANTIGEN UPTAKE, DISSEMINATION, AND PRESENTATION

Potentially immunogenic proteins are first subject to denaturation and degradation by means of digestion in the gut. The fact that these processes might play a role in preventing sensitization to food antigens has been shown in several models. Coadministration of antacids with fish proteins to mice resulted in increased levels of IgE reactive with fish proteins and increased T-cell reactivity compared with that seen in mice administered fish proteins alone,[6] implying that acid either directly decreases protein antigenicity by denaturing the protein or indirectly influences antigenicity by affecting protein proteolysis or uptake.[7] Similar observations were made in a mouse model of hazelnut allergy, and there is a positive correlation in human subjects with antacid use and sensitization to food allergens.[8] Antigens placed in acrylic microspheres and thereby protected from both acid denaturation and enzymatic proteolysis can induce allergy in animals previously tolerant to ***ovalbumin*** (OVA).[9]

Proteins and peptides that survive denaturation and digestion in the gut can pass through the epithelial barrier through several potential mechanisms, including paracellular diffusion, transcytosis through intestinal epithelial cells, endocytosis by ***microfold cells (M cells)***, and sampling by luminal processes of ***CX3CR1***$^+$ cells (Fig 1, *A*).[10-14] Intestinal epithelial cells might also directly present antigens to T cells in the gut because they can express ***MHC class II*** on basolateral surfaces under some conditions.[15-17] It is unclear at this point which mechanisms are the most important in promoting oral tolerance and food sensitization.

Certain specialized cells are implicated in antigen sampling from the gut through distinct mechanisms. M cells are a type of epithelial cell overlying the GALT (including Peyer patches) that have a reduced glycocalyx, irregular brush border, and reduced microvilli. M cells actively engage in phagocytosis and transcytosis of particulate antigens (including microbes) and, less efficiently, soluble macromolecules from the gut lumen.[15,18,19] Although one study demonstrated that targeting of soluble protein antigens to M cells facilitated tolerance induction to OVA,[20] other studies have demonstrated that tolerance to soluble antigens could be induced even in the absence of Peyer patches found beneath M cells, implying that M cell–facilitated transport to Peyer patches does not play an essential role in oral tolerance.[21-23] A population of ***CD11c***$^+$ myeloid cells in the lamina propria that express CX3CR1 can extend cellular processes into the intestinal lumen and sample antigens without compromising tight junctions or epithelial integrity.[10,12] These cells do not migrate to mesenteric lymph nodes (MLNs) and cannot activate naive T cells but might pass antigens to neighboring migratory ***dendritic cells (DCs)***.[13,24-26]

It has been estimated that 2% of gut luminal proteins can pass through the epithelial barrier intact[27] and then be disseminated locally or systemically through blood or lymph. Food proteins can be detected in the blood of mice and human subjects shortly after eating.[28,29] Food antigens can then be presented by conventional ***antigen-presenting cells*** (***APCs***; eg, DCs) or unconventional APCs (eg, liver-sinusoidal endothelial cells, Kupffer cells, or plasmacytoid DCs), where, in the absence of costimulatory signals, antigen is likely to induce tolerance.[13,30,31] A potential role for systemic dissemination of antigens in tolerance is supported by studies demonstrating that transfer of serum from fed mice can induce tolerance[32] and that shunting of the portal blood flow can inhibit development of oral tolerance.[33,34] Locally, in the gut CD11c$^+$CD103$^+$ DCs migrate from the lamina propria to the MLNs in a ***CCR7***-dependent manner, carrying antigens that appear critical for the development of oral tolerance.[35-37] Inhibition of normal lymph node drainage and lymphocyte trafficking through mesenteric lymphadenectomy, small-bowel transplantation (without ligation of lymphatic vessels), or CCR7 deficiency all prevented induction of oral tolerance in mice.[37]

How antigen uptake, dissemination, and/or presentation can differ in those with food allergy rather than food tolerance and the importance of such differences in the development of the disorder are not well understood. Patients with food allergy are known to have increased gut permeability at baseline,[38] which can be further exacerbated by cytokines and chemokines produced during an allergic reaction (eg, TNF-α), which can act to reduce tight junction integrity or otherwise increase gut permeability.[39-41] In rodent models of food allergy, increased specific antigen uptake can occur through IgE binding to antigen in the lumen, followed by transcytosis through intestinal epithelia caused by the low-affinity IgE receptor CD23.[42,43] Notably, although CD103$^+$ DC migration is necessary for induction of oral tolerance, adoptive transfer of CD11c$^+$***B220***$^-$ splenic and Peyer patch cells (including DC populations) from mice with cow's milk allergy to naive recipient mice was sufficient to induce milk-specific IgE production.[44] These observations indicate that DCs are likely to play critical roles in the induction of both oral tolerance and allergic sensitization.

