

# Mechanisms of food allergy



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**Although oral tolerance is the normal physiologic response to ingested antigens, a breakdown in this process appears to have occurred in the past 2 decades, leading to an increasing prevalence of sensitization to food allergens. Over the past decade, basic research has intensified in an attempt to better understand the mechanisms leading to sensitization and disease versus desensitization and short- and long-term tolerance. In this review we assess various factors that can influence tissue and immune responses to food antigens, the current understanding of immune tolerance development, the role of the gastrointestinal microbiota, and current knowledge regarding immunologic mechanisms involved in desensitization and sustained unresponsiveness, although perhaps the latter is more appropriately termed remission. (J Allergy Clin Immunol 2018;141:11-9.)**

**Key words:** Food allergy, sensitization, tolerance, desensitization, sustained unresponsiveness, remission, allergen immunotherapy, microbiota

The prevalence of food allergy is increasing, and the development of more accurate diagnostic methods, prevention, and treatment require a better understanding of the underlying mechanisms. Oral tolerance is the normal physiologic response to ingested antigens, and a breakdown in this process results in sensitization to food allergens.<sup>1</sup>

Studies for a better understanding of the mechanisms leading to sensitization and disease versus desensitization and short- and long-term tolerance are being pursued intensively.<sup>2</sup> A number of animal models have been developed to investigate cellular and molecular events, which lead to food allergen sensitization and

### Abbreviations used

AD: Atopic dermatitis  
Breg: Regulatory B  
DC: Dendritic cell  
EC: Epithelial cell  
FoxP3: Forkhead box P3  
ILC: Innate lymphoid cell  
ILC3: Type 3 innate lymphoid cell  
OIT: Oral immunotherapy  
Treg: Regulatory T

anaphylaxis.<sup>2-5</sup> One key finding has been that oral administration of a protein to an animal normally induces tolerance but can result in sensitization and allergic disease.<sup>6</sup> The responses in animal models have been shown to be influenced by a long list of factors that damage the epithelial barrier (Box 1).<sup>7-10</sup> These models also suggest that sensitization to food allergens can actually occur through other sites, such as the airways or skin, in contrast to the intestine, where oral tolerance is typically the default response.

The results of these murine models also support the observation that early skin barrier disruption caused by inflammation or genetic defects (eg, filaggrin gene mutations) are associated with increased rates of food sensitization in human subjects.<sup>11</sup> However, studies on IgE responses and digestibility of food proteins suggest that the oral route of exposure is also an important path for sensitization to food allergens.

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Disclosure of potential conflict of interest: H. A. Sampson has received grants from the National Institute of Allergy and Infectious Diseases (AI-44236, CoFar, ITN); has consultant arrangements with Allertein Therapeutics, LLC, Hycor, and UCB; is Chief Scientific Officer of DBV Technologies; has received royalties from UpToDate; and has stock/stock options with DBV Technologies. A. W. Burks reports personal fees from NIH AITC Review Panel, Allertein, American Society for Microbiology, Elsevier, FARE, World Allergy Organization, Adept Field Solutions, Aimmune Therapeutics, Inc, Astellas Pharma Global Development, Inc, Biomerica, Inc, Evelo Biosciences, Inc/Epiva Biosciences, Inc, First Manhattan Co, Genentech, GLG Research, Inc, Insys Therapeutics, Intrimmune Therapeutics, PPD Development, LP, Regeneron Pharmaceuticals, Inc, Sanofi US Services, SRA International, Stallergenes, UKKO, Inc, and Valeant Pharmaceuticals North America, LLC and reports

grants from Food Allergy Research and Education (FARE), National Institutes of Health, and Wallace Research Foundation. G. Lack has received grants from the National Institutes of Allergy and Infectious Diseases (NO1-AI-15416 [contract] and UM1AI109565 [grant]), Food Allergy Research and Education (FARE), MRC & Asthma UK Centre, UK Department of Health through the National Institute for Health Research, the National Peanut Board, and Osem; and has consultant arrangements and stock/stock options with DBV Technologies. C. A. Akdis has received grants from Actellion, the European Union projects Medall and Predicta, Allergopharma, the Swiss National Science Foundation, and the Christine Kühne Center for Allergy Research and Education. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication August 11, 2017; revised November 10, 2017; accepted for publication November 14, 2017.

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0091-6749/\$36.00

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<https://doi.org/10.1016/j.jaci.2017.11.005>

Terms in boldface and italics are defined in the glossary on page 12.

This review presents an overview of mechanisms of food allergy, focusing in particular on pathways leading to immune tolerance. In addition, it includes suggestions for new nomenclature on the definition of sustained unresponsiveness, desensitization, and tolerance.

## ANTIGEN-SPECIFIC IMMUNE RESPONSE DEVELOPMENT TO FOOD ANTIGENS

Sensitization to food antigens can take place in the gastrointestinal tract, oral cavity, and skin and occasionally in the respiratory tract. After ingestion, the vast majority of food

proteins are broken down largely by gastric acid and digestive enzymes in the stomach and intestine. Subsequently, the remaining intact food proteins and peptides are transported from the lumen to the mucosa through gut epithelial cells (ECs) and by specialized ECs called M cells that are localized above Peyer patches.

