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Narrative Review

FOXP3-Positive Regulatory T Cells and Kidney Allograft Tolerance

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Normal immune homeostasis is achieved by several mechanisms, and prominent among them is immunoregulation. Although several types of regulatory lymphocyte populations have been described, CD4 T cells expressing the FOXP3 transcription factor (FOXP3-positive regulatory T cells [FOXP3$^+$ Tregs]) are the best understood. This population of cells is critical for maintaining self-tolerance throughout the life of the organism. FOXP3$^+$ Tregs can develop within the thymus, but also under select circumstances, naive peripheral T cells can be induced to express FOXP3 and become stable Tregs as well. Abundant evidence from animal systems, as well as limited evidence in humans, implicates Tregs in transplant tolerance, although whether these Tregs recognize allo- or self-antigens is not clear. New translational approaches to promote immunosuppression minimization and/or actual tolerance are being designed to exploit these observations. These include strategies to boost the generation, maintenance, and stability of endogenous Tregs, as well as adoptive cellular therapy with exogenous Tregs.
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INDEX WORDS: Regulatory T cells; Foxp3$^+$ Tregs; kidney transplant tolerance; immunotherapy; kidney allografts; immunoregulation; self-tolerance; transplant tolerance; renal transplantation; end-stage renal disease (ESRD); cellular therapy; review.

INTRODUCTION

The role of the immune system is to discriminate between self- and non–self-antigens, protecting the host from foreign pathogens and at the same time maintaining tolerance to self. Immune tolerance is an active process that is characterized by a central and peripheral component.[1-7] Central tolerance is a thymic-dependent process that involves the deletion of autoreactive clones through the induction of apoptosis. Peripheral tolerance can be subdivided into at least 3 major categories: clonal deletion, anergy, and suppression. A subset of T cells has been identified that specifically regulate the suppression process. These cells are known as regulatory T cells (Tregs). Although these cells are frequently grouped under one category, they can be divided into 2 developmental subsets, thymic-derived (natural) Tregs (tTregs or nTregs), and induced (adaptive) peripheral Tregs (iTregs or pTregs)[8] (Fig 1). Although these cells are predominantly CD4-positive (CD4$^+$), CD8$^+$FOXP3$^+$ cells have been identified that are also suppressive in nature. Natural Tregs are produced in the thymus and most express the interleukin 2 (IL-2) receptor alpha chain (CD25).[9] Their development and functionality depend on the expression of the transcriptional factor forkhead box P3 (FOXP3).[10,11] Induced Tregs are derived in the periphery from naive T cells following specific antigenic stimulation. There are also several populations of CD4$^+$FOXP3$^-$ T cells that are suppressive, including IL-10 producing T regulatory 1 (Tr1) cells, transforming growth factor β (TGFβ) T helper 3 (T_H3) cells, and T regulatory type 35 (Tr35) cells that produce IL-35, which is related to the IL-12 superfamily[1,3,12] (Fig 2). This review focuses on the biology of Tregs, the role that they play in kidney allograft acceptance, and the ways that our knowledge about Tregs is being leveraged in the clinic.

TREG BIOLOGY

Basic Concepts

FOXP3$^+$ Tregs constitute 5% to 10% of peripheral CD4$^+$ T cells in both mice and humans[13] and are critical for maintaining immune homeostasis. Mutations in *Foxp3* leading to an absence of functional Tregs is the cause of severe autoimmunity as observed in *scurfy* mice and humans with IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked).[11,14] Importantly, Tregs also are critical for maintaining immune homeostasis throughout the lifespan of an animal. This was elegantly demonstrated by Kim et al[15] using a mouse in which the diphtheria toxin receptor was knocked into the *Foxp3* locus (*Foxp3^{DTR}* mouse). In these adult animals, administration of diphtheria toxin leads