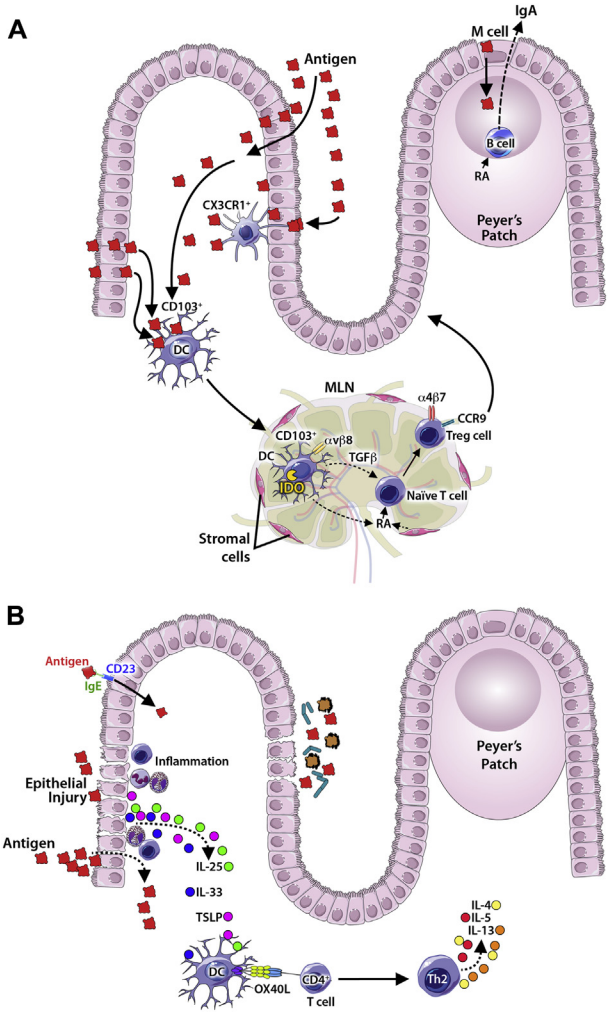

FIG 1. Model of how the gut promotes tolerance or sensitization. Protein antigens pass through the epithelial barrier through multiple mechanisms, including capture by transluminal processes of CX3CR1$^+$ cells. CD103$^+$ DCs then capture antigens, migrate to the MLNs, and present antigens to naive T cells. **A,** In tolerance this interaction promotes the generation of Treg cells through (1) production of retinoic acid by MLNs, (2) DC expression of indoleamine 2,3-dioxygenase, (3) DC secretion of TGF-β, and (4) DC upregulation of αvβ8 to activate latent TGF-β. The gut-homing receptors CCR9 and α4β7 are upregulated on newly formed Treg cells. Retinoic acid and DC interactions also stimulate differentiation of IgA-producing B cells. *AMPs*, Antimicrobial peptides. **B,** In sensitization epithelial disruption allows increased antigen penetration and promotes production/release of epithelial cytokines (IL-33, thymic stromal lymphopoietin *[TSLP]*, and IL-25) that upregulate OX40 ligand (OX40L) on DCs. DCs then promote differentiation of naive T cells to T_H2 cells producing cytokines that recruit eosinophils (IL-5) and promote IgE class-switching in B cells (IL-4 and IL-13). IgE can facilitate antigen uptake through CD23.

Even before introduction to complementary or solid foods, infants can be exposed to food proteins (eg, in household dust) through cutaneous contact.[45,46] Like the gut, the skin is one of the largest immune organs and provides not only a physical and chemical barrier but also serves as a protective immunologic barrier in maintaining immune homeostasis between the environment and the host's deeper tissues. The ability of the skin to constitute a protective barrier to environmental insults and antigen exposure is the result of a complex constellation of its properties, including: proper epidermal cell differentiation; a hydrolipidic milieu caused by lipids, sebum, sweat, and antimicrobial peptides; and features of the normal dermis (Fig 2, *A*).[47] Healthy skin normally represents a noninflammatory environment with numerous resident APCs. One such APC, the ***CD14***$^+$ DC, shares features of both CX3CR1$^+$ and CD103$^+$ cells in the lamina propria of the gut. It phagocytoses large quantities of antigens (similar to CX3CR1$^+$ cells) but produces large amounts of ***IL-10*** and effectively induces regulatory T (Treg) cell differentiation.[48] Loss of epithelial integrity and skin inflammation can predispose subjects to allergic sensitization (Fig 2, *B*), as discussed below.

ORAL TOLERANCE

DCs in oral tolerance

It is still unclear whether there is a critical time period for encountering antigen and an ideal type of antigen exposure for the development of tolerance. Many believe that first encountering antigen at the gastrointestinal mucosa or GALT can promote tolerogenic responses to food proteins.[13] Indeed, a recent clinical trial has indicated that earlier exposure in infancy to one potentially allergenic food (ie, peanut) can decrease rates of clinical food allergy.[49] Tolerance can be driven largely by APCs within the lamina propria that sample antigens in the lumen and promote T-cell differentiation. Intriguingly, diet can influence the development of APCs in the lamina propria because mice fed an elemental diet exhibited differences in lamina propria DC subsets.[50] As mentioned above, mouse studies have revealed that at least 2 distinct CD11c$^+$ APC populations exist: CX3CR1$^+$CD103$^-$ and CX3CR1$^-$CD103$^+$ phagocytes. CX3CR1$^+$CD103$^-$ phagocytes are derived from monocytes and extend processes through the epithelium to sample antigens. They do not migrate or activate naive T cells, but they influence early immunologic responses to antigens and are involved in the restimulation of T cells.[51] In contrast, CX3CR1$^-$CD103$^+$ DCs capture antigens in the lamina propria and migrate to draining MLNs to present antigen to T cells.

In the MLNs migratory CD103$^+$ DCs from the lamina propria can promote the development of gut-homing Treg cells through multiple mechanisms. CD103$^+$ DCs produce ***TGF-β*** and ***retinoic acid*** (derived from vitamin A), driving Treg cell differentiation.[52] Moreover, upregulation of ***αvβ8*** on CD103$^+$ DCs is important for activating latent TGF-β and generating Treg cells during the induction of tolerance to intestinal antigens in mice.[53,54] Additionally, CD103$^+$ DCs can express indoleamine 2,3-dioxygenase, an enzyme involved in tryptophan catabolism. Inhibition of indoleamine 2,3-dioxygenase diminishes Treg cell conversion and favors T_H1 and T_H17 induction.[55]

Cooperation of CD103$^+$ DCs and MLN stromal cells is important for inducing expression of gut-homing receptors on activated T cells.[56] CD103$^+$ DCs induce expression of the gut-homing receptors ***CCR9*** and ***α4β7*** on T cells primed in MLNs to facilitate migration of T cells to the small intestine.[57] However, other mechanisms might also be capable of inducing gut tropism. *In vitro* activation of T cells by intestinal DCs, retinoic acid alone, and stromal cells isolated from MLNs was sufficient for induction of gut tropism. High levels of retinoic acid–producing enzymes are unique to MLNs (compared with peripheral lymph nodes) and support induction of the chemokine receptor CCR9 on activated T cells; CCR9 expression is further enhanced by bone marrow–derived DCs *in vitro*.[58]

In addition to driving gut tropism of T cells, retinoic acid derived from GALT-associated DCs has also been shown to