In addition, direct sampling of ingested antigens/allergens can occur when mucosal dendritic cells (DCs) extend dendrites into the gut lumen. In the mucosa DCs internalize and process these proteins and peptides and move to T-cell areas of draining lymph nodes, where the DCs can interact with naive T cells and present antigen on *MHC class II* molecules (Fig 1).<sup>12</sup> The activation of

### GLOSSARY

**CD28:** A protein expressed on T cells that provides costimulatory signals required for T-cell activation and survival, cytokine production, and T<sub>H</sub>2 development.

**CD80 AND CD86:** Also known as B7-1 and B7-2, CD80 and CD86 are proteins expressed on dendritic cells, activated B cells, and monocytes that work in tandem to provide a costimulatory signal necessary for T-cell activation and survival. They are ligands for the T-cell proteins CD28 and cytotoxic T lymphocyte-associated protein 4.

**CCR6:** A protein that belongs to family A of the G protein-coupled receptor superfamily that is expressed preferentially by immature dendritic cells and memory T cells. The ligand of this receptor is macrophage inflammatory protein 3 $\alpha$ . CCR6 is known to be important for B-lineage maturation and antigen-driven B-cell differentiation and is thought to regulate the migration and recruitment of dendritic and T cells during inflammatory and immunologic responses.

**CYTOTOXIC T LYMPHOCYTE-ASSOCIATED PROTEIN 4 (CTLA-4):** A receptor that functions as an inhibitory signal that downregulates immune responses when bound to CD80 and CD86. CTLA-4 is constitutively expressed in regulatory T cells but is only upregulated in conventional T cells after activation.

**GM-CSF:** Also known as colony-stimulating factor 2, GM-CSF is a monomeric glycoprotein secreted by macrophages, T cells, mast cells, natural killer cells, endothelial cells, and fibroblasts and functions as a cytokine. GM-CSF functions as a white blood cell growth factor and stimulates stem cells to produce granulocytes (neutrophils, eosinophils, and basophils) and monocytes.

**GRANZYMES A AND B:** Serine proteases that are released by cytoplasmic granules within cytotoxic T cells and natural killer (NK) cells. They induce programmed cell death in the target cell, thus eliminating cancerous or infected cells. In NK cells and T cells the granzymes are packaged in cytotoxic granules with perforin. Granzyme A is the most abundant and activates a novel programmed cell death pathway, whereas granzyme B activates apoptosis through activation of caspases (especially caspase-3), which in turn cleaves many substrates, including caspase-activated DNase to execute cell death.

**IL-1 $\beta$ :** A member of the IL-1 cytokine family that is produced by activated macrophages as a proprotein, which is proteolytically processed to its active form by caspase 1. IL-1 $\beta$  is an important mediator of the inflammatory response and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis.

**IL-6:** A cytokine also known as IFN- $\beta$ 2, IL-6 is implicated in a wide variety of inflammation-associated disease states, has been associated with the maturation of B cells, and has been shown to act as an endogenous pyrogen capable of inducing fever in patients with autoimmune diseases or infections.

**IL-22:** A cytokine that has important functions in host defense at mucosal surfaces, as well as in tissue repair. It is unique in that it is produced by immune cells, including T<sub>H</sub> cell subsets and innate lymphocytes, but acts

only on nonhematopoietic stromal cells, in particular epithelial cells, keratinocytes, and hepatocytes.

**IL-35:** An IL-12 family cytokine produced by regulatory, but not effector, T and B cells and plays a role in immune suppression.

**MHC CLASS II:** A complex critical in initiating immune responses, MHC class II is found on antigen-presenting cells and presents antigen derived from extracellular proteins to T-cell receptors.

**OX40 AND OX40 LIGAND:** Members of the tumor necrosis factor superfamily expressed on a variety of cells, including activated CD4<sup>+</sup> and CD8<sup>+</sup> T cells. The OX40–OX40 ligand complex has been shown to regulate cytokine production from T cells, antigen-presenting cells, natural killer cells, and natural killer T cells while modulating cytokine receptor signaling. This complex plays a central role in the development of multiple inflammatory and autoimmune diseases, making them ideal therapeutic candidates.

**PROGRAMMED CELL DEATH 1 (PD-1):** A cell-surface receptor that plays an important role in downregulating the immune system and suppressing inflammatory T-cell activation. PD-1 is an immune checkpoint that serves the dual role of promoting apoptosis in antigen-specific T cells while simultaneously reducing apoptosis in regulatory T cells.

**$\gamma/\delta$  T CELLS:** A small subset of T cells comprising the highest abundance of T cells in the gut mucosa that possess a distinct T-cell receptor (TCR) on their surface. These T cells have a TCR that is made up of one  $\gamma$  chain and one  $\delta$  chain, unlike most T cells, which are  $\alpha\beta$  T cells.

**TGF- $\beta$ :** A cytokine secreted by many cell types, including macrophages, that controls proliferation, cellular differentiation, and inflammatory processes in a variety of cells. It also plays a role in T-cell regulation and differentiation.

**TOLL-LIKE RECEPTOR 4 (TLR4):** A member of the Toll-like receptor family, TLR4 is a human transmembrane protein that belongs to the pattern recognition receptor family. Its activation leads to activation of the innate immune system through an intracellular signaling pathway, nuclear factor  $\kappa$ B, and inflammatory cytokine production. TLR4 recognizes LPS, which is a component present in many gram-negative bacteria and select gram-positive bacteria. Its ligands also include several viral proteins, polysaccharides, and a variety of endogenous proteins.

