



Published in final edited form as:

Immunity. 2018 April 17; 48(4): 745–759.e6. doi:10.1016/j.immuni.2018.03.025.

T cell receptor-regulated TGF β type I receptor expression determines T cell quiescence and activation

Eric Tu^{1,5}, Cheryl P.Z. Chia^{1,5}, Weiwei Chen², Dunfang Zhang¹, Sang A Park¹, Wenwen Jin¹, Dandan Wang², Maria-Luisa Alegre³, Ying E. Zhang⁴, Lingyun Sun^{2,6}, and WanJun Chen^{1,6,*}

¹Mucosal Immunology Section, NIDCR, NIH, Bethesda, MD 20892

²Department of Rheumatology and Immunology, The Affiliated Drum Tower Hospital of Nanjing University Medical School, Nanjing, China

³Department of Medicine, University of Chicago

⁴Laboratory of Cellular and Molecular Biology, Center for Cancer Research, NCI, NIH

Summary

It is unclear how quiescence is enforced in naïve T cells, but activation by foreign antigens and self-antigens is allowed, despite the presence of inhibitory signals. We showed that active transforming growth factor-beta (TGF β) signaling was present in naïve T cells and T cell receptor (TCR) engagement reduced TGF β signaling during T cell activation by downregulating TGF β type I receptor (T β RI) through activation of caspase recruitment domain-containing protein 11 (CARD11) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF κ B). TGF β prevented TCR-mediated T β RI downregulation but this was abrogated by interleukin-6 (IL-6). Mitigation of TCR-mediated T β RI downregulation through overexpression of T β RI in naïve and activated T cells rendered T cells less responsive and suppressed autoimmunity. Naïve T cells in autoimmune patients exhibited reduced T β RI expression and increased TCR-driven proliferation compared to healthy subjects. Thus, TCR-mediated regulation of T β RI-TGF β signaling acts as a crucial criterion to determine T cell quiescence and activation.

eTOC Blurbs

It is unclear how quiescence is enforced in naïve T cells. Tu et al. show that TGF β signaling maintains T cell quiescence, preventing aberrant responses to self-antigens. Strong TCR stimuli

⁶Correspondence should be addressed to: wchen@mail.nih.gov, lingyunsun@nju.edu.cn.

⁵These authors contributed equally

*Lead contact

Author contributions

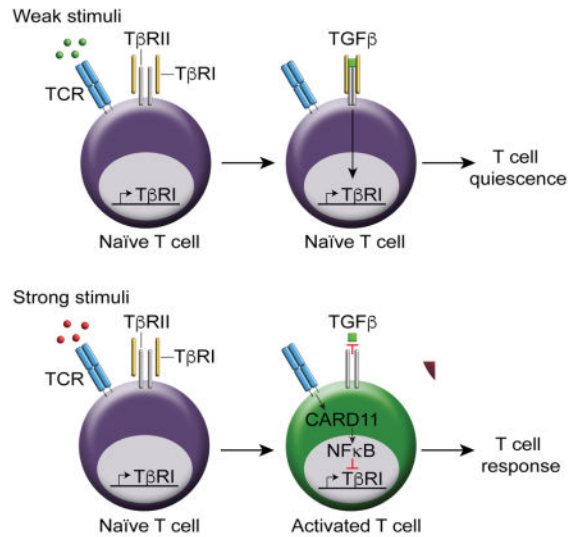
E. T designed and performed experiments, analyzed data and wrote the manuscript; C.P.Z. C, D.Z, S.A. P, and W. J designed and performed experiments; W.W. C and D. W designed and performed experiments and analyzed data for human studies; M-L. A and Y.E. Z provided critical materials and scientific input; L. S designed and supervised human studies and provided critical scientific input; W.J. C conceived, initiated and supervised the whole study, designed experiments and wrote the manuscript.

Declaration of Interests

The authors declare no competing interests.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

reduce T β RI expression and consequently abolish TGF β signaling in T cells. TCR-mediated T β RI downregulation acts as a “third criterion” to fully activate T cells in addition to the “two-signal” model.



Introduction

The initiation and magnitude of the T cell response is dependent on the balance of stimulatory and inhibitory signals. Naïve T cells are present in blood and peripheral lymphoid organs in their quiescent state, characterized by small cell size and reduced metabolic activity. The quiescent state of naïve T cells was thought to occur by default due to the lack of activation signals. However, accumulating studies have shown that survival of naïve T cells in the steady state requires TCR tickling by self-MHC molecules (Takada and Jameson, 2009). TCR tickling does not lead to autoimmunity in healthy individuals as T cell quiescence is actively reinforced by extrinsic factors such as regulatory T (Treg) cells, and intrinsic mechanisms such as transcription factors Peli1, TRIM28, Foxp1, Tsc1, and Tob (Chang et al., 2011; Chikuma et al., 2012; Feng et al., 2011; Sakaguchi et al., 2008; Tzachanis et al., 2001; Yang et al., 2011).