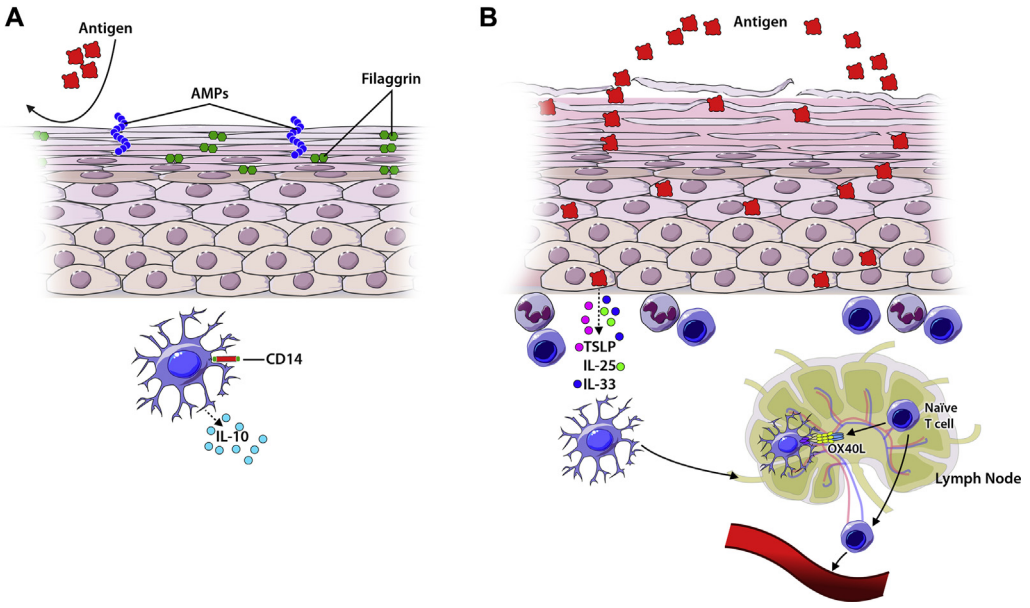

FIG 2. Model of how the skin promotes tolerance or sensitization. **A,** Keratinocyte differentiation, an intact uppermost stratum corneum layer with epidermal proteins that maintain barrier function (eg, filaggrin), antimicrobial peptides, and tolerogenic APCs, such as $CD14^+$ DCs producing IL-10, are important for promoting tolerance in the skin barrier. Loss of barrier function in the stratum corneum, allowing increased antigen penetration, can occur as a result of genetically determined defects in factors necessary for keratinocyte differentiation (eg, mutations in filaggrin) or as a result of inflammatory skin diseases (eg, atopic dermatitis). *RA*, Retinoic acid. **B,** In response to injury, activation by microbial or food antigens, or inflammatory signals, thymic stromal lymphopoietin *(TSLP)*, IL-33, and/or IL-25 produced by keratinocytes can upregulate OX40L on APCs to promote T_H2 differentiation.

imprint gut tropism on B cells and to act synergistically with DC-derived ***IL-6*** or ***IL-5*** to induce IgA secretion; mice deprived of vitamin A and thus retinoic acid lacked IgA-secreting cells in the small intestine.[59] Clearly, such studies indicate that retinoic acid, as derived from DCs and MLN stromal cells, is important for inducing tolerogenic responses in B and T cells and for directing these cells to the small intestine in mice.

Treg cells in oral tolerance

Treg cells play a central role in oral tolerance. In patients with immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome, a rare disease linked to dysfunction of the transcription factor forkhead box protein 3 (Foxp3), which is essential for Treg cell development, there is an increased incidence of food allergies.[60] Several mouse models support a role for $Foxp3^+$ T cells in oral tolerance. In a model using the ***hapten*** 2,4-dinitrofluorobenzene (DNFB), antibody depletion of $CD25^+$ cells (a marker for Treg cells) impaired the oral tolerance normally induced by feeding DNFB.[61] Transfer of $CD4^+CD25^+$ cells to $CD4^+$ T cell–deficient mice, which do not normally have oral tolerance after DNFB feeding, is sufficient to restore oral tolerance induced by feeding.[61] Similarly, in a model of OVA-induced allergic diarrhea, $Foxp3^+$ antigen-specific cells proliferated in the lamina propria during oral tolerance induction, and depletion of $Foxp3^+$ cells abrogated oral tolerance.[62] Dietary antigens can promote differentiation of Treg cells and development of oral tolerance. Mice fed an elemental diet had reduced numbers of lamina propria Treg cells, increased proliferation of antigen-specific T cells on antigen feeding, and increased susceptibility to a model of allergic diarrhea when compared with control mice fed normal chow.[50]

Although it is now appreciated that there are multiple subtypes of Treg cells[63,64] and that these populations can exhibit phenotypic plasticity, the roles of individual Treg cell subtypes in oral tolerance is less well defined. Both $Foxp3^+$ and $Foxp3^-$ Treg cells producing IL-10 can be found in the gut, and many of the $Foxp3^-$ Treg cells are likely peripherally induced ***type 1 regulatory cells*** that can produce large amounts of IL-10 and TGF-β.[34,65,66] In 2 models of oral tolerance to OVA, peripheral conversion of naive T cells to $Foxp3^+$ inducible Treg cells was necessary for tolerance induction.[62,67] Deficiency of CCR9 or $\alpha_4\beta_7$ integrin on T cells or deficiency of the $\alpha_4\beta_7$ ligand mucosal addressin cell adhesion molecule 1 on gut endothelial cells inhibited Treg cell homing to the gut, which is essential for induction of oral tolerance.[62,68] These observations indicate that Treg cells can act locally in the gut and GALT rather than (or in addition to) acting at peripheral sites. As a prominent source of TGF-β, Treg cells that have homed to the GALT might also promote B-cell production of noninflammatory IgA.[69]

ALLERGIC SENSITIZATION: A BREAKDOWN IN ORAL TOLERANCE

The inciting events leading to the breakdown of oral tolerance, allergic sensitization, and development of food allergies in a subset of sensitized subjects are poorly understood. It is likely that multiple pathways could ultimately lead to a failure to develop or loss of oral tolerance (Fig 1, *B*). Epithelial cells produce

thymic stromal lymphopoietin, ***IL-25***, and/or ***IL-33*** in response to injury, inflammation, and innate immune activation, and these cytokines can drive T_H2 inflammation.[70] Such epithelial cytokines promote T_H2 inflammation through activating ***innate lymphoid cells***, mast cells, basophils, and DCs to produce cytokines that drive T_H2 immunity.[70] IL-33 was shown to be critical in the development of allergy in a ***cholera toxin***–induced mouse model of peanut allergy.[71] Intriguingly, both cholera toxin and IL-33 upregulate ***OX40 ligand*** (OX40L) on DCs, driving differentiation of naive $CD4^+$ T cells to T_H2 cells.[71,72] We are only beginning to understand the full spectrum of factors that interact to regulate epithelial cell production of cytokines in food allergy.