**TOLL-LIKE RECEPTOR 8 (TLR8):** A member of the Toll-like receptor family, TLR8 is an endosomal receptor that recognizes single-stranded RNA (ssRNA) and can recognize ssRNA viruses, such as influenza, Sendai, and Coxsackie B viruses.

**TYPE 3 INNATE LYMPHOID CELLS (ILC3s):** Defined by their production of the cytokines IL-17A, IL-22, or both. They are the innate counterpart to T<sub>H</sub>17 cells, sharing the common transcription factor of retinoic acid-related orphan receptor  $\gamma$ t.

*The Editors wish to acknowledge Kristina Bielewicz, MS, for preparing this glossary.*

**Box 1.** Factors that can influence tissue responses and immune response to food antigens

Food-related factors

- Epithelial barrier–damaging factors (alcohol, toxins, unknown ingredients, detergents)
- Allergen type and exposure dose
- Type of adjuvants in the allergen and their dose, microbial products, and contamination of microorganisms
- Route of exposure
- Food matrix effects, such as aggregated or repetitive proteins, lipids, and glycosylated sugars
- Cooking temperature

Individual factors

- Age and immune status
- Microbiome
- Barrier defects (filaggrin mutations)
- Certain drugs (antacids)
- Underlining disease (atopy, dermatitis, rhinitis, and immune deficiency)

different DC subsets and expression of costimulatory molecules are important in determining the subsequent immune response.<sup>13</sup> For example, the interaction between *CD28*, which is present on T cells, with *CD80* and *CD86*, which are expressed on DCs, induces T-cell activation, whereas interaction of CD80 and CD86 with cytotoxic T lymphocyte–associated protein 4 present on T cells downregulates T-cell activation.

Several factors play a role in the development of T<sub>H</sub>2 polarization. IL-4 secreted from innate lymphoid cells (ILCs), basophils, and natural killer T cells is a major player in the further development of type 2 immune responses.<sup>2</sup> Recently, Wambre et al<sup>14</sup> identified a unique subset of antigen-specific T<sub>H</sub>2 cells (T<sub>H</sub>2A cells) in allergic patients that drive IgE class-switching and expansion of allergic effector cells. In addition, activation of DCs mediated by OX40 ligand (*OX40L*) appears to be important in the induction of allergic sensitization to food allergens.<sup>15</sup> Advanced glycation end-products in foods (generated by high heat treatment or in the presence of a high concentration of sugars) might inadvertently activate DCs and lymphocytes, resulting in a “false alarm,” ultimately leading to sensitization and food allergy.<sup>16</sup>

IgE is a fundamental antibody in patients with atopic diseases and a hallmark of allergic sensitization. T<sub>H</sub>2 lymphocytes and associated cytokines support B-cell proliferation, promote immunoglobulin isotype class-switch recombination toward IgE, and drive their differentiation into antibody-secreting plasma cells. IgE mediates immediate-phase reactions by inducing mast cell and basophil degranulation. After sensitization and re-exposure to the allergen, mast cell–derived mediators, such as histamine, prostaglandin, and proteases, change the bioavailability of food proteins, whereas ECs upregulate their secretion of T<sub>H</sub>2-promoting mediators, including thymic stromal lymphopoietin, IL-25, and IL-33. These cytokines also upregulate OX40 ligand on DCs and support the expansion of the IL-4– and IL-13– producing type 2 ILC subset, which further stimulates DCs, reduces allergen-specific regulatory T (Treg) cells, and activates mast cells.<sup>17,18</sup> Although demonstrated in murine models of asthma but not in food allergy, IL-33 dysregulates Treg cells and impairs established immunologic tolerance.<sup>19</sup> However, in epicutaneously sensitized mice IL-33 promotes orally induced

anaphylaxis by targeting mast cells.<sup>5</sup> Migration and activation of intraepithelial lymphocytes, including  $\gamma\delta$  T cells, also occurs in response to allergic sensitization in mice. Recently, it was reported that injection of an mAb to IL-25, IL-33 receptor, or thymic stromal lymphopoietin strongly inhibited food allergy development in mice. Administration of a single mAb targeting either of these cytokines could not suppress established food allergy and optimal food allergy suppression required treatment with a cocktail of all 3 anti-pro-T<sub>H</sub>2 mAbs.<sup>20</sup>

## THE SKIN BARRIER AND FOOD ALLERGEN SENSITIZATION

Skin barrier dysfunction is predictive of food allergy and supports the concept of transcutaneous allergen sensitization, particularly in patients with atopic dermatitis (AD).<sup>21</sup> Several different lines of evidence have been published recently in this area. Worsening of the skin on air-exposed skin sites, but not covered sites, during aeroallergen challenge in sensitized patients with AD suggests dysfunction of the epidermal barrier.<sup>22</sup> There is evidence that a lack of filaggrin breakdown products favors trans-epidermal water loss, allergen penetration, and skin colonization with *Staphylococcus aureus*. This explains why filaggrin loss-of-function mutations are associated with higher total IgE levels, sensitization to more allergens, and a more severe course of AD, as well as allergic asthma.<sup>21</sup>

