Regulatory T cells in allergic diseases

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Overall Purpose/Goal: To provide excellent reviews on key aspects of allergic disease to those who research, treat, or manage allergic disease.

Target Audience: Physicians and researchers within the field of allergic disease.

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Activity Objectives:

1. To understand the distinction between natural regulatory T (nTreg) and induced regulatory T (iTreg) cells and their role in induction of tolerance.
2. To describe how regulatory T (Treg) cells are beneficial in promoting tolerance.
3. To describe how a proallergic environment can derange the Treg cell response to aggravate and perpetuate disease.
4. To understand the influence of inflammation on Treg cell subsets.
5. To describe the relationship between type 2 innate lymphoid cells (ILC2s), T_H2 cells, and Treg cells.

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The pathogenesis of allergic diseases entails an ineffective tolerogenic immune response to allergens. Regulatory T (Treg) cells play a key role in sustaining immune tolerance to allergens, yet mechanisms by which Treg cells fail to maintain tolerance in patients with allergic diseases are not well understood. We review current concepts and established mechanisms regarding how Treg cells regulate different components of allergen-triggered immune responses to promote and maintain tolerance. We will also discuss more recent advances that emphasize the "dual" functionality of Treg cells in patients with allergic diseases: how Treg cells are essential in promoting tolerance to allergens but also how a proallergic inflammatory environment can skew Treg cells toward a pathogenic phenotype that aggravates and perpetuates disease. These advances highlight opportunities for novel therapeutic strategies that aim to re-establish tolerance in patients with chronic allergic diseases by promoting Treg cell stability and function. (J Allergy Clin Immunol 2016;138:639-52.)

Key words: *Asthma, food allergy, regulatory T cells, forkhead box P3, IL-4, T_H2 cells*

The increased prevalence in allergic diseases has become a major health problem in affluent and rapidly developing societies. Over the last 150 years, accelerated social and environmental changes augured by the industrial revolution that profoundly altered patterns of human activity, living arrangements, diet, and infections all came to influence the increase in and severity of allergic disorders.[1] In the United States food allergy prevalence reaches up to 8% among children and 5% of the adult population, whereas 8.6% of children and 7.4% of adults are affected by asthma.[2,3] The dramatically increased burden of allergic diseases has exacted considerable morbidity on those with these disorders and resulted in substantial financial costs incurred by affected

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Abbreviations used
CNS: Conserved noncoding region
CTLA-4: Cytotoxic T-lymphocyte-associated protein 4
DC: Dendritic cell
FOXP3: Forkhead box P3
GF: Germ-free
ICOS: Inducible T cell costimulator
ILC2: Type 2 innate lymphoid cell
IL-4Rα: IL-4 receptor α chain
iTreg: Induced regulatory T
LAG-3: Lymphocyte activation gene 3
MyD88: Myeloid differentiation primary response gene 88
nTreg: Natural regulatory T
ROR: Retinoic acid–related orphan receptor
SCFA: Short-chain fatty acid
STAT: Signal transducer and activator of transcription
Tconv: Conventional T
TCR: T-cell receptor
TLR: Toll-like receptor
T_R1: Type 1 regulatory T
Treg: Regulatory T

subjects and their health care systems. Although therapies for allergic diseases have improved over the years with the introduction of agents aimed at combating inflammatory processes, as well as providing symptomatic relief, those therapies have remained, for the most part, noncurative.

Allergic diseases arise in response to normally innocuous environmental agents, including aeroallergens and foods. They involve the participation of components of the innate and adaptive immune responses, such as type 2 innate lymphoid cells (ILC2s), mast cells, basophils, and eosinophils, as well as activated T_H2 cells and B cells switched to the production of IgE.[4,5] Immune regulatory mechanisms normally operating to maintain allergen tolerance break down for reasons that still remain unknown.

