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# Can we produce true tolerance in patients with food allergy?

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**Immune tolerance is defined as nonresponsiveness of the adaptive immune system to antigens. Immune mechanisms preventing inappropriate immune reactivity to innocuous antigens include deletion of reactive lymphocytes and generation of regulatory T (Treg) cells. The normal response to food antigens is the generation of antigen-specific Treg cells. In patients with food allergy, the dominant immune response is a $T_H2$-skewed T-cell response and the generation of food-specific IgE antibodies from B cells. It is not known whether a failure of the Treg cell response is behind this inappropriate immune response, but interventions that boost the Treg cell response, such as mucosal immunotherapy, might lead to a restoration of immune tolerance to foods. Tolerance has been notoriously difficult to restore in animal disease models, but limited data from human trials suggest that tolerance (sustained nonresponsiveness) can be re-established in a subset of patients. Furthermore, studies on the natural history of food allergy indicate that spontaneous development of tolerance to foods over time is not uncommon. The current challenge is to understand the mechanisms responsible for restoration of natural or induced tolerance so that interventions can be developed to more successfully induce tolerance in the majority of patients with food allergy. (J Allergy Clin Immunol 2013;131:14-22.)**

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Immune tolerance is defined as a nonresponsiveness of the adaptive immune system to an antigen and can be mediated either by deletion or inactivation of antigen-specific lymphocytes or deviation of antigen-specific T lymphocytes into ***regulatory T (Treg) cells***. Immune tolerance is the basis of nonresponsiveness to self-antigens, and disruption of normal tolerance pathways leads to autoimmunity. In addition to discriminating self-antigens from non–self-antigens, the immune system must discriminate harmful non–self-antigens from innocuous antigens, such as those derived from food or the commensal flora. There is some overlap between immune mechanisms responsible for tolerance to self-antigens and innocuous non–self-antigens, which can also be mediated by deletion, anergy, or generation of antigen-specific Treg cells.

*Abbreviations used*

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| CT: | Cholera toxin |
| CTLA-4: | Cytotoxic T lymphocyte–associated antigen 4 |
| DBPCFC: | Double-blind, placebo-controlled food challenge |
| DC: | Dendritic cell |
| FoxP3: | Forkhead box protein 3 |
| iTreg: | Induced regulatory T |
| nTreg: | Natural regulatory T |
| OIT: | Oral immunotherapy |
| SCIT: | Subcutaneous immunotherapy |
| SLIT: | Sublingual immunotherapy |
| Treg: | Regulatory T |

Removal of autoreactive lymphocytes is a process that occurs in the thymus and bone marrow and is known as central tolerance. Receiving a strong signal through the lymphocyte receptor at this early stage of lymphocyte development leads to apoptosis of the cell. The thymus has a specialized population of medullary epithelial cells that express a wide range of peripheral tissue antigens under the control of the transcription factor autoimmune regulator *(AIRE)*.[1] Mutations in *AIRE* lead to autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy (APECED) in human subjects, showing the importance of this pathway in tolerance to self-antigens.[2,3] The thymus is also the origin of a population of Treg cells that express the transcription factor forkhead box protein 3 ***(FoxP3)*** and are termed natural regulatory T (nTreg) cells. These are distinct from another population of regulatory $CD4^+$ T cells that are induced in the periphery and also express FoxP3 termed induced regulatory T (iTreg) cells. iTreg cells will be discussed at a later point in this review. Deletion of autoreactive T cells during development in the thymus is incomplete, and nTreg cells are involved in the suppression of autoreactive effector T cells in the periphery. Human subjects and mice lacking Treg cells caused by mutations in the FoxP3 gene have severe autoimmunity, which is known as immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) in human subjects. Ablation of $FoxP3^+$ Treg cells, even in adulthood, leads to rapid onset of autoimmunity in mice, showing that continued presence of $FoxP3^+$ Treg cells is necessary for maintenance of self-tolerance.[4]

The paradigm of deletion of antigen-specific lymphocytes and generation of Treg cells also applies to tolerance induced in

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Terms in boldface and italics are defined in the glossary on page 15.

mature lymphocytes outside the thymus or bone marrow, and this process is known as peripheral tolerance. Exposure of naive T cells to antigens presented in the absence of costimulatory signals results in inactivation or anergy of the responder cell. In the absence of activation of the innate immune system by microbial signals (pathogen-associated molecular patterns) or damage signals (damage-associated molecular patterns), presentation of self-antigens or environmental antigens does not generate an effector T-cell response but rather deletion or anergy.

The site of antigen presentation also plays a significant role in determining the nature of the T-cell response. We know that antigen presentation in the gastrointestinal tract under homeostatic conditions results in the generation of an active regulatory response, which is termed oral tolerance, that is mediated by the generation of antigen-specific Treg cells. The preferential induction of T cells with regulatory activity is provided by tissue-specific factors, suggesting that the route of antigen exposure might be a critical factor in the development of immune tolerance.

