Editorial
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Immune Tolerance by Induced Regulatory T Cells in Asthma

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Dr. Ralph M. Steinman, who discovered the dendritic cells (DCs) that play a key role in the adaptive immune response, was awarded the 2011 Nobel Prize in Physiology or Medicine 3 days after his untimely death.[1] Dr. Steinman used autologous DCs, cultured with tumor-specific antigens, to treat his own pancreatic cancer; the efficacy of this so-called "DC-based tumor vaccination" has recently been proven, and many researchers are now actively investigating this field. In contrast to the strategy of treating diseases by inducing active immunity, a strategy that involves suppressing unnecessary active immunity is required to avoid the development of autoimmune or allergic diseases, as these ailments occur due to failure to achieve immune tolerance to self or innocuous foreign materials. An article by Jang et al.,[2] which is published in this issue, demonstrates that *Lactobacillus rhamnosus* prevents the development of asthmatic reactions by inducing immune tolerance via regulatory T (Treg) cells in a mouse asthma model. This study highlights the possibility of preventing or treating asthma using cutting-edge immunological techniques.

Mechanisms of immune tolerance include the deletion of self-reactive T cells in the thymus (central tolerance) and the deletion, ignorance, anergy, inhibition, suppression, or deviation of the cells in peripheral organs (peripheral tolerance); Treg cells mediate immune suppression.[3] Since Sakaguchi et al.[4] first described in 1995 that T cells expressing interleukin (IL)-2 receptor α chain (CD25) are self-tolerant, it has been shown that the transcription factor forkhead box P3 (Foxp3) is a prerequisite for the development and function of immune suppression of such Treg cells. In fact, immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome, a disease that arises due to a mutation in the *Foxp3* gene, results in various severe autoimmune and allergic diseases. As both innate and adaptive immunity work in response to bacterial infection, Treg cells come in two varieties: natural Treg (nTreg) cells, which are produced in the thymus, and adaptive or induced Treg (iTreg) cells, which are induced by antigen and transforming growth factor (TGF)-β, mainly in mucosal tissue.[5,6] nTreg cells make up 5%-10% of peripheral $CD4^+$ T cells and express CD4, CD25, and Foxp3 on their surface, but their IL-7 receptor (CD127) expression is low, and they develop early and play a primary role in self-tolerance. There are three types of iTreg cells: those that transform from peripheral naïve ($CD4^+CD25^-Foxp3^-CD127^{high}$) T cells by acquiring CD25 and Foxp3; Th3 cells ($CD4^+CD25^-Foxp3^-$ latency-associated peptide$^+$), which were discovered in 1994 by a group researching the induction of oral tolerance and which secrete TGF-β;[7] and type 1 regulatory T cells (Tr1; $CD4^+CD25^-Foxp3^-$), which were discovered in 2001 and which secrete IL-10 and TGF-β.[8]

Immune tolerance is not simply the absence of an immune reaction to an antigen, but an active immune process that prevents inappropriate immune responses. In healthy humans, Tr1 cells are generated in response to inhaled allergens in the presence of partially matured DCs or plasmacytoid DCs, and, thus, Tr1 cells are the dominant T cell subset in healthy persons, while Th2 cells dominate in allergic individuals.[9,10] The generation of Tr1 cells depends on DC-derived IL-10, IL-27, and TGF-β along with inducible costimulatory molecule (ICOS) signaling by DCs;[11] however, IL-10 expression is diminished in allergic airways.[12] Inhaled allergens induce indoleamine 2,3-dioxygenase (IDO) in airway DCs, which catalyze tryptophan metabolism to produce TGF-β, suppress IL-6, and promote the generation of $Foxp3^+$ iTreg cells.[11] Because the function, but not the number, of $CD4^+CD25^+Foxp3^+$ Treg cells in atopy and allergic rhinitis,[13] the number of Tr1 cells in allergic rhinitis,[14] and both the function and number of $CD4^+CD25^+Foxp3^+$ Treg cells all decrease in asthma,[15] and because the lung is normally a Th2-biased compartment,[10] atopic individuals develop allergic diseas-

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es via Th2 responses, instead of developing tolerance to innocuous allergens. A recent study reported *Foxp3* gene polymorphisms in allergic rhinitis.[16] IL-6 suppresses Foxp3 expression and iTreg cell generation to release the suppression of active immunity by Treg cells and induces Th17 cell differentiation, resulting in neutrophilic asthma.[6,10]

Allergen-specific immunotherapy (SIT) is a well-known strategy to develop immune tolerance to innocuous allergens that is naturally present in healthy persons and, thus, to modify the natural course of asthma. This treatment induces Tr1 cells[17] and Foxp3$^+$ iTreg cells[18] and thus suppresses pathogenic immune responses through secretion of IL-10 and TGF-β. In addition, sublingual immunotherapy (SLIT) may induce Th3 cells, as can be seen in oral tolerance.[19] The vitamin A metabolite retinoic acid, another agent that promotes the differentiation of Foxp3$^+$ iTreg cells, suppresses IL-6 production by DC and CD44^{hi} effector memory T cells that suppress TGF-β. Vitamin D (calcitriol) and glucocorticoid also induce Foxp3$^+$ iTreg cells by promoting TGF-β production by DC.[11] In addition, both calcitriol and glucocorticoid convert CD4$^+$ T cells into Tr1 cells, and the effect by calcitriol, but not glucocorticoid, is associated with programmed death ligand (PDL)-1 expression in DCs.[20] CpG induces Foxp3$^+$ iTreg cells by binding to Toll-like receptor (TLR) 9 and activating IDO,[21] and bacille Calmette-Guérin vaccination induces those cells in association with ICOS signaling.[22]

