The nuclear orphan receptor Nr4a family is essential for nTreg development and immune homeostasis

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ABSTRACT

Regulatory T cells (Tregs) play a central role in maintaining immune homeostasis through various mechanisms. Although the Forkhead transcription factor Foxp3 defines the Treg cell lineage and functions, the molecular mechanisms of Foxp3 induction and maintenance remain elusive. We have screened an expression library consisting of cDNAs highly expressed in thymic natural-occurring Treg (nTreg) cells to isolate genes that can directly activate *Foxp3*-promoter in 293 cells. Over 100 genes, we identified NR4a family receptors, NR4a1, NR4a2, and NR4a3 as a strong activator of the *Foxp3*-promoter, which are highly expressed in nTregs compared with conventional T cells.

The members of the NR4A subgroup are expressed in a wide variety of tissues, such as skeletal muscle, adipose, heart, kidney, T-cells, liver and the brain. The ability to sense and rapidly respond to changes in the cellular environment appears to be a hallmark of this family. Expression of Nr4a receptors are induced by TCR signaling in $CD4^+$T cells, including immature T cells. Expression levels of Nr4a receptors are stably high in Tregs, which is possibly mediated by constitutive activation of their self-reactive TCR during development in the thymus. Nr4a receptors are also suggested to play roles in negative selection of self-reactive T cell clones, and thymocytes undergoing negative selection have been shown to express higher levels of Nr4a1 (Moran, A.E. *et al. J Exp Med* **208**, 1279-1289 (2011). Fassett et al.. *Proc Natl Acad Sci U S A* **109**, 3891-3896 (2012).)

We found that that Nr4a receptors have strong ability to induce Foxp3 in $CD4^+$T cells. Nr4a receptors bind directly to the *Foxp3* proximal promoter and activate its transcription by incorporating permissive histone modifications. Ectopic expression of Nr4a2 imparted Tregs-like suppressive activity to naïve $CD4^+$T cells by inducing Foxp3 and by repressing cytokines production, including IFN-γ and IL-2 (Sekiya et al. *Nat Commun* **2**, 269 (2011)). While, deletion of all three NR4a receptors in T cells in mice resulted in complete loss of Treg development and severe systemic autoimmunity. Along with their proposed roles in negative selection, our results suggest that Nr4a receptors are key factors for $CD4^+$T cell fate decision in the thymus. Activation of NR4a receptors could be a novel strategy to generate Tregs artificially for suppression of autoimmune diseases and allergy.