

VIEWPOINT

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Taking regulatory T cells into medicine

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Regulatory T cells (Tregs) are indispensable for the establishment and maintenance of immunological self-tolerance. Their genetic anomalies or variations in function are causative of various monogenic and polygenic autoimmune diseases. Treg-based reestablishment of self-tolerance is envisioned to cure autoimmune diseases in the clinic.

How immunological unresponsiveness to self-antigens (i.e., immunological self-tolerance) is established and maintained to prevent pathological autoimmunity and how self-tolerance can be reestablished to treat autoimmune diseases are among the most fundamental issues in immunology. Regulatory T cells (Tregs) were first discovered in rodents as naturally occurring suppressive CD25-expressing CD4⁺ T cells actively engaged in the maintenance of peripheral self-tolerance: specific removal of CD25⁺CD4⁺ T cells (constituting ~10% of CD4⁺ T cells) from the normal immune system resulted in spontaneous development of a variety of autoimmune diseases similar to the human counterparts, which could be successfully prevented by reconstitution of the same cell population (Sakaguchi et al., 1985, 1995; Powrie and Mason, 1990; see figure, panel A). These CD25⁺CD4⁺ Tregs were later found to specifically express the transcription factor FoxP3 as a “master controller” of Treg function (Hori et al., 2003; Khattri et al., 2003; Fontenot et al., 2003). With the establishment of Foxp3⁺CD25⁺CD4⁺ Tregs as a functionally and phenotypically distinct T cell subpopulation, active research over the last 25 yr has elegantly shown that FoxP3⁺ Tregs are essential in controlling a wide variety of pathological and physiological immune responses (e.g., autoimmunity, allergy, tumor immunity, transplantation or

feto-maternal tolerance, and microbial immunity) and can be targeted to suppress or enhance the immune responses in clinical settings. Here, with recent advances in our understanding of the cellular and molecular basis of Treg development and function, I discuss the roles of Tregs in self-tolerance and autoimmune disease, the archetypal function of Tregs, and the prospects of applying Tregs to treating autoimmune and other inflammatory diseases.

Development and function of Tregs: Transcriptional and epigenetic control of Treg function-associated genes

A majority of Tregs are physiologically produced by the normal thymus as a functionally mature and distinct T cell subpopulation (thymus-derived Tregs, or tTregs), persisting in the periphery with stable function (see figure, panel B). In the thymus, developing T cells with intermediate affinity TCRs for self-peptide/MHC ligands are driven to differentiate into tTregs, with deletion of T cells with high-affinity TCR for the ligands and differentiation of those with low affinity TCRs into naive conventional T cells (Tconvs). In addition to possessing self-skewed TCR repertoire, tTregs are phenotypically in an “antigen-primed” state already in the thymus, as indicated by their expression of CD25 and CTLA-4. These properties enable them to be swiftly activated and functionalized upon self-antigen



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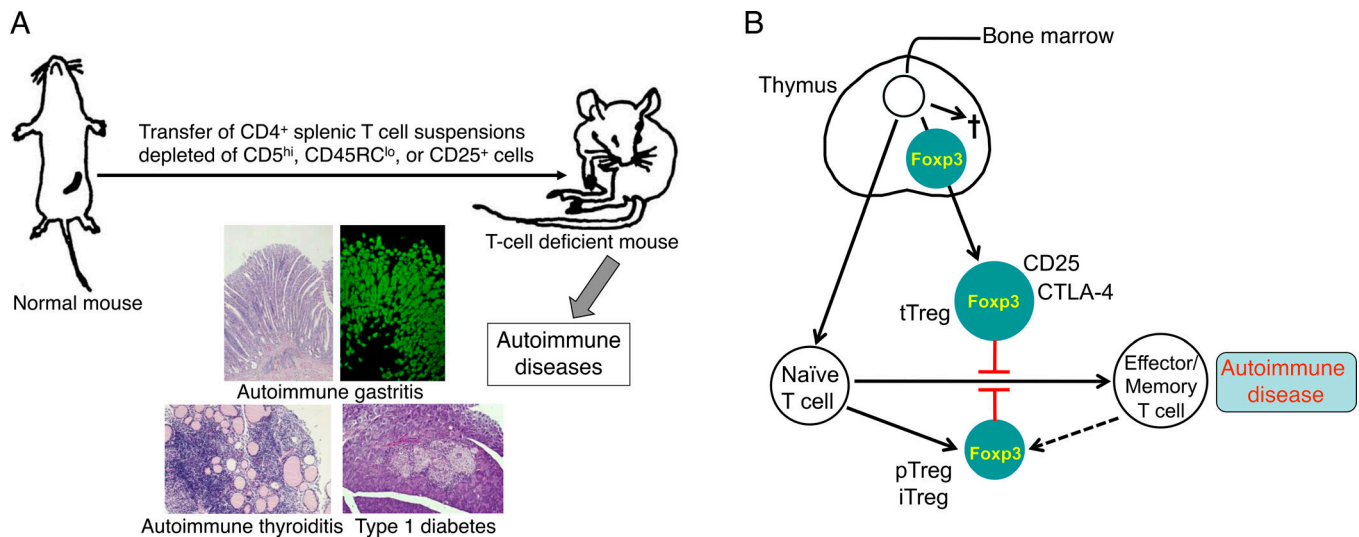
recognition in the periphery to maintain dominant self-tolerance.