More than your average food protein: A role for allergens in sensitization

Although ample evidence supports a role for aeroallergens in promoting allergic sensitization and type 2 immunity, similar evidence for food allergens is more limited. Aeroallergens have been shown to foster allergic sensitization and production of epithelial cell cytokines through multiple mechanisms, including activation of innate pattern recognition receptors, activation of protease-activated receptors, and through direct injury to epithelia.[73] For example, the proteolytic activity of the dust mite protein Der p 1 has been extensively studied and shown to disrupt bronchial epithelial integrity and enhance allergen uptake from the lumen,[74] cleave receptors from the surfaces of immune cells (CD25 and CD23), and enhance IgE production.[75] Among food antigens, ***Ara h 1*** binds to CD209 on DCs, and milk sphingomyelin activates invariant natural killer T cells, effectively acting as adjuvants that enhance type 2 cytokine production.[76,77]

Many have tried to identify other characteristics of proteins that promote loss of tolerance, allergic sensitization, or both. Features of proteins, such as disulfide bonds, resistance to enzymatic proteolysis or thermal degradation, biological functional activity, and protein glycosylation, can contribute to allergenicity and elicit more avid IgE binding.[78] Disulfide bonds preserve protein structure and stability. For example, among aeroallergens, disrupting the disulfide bonds in the dust mite proteins Der p 1 and Lep d 2 reduced binding of IgE derived from allergic patients.[79,80] Similarly, common food allergens resisted proteolysis in gastric fluid,[81] although there are many proteins resistant to digestion that are not ordinarily allergenic. Sensitization to food proteins that are resistant to thermal degradation during cooking is associated with more severe and persistent milk and egg allergies,[82-85] whereas sensitization to heat-labile pathogenesis-related 10 proteins in patients with oral allergy syndrome is associated with symptoms that are generally more mild and rarely systemic.[86] Finally, glycosylation of antigens can prevent proteolysis and form neoantigens, potentially affecting food protein allergenicity. The Maillard reaction is a nonenzymatic chemical reaction between amino acids and reducing sugars occurring at high temperatures, which leads to the "browning" of food. Studies of peanut allergenicity have shown that dry roasting can increase sensitization to peanut in mouse models.[87] Indeed, when the major peanut epitopes Ara h 1 and ***Ara h 2*** were subjected to Maillard reactions, they bound higher levels of IgE from patients with peanut allergy and were resistant to digestion.[88] It might be that the allergenicity of various proteins from the environment and in food operate in concert to induce allergenic responses.

Strange encounters: Food allergen sensitization through skin

Atopic dermatitis is a chronic inflammatory disorder of the skin in which defects in the epidermal epithelium can lead to systemic allergen sensitization often preceding other atopic diseases, such as food allergy, asthma, and allergic rhinitis, a phenomenon known as the atopic march. Recent studies have highlighted the effect of both defective skin epithelium and the presence of food antigens in household dust in leading to sensitization to food allergens. Filaggrin is an epidermal protein involved in maintenance of skin barrier function, and patients with loss-of-function filaggrin mutations are at higher risk of eczema and atopy.[89,90] Patients with loss-of-function filaggrin mutations had a higher prevalence of food sensitization and development of food allergy by age 10 years.[91] Given this observation, Brough et al[45] investigated exposure of peanut protein in the dust of households consuming peanut and the development of peanut allergy in patients with skin barrier defects. In children with filaggrin loss-of-function mutations, peanut allergy was 3-fold higher in 8- to 11-year-olds compared with those without filaggrin mutations. As with filaggrin, polymorphisms of SPINK5, a protease regulating keratinocyte differentiation and affecting epithelial integrity, are associated with severe atopic dermatitis and increased incidence of food allergy.[92] Further supporting a role for cutaneous sensitization in food allergies, increased exposure to peanut protein in dust increased the risk of peanut sensitization in children, especially those with atopic dermatitis.[93]

Because atopic dermatitis has been identified as an instigating event predisposing to further sensitization, many have tried to find therapies to prevent eczema and potentially food sensitization. Simply applying moisturizers or emollients from birth has been shown to reduce the development of atopic dermatitis in 2 studies,[94,95] one of which also examined whether emollient use would influence allergic sensitization to egg but observed no differences between those receiving emollient and control groups.[95] Larger long-term studies are needed to determine whether this approach can prevent the further development of additional atopic diseases in patients with atopic dermatitis.

Just as some allergens can have an adjuvant effect in the respiratory and gastrointestinal tracts, some allergens can have adjuvant effects in the skin. Peanut extracts, specifically Ara h 2, were shown to have adjuvant activity in mouse skin.[96] *In vitro* peanut extract induced IL-33 and IL-6 expression in keratinocytes and upregulated OX40L expression on bone marrow–derived DCs, whereas *in vivo* peanut extract enhanced cutaneous responses to a bystander antigen, OVA, and promoted T_H2 T-cell development.[96] These observations suggest that future studies examining food allergenicity and the propensity to develop clinical food allergies might need to examine both the skin and gut.