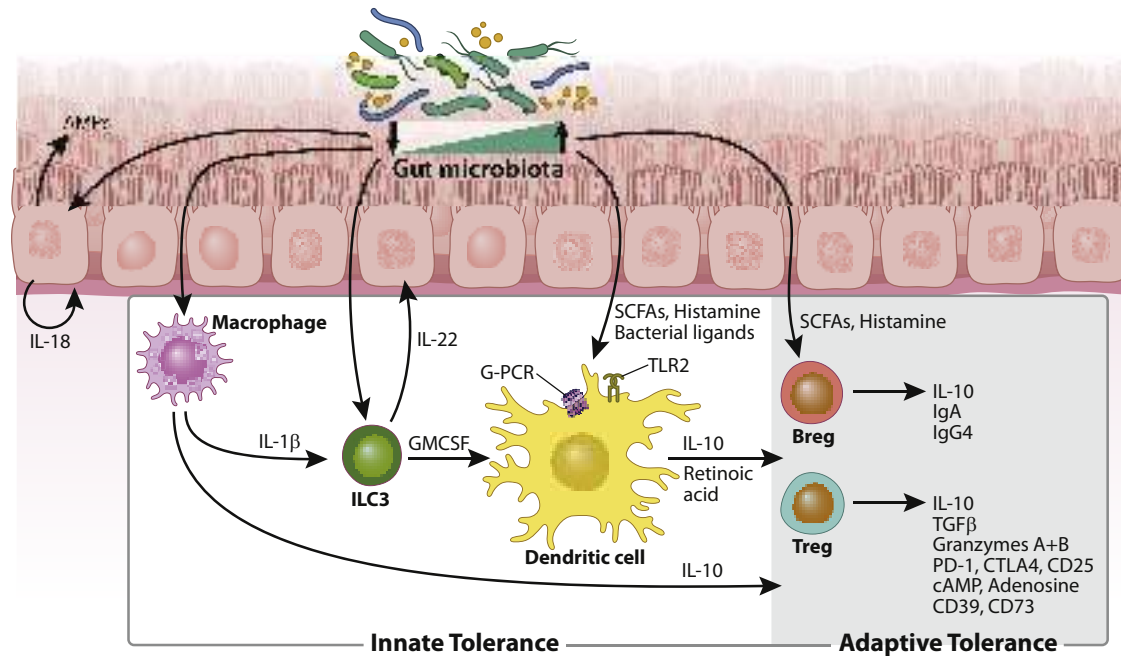
In addition to the stratum corneum barrier, the tight junction barrier is located in the granular layer of the epidermis and contributes to the barrier dysfunction and immune dysregulation observed in patients with AD.<sup>23,24</sup> Impaired skin barrier function at birth and 2 months has been shown to precede clinical AD, demonstrating that an early barrier defect is predictive of increased allergen sensitization.<sup>11</sup>

In addition, it was demonstrated that commercial detergents and surfactants induce skin barrier leakiness at extremely low doses.<sup>25</sup> Molecular mechanisms that decrease this barrier-damaging effect by novel ingredients included in detergents are currently emerging.<sup>26</sup>

In addition, several new molecular mechanisms that influence the tight junction barrier of the skin, nose, lung, and gut have been reported, such as platelet-activating factor and ILCs.<sup>23-30</sup> Leakiness in the gut might play a role in the pathogenesis of not only allergic but also autoimmune diseases.<sup>31</sup>

## MECHANISMS OF DEVELOPMENT OF NATURAL AND THERAPEUTICALLY INDUCED TOLERANCE

Overall, oral tolerance is thought to involve suppression of T<sub>H</sub>2 cells, generation of Treg cells, decreased production of IgE by B cells, increased IgA and IgG<sub>4</sub> production by B cells, suppression of effector T-cell migration to tissues, induction of IL-10–producing DCs, and suppression of basophil, eosinophil, and mast cell activation.<sup>32</sup> Although there might be some differences, in principle, the same mechanisms that have been reported to be responsible for the development of immune tolerance to other allergens through different routes, such as bee venom injected into the skin and grass pollen and house dust mite inhaled into the upper and lower respiratory tracts, likely apply in oral tolerance.<sup>33,34</sup> However, the reasons for a loss of tolerance to foods in allergic patients is still not clear.



**FIG 1.** Interactions between the microbiota and innate and adaptive immune systems in tolerance induction within the mucosa. The gut microbiota has been shown to interact with the mucosal immune system at many levels to support the induction of tolerance. Microbially derived metabolites induce inflammasome activation in ECs, leading to release of IL-18 and antimicrobial peptide (AMP) secretion, thereby strengthening the epithelial barrier. ILC3-derived IL-22 also promotes the epithelial barrier. Macrophage-derived IL-1 $\beta$  promotes GM-CSF release from ILC3s, further promoting IL-10 and retinoic acid secretion by DCs, which are essential for induction of Breg and Treg cells. Mucosal DCs can be influenced directly by microbially associated metabolites, such as short-chain fatty acids (SCFAs) and histamine, which polarize cytokine production through G protein-coupled receptor (G-PCR) signaling. Bacterially derived ligands can directly activate DC pattern recognition receptors, in particular Toll-like receptor 2 (TLR2), also promoting IL-10 and retinoic acid secretion. Mucosal macrophages secrete large amounts of IL-10, thereby contributing to the tolerance state. In addition to the influence of immunoregulatory factors released by microbiota-exposed innate immune cells, on Breg and Treg polarization, the microbiota can also have direct effects on both Breg and Treg cells. Metabolites, such as SCFAs and histamine, promote polarization of these regulatory cells, and activation of Toll-like receptor 9 supports expansion of IL-10<sup>+</sup> Breg cells. cAMP, Cyclic AMP; CTLA4, cytotoxic T lymphocyte-associated protein 4; PD-1, programmed cell death 1.

