For Antigen-Specific Effector or Foxp3 + Regulatory T Cell Fate, Cyclin-Dependent Kinases Hold the Trump Card

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Research Highlight

Antigen-specific effector or Foxp3+ regulatory T cells, cyclin-dependent kinases hold the trump card

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

Several lines of evidences clearly demonstrate that Foxp3+ Tregs are not homogenous population and several subsets of Tregs like 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 a key role in maintaining Treg identity, function and switching between effector T cells and Tregs.[6-8] In fact, Tregs require phosphatase and tensin homolog (PTEN) for their stability and for the maintenance of metabolic balance between glycolysis and mitochondrial functions.[9] A recent report advocates that mitochondrial complex III is indispensable for the suppressive function of Tregs as loss of complex III in Tregs triggered enhanced levels of metabolites 2-hydroxyglutarate (2-HG) and succinate that blocked the ten-eleven translocation (TET) family of DNA demethylases resulting in DNA hypermethylation and repression of several transcripts required for Treg functions.[10,11]

The 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 with a concommittant enhancement in autocrine TGF-β signaling to promote Foxp3+ iTreg

generation.[12] A metabolic regulator hypoxia-inducible factor 1 (HIF-1) controls the balance between effector Th17 cells and Tregs. Glutamine transamination, mainly catalysed by glutamic-oxaloacetic transaminase 1, determines the fate of $CD4^+$ T cell differentiation towards Th17 at the expense of iTregs. Increased transamination leads to abundancy of 2-HG that promotes hypermethylation of Foxp3 gene locus and inhibition of its transcription.[13]

A recent study by Prof. Shimon Sakaguchi group aimed at identifying the molecular regulator that controls the generation of antigen-specific Tregs.[14] They 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 as Mediator kinases. CDK8 in association with cyclin C (CCNC), Mediator complex subunit 12 (MED12), and MED13 forms a part of the mediator complex. These kinases in association with Mediator complex are implicated in several cellular processes like 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 the innate immune responses, CDK8/19 consort with NF-κB to regulate respective inflammatory gene expression. Due to involvement of NF-κB in the inflammatory disorders, inhibition of associated factors (CDK8/19) represents an attractive strategy to target inflammatory processess.[17]

Until now, many clinical studies have explored the therapeutic use of polyclonal Treg therapy in autoimmune and inflammatory diseases. Cllinical studies and experimental models have also considered alternative strategy of expanding Tregs via IL-2-based therapies (low dose IL-2 or specific targeting of IL-2 by monoclonal antibody), TGF-β, rapamycin, retinoic acid, (aminooxy)acetic acid.[13,18-20] Interestingly, Akamatsu et al. identified a novel molecule

AS2863619 (AS) that converts the naïve and effector/memory antigen-specific CD4+ and CD8+ T cells into Foxp3+ Tregs *in vitro* by an IL-2-dependent but TGF-β-independent mechanism (Figure 1).[14] However, AS-induced Tregs showed characteristics similar to that of TGF-β-induced Tregs. Like in the case of TGF-β-induced Tregs, AS-induced Tregs lacked Treg-specific DNA hypomethylation. Surprisingly, AS-transformed Tregs were not negatively regulated by the inflammatory cytokines like IL-12 and IL-6.[14] These data together provided an indicator that AS-generated Tregs might have a antigen-specific beneficial effect in autoimmune diseases.

Mass spectrometry analyses of mouse T lymphoma cells with or without treatment of AS identified CDK8 and CDK19 as the target proteins for AS.[14] In addition, inhibition of CDK8 and CDK19 was correlated with the enhanced Foxp3 expression. Further, RNA interference studies and retroviral overexpression studies confirmed the repressive role of CDK8/19 on Foxp3+ T cells. CDK8/19 inactivates the signal transducer and activator of transcription 5 (STAT5) by phosphorylating serine at PSP (pro-ser-pro) motif, thereby hindering the Foxp3 expression. Immunoprecipitation studies to identify the downstream signaling confirmed that AS suppresses STAT5 serine phosphorylation while promoting the C-terminal domain tyrosine phosphorylation. Data from whole-genome chromatin immunoprecipitation sequencing revealed that AS enhances the binding of STAT5 mainly to the CNS0 region of Foxp3 and to a minor extent Foxp3 promoter and CNS2 region to induce Foxp3+ Tregs.[14] Notably, AS enhanced the expression of several genes that are critical for Treg functions including *Il2ra*, *Tnfrsf18*, *Foxo1*, *Ccr4*, and *Icos* (Figure 1).