THE MICROBIOME IN TOLERANCE AND ALLERGY

The communities of bacteria comprising the gut microbiome are complex and dynamic. They are influenced by the environment in which subjects live, and they evolve as persons age from infancy to adulthood.[97-99] Living in a rural versus urban environment likely influences the composition of an individual's microbiome,[97,100] but the underlying causes of these differences are not fully understood. An increased diversity of bacteria in

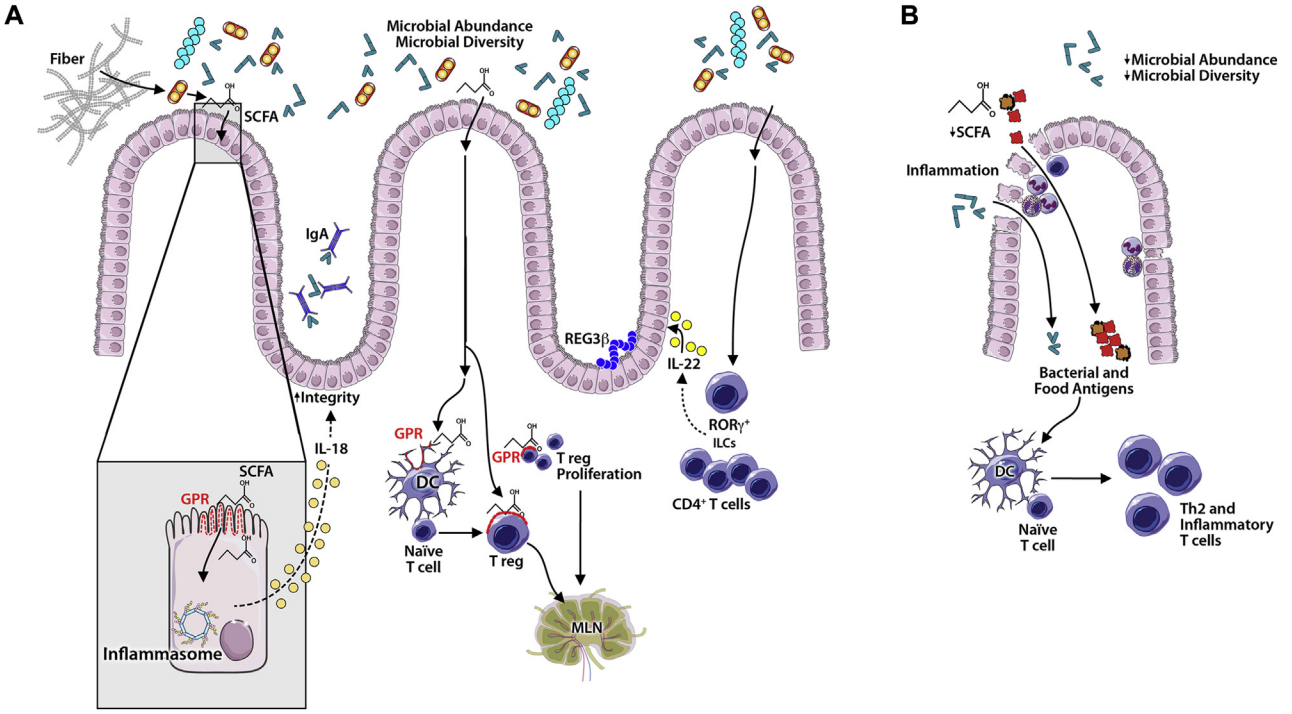

FIG 3. Microbial mechanisms contributing to oral tolerance and allergic sensitization in the colon. **A,** Microbial diversity and abundance promote tolerance. Microbes ferment fiber to produce SCFAs that bind GPRs on (1) intestinal epithelial cells to activate inflammasome production of IL-18 that promotes epithelial barrier integrity, (2) DCs to drive naive T cells to become Treg cells, and (3) Treg cells to induce proliferation. Additionally, SCFAs promote acetylation of histone H3 to preserve or induce Foxp3$^+$ Treg cells. Microbe-induced IL-22 production by RORγt$^+$ innate lymphocytes and CD4$^+$ T cells promotes barrier integrity and intestinal epithelial cell synthesis of antimicrobial peptides and mucus. Tolerogenic colonic DCs and lymphocytes likely migrate to MLNs. **B,** In allergic sensitization changes in microbial abundance and diversity (eg, after antibiotic exposure) decrease SCFA, IL-18, and IL-22 levels, compromising epithelial integrity and thereby facilitating epithelial passage of microbial and food antigens. DC activation promotes inflammation, development of T_H2 cell–associated immune responses (including production of allergen-specific IgE antibodies), and allergic sensitization.

household dust in farm homes inversely correlated with the risk of asthma and atopy,[101] but it is unclear whether this was related to any differences in the subjects' microbiomes. It is possible that additional factors can contribute to variations in microbial communities between urban and rural dwellers, such as diet.[102,103] For instance, plant-based diets promote growth of phyla capable of fermenting plant polysaccharides.[102] As factors affecting the diversity and development of the gut microbiome are being elucidated, it is also becoming clear that the microbiome can dramatically influence the development of immune responses in the gut, including those to food antigens (Fig 3).

Data suggest that particular bacteria, most notably from the Clostridia class, can promote the development of tolerance in the gut. Colonization of antibiotic-treated mice with Clostridia-enriched microbiota prevented allergen absorption and allergic sensitization, restoring oral tolerance.[104] Clostridia can promote tolerance in the gut through several mechanisms. Colonization of germ-free mice with Clostridia-enriched microbiota promoted IgA production and Foxp3$^+$ cell numbers in the colon.[104] IgA in the intestinal lumen (Fig 3, *A*) can regulate the composition of the microbiome and inhibit inflammation induced by the bacterial species and antigens to which it binds.[105,106] Although the mechanisms underlying these changes are unclear, *Clostridium* species clusters IV, XIVa, and XVIII are known to promote a TGF-β– and IL-10–rich environment in the mouse colon.[107,108] In addition, Clostridia promote ***IL-22*** production by RAR-related orphan receptor γ–positive innate lymphoid cells and T cells in the intestinal lamina propria, promoting epithelial integrity and upregulating expression of antimicrobial peptides, including REG3β and mucus (Fig 3, *A*).[104] Injection of anti–IL-22 in mice enhanced absorption of peanut antigens but did not lead to significantly increased levels of peanut-specific IgE or IgG.[104]

