RESEARCH HIGHLIGHT

For antigen-specific effector or Foxp3$^+$ regulatory T cell fate, cyclin-dependent kinases hold the trump card

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Forkhead box p3$^+$ (Foxp3$^+$) regulatory T cells (Tregs) are indispensable for immune homeostasis and for maintaining immune tolerance. Several studies have confirmed that injection of a T cell population depleted of Tregs causes autoimmunity, rejection of grafts and inflammatory disorders, whereas reconstitution with Tregs inhibits these pathogenic processes. Over the last two decades, intense efforts have been made to identify Treg subsets, Treg differentiation processes, and the molecular signatures and regulators that determine Treg lineage specificity and stability.[1–6]

Several lines of evidence clearly demonstrate that Foxp3$^+$ Tregs do not constitute a homogeneous population, and various subsets of Tregs, such as thymic Tregs (tTregs), in vitro-generated Tregs (iTregs), and peripherally induced Tregs (pTregs), have been identified.[5] In addition to Foxp3, epigenetic factors and metabolic processes play key roles in maintaining Treg identity and function, and mediate the switch between effector T cells and Tregs.[6–8] In fact, Tregs require phosphatase and tensin homolog (PTEN) for stability and for maintaining the metabolic balance between glycolysis and mitochondrial functions.[9] A recent report argues that mitochondrial complex III is indispensable for the suppressive function of Tregs, citing loss of complex III in Tregs triggering enhanced levels of metabolites 2-hydroxyglutarate (2-HG) and succinate, thereby blocking the ten-eleven translocation (TET) family of DNA demethylases and resulting in DNA hypermethylation and the repression of several transcripts required for Treg function.[10,11]

iTreg generation is also governed by several factors. Inhibition of C3aR (complement component 3a receptor)- and C5aR (complement component 5a receptor)-mediated signaling in CD4$^+$ T cells has been reported to reduce PI3K-Akt-mTOR pathway activity with a concomitant enhancement in autocrine TGF-β signaling to promote Foxp3$^+$ iTreg generation.[12] Metabolic regulator hypoxia-inducible factor 1 (HIF-1) controls the balance between effector Th17 cells and Tregs. Glutamine transamination, which is primarily catalyzed by glutamic-oxaloacetic transaminase 1, determines the fate of CD4$^+$ T cell toward Th17 instead of toward iTreg differentiation. Increased transamination leads to abundant 2-HG, which promotes the hypermethylation of the Foxp3 gene locus and the subsequent inhibition of its transcription.[13]

A recent study by the Prof. Shimon Sakaguchi group was aimed at identifying the molecular regulator that controls the generation of antigen-specific Tregs.[14] This group reported that cyclin-dependent kinase (CDK)8 and CDK19 (a homolog of CDK8 with 91% sequence homology) are the key factors that control switching between antigen-specific effector and Foxp3$^+$ Treg polarization.[14] CDK8 and CDK19 are collectively called mediator kinases. CDK8, in association with cyclin C (CCNC), mediator complex subunit 12 (MED12), and MED13 form a part of the mediator complex. These kinases in association with the mediator complex are implicated in several cellular processes, such as transcription, cell signaling, metabolism, and immunity to infection (especially antiviral immunity). Contradictory and context-dependent roles of CDK8/19 have been observed in cancer.[15,16] During innate immune responses, CDK8/19 associates with NF-κB to regulate the expression of inflammatory genes. Because of the involvement of NF-κB in inflammatory disorders, inhibition of these associated factors (CDK8/19) represents an attractive strategy for targeting inflammatory processes.[17]

To date, many clinical studies have explored the therapeutic use of polyclonal Treg therapy in treating autoimmune and inflammatory diseases. Some clinical studies and experimental models have been based on alternative strategies that expand Tregs via IL-2-based therapies (low-dose IL-2 or specific targeting of IL-2 by a monoclonal antibody), TGF-β, rapamycin, retinoic acid, or aminooxyacetic acid.[13,18–20] Interestingly, Akamatsu et al. identified a novel molecule, AS2863619 (AS), that converts naïve and effector/memory antigen-specific CD4$^+$ and CD8$^+$ T cells into Foxp3$^+$ Tregs in vitro through an IL-2-dependent but TGF-β-independent mechanism (Fig. 1).[14] However, the AS-induced Tregs showed characteristics similar to those of TGF-β-induced Tregs. Similar to TGF-β-induced Tregs, the AS-induced Tregs lacked Treg-specific DNA hypomethylation. Surprisingly, the AS-transformed Tregs were not negatively regulated by inflammatory cytokines such as IL-12 and IL-6.[14] These data taken together indicate that AS-generated Tregs might have an antigen-specific beneficial effect in autoimmune diseases.