However, a few unresolved issues have arisen from these studies. First, it is not understood how T cell activation can still occur upon antigen stimulation when these mechanisms are in place to maintain T cell quiescence and tolerance. The “two-signal” model of T cell activation has been widely accepted: the first signal provided by the engagement of TCR to peptide-MHC complexes on antigen presenting cells (APCs) and the second signal provided by co-stimulation (Smith-Garvin et al., 2009). It is plausible that an additional signal is required to release T cells from quiescence programs to achieve T cell activation. Secondly, although hyperactivation and hyperproliferation of T cells were observed in mice with deletion of any of the quiescence-associated factors, none of these mice developed early onset lethal autoimmune diseases like mice with deficiency in forkhead box P3 (Foxp3), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) or TGF β (Fontenot et al., 2003; Hori et al., 2003; Shull et al., 1992; Waterhouse et al., 1995). However, Foxp3 and CTLA-4

are unlikely to regulate quiescence in naïve T cells intrinsically as they are not expressed in naïve T cells (Egen and Allison, 2002; Josefowicz et al., 2012). These findings collectively suggest that there must be other mechanism(s) that play a major role in governing quiescence of naïve T cells, and TGF β signaling is one such candidate. TGF β is involved in the development, survival and function of various immune cells, especially T cells (Tu et al., 2014). Bioactive TGF β binds to TGF β type II receptor (T β RII) and induces the assembly of a tetrameric TGF β receptor complex (T β R) composed of T β RII and T β RI, which phosphorylates transcription factors mothers against decapentaplegic (SMAD)2 and SMAD3. Phosphorylated SMAD2 and/or SMAD3 form complexes with SMAD4 and are translocated into the nucleus, where they associate with DNA-binding cofactors to regulate the transcription of target genes (Shi and Massague, 2003). In addition, SMAD-independent pathways are also involved in mediating TGF β signaling (Derynck and Zhang, 2003). The roles of TGF β in suppressing activation of T cells have been well demonstrated by either addition of exogenous TGF β to T cells *in vitro* (Ruegamer et al., 1990) or by genetic mutation of TGF β ligands or receptors in T cells (Li et al., 2006; Liu et al., 2008; Marie et al., 2006; Shull et al., 1992). However, few studies have investigated the impact and mechanisms of TCR stimulation in TGF β signaling and the consequential effects on the balance between T cell quiescence and T cell activation.

Here we showed that active TGF β signaling was present in naïve T cells and strong TCR stimulation abolished TGF β signaling to overcome its ongoing inhibition, through downregulation of T β RI expression. T β RII did not play an important role in the process. Accordingly, overexpression of T β RI in naïve T cells and restoration of T β RI in activated T cells constrained T cell responses. TCR drove the downregulation of T β RI through activation of the CARD11 and NF κ B pathway. We demonstrated that T β RI expression was significantly lower in naïve CD4⁺ T cells of systemic lupus erythematosus (SLE) patients compared to healthy subjects. These results reveal a TCR-NF κ B-T β RI axis that acts as a switch between T cell quiescence and T cell activation.

Results

TCR signals downregulate TGF β signaling and T β R expression in naïve T cells

We first examined TGF β signaling in naïve CD4⁺ T cells isolated from normal mice and humans. The amount of phosphorylated SMAD2 (p-SMAD2) protein was used as a readout for the degree of TGF β signaling in T cells as SMAD2 is phosphorylated when T β R is activated by TGF β . Freshly isolated naïve CD4⁺ T cells (0 hr) exhibited p-SMAD2 protein, indicating the presence of active TGF β signal in naïve CD4⁺ T cells (Figure 1A). We next stimulated naïve CD4⁺ T cells *in vitro* with anti-CD3 and anti-CD28 to determine TGF β signaling during T cell activation. We found that the amount of p-SMAD2 but not total SMAD2 protein decreased rapidly when T cells were activated (Figure 1A). A similar phenomenon was observed when human naïve and/or resting CD4⁺ T cells were stimulated (Figure 1A). To exclude the possibility that the reduction of p-SMAD2 in TCR-stimulated T cells might be caused by lack of sufficient amounts of TGF β in cultures, we treated naïve and previously-activated (24 hr stimulation) T cells with a high concentration of TGF β (2 ng/ml). We still detected higher levels of p-SMAD2 in naïve T cells than that in TCR-

activated T cells in the presence of exogenous TGF β , indicating an intrinsic feature of TCR stimulation in reducing TGF β signals (Figure 1B). These results altogether indicate that active TGF β signaling is present in naïve T cells but is reduced during T cell activation.