Exactly how Clostridia promote these effects is unknown, but evidence now supports a role for bacterial metabolites in regulating epithelial integrity and immune responses in the gut. The clostridial families Lachnospiraceae and Ruminococcaceae are among prominent bacterial groups in the proximal colon that ferment dietary fiber to produce short-chain fatty acids (SCFAs), including acetate, propionic acid, and most notably butyric acid, which can have multiple effects on the immune response (Fig 3, *A*).[109-112] SCFAs bind to the G protein–coupled receptors (GPRs) GPR43 and GPR109A on mouse enterocytes, activating the inflammasome and promoting production of IL-18; IL-18 fosters epithelial integrity, repair, and homeostasis.[113,114] SCFAs, particularly butyric acid, can increase numbers of colonic Foxp3$^+$ Treg cells when administered to mice in drinking water, as an enema, or as dietary precursors.[115-117] Inhibition of histone

deacetylase activity by SCFAs can promote acetylation of histone H3 at the Foxp3 promoter, thereby promoting Treg cell differentiation.[115,116] Signaling through GPR43 by ***propionate*** might promote expansion of Treg cells,[117] whereas there is conflicting evidence for whether GPR109a expression on APCs has a role in promoting Treg cell differentiation.[114,115] How the gut microbiome, which is largely resident in the colon, affects immune responses in the small intestine is unclear. Possible mechanisms include migration of colonic immune cells (APCs, T cells, and B cells) to the MLNs, where they interact with cells from the small intestine or transport bacterial metabolites or cytokines to distant sites through blood or lymph, where they can exert their effects; there also might be direct effects of the less abundant microbiota in the small intestine.[110]

Consistent with observations that the gut microbiome can normally promote oral tolerance, growing evidence suggests that perturbations of the microbiome might correlate with or even predispose to food allergy (Fig 3, *B*). Most notably early antibiotic use in human subjects has been linked to alterations in microbiome composition and development of food allergies. Intrapartum antibiotics were associated with changes in infant microbiome composition at 3 and 12 months.[118] Similarly, other studies have shown that effects of antibiotics on human microbiome composition can be persistent, even in older subjects.[119,120] Dysbiosis has also been observed in neonatal mice treated with antibiotics, and these perturbations also can persist.[104,121] Maternal use of antibiotics during human pregnancy or infant antibiotic use in the first month of life is associated with increased risk of cow's milk allergy,[122] and higher urinary levels of triclosan are found in children sensitized to food allergens and aeroallergens.[123] In mice it has been similarly observed that neonatal antibiotic administration promotes allergic sensitization to peanut.[104] In one study disturbances in the microbiome occurred at doses of antibiotics of only 1/50th to 1/100th of treatment doses,[121] raising the possibility that even exposure to low doses of antibiotics can affect human microbiome composition.

Given the large number of other factors that can affect microbial composition, as discussed above, identification of consistent patterns of microbiome features in patients with food allergy has been difficult. In a prospective study an increased Enterobacteriaceae to Bacteroidaceae ratio and low Ruminococcaceae abundance in the context of low microbiota richness at 3 months was associated with sensitization to 1 or more foods at 12 months of age.[118] In contrast, 4-month-old infants with milk allergy identified by means of double-blind oral food challenge were found to have increased microbial diversity with an increased abundance of Ruminococcaceae and Lachnospiraceae compared with that seen in healthy age-matched control subjects.[124] In that study the microbiota of healthy infant control subjects had lower diversity dominated by Bifidobacteriaceae, Enterobacteriaceae, and Enterococcaceae.[124] Chinese infants with both IgE- and non–IgE-mediated food allergy had similar overall microbial diversity when compared with control infants but showed alterations in particular phylotypes.[125] Notably, infants with IgE-mediated allergy had decreased levels of *Bacteroides* and *Clostridium* species XVIII.[125] Given the many factors that can influence microbiome composition in the gut, many more large longitudinal studies will be needed to identify whether reproducible patterns are associated with type I food allergy.

Skin microbiota in patients with food allergy

With growing evidence that food sensitization can occur through the skin, as discussed above, it is possible that dysbiosis of skin microbiomes also can contribute to food sensitization. Alterations of the skin microbiome have been observed in patients with atopic dermatitis and can drive atopic dermatitis in a mouse model;[126,127] therefore it is reasonable to hypothesize that such changes can promote food sensitization through the skin. ***Staphylococcal enterotoxin B*** acts as an adjuvant in the skin to drive T_H2 responses and ***follicular helper T cell*** development.[96] Indeed, staphylococcal enterotoxin B is used in animal models of food allergy as an oral adjuvant to promote allergic sensitization in the gut and can enhance T_H2 responses to peanut in mouse skin.[128,129] Clearly, the relationship between dysbiosis of the skin microbiota and food allergy needs to be more closely examined.

Probiotic therapy

Probiotic administration for the prevention or treatment of allergic disease has yielded conflicting results to date. A meta-analysis of trials of prenatal and neonatal probiotic treatment found reduced total IgE levels and atopic sensitization but no reductions in asthma or wheezing.[130] Few trials of probiotics in the prevention or treatment of food challenge–verified food allergies have been published. In patients with food challenge–proved cow's milk allergy, treatment with *Lactobacillus casei* and *Bifidobacterium lactis* for 12 months did not affect rates of milk allergy resolution; however, treatment with *Lactobacillus rhamnosus* in combination with extensively hydrolyzed casein formula increased rates of milk allergy resolution compared with those in a control group receiving hydrolyzed formula alone.[131-133] Notably, treatment with *L rhamnosus* correlated with increased levels of fecal ***butyrate***.[124] Similarly, coadministration of peanut oral immunotherapy (OIT) with *L rhamnosus* for 18 months resulted in nonresponsiveness in 82% of treated subjects at 2 to 5 weeks after cessation of OIT versus 3% of those receiving placebo.[134] However, because no OIT-only or probiotic-only control groups were included, it is unclear what the benefit of probiotic plus OIT would be over that of OIT alone or probiotic alone. It is likely that the benefits of probiotic supplementation are phyla specific, but insufficient data are currently available to support the use of probiotics containing particular phyla at this time. Additional data on microbiota dysbiosis in patients with food allergy will allow the design of appropriate randomized controlled trials of probiotics and prebiotics (dietary substances promoting the growth of beneficial microorganisms) in patients with food allergy. Given current data discussed above, it will be important to evaluate critically whether probiotic supplementation with specific Clostridia can have benefits for treating or preventing food allergy.