Among various molecules expressed by Tregs, deficiency of Foxp3, CD25, or CTLA-4 in mice results in severe autoimmune diseases similar to those produced by Treg deficiencies, illustrating their critical roles in Treg function. For example, Treg expression of the high-affinity IL-2 receptor (with CD25 as its component) and their intrinsic IL-2 nonproduction (see below) together render Tregs highly sensitive to and dependent on exogenous IL-2 for their survival, and at the same time confer them with the ability to deprive IL-2 from Tconvs through direct competition. CTLA-4 on Tregs acts as a critical mediator of immune

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Tregs in immunological self-tolerance and autoimmunity. **(A)** Various autoimmune diseases can be induced in T cell-deficient rodents by simply transferring CD4⁺ T cell suspensions depleted of a subpopulation, for example, in mice, by depleting CD5^{hi}CD4⁺ T cells (Sakaguchi et al., 1985) and in rats, by depleting CD45RC^{lo}CD4⁺ T cells (Powrie and Mason, 1990). Further efforts to delineate Tregs by more specific molecular markers led to the discovery of Tregs as CD25⁺CD4⁺ T cells, which are included in CD5^{hi} and CD45RC^{lo}CD4⁺ T cells (Sakaguchi et al., 1995). Depletion of CD25⁺CD4⁺ T cells indeed resulted in spontaneous development of a wide spectrum of histologically evident autoimmune diseases such as autoimmune gastritis with anti-parietal cell autoantibody (shown here by indirect immunofluorescence staining of normal mouse gastric mucosa), autoimmune thyroiditis, and type 1 diabetes. **(B)** The majority of FoxP3⁺ Tregs constitutively expressing CD25 and CTLA-4 are produced by the thymus (tTregs), while some differentiate from Tconvs in the periphery (pTregs). Foxp3⁺ Tregs can also be induced in vitro from naive Tconvs (iTregs). Conversion of disease-mediating effector/memory T cells into functionally stable pTregs or iTregs (dotted line) can be ideal for antigen-specific suppression of pathological immune responses.

suppression by down-regulating surface expression of CD80/CD86 costimulatory molecules on antigen-presenting cells, thereby suppressing Tconv activation. Activated Tregs can also secrete immunosuppressive cytokines such as IL-10. Tregs thus exert effective immune suppression via humoral and cell contact-dependent mechanisms.

Treg development and function is coordinated by the establishment of the Treg-specific epigenetic landscape and the transcription factor network involving FoxP3. While FoxP3 functionally acts as a strong repressor of *Il2*, *Ifng*, and other genes upon Treg activation, the Treg epigenome encompassing Treg-specific CpG hypomethylation, chromatin accessibility, and histone modifications reinforces activation of enhancers, especially super-enhancers, associated with Treg signature genes such as *Foxp3*, *Cd25*, and *Ctla4* and stabilizes their expression. Notably, the establishment of such Treg-specific epigenome is Foxp3-independent, and a large portion of Treg-specific super-enhancers are concurrently activated at the precursor stage, before FoxP3 expression (Kitagawa et al., 2017). These findings suggest that genetic and epigenetic alterations at Treg function-associated genes may be causative of autoimmune and other inflammatory diseases by affecting Treg development and function.

Further, future strategies looking to prepare functionally stable Tregs for therapeutic use will require assessment of FoxP3-independent Treg-specific epigenetic changes and not just the sole expression of FoxP3.

Tregs in human monogenic and polygenic autoimmune diseases

Mutations of Treg signature genes, such as *FOXP3*, *IL2RA* (*CD25*), and *CTLA4*, impair Treg development and function and consequently produce in humans severe autoimmune diseases reminiscent of murine FoxP3 deficiency. *FOXP3* mutations, in particular, cause profound Treg-specific deficiency/dysfunction, resulting in IPEX (immune dysregulation, poly-endocrinopathy, enteropathy, X-linked) syndrome accompanying a variety of autoimmune diseases (e.g., type 1 diabetes and autoimmune thyroiditis), inflammatory bowel disease, and allergy in infants and even in utero. This is unequivocal evidence that Treg-mediated dominant self-tolerance is relevant in humans and that autoimmune diseases can develop as a pure intrinsic defect of the immune system.