We next examined the components of the TGF β signaling pathway affected upon TCR stimulation. We showed that activation of naïve CD4⁺ T cells by anti-CD3 and anti-CD28 led to downregulation of T β RI and T β RII mRNA expression. This was observed in both mouse (Figure 1C) and human (Figure 1D) CD4⁺ T cells, as well as in mouse CD8⁺ T cells (data not shown). There was a time dependency in T β RI and T β RII downregulation, as indicated by much lower T β RI and T β RII expression in T cells that received prolonged stimulation (24 hr) compared to T cells that received short stimulation (1 hr). The downregulation of T β RI and T β RII was ascribed to TCR signals since stimulation of naïve CD4⁺ T cells with anti-CD3 alone was sufficient to induce the downregulation (Figure 1E), whereas stimulation with anti-CD28 alone did not cause T β R downregulation (data not shown). We further demonstrated downregulation of T β RI and T β RII in T cells activated *in vivo* by challenging DO11.10 TCR transgenic mice with OVA peptide that was specifically recognized by T cells in these mice, in which downregulation of T β RI and T β RII correlated with IL-2 mRNA upregulation (Figure 1F). Thus, TCR stimulation downregulates T β RI and T β RII transcription in naïve CD4⁺ T cells.

We next investigated whether T β RI and T β RII proteins were also regulated by TCR signals. We showed that TCR stimulation reduced the amount of T β RI protein (Figure 1G). However, the level of T β RII protein was not affected by TCR stimulation (Figure 1G), despite reduced T β RII mRNA expression. The data indicate that the reduction of T β RI, but not T β RII, correlates with the downregulation of TGF β signaling in T cells upon T cell activation.

Strength of TCR stimuli determines T β RI expression

As the change in T β RI reflects the change in TGF β signaling upon TCR stimulation, we next sought to determine the effect of TCR stimulation strength on T β R expression. When naïve T cells were stimulated with different concentrations of anti-CD3, it was evident that the degree of T β RI and T β RII downregulation was regulated by the strength of TCR signals, with a high concentration of anti-CD3 causing more T β RI and T β RII mRNA downregulation (Figure 1H). In contrast, weak TCR stimulation (e.g. 0.01 μ g/ml anti-CD3) failed to reduce, and instead, upregulated T β RI and T β RII expression compared to naïve T cells. The strength of TCR signals also had a similar effect on T β RI and T β RII expression *in vivo* when DO11.10 T cells were stimulated with different doses of OVA peptide (Figure 1I). Furthermore, we showed that the TCR affinity to antigens also differentially regulated the expression of T β R. When 5CC7 TCR transgenic T cells were stimulated with their natural ligand moth cytochrome C (MCC) or the superagonist K5 (Krogsgaard et al., 2003), significantly lower T β RI and T β RII expression was detected compared to 5CC7 T cells stimulated with weak agonist peptide, 102S (Figure 1J). We also extended this finding to CD8⁺ T cells by stimulating OT-I TCR transgenic T cells with OVA peptide variants (Daniels et al., 2006). Significant T cell activation and T β RI downregulation were detected when OT-I T cells were stimulated with their high-affinity cognate antigen, N4 peptide

(Figure S1A and S1B). In comparison, T4 peptide which has intermediate affinity for OT-I TCR, induced T cell activation and T β RI downregulation only at a high dose and the weak peptide E1 was unable to induce T β RI downregulation even at a high dose.

As T β RI expression in T cells negatively correlated with the strength of TCR stimuli, we hypothesized that strength of TCR stimuli could regulate T cell differentiation, especially the T cell subsets that require TGF β for their generation (Tu et al., 2014). We adoptively transferred naïve 5CC7 T cells to the syngeneic B10.A mice and challenged the mice with MCC peptide (pMCC) at different doses (Figure 2A). Consistent with our findings *in vitro*, low-dose pMCC (0.1 μ g) stimulation led to a higher T β RI expression in 5CC7 T cells compared to high-dose (10 μ g) (Figure 2B), whereas T β RII was not affected regardless of dose. Low-dose antigen stimulation also induced a higher frequency of Foxp3⁺ Treg cells but a lower frequency of IFN γ -producing T helper 1 (Th1) cells compared to high-dose antigen stimulation (Figure 2C and 2D). In contrast, a high dose of pMCC led to more Th1 cells, but fewer Treg cells (Figure 2C and 2D). We next investigated if reduction of T β RI expression with a high dose of antigen stimulation could affect Th17 cell differentiation *in vivo*. When 5CC7 T cells were challenged with a high dose (10 μ g) or a low dose (0.1 μ g) of pMCC with complete Freund's adjuvant (CFA), a decrease in T β RI but not T β RII was observed in high-dose antigen-challenged transgenic T cells compared to low-dose antigen-challenged T cells (Figure 2E). Indeed, an increase in the frequency of Th17 cells in these T cells correlated with decreased T β RI expression in T cells that were challenged with a high dose of pMCC (Figure 2F). These data altogether indicate that the strength of TCR stimulation regulates T β RI expression, which correlates with T cell lineage decisions.