IMMUNOTHERAPY: MECHANISMS OF DESENSITIZATION AND LONG-TERM TOLERANCE

Multiple approaches have been attempted to regain or induce tolerance to foods. OIT was first reported by Schofield[135] in 1908, with successful desensitization through incorporation of egg into a child's diet. Note that we are using the term "desensitized" here to refer to the ability of a subject to ingest the offending food

without clinical reactivity to it but requiring continued consumption of that food to maintain this state of nonreactivity. Nelson et al[136] attempted subcutaneous desensitization to peanut in 1997 in a small cohort and noted significantly increased systemic reactions requiring epinephrine during buildup and maintenance periods, directing others to find alternative routes and safer approaches. Further attempts at desensitization have involved trials of OIT, sublingual immunotherapy (SLIT), or epicutaneous immunotherapy (EPIT). Although SLIT and EPIT are regarded as safer approaches compared with OIT because subjects typically only experience mild local oral and cutaneous reactions, respectively,[137-140] 1 subject undergoing SLIT for peanut allergy required epinephrine for urticaria and coughing.[141] OIT can be done safely, but subjects experience increased reactions (the majority are mild gastrointestinal complaints) with daily dosing during buildup and maintenance periods.[142] However, these approaches differ significantly in the amount of food to which the subject is effectively desensitized, with OIT achieving desensitization to serving sizes and SLIT and EPIT achieving desensitization to amounts substantially smaller than a typical serving size.[138,140,142-144] Recently, omalizumab, an mAb against IgE, has been explored as an adjunctive therapy with OIT, with multiple studies showing safe and faster desensitization rates to milk, peanut, or multiple foods simultaneously.[145-148] Although many questions remain in optimizing OIT regarding the optimal dose of the offending food allergen to be used for maintenance, the maintenance time period, and the sustainability of the desensitization process, OIT can achieve average desensitization rates of 80% to 85%.[149]

Whether these therapies produce long-lasting nonreactivity to the offending allergen (either with or without the continued intentional ingestion of those allergens) has only been analyzed in a few studies. Rechallenge after varying periods of avoidance has shown rates of sustained unresponsiveness (defined here as nonreactivity to a food challenge after avoidance of the offending allergen for periods of 1 week to 6 months) ranging from 13% to 36%.[149] Although these rates seem suboptimal, most subjects still maintained a state of desensitization to a threshold level higher than their screening challenge. Whether any of these patients will maintain "long-term tolerance" to that allergen, which we propose to define as experiencing years of unresponsiveness to the food in the absence of intentional ingestion of the offending allergens, remains to be seen. It also will be important to determine the extent to which the mechanisms underlying "desensitization" versus "long-term tolerance" are similar or different and to develop tests that can reliably determine the immune status of patients with food allergy.

Early responses to antigen-specific immunotherapy

Mechanisms of action in allergen-specific immunotherapy have been explored for allergic rhinitis and stinging insect hypersensitivity[150] and are likely to be similar in food allergy immunotherapy. Protection from reactions in the early stages of immunotherapy is associated with decreased activation of mast cells and basophils, which has been seen as early as in the first 3 to 4 months of OIT.[143,151-153] This might in part be due to reduced levels of antigen-specific IgE on the surfaces of these effector cells.[154,155] OIT in a mouse model of egg allergy did not result in desensitization of blood basophils or peritoneal mast cells or protection from challenge by means of intraperitoneal injection despite protection from oral challenge, implying that desensitization can occur locally in the gastrointestinal tract.[156] However, other mechanisms also might be involved. It has long been speculated that desensitization might deplete certain mediators of effector cells (eg, by inducing the release of histamine from granules) and stimulate leukotriene release but in amounts that are small and less than the threshold for causing anaphylaxis. In patients undergoing rush desensitization for venom allergy, decreased histamine levels were observed in whole blood, suggesting degranulation of basophils or decreased basophil numbers.[157] In contrast, patients undergoing standard (not rush) immunotherapy for venom allergy had normal histamine content in blood leukocytes.[158] Notably, mouse studies of oral desensitization for penicillin demonstrated antigen-specific desensitization with no evidence for mediator depletion.[156,159]

In addition to basophils and mast cells, many other cell types can contribute to early immunotherapy responses. During venom desensitization, monocytes increased expression of immunoglobulin-like transcript 3 and 4 receptors, which are crucial to the tolerogenic function of monocytes.[160] Tolerogenic APCs, such as $CD103^+$ DCs, as described above, can aid in the differentiation of T cells into Treg cells. Oral mucosal Langerhans cells have been shown to bind grass pollen in an *ex vivo* model, which enhanced their migratory capacity and promoted the secretion of the tolerogenic cytokines TFG-β1 and IL-10.[161] Grass pollen SCIT has been shown to mitigate seasonal increases in the numbers of peripheral ***type 2 innate lymphoid cells***,[162] potent potential producers of the type 2 cytokines IL-4, IL-13, and IL-5 that can enhance inflammation in patients with asthma, allergic rhinitis, and atopic dermatitis through activation of mast cells, basophils, and eosinophils and promotion of B-cell class-switching to produce IgE antibodies.[163-166]

Skin-derived APCs are important in directing the initial immune response. When OVA is applied to the intact skin of mice, it is taken up in the superficial layers of the stratum corneum and transported to the draining lymph nodes. After repeated epicutaneous delivery of OVA, local and systemic T_H2 responses are downregulated, with associated upregulation of Treg cells.[167] Consistent with this observation in mice, EPIT requires an intact stratum corneum layer.[168]