Mass spectrometry analyses of mouse T lymphoma cells with or without AS treatment identified CDK8 and CDK19 as the target proteins for AS.[14] In addition, the inhibition of CDK8 and CDK19 was correlated with enhanced Foxp3 expression. Further, RNA interference studies and retroviral overexpression studies confirmed the repressive role of CDK8/19 on Foxp3$^+$ T cells. CDK8/19 inactivate the signal transducer and activator of transcription 5 (STAT5) by phosphorylating serine at the PSP (pro-ser-pro) motif, thereby hindering Foxp3 expression. The evidence from immunoprecipitation studies used to identify downstream signaling

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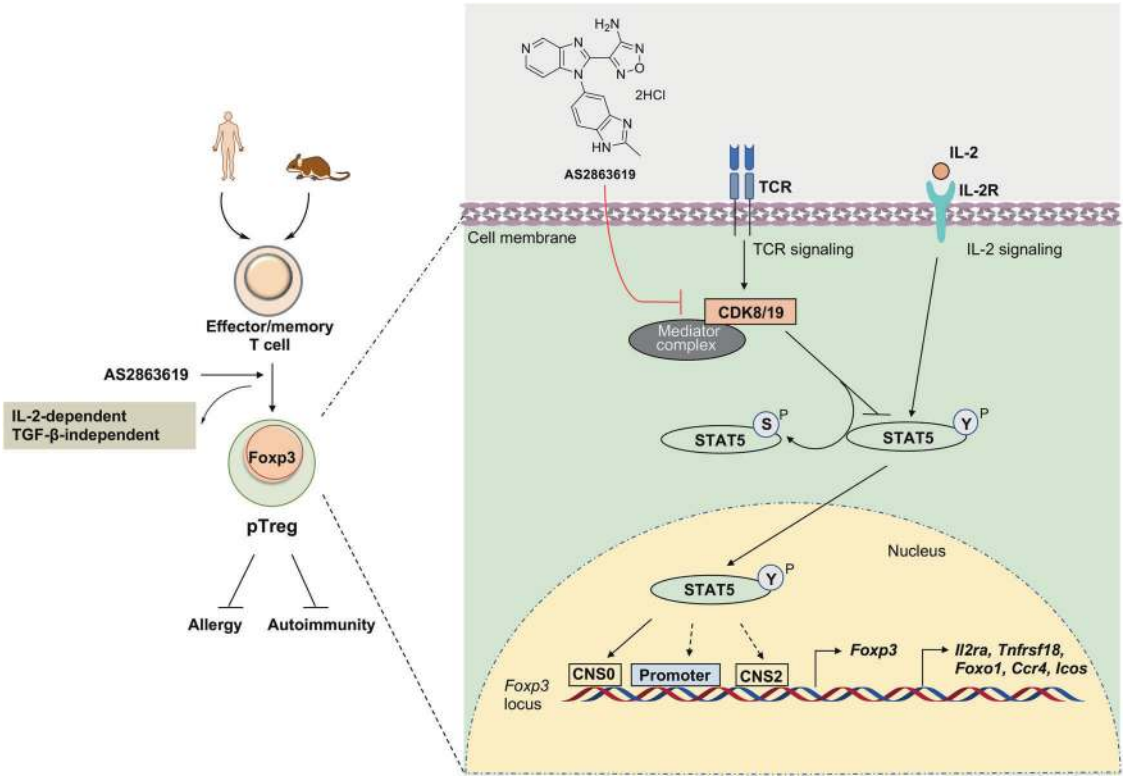