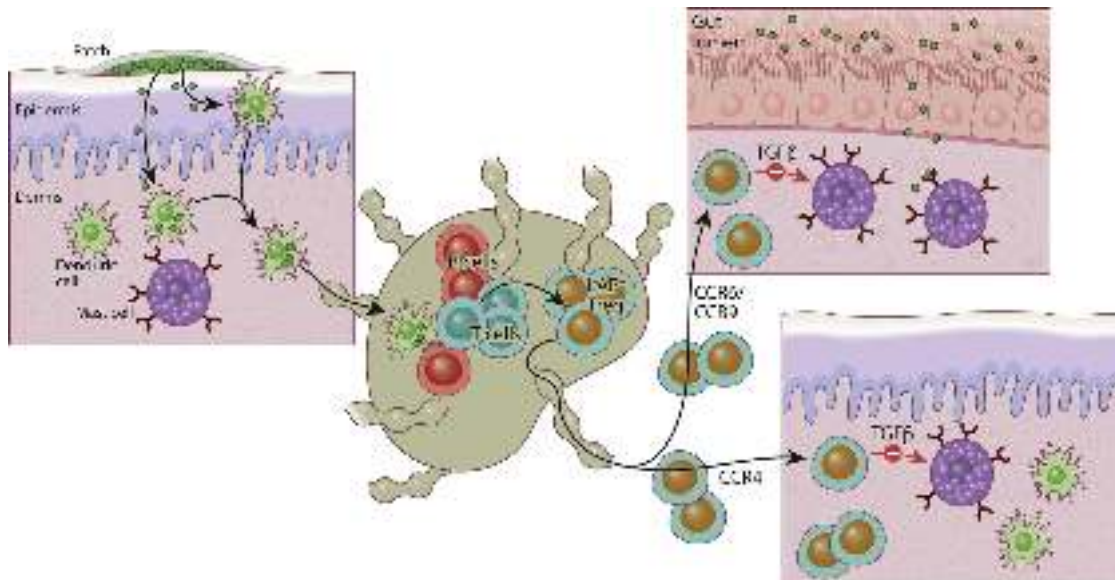
## ROLE OF TREG AND REGULATORY B CELLS

Oral tolerance to food antigens requires the robust induction of Treg cells within the mucosa. The gut microenvironment supports and promotes expansion of Treg cells through multiple mechanisms, including the presence of retinoic acid- and bacteria-derived metabolites, such as short-chain fatty acids.<sup>35</sup> The main mechanisms underpinning Treg cell immune effects include production of suppressor cytokines (IL-10, TGF- $\beta$ , and IL-35), effector cell cytotoxicity (through secretion of *granzymes A and B*), direct targeting of DCs through inhibitory *programmed cell death 1* and cytotoxic T lymphocyte-associated protein 4 cell-surface molecules, and metabolic disruption of effector cells (CD25, cyclic AMP, adenosine, CD39, and CD73).<sup>36</sup> Forkhead box P3 (FoxP3)<sup>+</sup> induced Treg cells have been shown to be mandatory for oral tolerance, whereas thymus-derived natural Treg cells, which also express FoxP3, are not directly involved in oral tolerance induction. In addition, induced Treg cells, rather than natural Treg cells, are involved in control of mucosal T<sub>H</sub>2 responses.<sup>37</sup> Interestingly, an increased frequency of CD4<sup>+</sup>CD25<sup>+</sup> Treg cells was observed in children who outgrew milk allergy, suggesting that development of oral tolerance to food allergens in human subjects involves Treg cells, at least early on.<sup>38</sup>

Atopic children with food allergy have lower percentages of CD25<sup>+</sup>CD127<sup>lo</sup>FoxP3<sup>+</sup> Treg cells compared with healthy control subjects of similar age.<sup>39</sup> In addition, age-related increases in Treg cell expression of *CCR6* were observed in healthy control subjects but not children with food allergy, which might suggest a delay in Treg cell migration to peripheral sites of inflammation, a likely handicap in the maintenance of immune tolerance.

Activation of ILCs by local epithelial cytokines has been shown to play a major role in the development of T<sub>H</sub>2-driven inflammatory and allergic responses in the skin, lung, and gastrointestinal tract, such as AD, asthma, and eosinophilic esophagitis, respectively.<sup>40</sup> Evidence has been accumulating that these inflammatory responses can be initiated and regulated independently of the adaptive immune system. Recently, Wang et al<sup>41</sup> identified a new subset of ILCs, regulatory ILCs, in the intestine that could inhibit inflammation. Such regulatory ILCs are likely to be identified in other inflamed tissues.