The dramatic increase in the prevalence of allergic disease during past decades indicates a strong influence of environmental factors acting on genetically susceptible hosts to promote disease.[6,7] Emerging studies emphasize the interaction of environmental factors, including diet, antibiotic use, and others, with components of the immune system affecting their function and modifying the outcome of the immune response.[8] They also support the idea that commensal bacteria play a central role in the regulation of allergic diseases and that they dynamically interact with host genetic background and environmental factors to promote or disrupt oral tolerance.[9-11] Genetic and immunologic evidence also reinforce the idea of a pivotal role for regulatory T (Treg) cells in promoting tolerance to allergens and preventing allergic disorders.[12-16] In this review we will discuss recent advances demonstrating the "dual potential" of Treg cells in patients with allergic diseases: how Treg cells are beneficial in promoting tolerance but also how the proallergic environment can derange the Treg cell response to aggravate and perpetuate disease.

NATURAL AND INDUCED FORKHEAD BOX P3–POSITIVE Treg CELLS

Treg cells were initially described as a population of $CD4^+$ T cells expressing the IL-2 receptor α chain (CD25) and CD45RB, which are able to protect mice from autoimmune diseases.[17,18] Afterward, the establishment of Treg cells as a distinct $CD4^+$ T-cell subpopulation was empowered by identification of the forkhead winged helix transcription factor forkhead box P3 (FOXP3) as a specific Treg cell maker essential to their function.[19,20] FOXP3 is required for the differentiation of Treg cells, as evidenced by the generation of aberrant Treg cells lacking in regulatory function in mice with loss-of-function mutations in *Foxp3*.[21,22] FOXP3 deficiency results in the development of a multiorgan lymphoproliferative autoimmune disease, referred to as immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome, in human subjects and *scurfy* in mice.[12,23-26] Expression of FOXP3 into human and murine conventional $CD4^+Foxp3^-$ non-Treg cells by means of retroviral gene transfer converts naive T cells into Treg cells.[19] It is now well established that Treg cells enforce tolerance to both self-antigens and the "extended self," the latter encompassing commensal flora and innocuous environmental antigens, such as allergens.[27-30]

A major population of Treg cells arises in the thymus and is known as $CD4^+FOXP3^+$ natural regulatory T (nTreg) cells (also known as thymus-derived regulatory T cells), which chiefly mediate tolerance to self-antigens (Fig 1).[31] A second population of $CD4^+FOXP3^+$ Treg cells arises extrathymically in peripheral lymphoid tissues from a pool of naive conventional $CD4^+FOXP3^-$ T cells (conventional T [Tconv] cells) after exposure to antigens and in the presence of TGF-β.[32] These induced regulatory T (iTreg) cells (also known as peripheral regulatory T cells) are particularly enriched in the gastrointestinal tract and in the lungs during chronic inflammation, with specificities directed against microbial antigens or environmental allergens (Fig 1).[33-35] The generation of iTreg cells at the intestinal mucosa is facilitated by the large abundance of TGF-β and retinoic acid, a vitamin A metabolite, both secreted by the $CD103^+CD11c^+$ dendritic cells (DCs).[36-38] In lung tissues resident macrophages ($CD45^+CD11c^+$MHC class $II^{low}F4/80^+$) constitutively expressing TGF-β and retinoic acid are the main subset of cells driving iTreg cell induction from naive $CD4^+$ Tconv cells (Fig 1).[39] Both $FOXP3^+$ nTreg and iTreg cell subsets play a key function in the maintenance of peripheral tolerance by suppressing reactivity to self-antigens and by containing the amplitude of immune responses to foreign antigens.

Because of their different origins, the T-cell receptor (TCR) repertoires of thymic nTreg and peripheral iTreg cells are largely nonoverlapping and biased toward self and nonself antigens, respectively.[40] However, iTreg cells are known to be less stable than nTreg cells and can lose FOXP3 expression and produce cytokines, such as IFN-γ and IL-17, under inflammatory conditions.[41,42] This lack of stability can be explained by the methylation status of the conserved noncoding region (CNS) 2 of the *Foxp3* gene. The *FOXP3* CNS2 locus, which acts to maintain Treg cell lineage identity under inflammatory conditions, is known to be stably hypomethylated in nTreg cells, whereas it is incompletely demethylated in iTreg cells.[43-46]

One difficulty for the functional and genetic study of iTreg and nTreg cells is the lack of unique and specific markers allowing the distinction between those 2 populations and their identification *in vivo*. nTreg and iTreg cells express similar levels of shared Treg cell markers, such as FOXP3, cytotoxic T-lymphocyte antigen 4 (CTLA-4), glucocorticoid-induced TNFR-related protein