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## Hindsight

During the past 20 years, major advances have been made in understanding the molecular and cellular mechanisms of allergen tolerance in humans. The demonstration of T cell tolerance, particularly that mediated by the immune-suppressive functions of IL-10, led to a major conceptual change in this area. Currently, the known essential components of allergen tolerance include the induction of allergen-specific regulatory subsets of T and B cells, the immune-suppressive function of secreted factors, such as IL-10 and TGF- $\beta$ , the production of IgG4 isotype allergen-specific blocking antibodies, and decreased allergic inflammatory responses by mast cells, basophils, and eosinophils in inflamed tissues.

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# Mechanisms of immune tolerance to allergens: role of IL-10 and Tregs

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During the past 20 years, major advances have been made in understanding the molecular and cellular mechanisms of allergen tolerance in humans. The demonstration of T cell tolerance, particularly that mediated by the immune-suppressive functions of IL-10, led to a major conceptual change in this area. Currently, the known essential components of allergen tolerance include the induction of allergen-specific regulatory subsets of T and B cells, the immune-suppressive function of secreted factors, such as IL-10 and TGF- $\beta$ , the production of IgG4 isotype allergen-specific blocking antibodies, and decreased allergic inflammatory responses by mast cells, basophils, and eosinophils in inflamed tissues.

## T cells and the allergic response

Immune tolerance can develop against any immune-activating substance, and multiple mechanisms mediate this process. Deregulation of immune tolerance may lead to the development of allergies, asthma, tumors, chronic infections, rejection of transplanted organs, graft-versus-host disease, and many autoimmune diseases. Allergic diseases are characterized by the induction of a type 2 immune response that includes Th2 cells and type 2 innate lymphoid cells (ILC2s), together with the production of allergen-specific IgE antibodies and increased eosinophil numbers in the affected tissues and sometimes in peripheral blood (1). Although there are many different ways to treat allergic disease-associated symptoms, currently, the only long-term curative treatment is allergen-specific immunotherapy (AIT), which involves the administration of increasing doses of the causative allergen. Over time, AIT induces a state of allergen-specific immune tolerance. AIT has been performed for more than 100 years, though it has only been within the past two decades that the mechanisms that mediate the action of AIT have been

slowly uncovered (2). After the discovery of Th1 and Th2 cells in 1986 (3), it was suggested that a Th2 response underlies the development of allergic diseases and that Th1 responses are predominant in infections and autoimmunity. Following these initial findings, the general dogma was that a switch toward a Th1 response would be required for the successful treatment of allergies by AIT, and a switch toward a Th2 response would be beneficial for the treatment of autoimmunity.

In the mid-1990s, our group was working on the mechanisms of action of AIT in conventional and T cell epitope peptide immunotherapies (4, 5). We were using honey bee venom allergy and its major allergen phospholipase A<sub>2</sub> as a model to investigate antigen/allergen-specific immune response development in humans (4). In a 1996 article published in the *JCI*, we demonstrated that allergen-specific T cell tolerance was induced during the course of AIT (4). Two months after the beginning of venom immunotherapy, the proliferation of T cells specifically targeting phospholipase A<sub>2</sub> and its T cell epitope peptides as well as the secretion of both Th2 (IL-4, IL-5, and IL-13) and Th1 (IL-2 and IFN- $\gamma$ ) cytokines were abolished.

