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Foxp3 controls autoreactive T cell activation through transcriptional regulation of early growth response genes and E3 ubiquitin ligase genes, independently of thymic selection

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Abstract To elucidate the mechanisms of autoreactive T cell activation and expansion, we used endogenous viral superantigens (VSAg)-reactive T cells as a model of self-antigens in two strains of *Foxp3*-mutant mice. These two strains, together with wild-type mice, provided us with an advantage to simultaneously study the positively and negatively selected as well as rescued autoreactive T cells. We show here that while both VSAg-reactive and non-VSAg-reactive T cells are equally activated in *Foxp3*-mutant mice, only the VSAg-reactive T cells are preferentially expanded independently of their selected states in the thymus. The T cell activation appears to be controlled by *Foxp3* through transcriptional regulation of early growth response (Egr) genes *Egr-2* and *Egr-3*, and E3 ubiquitin (Ub) ligase genes *Cblb*, *Itch* and *GRAIL*, subsequently affecting degradation of two key signaling proteins, PLCγ1 and PKC-θ. Physiologically, the positively, but not negatively selected VSAg-reactive T cells are spontaneously activated without significant expansion. The results suggest that autoreactive T cell activation is controlled by *Foxp3* through transcriptional regulation of early growth response genes and E3 ubiquitin ligase genes, independently of thymic selection.

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Introduction

IPEX (Immunodysregulation, polyendocrinopathy, enteropathy, X linked) in human [1] is caused by the mutation in forkhead-box transcription factor *Foxp3* [1–5]. A mouse model of human IPEX exhibits a Scurfy (sf) phenotype characterized by severe lymphoproliferation, eczema, diarrhea, anemia and cytokine overproduction, resulting in death of hemizygous males (sf/Y) by 3–4 weeks of age [6–8]. The *Foxp3* gene appears to encode a suppressor transcription factor and regulates T cell activation through acting on genes regulated by NFAT (nuclear factor of activated T cells) and NF-κB [9,10]. In the sf mice, the function of $CD4^+CD25^+$ regulatory T cells (T_{reg}) is defective [11–14]; cytokines are overproduced by activated T cells [7,15]; and the disease is transferable by CD4, but not CD8 T cells [16].

While the defect of T_{reg} has been ascribed to the cause of autoimmune syndrome [11–14], it remains to be elucidate why the complete absence of T_{reg} only induces limited organ-specific autoimmune disease in thymectomic mice [17,18]. Treatment of sf mice with $CD25^+$ T_{reg} cells only exhibited limited beneficial effect [19]. Thus, it is unlikely that functional deficiency of T_{reg} is the sole cause for the development of scurfy disease. Our and other's recent study also suggests that *Foxp3* expression might be beyond $CD25^+$ T_{reg} cells [14,20,21].

To investigate how autoreactive T cells are activated and expanded in *Foxp3* mice, we used endogenous viral superantigens (VSAgs) as model antigens of self-antigens in two strains (*Foxp3*^{sf} and *Foxp3*^{sf}*Otc*^{spf}) of sf mice with BALB/c background. BALB/c mice have the integration of mouse mammary tumor virus (*mtv*) types 6, 8 and 9 in the genome, and the VSAg-reactive T cells bearing TCR Vβ 3, 5, 11 or 12 are normally deleted [22,23]. The mutation of spare fur (*spf*) gene encoding ornithine transcarbamylase (*Otc*) [24] may disturb the thymic selection of VSAg-reactive T cells, but does not cause significant peripheral T cell activation. Therefore, we could simultaneously investigate the effects of thymic selection states on *Foxp3*-mediated VSAg-reactive T cell activation and expansion by analyzing *Foxp3*^{sf}, *Foxp3*^{sf}*Otc*^{spf} and wild-type (wt) mice. We have found that while all T cells are activated in *Foxp3*-mutant mice, VSAg-reactive T cells rather than non-VSAg-reactive T cells are predominantly expanded. The T cell activation appears to be controlled by *Foxp3* probably through regulating T cell anergy-associated early growth response (Egr) genes *Egr-2* and *Egr-3* [25], and E3 ubiquitin (Ub) ligase genes *Cblb*, *Itch* and *Grail* [26–28], independently of their selected states in the thymus. Physiologically, the positively, but not negatively selected VSAg-reactive T cells are spontaneously activated without significant expansion. The results suggest that autoreactive T cell activation is controlled by *Foxp3* through transcriptional regulation of Egr and E3 ubiquitin ligase genes, independently of thymic selection.