T-cell responses to antigen-specific immunotherapy

Induction of peripheral T-cell tolerance is a crucial step induced by immunotherapy, and in different models various changes in antigen-specific T-cell populations correlated with tolerance, including increased Treg cell numbers,[169] decreased T_H2 cell numbers,[170] and increased anergic T-cell numbers.[171] The proportion of allergen-specific T-cell subsets and the change in the dominant subset might skew toward allergy versus tolerance.[169] In a cholera toxin–induced mouse model of milk allergy, treatment with milk OIT increased levels of IL-10 and TGF-β in the jejunum, which were likely produced by Treg cells within the gut.[170] Using allergen-***MHC tetramers*** to track allergen-specific T cells during the course of wasp venom immunotherapy, clinical tolerance was associated with a loss of IL-4–producing T cells and increased IL-10–producing $Foxp3^+$ antigen-specific T cells that might share a common precursor with IL-4–producing T cells specific for the same epitope.[171] Recently, we used peanut-MHC dextramers to sort peanut-specific T cells from

patients with peanut allergy and analyzed changes in gene expression of individual $CD4^+$ T cells during the course of OIT. Our evidence indicated that increased length of treatment with OIT induced peanut-specific T cells to shift toward an anergic, memory T-cell phenotype ($CD28^{lo}Ki67^{lo}$). Moreover, sustained nonresponsiveness to peanut, even after a 3-month period of withdrawal from peanut, was associated with induction and maintenance of naive and memory peanut-specific T cells that were detectable even 3 months after therapy.[172] The failure to maintain tolerance to the offending allergen might be due to the induction of Treg cells that are short lived or epigenetically modified. In our cohort of subjects who completed 24 months of peanut OIT (20/23 subjects), we have shown that 7 of 20 subjects were still "immune tolerant" after a 3-month period of withdrawal, and 3 of 7 remained "immune tolerant" after an additional 3-month withdrawal period (totaling 6 months of withdrawal from therapy).[151] All subjects were found to have an increase in numbers of peanut-specific Treg cells after 12 months of OIT; in those who were immune tolerant, there was significant hypomethylation of ***CpG sites*** in peanut-specific Treg cells at 24 and 27 months.[151] In the 4 subjects who "lost" their tolerant status after 6 months of peanut withdrawal, there was increased methylation of their peanut-specific Treg cells.[151] These findings suggest that epigenetic changes of antigen-specific immune cells might, at least in part, explain desensitization and tolerance. However, achieving sustained responses to therapy might depend on whether such epigenetic changes can be maintained.

B-cell responses to antigen-specific immunotherapy

OIT can induce changes in immunoglobulin subsets in patients with peanut allergy. Patients undergoing peanut OIT for a median of 41 months exhibited increased levels of peanut-specific ***IgG$_4$*** with *de novo* specificities associated with reduced serum levels of peanut IgE.[173] In our own cohort of patients, peanut OIT was associated with increases in the frequency of peanut-specific B cells in the blood.[174] The allergen-specific B cells were mainly of the memory phenotype, with lower numbers of plasmablasts, and predominantly expressed somatically mutated class-switched antibodies of IgG and IgA subtypes, with lower numbers of IgM-expressing cells also noted.[174] Antibodies from these cells recognized both conformational and linear epitopes.[174] Notably, during the course of OIT, more highly mutated IgG_4-expressing members of a peanut-specific clone were observed; in contrast, the somatic mutation levels in IgE members of the clone did not increase, suggesting that ongoing somatic mutation of IgG_4-expressing B cells might contribute to the increased effectiveness of peanut OIT over time, perhaps by increasing IgG_4 affinity for allergen.[174]

Although increasing evidence supports a role for antigen-specific IgG_4 in directly promoting tolerance, IgG_4 levels can also correlate with other mechanisms responsible for inducing tolerance. Ratios of peanut-specific IgG_4 to peanut-specific IgE were higher in sensitized (ie, positive skin test or specific IgE results) but clinically tolerant patients than in patients with clinical peanut allergy.[175] Sera from sensitized but clinically tolerant patients or patients undergoing peanut OIT inhibited activation of peanut-sensitized mast cells or basophils by peanut extract, and the inhibitory activity of sera was decreased if IgG_4 was depleted.[175,176] The mechanisms by which IgG_4 inhibited mast cell or basophil activation might include IgG_4 activity as a "blocking antibody," binding allergen before it encounters IgE bound to the surfaces of basophils or mast cells, or IgG_4-dependent activation of ***inhibitory Fcγ receptors***.[150,177] Studies using blocking antibodies for CD32 with human basophils or in a mouse model of peanut OIT demonstrated that IgG binding to inhibitory Fcγ receptors is at least partially responsible for the observed inhibitory effects.[176] In a study of egg OIT, children with egg allergy had lower egg white–specific IgA levels compared with healthy control subjects; in most who became tolerant to egg, there was a significant increase of greater than 28% in egg white–specific IgA levels over time, suggesting a role for allergen-specific IgA in food tolerance.[178] Analysis of tolerant beekeepers and patients after venom immunotherapy demonstrated that venom-specific IgG_4 production was predominately through IL-10–secreting regulatory B1 cells, a population that was observed to increase after immunotherapy and to express high levels of IL-10 on a per-cell basis.[179] Large amounts of IL-10 produced by B cells could suppress effector T-cell function or promote differentiation of Treg cells. Overall, global epitope-specific shifts from IgE to IgG_4 binding occur over the course of immunotherapy and can often be attributed to B cells of a regulatory type. Such changes can act in concert with Treg cell alterations and tolerogenic APC functions to promote long-term tolerance.

SUMMARY

Oral tolerance to food is a result of complicated interactions among antigens in the food we consume, the microbiome inhabiting our guts, nonimmune cells in the gut, and specialized APCs and lymphocytes found in the gut and associated lymphatic tissues. A failure to develop or breakdown in tolerance leading to food allergy could occur at multiple points and possibly in multiple tissues, including the gut or skin. Future progress in our understanding of oral tolerance in human subjects will be greatly advanced by new techniques allowing detailed analysis of innate and antigen-specific responses in the blood and in small samples of affected tissues complemented by using genetic models in experimental animals to analyze in mechanistic detail immune responses in the gut that contribute to sensitization, desensitization, and tolerance. Such studies will offer crucial insights into factors determining development of natural or therapy-induced long-lasting tolerance.

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