TCR signals downregulate T β RI expression through the NF κ B pathway

TCR-mediated downregulation of T β R was likely to depend on transcriptional regulation since both total and nascent T β RI and T β RII mRNA were significantly lower in activated T cells compared to naïve T cells (Figure 3A). We next investigated the underlying molecular mechanisms by which TCR signals regulate T β R expression. SMAD7 has been previously reported to act as an inhibitor for TGF β signaling and T β R expression in epithelial cells and cancer cell lines (Nakao et al., 1997). However, we found that CD4⁺ T cells from *Smad7^{f/f}Cd4-cre* and *Smad7^{+/+}Cd4-cre* mice showed similar degrees of T β R downregulation upon TCR activation (Figure S2A), suggesting non-involvement of SMAD7. Furthermore, TGF β signaling in T cells was not significantly affected by the absence of SMAD7 when T cells were stimulated in the presence or absence of TGF β (Figure S2B). Thus, downregulation of T β R during T cell activation is independent of SMAD7.

Given that downregulation of T β RI is mediated by TCR signals, it is likely that transcription factors that are downstream of TCR act as repressors for T β RI expression during T cell activation. Indeed, predicted binding sites for transcription factors, such as NF κ B and nuclear factor of activated T cells (NFAT), were detected in the promoter region of T β RI using bioinformatics tools (BIOBASE, ALGGEN-PROMO). NF κ B and NFAT play important roles in TCR signaling (Smith-Garvin et al., 2009). As expected, we found that chemical inhibitors for NF κ B (IKK inhibitor VII), and NFAT (cyclosporin A) were both able to suppress the upregulation of IL-2 mRNA in activated T cells. However, only NF κ B

inhibition prevented TCR-mediated T β RI downregulation, but not inhibition of NFAT (Figure 3B). Neither NF κ B nor NFAT inhibitors had any effect on the downregulation of T β RII mRNA induced by TCR stimulation (Figure 3B). Despite not finding any transcription factor that regulates T β RII mRNA during T cell activation, we found that TCR signals significantly accelerated T β RII mRNA decay (Figure S3A), providing a possible mechanism for TCR-mediated T β RII mRNA downregulation.

We further confirmed the role of NF κ B as the repressor of T β RI expression using T cells from *Card11*^{-/-} mice and *Nfkb1a* N-Tg mice. The NF κ B pathway is inhibited in T cells from *Card11*^{-/-} mice since TCR mediates NF κ B activation through CARD11 (Egawa et al., 2003). *Nfkb1a* N-Tg mice express, selectively in T cells, a super-repressor form of I κ B α , which constitutively inactivates NF κ B (Boothby et al., 1997). Naïve CD4⁺ T cells from *Card11*^{-/-} and *Nfkb1a* N-Tg mice did not show downregulation of T β RI expression after TCR stimulation (Figure 3C). Inhibition of NF κ B activity in *Card11*^{-/-} and *Nfkb1a* N-Tg mice could not rescue T β RII mRNA during T cell activation (Figure 3C). We next determined which NF κ B subunits were required for the reduction of T β RI. p50, p52 and cRel were not required for the downregulation of T β RI by TCR signals as TCR stimulation induced a similar degree of T β RI downregulation in T cells from mice that lacked these NF κ B subunits compared to wild-type mice (Figure 3D). As p65 (RelA) deletion is embryonically lethal, we inhibited p65 activity in T cells using caffeic acid phenethyl ester (CAPE) that prevented the translocation of p65 to the nucleus (Natarajan et al., 1996). CAPE prevented T β RI downregulation in stimulated T cells (Figure 3E). Using chromatin immunoprecipitation (ChIP) assays, we also detected significantly higher binding of p65 at the predicted NF κ B binding site in the promoter region of T β RI in activated T cells compared to naïve T cells (Figure 3F). In contrast, there was no significant enrichment of other NF κ B subunits at the same region of T β RI gene in activated T cells (Figure S3B). Together, these experiments indicate that NF κ B is essential for TCR-mediated downregulation of T β RI.