Could AS induce Foxp3 in the antigen specific T cells *in vivo* ? To explore this authors used DO11.10 TCR transgenic mice on the RAG2-deficient background.[14] These mice lack tTregs and hence pTreg generation following immunization with model antigen (ovalbumin;

OVA) could be monitored. In line with *in vitro* results, authors found that AS induced Foxp3+ T cells when T cell receptor (TCR) is activated by the antigen interaction, but not naïve T cells. Based on the expression of killer cell lectin like receptor G1 (KLRG1) and Treg-specific DNA demethylation, authors confirmed the complete differentiation of Tregs induced by AS.[14]

Preclinical studies in mouse models revealed the therapeutic effect of AS in allergy and autoimmune disease. AS treatment in the aforementioned mouse models reduced the skin hypersensitivity reactions, OVA-induced delayed-type hypersensitivity reaction, spontaneous diabetes, and experimental allergic encephalomyelitis by increasing the Foxp3+ T and KLRG1+ cells and reciprocally reducing the effector T cell populations.[14] However, AS-induced pTregs were relatively less activated as compared to tTregs as assessed by the expression pattern of CD25, Glucocorticoid-Induced tumor necrosis factor receptor-related (GITR), Cytotoxic T-lymphocyte antigen-4 (CTLA-4). All these data together suggested that AS could suppress both acute and chronic immune responses by promoting the generation of antign-specific pTregs.

This study thus identifies a novel molecular regulator that determines the balance between antigen-specific effector T cells and Foxp3+ Tregs. Further, authors have also identified a small molecule (AS) that could promote the generation of antigen-specific pTregs and protect the mice from allergic and autoimmune diseases. Devoid of toxicity is one the great virtues of this small molecule though CDK8/19 inhibitors have shown unexpected toxicity in other animal species (rats and dogs).[15,21] This work also attracts the focus of chemists to prepare structural mimics and analogs. Further work is necessary regarding the relative stability of AS-induced pTregs *in vivo*. Whether long-term (chronic) inflammatory microenvironment affects the efficacy of AS-induced Tregs need to determined.[22,23]

Since targeting CDK8/19 by AS induces antigen-specific pTregs, possible systemic immunosuppression by the polyclonal Treg therapy could be avoided. If successful, therapeutic use of AS would be more economical than conventional polyclonal Treg therapy. However, caution should be exercised as depending on the avialibility of antigens, AS could also convert effector T cells specific for the pathogens, tumors or vaccine antigens into pTregs and as a consequence might reduce protective immune responses.

CONFLICT OF INTEREST

Authors declare no conflict of interests.

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AUTHOR CONTRIBUTIONS

SRB and JB performed literature seaerch, analyses and drafted the manuscript

REFERENCES

1. Knochelmann, H.M. *et al*. When worlds collide: Th17 and Treg cells in cancer and autoimmunity. *Cell. Mol. Immunol.* **15**, 458-469 (2018).
2. Sakaguchi, S., Miyara, M., Costantino, C.M. & Hafler, D.A. FOXP3+ regulatory T cells in the human immune system. *Nat. Rev. Immunol.* **10**, 490-500 (2010).
3. Lu, L., Barbi, J. & Pan, F. The regulation of immune tolerance by FOXP3. *Nat. Rev. Immunol.* **17**, 703-717 (2017).
4. Ferreira, L.M.R., Muller, Y.D., Bluestone, J.A. & Tang, Q. Next-generation regulatory T cell therapy. *Nat. Rev. Drug Discov.* **18**, 749-769 (2019).
5. Wing, J.B., Tanaka, A. & Sakaguchi, S. Human FOXP3+ regulatory T cell heterogeneity and function in autoimmunity and cancer. *Immunity* **50**, 302-316 (2019).
6. Ohkura, N., Kitagawa, Y. & Sakaguchi, S. Development and maintenance of regulatory T cells. *Immunity* **38**, 414-423 (2013).
7. Zhang, X., Liu, J. & Cao, X. Metabolic control of T-cell immunity via epigenetic mechanisms. *Cell. Mol. Immunol.* **15**, 203-205 (2018).
8. Wei, G. *et al.* Global mapping of H3K4me3 and H3K27me3 reveals specificity and plasticity in lineage fate determination of differentiating CD4+ T cells. *Immunity* **30**, 155-167 (2009).
9. Shrestha, S. et al. Treg cells require the phosphatase PTEN to restrain TH1 and TFH cell responses. *Nat. Immunol.* **16**, 178-187 (2015).
10. Weinberg, S.E. *et al*. Mitochondrial complex III is essential for suppressive function of regulatory T cells. *Nature* **565**, 495-499 (2019).