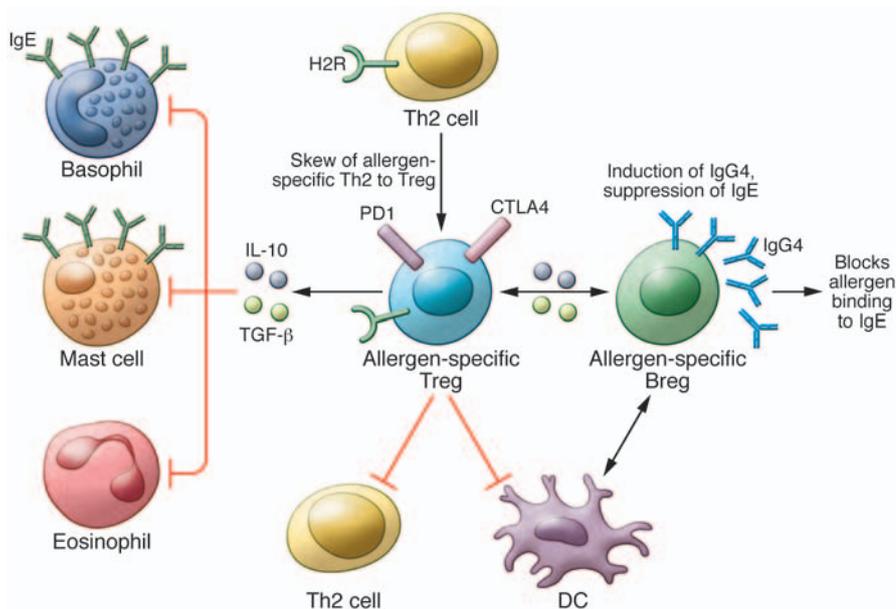
These data directly challenged the dogma that switching the balance between Th1 and Th2 responses is playing a role in treatment and suggested that the development of full T cell tolerance and suppression of both Th1 and Th2 subsets are taking place. As a control antigen, we demonstrated that tetanus toxoid-induced T cell proliferation and cytokine production remained unchanged following venom-specific AIT, demonstrating that the immune tolerance was specific to venom antigens and did not extend to other antigens. In addition, the state of T cell tolerance induced by AIT was altered in vitro by treatment with IL-2, IL-4, and IL-15. This was one of the first demonstrations that antigen-specific effector T cells have plasticity, suggesting that microenvironmental cytokines are important for determining success or failure in AIT (4). During these experiments, we were fortunate to be working with highly pure synthetic peptides and purified bee venom antigens collected in a sterile way. This enabled us to demonstrate immune tolerance, because any lipopolysaccharide contamination during collection and extraction of environmental allergens, such as house dust mites and pollens, would have led to results showing a skew from Th2 toward Th1.

## IL-10-producing Tregs in immune tolerance

In hindsight, our research continued to elucidate the molecular and cellular mechanisms of T cell tolerance to allergens, and in our 1998 study published in the *JCI*, we demonstrated that human IL-10-producing Tregs (Tr1 cells) are linked to an antigen-specific suppressor function (Figure 1 and ref. 6). Intracytoplasmic cytokine staining of circulating lymphocytes revealed that IL-10 was initially produced by CD4<sup>+</sup>CD25<sup>+</sup> allergen-specific Tr1 cells and then by B cells and monocytes. These IL-10-secreting Tr1 cells were substantially increased as early as seven days after the start of bee venom AIT in allergic individuals. Fol-

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**Figure 1. Mechanisms of allergen tolerance.** The induction of allergen-specific Tregs, which switch from allergen-specific Th2 cells is one of the initial events in the development of allergen tolerance. The effector cells of allergic inflammation – mast cells, basophils, and eosinophils – are regulated by suppressive and regulatory functions of Tregs in several ways. Treg-secreted IL-10 and TGF- $\beta$  suppress these cells. Tregs also suppress Th2 cells and their cytokines, preventing the provision of survival factors for these allergy effector cells. IL-10 and TGF- $\beta$  suppress IgE production by B cells, and meanwhile, IL-10 induces IgG4. IL-10–producing regulatory B cells (Bregs) play a role in suppression of allergen-specific T cells and mainly switch to IgG4-producing plasma cells. H2R plays a role in the suppression of Th2 cells, inflammatory dendritic cells, and basophils.

lowing these initial findings, one major question was whether these Tr1 cells also develop during other types of AIT against different allergens. It took many years to demonstrate that Tr1 cells are generated in response to other immunotherapies such as sublingual immunotherapy, other allergens such as grass pollen and house dust mites, and peptide immunotherapies in allergy and autoimmune diseases (7–12). In addition, Tr1 cells were demonstrated to be present in high-dose exposure to allergen models, such as nonallergic beekeepers and cat owners (6, 13, 14).