B cells can also limit aggressive immune reactivity. Regulatory B (Breg) cells regulate immune responses, mainly through IL-10, which has been shown in experimental models of infection, allergic inflammation, and tolerance.<sup>42,43</sup> Within the mesenteric lymph nodes, IL-10-producing CD5<sup>+</sup> Breg cells might play a



**FIG 2.** Mechanisms of epicutaneous immunotherapy (EPIT) in protection of mice from anaphylaxis. By using an adjuvant-free model of food allergy generated by means of epicutaneous sensitization and reactions triggered by oral allergen challenge, EPIT induced sustained protection against anaphylaxis. The gastrointestinal tract is deficient in *de novo* generation of Treg cells in allergic mice. This defect is tissue specific, and epicutaneous application of antigen generated a population of gastrointestinal tract-homing LAP<sup>+</sup>FoxP3<sup>-</sup> Treg cells. This mechanism of protection represents a novel pathway of direct TGF- $\beta$ -dependent Treg cell suppression of mast cell activation.<sup>3</sup> Artwork adapted from Tordesillas et al.<sup>3</sup> Figure credit to Molecule Medical Arts, LLC.

role in regulation of IgE-mediated anaphylaxis after challenge with cow's milk allergen in mice.<sup>44</sup>

By using an adjuvant-free model of food allergy generated by means of epicutaneous sensitization and reactions triggered by oral allergen challenge, it was found that epicutaneous immunotherapy induced sustained protection against anaphylaxis.<sup>3</sup> In allergic mice the gastrointestinal tract was deficient in *de novo* generation of Treg cells, and epicutaneous application of antigen generated a population of gastrointestinal tract-homing latency-associated peptide (LAP)<sup>+</sup>FoxP3<sup>-</sup> Treg cells, a novel pathway of direct TGF- $\beta$ -dependent Treg cell suppression of mast cell activation in the absence of modulation of T- or B-cell responses (Fig 2).<sup>3</sup>

## ROLE OF THE MICROBIOTA

The balance between immune tolerance and inflammation is regulated in part by the crosstalk between innate and adaptive immune cells and the intestinal microbiota.<sup>45-47</sup> Many studies now provide clear and strong associations between the composition and metabolic activity of the bacterial microbiota and the development of allergic disease and protective tolerogenic pathways.<sup>48,49</sup> Studies of children with milk allergy have shown that infants with milk allergy have an altered microbiota, and longitudinal studies showed that Firmicutes, including *Clostridium* species, were enriched in the early infant gut microbiome of patients whose milk allergy resolved by 8 years of age.<sup>50</sup> In patients with peanut or tree nut allergy, reduced microbial richness and increased abundance of *Bacteroides* species were observed compared with nonallergic control subjects.<sup>51</sup> Germ-free mice display an exaggerated anaphylactic response to challenge with a food allergen, whereas transfer of the microbiota from food allergy-prone

mice (with a gain-of-function mutation in the IL-4 receptor  $\alpha$  chain) to wild type germ-free animals transfers the food allergy phenotype.<sup>52</sup>

In another study it was shown that microbial signals sensed by intestinal macrophages promoted *IL-1 $\beta$*  secretion and that this IL-1 $\beta$  supported *GM-CSF* release by local *type 3 innate lymphoid cells (ILC3s)*. ILC3-derived GM-CSF then triggered DC and macrophage secretion of retinoic acid and IL-10, which, in turn, promoted induction and expansion of mucosal Treg cells. Disturbance of this crosstalk significantly altered mucosal immune effector functions, resulting in impaired oral tolerance to dietary antigens.<sup>53</sup>

Gut microbes secrete histamine, which influences mucosal inflammatory responses through histamine receptor 2, and it was demonstrated recently that there are increased numbers of histamine-secreting microbes in the guts of asthmatic patients; however, it is not yet known whether bacteria-derived histamine can influence the allergic response to food allergens within the gut.<sup>54,55</sup>

The deliberate administration of specific bacterial strains, such as *Bifidobacterium* or *Clostridium* species to mice was shown to protect against food allergen sensitization because of the induction of Treg cells within the mucosa.<sup>56</sup> In addition, clostridia can also stimulate ILC3s to produce *IL-22*, which helps to reinforce the epithelial barrier and reduce the permeability of the intestine to dietary proteins.<sup>57</sup> In human subjects administration of *Bifidobacterium longum* 35624 has been shown to increase the number of FoxP3<sup>+</sup> Treg cells in peripheral blood,<sup>58</sup> whereas the combination of *Lactobacillus rhamnosus* GG and peanut oral immunotherapy (OIT) for 18 months induced a high rate of desensitization compared with placebo treatment.<sup>59</sup> However, the efficacy of the probiotic itself is unclear because of a lack of necessary controls (ie, there was no OIT-only or probiotic-only

group). There are many unanswered questions with respect to the role of the microbiota in food sensitization versus tolerance. Its role in desensitization, sustained unresponsiveness, and long-term tolerance needs to be extensively studied.

In addition to fecal microbial content, local microbiota in the tonsils, upper gastrointestinal tract (eg, the duodenum), and skin have not been adequately studied thus far. Further studies are required to determine whether manipulation of the microbiota during immunotherapy will lead to acquisition of tolerance (ie, a state of prolonged unresponsiveness in the absence of continuous exposure to the allergen). The potential mechanisms by which the microbiota can promote tolerance are illustrated in Fig 1.