11. Das, M., Alzaid, F. & Bayry, J. Regulatory T cells under the mercy of mitochondria. *Cell metabol.* **29**, 243-245 (2019).
12. Strainic, M.G., Shevach, E.M., An, F., Lin, F. & Medof, M.E. Absence of signaling into CD4+ cells via C3aR and C5aR enables autoinductive TGF-β1 signaling and induction of Foxp3+ regulatory T cells. *Nat. Immunol.* **14**, 162-171 (2013).
13. Xu, T. et al. Metabolic control of TH17 and induced Treg cell balance by an epigenetic mechanism. *Nature* **548**, 228-233 (2017).
14. Akamatsu, M. *et al.* Conversion of antigen-specific effector/memory T cells into Foxp3-expressing Treg cells by inhibition of CDK8/19. *Sci. Immunol.* **4**, eaaw2707 (2019).
15. Dannappel, M.V., Sooraj, D., Loh, J.J. & Firestein, R. Molecular and in vivo functions of the CDK8 and CDK19 kinase modules. *Front. Cell Dev. Biol.* **6**, 171 (2018).
16. Whittaker, S.R., Mallinger, A., Workman, P. & Clarke, P.A. Inhibitors of cyclin-dependent kinases as cancer therapeutics. *Pharmacol. Ther.* **173**, 83-105 (2017).
17. Chen, M. *et al*. CDK8/19 Mediator kinases potentiate induction of transcription by NFκB. *Proc. Natl. Acad. Sci. USA.* **114**, 10208-10213 (2017).
18. Sharabi, A. *et al.* Regulatory T cells in the treatment of disease. *Nat. Rev. Drug Discov.* **17**, 823-844 (2018).
19. Romano, M., Fanelli, G., Albany, C.J., Giganti, G. & Lombardi, G. Past, present, and future of regulatory T cell therapy in transplantation and autoimmunity. *Front. Immunol.* **10**, 43 (2019).
20. Abbas, A.K., Trotta, E., R. Simeonov, D., Marson, A. & Bluestone, J.A. Revisiting IL-2: biology and therapeutic prospects. *Sci. Immunol.* **3**, eaat1482 (2018).

21. Clarke, P.A. *et al*. Assessing the mechanism and therapeutic potential of modulators of the human Mediator complex-associated protein kinases. *Elife* **5**, e20722 (2016).

22. Zhao, H., Liao, X. & Kang, Y. Tregs: where we are and what comes next? *Front. Immunol.* **8**, 1578 (2017).

23. Sharma, M. *et al.* Regulatory T cells induce activation rather than suppression of human basophils. *Sci. Immunol.* **3**, eaan0829 (2018).

Figure Legend

Figure 1. Cyclin-dependent kinases check the switching of antigen-specific effector T cells into Foxp3$_{+}$ regulatory T cells. TCR signaling induces cyclin-dependent kinase 8 and its paralog 19 (CDK8/19). CDK8/19 inactivates the IL-2 induced signal transducer and activator of transcription 5 (STAT5) by phosphorylating serine at PSP (pro-ser-pro) motif, thereby hindering the 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 keeps the active state of STAT5 (phosphorylation at tyrosine in the C-terminal (C-ter) domain), which binds to regulatory regions of the Foxp3 locus majorily at conserved non-coding sequence (CNS)0, and to a lesser extent to Foxp3 promoter and CNS2 region to induce *Foxp3* and enhanced expression of numerous genes critical for the 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 *Foxp3* and *Helios* gene loci. Moreover, AS treatment rendered protective effects in the mouse models of allergy and autoimmune diseases by increasing the Foxp3$_{+}$ T cells and reciprocally reducing the effector T cell populations.