### Tr1 cells and recently discovered Br1 cells support a healthy immune response to allergens

Studies during the past two decades have demonstrated that there are two broad subsets of CD3<sup>+</sup>CD4<sup>+</sup> Tregs. One subset is the naturally occurring thymus-derived CD4<sup>+</sup>CD25<sup>+</sup>FOXP3<sup>+</sup> Tregs, also called natural Tregs (nTregs), and the other subset is the inducible Tregs (iTregs). Three main subsets of iTregs have been characterized: FOXP3-expressing iTregs, CD4<sup>+</sup>FOXP3<sup>+</sup>IL-10-producing Tr1 cells, and TGF- $\beta$ -

expressing Th3 cells. It has been repeatedly shown that all three subsets of iTregs coexist and overlap in many immune tolerance-related situations in humans. For example, antigen-specific CD4<sup>+</sup> T cells that express IL-10, TGF- $\beta$ , and FOXP3, increase in nasal biopsies (12, 15–17) and peripheral blood (7–9) in response to AIT at the healing phase.

Tr1 cells have been shown to be important for the maintenance of a healthy immune response in different diseases, such as allergy, many autoimmune conditions, transplantation tolerance, and graft-versus-host disease in both humans and mice (13, 18–26). Although IL-10 is the main cytokine produced by Tr1 cells, these cells also produce TGF- $\beta$  and low-to-medium levels of IFN- $\gamma$  and IL-5, but not IL-4 or IL-2 (27, 28). It took almost a decade to demonstrate that human Tr1 cells suppress effector T cell responses by multiple mechanisms that depend on IL-10, TGF- $\beta$  (7), PD-1, CTLA-4 (29), and histamine receptor 2 (H2R) (13).

IL-10 has a potent immunosuppressive capacity that is crucial not only for the establishment of peripheral tolerance

to allergens, but also in protecting the host from exaggerated inflammatory responses to pathogens as well as to autoimmune diseases (30). IL-10 directly inhibits T cells through suppression of CD28- and ICOS-dependent T cell costimulation (31). In addition, IL-10 inhibits the production of proinflammatory cytokines, chemokines, and chemokine receptors as well as the expression of MHC class II and costimulatory molecules CD80/CD86 on monocytes/macrophages and dendritic cells (30).

In addition to T cell regulation, IL-10 secreted from Tr1 cells plays a major role in the induction of IgG4 and suppression of IgE (6, 32). Moreover, it was recently demonstrated that IL-10–secreting B regulatory (Br1) cells essentially contribute to allergen tolerance (ref. 33 and Figure 1). Phospholipase A2–specific Br1 cells from nonallergic beekeepers and those that are induced after AIT showed increased expression of IL-10 with an antigen-specific suppressor capacity. A major finding of this study was that IgG4 production is specifically confined to Br1 cells. IgG4 represents a noninflammatory Ig isotype that does not activate complement and plays an IgE-blocking antibody role for the degranulation of mast cells and basophils (33).

### Conclusions

Specific Tr1 cells and immune tolerance development are essential for the induction and maintenance of healthy immune responses to allergens. The relationship between clinical allergen tolerance and immune tolerance has been observed by direct analysis of the affected tissues and skin during late-phase responses in humans who have undergone AIT with whole allergen and T cell epitope peptides as well as in individuals who have been exposed to naturally high doses of allergens. As of today, the concept that Tr1 cells mediate antigen-specific T cell tolerance has been demonstrated and generally accepted after the publication of several thousand studies on immune regulation.

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