Interestingly, it was shown recently that the majority of Treg cells induced in the small intestine are activated by dietary antigens from solid foods and repress underlying immunity to ingested protein antigens. These Treg cells are distinguishable from microbiota-induced Treg cells, which are generated primarily in the colon.<sup>60</sup>

## MECHANISMS OF DESENSITIZATION AND SUSTAINED UNRESPONSIVENESS

The mechanisms mediating sustained unresponsiveness (ie, short-term desensitization or temporary loss of allergic response to an allergen that returns after a variable period of time) in the absence of continued exposure to the allergen (remission) after immunotherapy are still unclear (Box 2). In particular, it is not known whether sustained unresponsiveness or desensitization are mediated by different mechanisms or whether these are sequential steps in the development toward immunologic tolerance (Fig 3). It is thought that one of the main mechanisms underlying OIT is the induction of Treg cells with subsequent increases in IL-10 and TGF- $\beta$ . The specific Treg cell subtypes required for successful immunotherapy are unknown currently, but interestingly, a recent study showed hypomethylation of CpG sites on FoxP3<sup>+</sup> Treg cells in patients who achieved sustained unresponsiveness, suggesting that epigenetic changes might be important for desensitization and tolerance.<sup>61</sup>

A role for Breg cells in immunotherapy outcomes has been suggested by the significant increases observed in levels of IgG<sub>4</sub> specific for food antigens after OIT.<sup>62</sup> The increase in IgG<sub>4</sub> levels associated with a subsequent decrease in IgE levels might be secondary to the downregulation of IL-4 (which induces IgE) and upregulation of IL-10 production (which induces IgG<sub>4</sub>). OIT has also been shown to increase the frequency of peanut allergen-binding B cells in peripheral blood and can stimulate somatic mutation of allergen-specific IgG<sub>4</sub>.<sup>63</sup> However, clinical improvement does not always correlate with IgG<sub>4</sub> levels in serum.

IgA can also be induced during immunotherapy, which might be important for blocking antigen binding and transport by ECs, but its role in preventing allergic responses requires further research. Indeed, IgA deficiency is associated with an increased risk of food allergy.<sup>64</sup>

Nowadays, it is well established that very rapid desensitization of mast cells and basophils occurs that nonspecifically impairs systemic anaphylaxis during immunotherapy to other allergens. This desensitization takes place quite early after the first administration. Although the involved mechanisms are not yet fully understood in food allergy, it is thought that events similar to those observed during rapid drug desensitization might be

**Box 2.** What is unknown in mechanisms of immune tolerance development to food allergens?

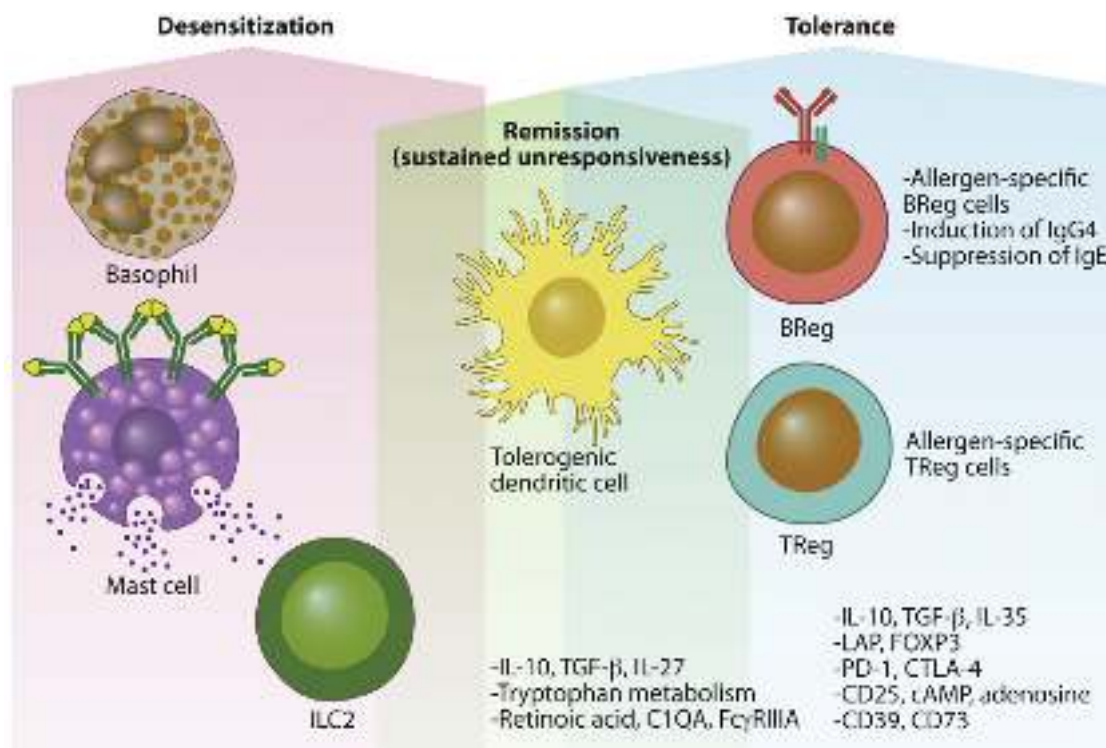
- Exact mechanisms of desensitization
- Host and environmental factors that facilitate induction of tolerance
- Food- and constituent-related factors that affect immune tolerance development
- Molecular mechanisms of Treg and Breg cell generation *in vivo*
- Adjuvants that promote Treg and Breg cells *in vivo*
- Lifespan of food immunotherapy-induced Treg and Breg cells *in vivo*
- Relationship of resident tissue cells with food immunotherapy-induced immune tolerance
- Early biomarkers and predictors for the success of food immunotherapy
- Local events in the microenvironment during different types of food immunotherapy, such as epicutaneous immunotherapy
- Identifying the optimal allergen dose and mechanisms of high-dose and low-dose immunotherapy
- Mechanisms that play a role in remission to long-term tolerance
- Mechanisms of long-term maintenance of allergen tolerance
- Is boosting needed for long-term effect? What should be the optimum time?
- Mechanisms of inducing high-affinity IgG<sub>4</sub> and low-affinity IgE antibodies and memory B-cell responses