## TOLEROGENIC CAPACITY OF THE GASTROINTESTINAL MUCOSA

The phenomenon of oral tolerance was first described by Wells and Osborne in 1911.[5,6] They used guinea pigs to show that inclusion of egg white, purified egg allergens, or oats in the diet rendered the animals hyporesponsive to sensitization and anaphylaxis to those proteins. Six decades later, a number of research groups showed that antigen feeding led to the development of suppressor T cells first in the gastrointestinal lymphoid tissue (Peyer patches and mesenteric lymph nodes) and at later time points in the spleen.[7-9] These suppressor cells, when transferred to naive animals, could inhibit IgE responses or delayed-type hypersensitivity responses in the recipient mice. IgE production is highly sensitive to oral tolerance, and feeding of antigen has been shown to prevent symptoms in experimental models of asthma[10,11] and food allergy or anaphylaxis.[12-15]

Weiner and colleagues initially showed that oral tolerance to myelin basic protein could be mediated by either CD4 or CD8 T cells,[16,17] and subsequent work from the group focused on a subset of Treg cells that they termed $T_H3$ cells.[18] $T_H3$ cells produce ***TGF-β*** and variable levels of IL-4 and ***IL-10*** and mediate their suppression in a TGF-β–dependent manner.[19] These cells are induced in both human subjects[18] and mice[20] after antigen feeding, and in mice they suppress the clinical severity of experimental autoimmune encephalitis (a model of multiple sclerosis). Regulatory cells other than $T_H3$ cells have been shown to be involved in oral tolerance. Similar to the early findings that CD8 T cells could transfer tolerance, feeding of mice with an MHC class I epitope of ovalbumin induced oral tolerance to ovalbumin in mice in a CD8-dependent manner.[21] Interestingly, these $CD8^+$ Treg cells could suppress $T_H1$ and ***$T_H17$*** responses but not $T_H2$ responses. Thymus-derived nTreg cells have been shown to be dispensable for oral tolerance induction,[10] but in contrast, iTreg cells ($CD4^+$***$CD25^+$***$FoxP3^+$ cells) are required for tolerance induction. This was shown by ablation of $FoxP3^+$ cells by using a transgenic mouse expressing the diphtheria toxin receptor under the control of the FoxP3 promoter (the DEREG mouse).[13,22] Injection of diphtheria toxin into the mice abolishes all $FoxP3^+$ Treg cells, including those induced after antigen feeding. After allowing the global Treg cell population to rebound, mice were immunized.

## GLOSSARY

**CD11c:** Also known as p150, CD11c is an integrin expressed on DCs (much less on macrophages) and is involved in leukocyte adhesion through ligands, such as intercellular adhesion molecule 1.

**CD25:** CD25 is the α chain of the IL-2 receptor and is expressed on activated T cells and Treg cells. Daclizumab, a humanized anti-CD25 antibody, has been used in the treatment of allograft rejection and adult T-cell leukemia.

**CD103:** Also known as integrin αE, CD103 binds to β7 to form αEβ7 on intraepithelial T cells that are retained in the intestinal mucosa (by binding to E cadherin).

**CD154:** CD154 is also known as CD40 ligand (CD40L), is expressed on activated T cells, and is required for isotype switching. Mutations in CD40L can cause X-linked hyper-IgM syndrome.

**CYTOTOXIC T LYMPHOCYTE–ASSOCIATED ANTIGEN 4 (CTLA-4):** Also known as CD152, CTLA-4 is upregulated by activation of T cells and is constitutively expressed by Treg cells. CTLA-4 is a member of the immunoglobulin superfamily and contains an immunoreceptor tyrosine-based inhibitory motif (ITIM). CTLA-4 binds to CD80 and CD86 on the antigen-presenting cell and counteracts activation delivered by the T cell receptor and CD28.

**$CX_3CR1$:** Part of the chemokine receptor family, all chemokine receptors are 7-transmembrane G protein–coupled receptors. $CX_3CR1$ binds fractalkine ($CX_3CL1$), a membrane-bound chemokine.

**FORKHEAD BOX PROTEIN 3 (FoxP3):** FoxP3 is expressed in some Treg cells. Congenital absence of Foxp3 Treg cells causes immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome, an immunodeficiency associated with polyorgan autoimmunity.

**IL-10:** IL-10 is associated with dampening immune responses by working through DCs and macrophages (decreased class II expression, costimulatory molecule expression, and costimulatory cytokine levels) and is produced by Treg cells ($T_R1$ cells).

**OX40 LIGAND (OX40L):** OX40L is a second signal molecule that is involved in multiple aspects of $T_H2$ inflammation, including eosinophilic inflammation.

**REGULATORY T (Treg) CELLS:** Some Treg cells can be $CD4^+CD25^+FoxP3^+$ and function to dampen the immune response to both allergenic and autoimmune antigens.

**RETINOIC ACID:** In the intestinal tract retinoic acid production promotes the development of $FoxP3^+$ Treg cells by inducing CD103.

**SIGNR1:** SIGNR1 is a C-type lectin that is expressed on DCs (DC-SIGN homologue), binds intercellular adhesion molecules 2 and 3, is a receptor for nonendosomal/nonlysosomal-mediated uptake, and is involved in T cell–mediated primary immune responses.

**TETRAMERS:** MHC peptide tetramers are used to stain antigen-specific T cells for flow cytometric analysis. Tetramers are multimers of peptide–MHC II molecules that can bind to the antigen-specific T-cell receptor.

**TGF-β:** TGF-β is a pleiotropic growth factor produced by epithelial cells and inflammatory cells, including eosinophils, mast cells, and T cells. TGF-β1 can have profibrotic effects, be a switch factor for IgA, and be a very immunosuppressant cytokine. TGF-β1 can also be produced by Treg cells.

**$T_H17$:** $T_H17$ cells are $CD4^+$ T cells that are defined by the production of IL-17A, IL-17F, IL-21, and IL-22. $T_H17$ cells are involved in autoimmunity and defense against bacteria, stimulated to produce IL-17 by IL-23, and maintained by the transcription factor retinoic acid–related orphan receptor γt.

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