In contrast to such monogenic “Tregopathies” with distinct Treg deficiency or dysfunction, functional Treg anomalies found in common polygenic autoimmune diseases (such

as type 1 diabetes and rheumatoid arthritis), which afflict ~10% of the population worldwide, have been tantalizingly equivocal and controversial, in particular, over whether a detected anomaly/variation is a “cause” or a “consequence” of autoimmunity. Meanwhile, genome-wide association studies of common autoimmune diseases have revealed that ~60% of autoimmune-causal single nucleotide polymorphisms (SNPs) are mapped to noncoding enhancer regions of immune cells, especially CD4⁺ T cells (Farh et al., 2015). A key question is then whether such autoimmune SNPs, for example, found at the *CD25* or *CTLA4* locus in type 1 diabetes (Wellcome Trust Case Control Consortium, 2007), should affect CD4⁺ helper T cells or CD4⁺ Tregs as a gain-of- or loss-of-function variant, respectively. A recent study addressing this issue characterized genome-wide epigenetic profiles of human Tregs and CD4⁺ Tconvs in naive and activated states, and revealed the presence of Treg-specific distinct CpG hypomethylated regions at Treg signature gene loci (e.g., *FOXP3*, *CD25*, and *CTLA4*), which are largely included in Treg-specific super-enhancers and closely associated with Treg-specific gene transcription and other epigenetic changes (Ohkura et al., 2020). Notably, autoimmune SNPs

are highly (approximately ninefold) enriched in Treg-specific DNA demethylated regions present in naive Tregs, compared with activation-induced demethylated regions. The findings indicate that many causative autoimmune SNPs are present at enhancer regions of Treg function-associated genes such as *CD25* (Simeonov et al., 2017) and *CTLA4* (Ohkura et al., 2020), acting as expression quantitative trait loci (eQTL) controlling endogenous Treg development and function. Genetic polymorphisms at particular HLA loci with autoimmune-protective or -susceptible alleles may also alter Treg generation and activation (Ooi et al., 2017). Further attempts to link genome-wide association studies and Treg immunobiology will elucidate a key contribution of Tregs in common autoimmune diseases.

Natural versus induced Tregs for the treatment of autoimmune diseases

Attempts at treating common autoimmune diseases such as type 1 diabetes are currently focused on the expansion of endogenous Tregs in vivo or via adoptive transfer of patient-derived Tregs after in vitro expansion (reviewed in Ferreira et al., 2019). For example, administration of low dose IL-2 (or IL-2 molecules modified to persist longer in circulation or more strongly bind to CD25) can selectively expand Tregs because of their constitutive expression of the high-affinity IL-2 receptor, while avoiding the unwanted expansion and activation of natural killer or effector T cells. In Treg-based adoptive cell therapy, circulating Tregs are first purified from the patient, expanded polyclonally in vitro, and then transferred back to the patient. This form of

therapy is predicated on the inherent assumption that transferred polyclonal Tregs retain the potential for recruitment to inflammation sites and that these disease-specific Tregs, including self-antigen-specific clones, will eventually become predominant and achieve disease-specific immune suppression. Other attempts have also been made using chimeric antigen receptor technology to generate chimeric antigen receptor-Tregs that express an antibody Fab region specific for a particular self-antigen to suppress autoimmune T cells.

Alternatively, Foxp3⁺ Tregs can be induced from Tconvs in vitro by antigenic stimulation in the presence of TGF- β and IL-2 (induced Tregs, or iTregs). In contrast to endogenous Tregs, iTregs lack the Treg-type epigenomic changes, particularly Treg-specific DNA demethylation, and are thus phenotypically and functionally unstable. Moreover, it is difficult in most cases to generate iTregs from activated or effector Tconvs, especially in the presence of inflammatory cytokines. A recent attempt to overcome these problems has shown that inhibition of CDK8/19, the regulatory modules of the Mediator complex, is able to efficiently induce Foxp3 in antigen-specific effector/memory as well as naive Tconvs, even in the presence of proinflammatory cytokines (Akamatsu et al., 2019). CDK8/19 inhibitors and TGF- β together can synergistically boost Foxp3 expression. In addition, abrogation of CD28 signaling via protein kinase C to NF- κ B during iTreg generation suffices to induce Treg-specific DNA hypomethylation at Treg signature gene loci in effector/memory as well as naive Tconvs (Mikami et al., 2020). Thus, such methods for Foxp3 induction

and installation of Treg-type DNA hypomethylation in combination will enable preparation of large quantities of functionally stable, antigen-specific iTregs from disease-mediating Tconvs for clinical use.

Conclusions

This viewpoint article has outlined the role of Tregs in immunological self-tolerance and autoimmunity. The findings will advance our understanding of Treg contribution in other pathological and physiological immune responses and help devising novel ways for Treg-based control of the responses in the clinic.

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