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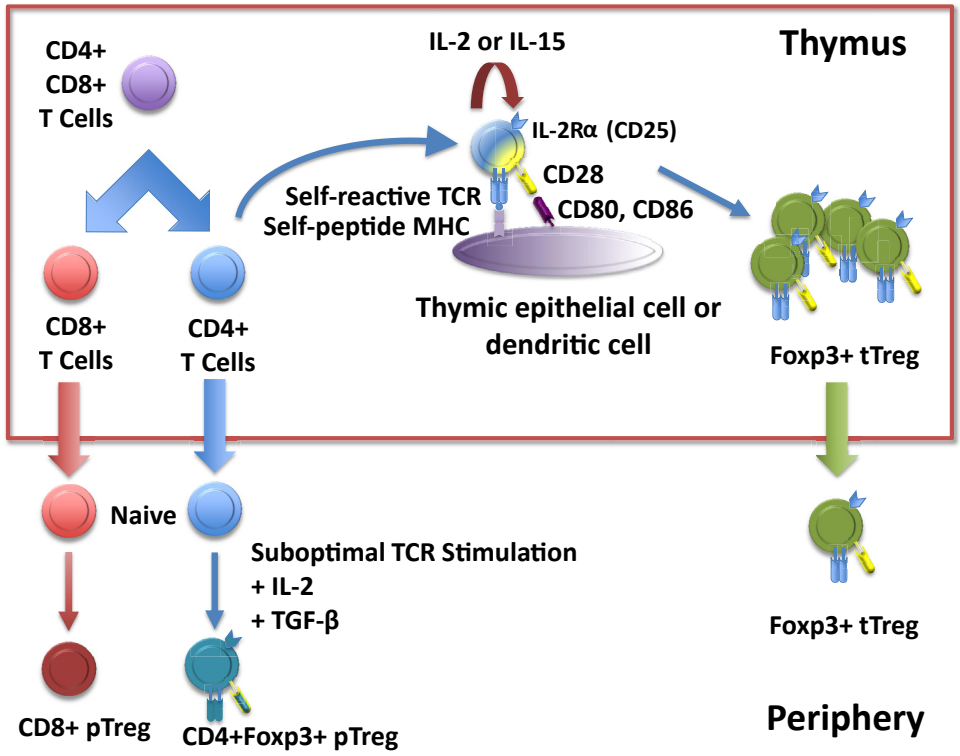

Figure 1. Origin of FOXP3$^+$ regulatory T cells (Tregs). During thymic ontogeny, double-positive (CD4$^+$CD8$^+$) thymocytes with low affinity for self-peptide plus self–major histocompatibility complex (MHC) undergo positive selection, becoming single-positive CD8$^+$ or CD4$^+$ thymocytes that eventually are exported into the peripheral lymphoid compartment as naive T cells. Under certain conditions (see Fig 2 for more details), these naive T cells can be induced to express FOXP3, and these cells are known as pTregs (for Tregs of peripheral origin). Double-positive thymocytes with moderate affinity for self-peptide plus self-MHC are induced to express FOXP3, and these cells, known as tTregs because of their thymic origin, are exported into the periphery as "fully formed" Tregs. Abbreviations: IL, interleukin; IL-2R, interleukin 2 receptor; TCR, T-cell receptor; TGF-β, transforming growth factor β.

to the selective ablation/depletion of Tregs, which then results in the induction of a wide variety of autoimmune diseases.[15] In addition to their role in preventing autoimmunity, Tregs have also been implicated in the resolution of inflammation and tissue repair.[16]

Two Types of FOXP3$^+$ Tregs

During thymic ontogeny, developing Tregs are subject to both positive and negative selection processes that together create a repertoire that is self–major histocompatibility complex (MHC) restricted (positive selection), but eliminates many autoreactive T cells (negative selection). Current data support a model in which developing thymocytes for which T-cell receptors have some modest affinity for self-peptide plus MHC are induced to express FOXP3 and differentiate into Tregs.[17] These tTregs (formerly nTregs), are believed to be important in suppressing the response of autoreactive T cells that escape negative selection. Despite having low-level self-reactivity, they have a relatively high rate of cycling in vivo and can be activated by self-antigens in the periphery.[18,19] When activated, they may be able to suppress in an antigen-nonspecific manner.

Another population of Tregs, termed pTregs, can be induced from naive T cells (ie, resting non-Tregs) in the periphery. In vitro, this population of inducible Tregs is created when T cells are stimulated in the presence of high TGFβ concentrations.[20] Other factors that support Treg induction include vitamin D, retinoic acid, vitamin C, and inhibition of phosphatidylinositol-4,5-bisphosphate 3-kinase (PI-3 kinase).[21,22] It has been more difficult to study this population in vivo, but pTreg induction appears to be favored by continual stimulation with low doses of antigen. Conversely, inflammatory signals, particularly IL-6, retard Treg induction.[23] We know less about the physiologic role of pTregs, although they appear to be required for maternal-fetal tolerance,[24] and thus it has been suggested that they may be more important for tolerance to foreign antigens, with tTregs focusing on self-antigens. It has been difficult to study these 2 populations of cells due to a lack of easily used molecular markers to distinguish them, although the transcription factor Helios and the surface protein neuropilin 1 have been proposed to be