working during allergen immunotherapy.<sup>34,65,66</sup> Decreased activation of mast cells and basophils can happen within a few hours in patients undergoing ultrarush venom immunotherapy; however, it takes 3 to 4 months in OIT.

The dose of allergen interacting with the responsible cells and their regulation by the immune microenvironment, which likely differs between different organs and tissues, could be decisive in different responses.<sup>67,68</sup> Different mechanisms of action and molecules, such as histamine receptor 2, have been shown to contribute to the rapid desensitization of effector cells.<sup>66</sup> Histamine receptor 2 plays a role in several immune tolerance-inducing events, such as suppression of DCs, natural killer T cells, and T cells. One likely mechanism inducing desensitization, which to date has only been demonstrated in mice, is targeting of Fc $\gamma$ RIIb on the surfaces of mast cells by IgG antibodies cross-linked by allergens.<sup>69-71</sup>

Several different molecular mechanisms have been suggested to play a role in the breaking of allergen tolerance, such as viral infections and proinflammatory cytokines, and triggering of receptors, such as rhinovirus infections, IL-1 $\beta$  and IL-6, and Toll-like receptor 4 and Toll-like receptor 8.<sup>72-74</sup> In addition, the remission state can be dependent on the dose of exposed food allergen; although low doses are tolerated, high doses can trigger allergic symptoms. Apparently, all of these are linked to individual thresholds and can be affected by several factors.<sup>75</sup>

In summary, dynamic interactions among a wide range of host immune cells, microbiota, dietary factors, and food allergens determine whether allergy or tolerance develops. However, significant gaps in our knowledge on the natural induction of tolerance have hampered the development of immunotherapeutic protocols that fully replicate this process. The addition of



**FIG 3.** Cells involved in desensitization, remission, and tolerance. There are overlaps between the states of desensitization and sustained unresponsiveness (remission); thus far, there are no distinctive biomarkers to show which state starts at which time period. Mast cells and basophils play a role in desensitization. Direct *in vivo* evidence has been demonstrated in murine models, and human findings suggest comparable associations. Similarly, “remission” and “tolerance” are overlapping, and thus far, there are no clear biomarkers. Tolerogenic DCs, Treg cells, Breg cells, and effector cell/Treg and Breg cell ratios are present during remission and long-term tolerance. Distinct mechanisms responsible for the immune response shifting from a state of remission into long-term tolerance are not known. *cAMP*, Cyclic AMP; *CTLA-4*, cytotoxic T lymphocyte-associated protein 4; *LAP*, latency-associated peptide; *PD-1*, programmed cell death 1.

tolerance-inducing adjuvants (eg, carefully selected probiotics/prebiotics) to OIT protocols might support the acquisition of sustained unresponsiveness in a greater number of patients. However, significant research is still required to fully appreciate and understand the complexities of development of tolerance to food antigens.

### NEW NOMENCLATURE SUGGESTIONS ON SUSTAINED UNRESPONSIVENESS, DESENSITIZATION, AND TOLERANCE

In the course of OIT trials for food allergy, it became apparent that desensitization to a food allergen resulted in loss of clinical reactivity to the allergen but that this loss of reactivity was often short-lived once exposure to the allergen was discontinued (ie, 2-24 weeks). The term sustained unresponsiveness was suggested,<sup>68</sup> but no clinical or immunologic criteria were specified for this term. Consequently, in the past 5 years, the term sustained unresponsiveness has been reported in various studies to indicate an allergen-tolerant state for periods ranging from 2 weeks to 6 months after discontinuation of immunotherapy.

Overall, given a shorter course of immunotherapy and longer period for patients to remain off therapy before evaluation of sustained unresponsiveness, fewer patients will remain clinically nonreactive. This loss of the tolerant state clearly indicates that

immunologic tolerance was not achieved but also leads to the recognition that we do not know how to identify tolerance (ie, the permanent state of nonresponsiveness to a foreign or host antigen). This loss of tolerance after immunotherapy is not unique to food allergy but also occurs after various forms of immunotherapy to airborne environmental allergens and insect stinging allergens. Therefore it might be more appropriate to refer to this temporary state of nonresponsiveness off therapy as a “remission,” as traditionally done with autoimmune disorders. Various therapies could then be compared for their ability to induce specified periods of remission, conceding that long-term tolerance has not been established.

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