It has been thought that immune tolerance to inhaled allergens in healthy persons, or when acquired after SIT in allergy patients, depends on Tr1 cells, not nTreg cells or Th3 cells, and that the transient expression of CD25 and Foxp3 in some iTreg cells may simply be a feature of activated effector T cells.[23] However, because allergy is severe in patients with IPEX syndrome, a disease due to a mutation in the *Foxp3* gene, it is apparent that Foxp3 plays an important role in immune tolerance to allergens. In addition, CD25$^+$ T cells that are activated nonspecifically without allergen suppress Th2 cells,[24] and helminth infestation is associated with an increased number of Foxp3$^+$ Treg cells and a low prevalence of allergic diseases.[25] Therefore, nTreg cells may prevent allergic diseases in an allergen-nonspecific manner, but iTreg cells that develop after encountering allergens in the periphery mediate allergen-specific immune tolerance.[25] However, among self-reactive T cells, only cells expressing Foxp3 can survive when they receive T cell receptor (TCR) signals in the thymus,[26] and nTreg cells proliferate when their TCRs recognize a specific antigen to become antigen-specific Treg cells.[27] More importantly, Foxp3$^+$ iTreg cells can be induced by the above-mentioned agents, including SIT;[11,18,21,22] consistent with this, *Lactobacillus rhamnosus* initiates immune tolerance to ovalbumin-induced allergic airway reactions by inducing Foxp3$^+$ iTreg cells, as presented later in this issue.[2]

Intestinal commensal bacteria have an immunomodulatory effect, and reduction in gut probiotics, such as *Lactobacillus* or *Bifidobacterium* species, is associated with atopy.[28] In 1997, Majamaa and Isolauri[29] first introduced probiotics for treating allergies by demonstrating that *Lactobacillus* GG ingestion for 1 month significantly improved atopic dermatitis in infants. In 2007, Feleszko et al.[30] demonstrated that oral administration of *Lactobacillus rhamnosus* GG or *Bifidobacterium lactis* for 8 weeks to newborn mice suppressed asthmatic airway reactions in association with increased Foxp3$^+$ T cells in peribronchial lymph nodes. In addition, Forsythe et al.[31] showed that *Lactobacillus reuteri*, but not *Lactobacillus salivarius*, attenuated asthmatic reactions, which was dependent on TLR9 and associated with increased IDO activity. The beneficial effects of probiotics seem to be strain-specific, and treatment may be most effective during the neonatal period. Orally administered probiotics induce IL-10 production and IDO activation systemically, and thus suppress allergic reactions in the airways.[28] Treg cells induced by probiotics cause "bystander suppression" in an antigen-nonspecific manner, and create a regulatory milieu that promotes antigen-specific Treg cell production. Moreover, combined treatment with *Bifidobacterium bifidum* and SLIT using birch pollen extract has been shown to be superior to SLIT alone in inducing Foxp3$^+$ iTreg cells and suppressing asthmatic reactions.[32] Therefore, probiotic therapy for inducing immune tolerance to innocuous allergens is promising, but appropriate selection of the strain(s), administration timing, selection of subjects, and allergen-specificity, among other things, should be carefully considered.

As treatments using autologous iTreg cells expanded *in vitro* are actively pursued in the field of transplantation and autoimmune diseases, further investigations of such treatments for asthma and allergies are warranted. nTreg cells show complete demethylation in CpG motif methylation of the *Foxp3* locus, conserved non-coding DNA sequence (CNS) 2 (formerly called the Treg cell-specific demethylated region [TSDR]),[33] and thus their Foxp3 expression is stable.[34] In contrast, iTreg cells show partial demethylation with TGF-β dependency, and thus their Foxp3 expression is unstable and progressively decreases over time. Therefore, to maintain the efficacy of Foxp3$^+$ iTreg cells, repeated induction of the cells should be done, or methods to stabilize their Foxp3 expression should be investigated. It has been reported that when Foxp3$^+$ iTreg cells are expanded *in vitro*, their function is occasionally lost or they are converted into effector T cells, resulting in the opposite of immune tolerance,[35] a problem that needs to be resolved. Because CD127 expression is high in effector T cells, but low in Treg cells, it has been suggested that the combination of CD127 with other markers, such as CD25, may be useful for distinguishing between these T cell subsets.[36] However, there is still no apparent marker that can distinguish between Foxp3$^+$ iTreg cells and nTreg cells, even though the TCR repertoire and induction signal for development of the cells (i.e., CD28 and CTLA-4) differ between nTreg and Foxp3$^+$ iTreg cells.[26,34,35]

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