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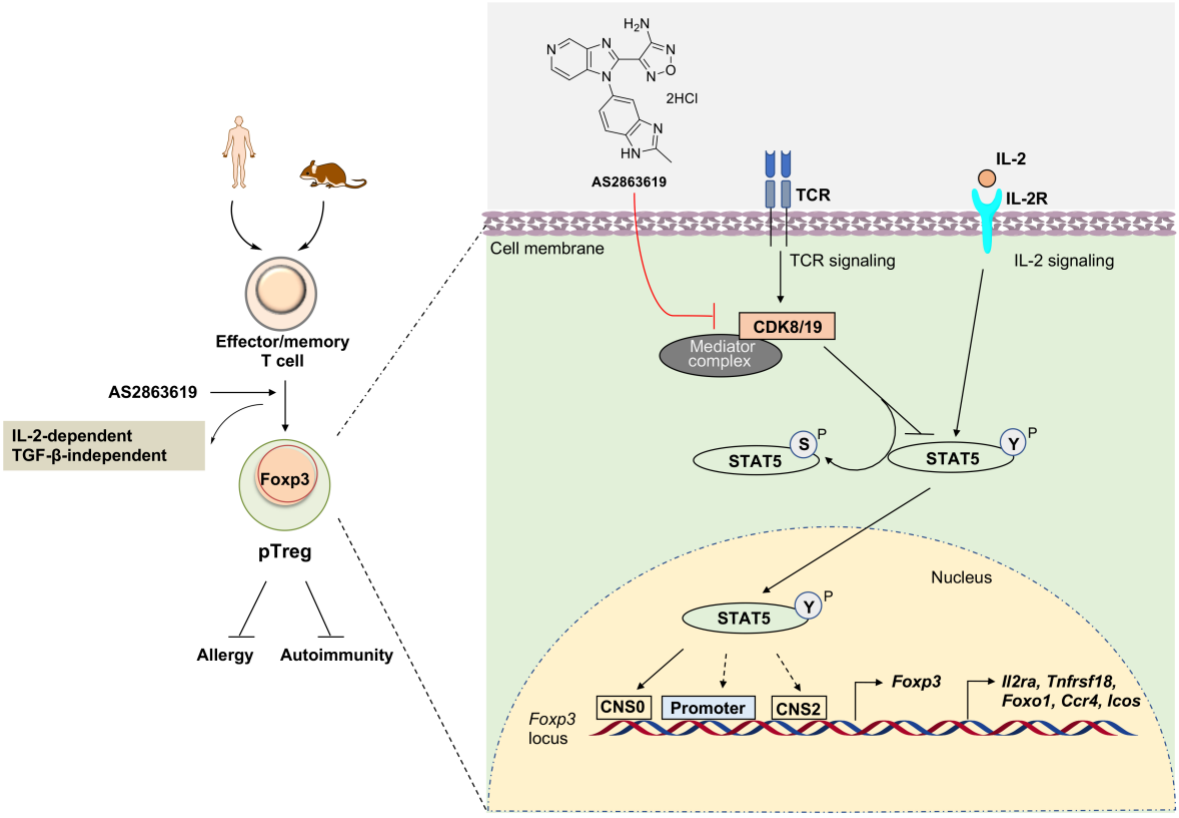
AS2863619
2HCl
IL-2
TCR
IL-2R
Cell membrane
TCR signaling
IL-2 signaling
CDK8/19
Mediator complex
STAT5
STAT5
Effector/memory T cell
AS2863619
IL-2-dependent TGF-β-independent
Foxp3
pTreg
Allergy
Autoimmunity
Nucleus
STAT5
CNS0
Promoter
CNS2
Foxp3
Il2ra, Tnfrsf18, Foxo1, Ccr4, Icos
Foxp3 locus