Results

Kinetics of clonal deletion of VSAg-reactive T cells in normal mice

To investigate the fate of VSAg-reactive T cells in the *Foxp3*-mutant mice, we initially investigated the kinetics of clonal deletion of VSAg-reactive T cells in the thymus of wild-type (wt) BALB/c mice. The frequency of TCR Vβ 3, 5, 11 and 12^+ $CD4^+$ $CD8^-$ single positive (SP) thymocytes in 7-, 15- and 30-day old mice was determined by flow cytometry. As shown in Fig. 1A, clonal deletion of Vβ 3 and 5^+ CD4 SP thymocytes occurred later than the Vβ 11 and 12^+ CD4 SP cells. The Vβ 3

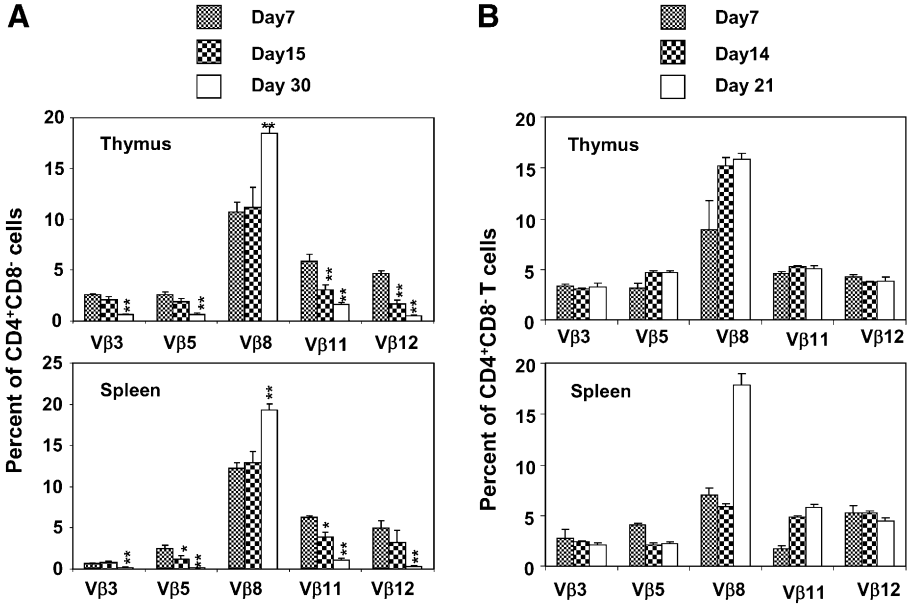

Figure 1 The kinetics of VSAg-reactive T cell development in the thymus of normal mice. BALB/c (A) and BALB.B (B) mice were sacrificed at day 7, 15 and 30 (A) or 21 (B) of age and single cells from thymus or spleens were stained for CD4, CD8 and TCR Vβ 3, 5, 8, 11 or 12. $CD4^+$ $CD8^-$ SP thymocytes or $CD4^+$ splenic T cells were gated and analyzed by flow cytometry for Vβ 3, 5, 8, 11 or 12 expression. Data are expressed as mean percentage ±SEM of Vβ 3, 5, 8, 11 or 12^+ cells among $CD4^+$ SP thymocytes or $CD4^+$ splenic T cells. (A) Day 7, $n=4$; day 15, $n=8$, day 30, $n=5$; (B) $n=3$ for all the groups. *$p \leq 0.05$; **$p \leq 0.01$, compared to day 7 of age.