Fig. 1 Cyclin-dependent kinases mitigate the conversion of antigen-specific effector T cells into Foxp3$^+$ regulatory T cells. TCR signaling induces cyclin-dependent kinase 8 and its paralog CDK19 (CDK8/19). CDK8/19 inactivate the IL-2-induced signal transducer and activator of transcription 5 (STAT5) by phosphorylating serine at the PSP (pro-ser-pro) motif, thereby hindering Foxp3 expression. In contrast, inhibition of CDK8/19 by AS2863619 (4-[1-(2-methyl-1*H*-benzimidazol-5-yl)-1*H*-imidazo[4,5-c]pyridin-2-yl]-1,2,5-oxadiazol-3-amine dihydrochloride) converts the antigen-specific effector/memory T cells into Foxp3$^+$ regulatory T cells. Mechanistically, AS2863619 maintains the active state of STAT5 (phosphorylation at a tyrosine in the C-terminal domain), which binds to a regulatory region of the Foxp3 locus primarily in the conserved noncoding sequence (CNS)0 and to a lesser extent to the Foxp3 promoter and CNS2 region to induce *Foxp3* and enhance the expression of numerous genes critical for Treg functions (*Il2ra*, *Tnfrsf18*, *Foxo1*, *Ccr4*, and *Icos*). Although not observed under in vitro conditions, in vivo AS2863619-induced Tregs also displayed stable Treg-specific demethylation features at the *Foxp3* and *Helios* gene loci. Moreover, AS treatment exerted protective effects on the mouse models of allergic and autoimmune diseases by increasing the Foxp3$^+$ T cells and reciprocally reducing the effector T cell population.

confirmed that AS suppresses STAT5 serine phosphorylation while promoting C-terminal domain tyrosine phosphorylation. Data from whole-genome chromatin immunoprecipitation sequencing revealed that AS enhances the binding of STAT5 primarily to the CNS0 region of Foxp3 and, to a lesser extent, to the Foxp3 promoter and CNS2 region to induce Foxp3$^+$ Tregs.[14] Notably, AS enhances the expression of several genes that are critical for Treg functions, including *Il2ra*, *Tnfrsf18*, *Foxo1*, *Ccr4*, and *Icos* (Fig. 1).

Can AS induce Foxp3 in antigen-specific T cells in vivo? To explore this possibility, the authors used DO11.10 TCR transgenic mice in a RAG2-deficient background.[14] These mice lack tTregs, and hence, pTreg generation after immunization with model antigen (ovalbumin; OVA) can be monitored in these mice. In line with the in vitro results, the authors found that AS induced Foxp3$^+$ T cells when the T cell receptor (TCR) was activated by the antigen interaction but not by naïve T cells. On the basis of killer cell lectin-like receptor G1 (KLRG1) expression and Treg-specific DNA demethylation, the authors confirmed that the Tregs were completely differentiated by AS .[14]

Preclinical studies in mouse models revealed the therapeutic effect of AS in allergic responses and autoimmune diseases. AS treatment in the aforementioned mouse models reduced skin hypersensitivity reactions, OVA-induced delayed-type hypersensitivity reactions, spontaneous diabetes, and experimental allergic encephalomyelitis by increasing the Foxp3$^+$ and KLRG1$^+$ T cells and reciprocally reducing the effector T cell population.[14] However, pTregs induced by AS were activated relatively lesser extent than that of tTregs, as assessed by the expression pattern of CD25, glucocorticoid-induced tumor necrosis factor receptor-related (GITR), and cytotoxic T-lymphocyte antigen-4 (CTLA-4). Together, these data suggested that AS could suppress both acute and chronic immune responses by promoting the generation of antigen-specific pTregs.

This study thus identified a novel molecular regulator that determines the balance between antigen-specific effector T cells and Foxp3$^+$ Tregs. Further, the authors have also identified a small molecule (AS) that can promote the generation of antigen-specific pTregs and protect mice from allergies and autoimmune diseases. Lack of toxicity is one of the great virtues of this small molecule; however, CDK8/19 inhibitors have shown unexpected toxicity in other animal species (rats and dogs).[15,21] This study also attracted the focus of chemists, who sought to prepare structural mimics and analogs of AS. Further work is necessary to determine the relative stability of AS-induced pTregs in vivo. Whether the long-term (chronic) inflammatory microenvironment affects the efficacy of the AS induction of Tregs needs to be determined.[22,23] Since targeting CDK8/19 by AS induces antigen-specific pTregs, possible systemic immunosuppression by polyclonal Treg therapy could be avoided. Successful therapeutic use of AS is expected to be more economical than conventional polyclonal Treg therapy. However, caution should be exercised as, depending on the availability of antigens, AS could also convert effector T cells that are specific for the pathogens, tumors, or vaccine antigens into pTregs and, as a consequence, could reduce protective immune responses.

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AUTHOR CONTRIBUTIONS

S.R.B. and J.B. performed the literature search and analyses and drafted the manuscript.

ADDITIONAL INFORMATION

Competing interests: Authors declare no competing interests.

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