TGF β prevents TCR-mediated T β RI downregulation

In addition to TCR stimuli, we also investigated the role of cytokines on T β R expression in CD4⁺ T cells. We added a panel of cytokines to TCR-stimulated naïve CD4⁺ T cell cultures and determined T β RI and T β RII mRNA expression. Only TGF β prevented TCR-mediated downregulation of T β RI, but not T β RII mRNA (Figure 4A and 4B). This was also reflected in T β RI protein levels (Figure 4C). In contrast, T β RII protein was detected at similar levels with or without TGF β (Figure 4C). Similarly, TGF β treatment of naïve T cells without TCR stimulation also led to an increase in T β RI, but not T β RII protein (Figure S4A). The TGF β -mediated maintenance of T β RI expression in activated T cells corresponded to substantial amounts of p-SMAD2 in these T cells (Figure 4D). We further confirmed the effect of TGF β on T β RI expression using *Tgfr2^{fl/fl}Esrl-cre* mice (Nakatsukasa et al., 2015). These mice express a mutated form of the mouse estrogen receptor and administration of tamoxifen induce efficient cre-mediated deletion of T β RII. T cells that were deficient in T β RII failed to upregulate their T β RI expression when stimulated in the presence of TGF β (Figure 4E). We also determined that the upregulation of T β RI by TGF β was, in part, through a SMAD-dependent pathway. *Smad3*^{-/-} T cells showed lower T β RI expression than wild-type T cells

when stimulated in the presence of TGF β . Nevertheless, increased T β RI expression was still observed in TGF β -treated *Smad3*^{-/-} T cells compared to untreated *Smad3*^{-/-} T cells (Figure 4F), suggesting involvement of SMAD3-independent pathways. Additionally, we showed that SMAD7 did not affect TGF β -mediated T β RI upregulation since similar degrees of T β RI upregulation were observed between T cells from *Smad7*^{+/+} *Cd4*-cre and *Smad7*^{f/f} *Cd4*-cre mice (Figure S2C).

As TGF β signaling in T cells is essential for the development of CD4⁺CD25⁺Foxp3⁺ Treg cells (Chen et al., 2003; Liu et al., 2008), we hypothesized that Treg cells, due to their exposure to TGF β , would express a higher level of T β RI than non-Treg cells (CD4⁺CD25⁻). Indeed, a significantly higher level of T β RI was detected in Treg cells compared to non-Treg cells before and after anti-CD3 and anti-CD28 stimulation. However, lower T β RII expression was detected in freshly-isolated Treg cells and no subsequent changes occurred upon TCR stimulation (Figure 4G). Overall, these data indicate that TCR-mediated reduction of T β RI is prevented by TGF β .

IL-6 abolishes TGF β -mediated prevention of T β RI reduction

As cytokines alone (with the exception of TGF β) did not influence TCR-mediated T β RI downregulation in naïve T cells (Figure 4A), we next investigated whether proinflammatory cytokines could interfere with the effect of TGF β on T β RI expression. We stimulated naïve CD4⁺ T cells with anti-CD3 and anti-CD28 in the presence of both TGF β and proinflammatory cytokines: IL-1 β , IL-2, IL-4, IL-6, IL-12, IL-23, IFN γ and TNF α . Only IL-6 blocked the upregulation of T β RI by TGF β (Figure S4B; data not shown). However, IL-6 had no effect on T β RII expression (Figure S4B).

We further confirmed the inhibitory effects of IL-6 on TGF β -mediated T β RI upregulation *in vivo*. Since bacteria antigens in CFA enhance IL-6 production in innate immune cells, we analyzed myelin oligodendrocyte glycoprotein (MOG)-specific T cells in mice that were immunized with MOG peptide with either CFA or incomplete Freund's adjuvant (IFA) (Figure S4C). The hypothesis was that IL-6 induced by CFA would reduce T β RI levels in MOG-specific T cells, compared to T cells from IFA-treated mice that received the same TCR stimulation in a TGF β -present microenvironment. Indeed, MOG-specific T cells in mice treated with MOG and CFA showed lower T β RI expression but not T β RII, compared to T cells in mice treated with MOG and IFA (Figure S4D). MOG and CFA also increased IL-17 production in MOG-specific T cells (Figure S4E), consistent with the previous notion that reduction of TGF β signaling favors Th17 cell differentiation (Zhou et al., 2008). These results suggest that IL-6 may affect TGF β signaling in T cells by antagonizing the effect of TGF β on T β RI expression.

T β RI downregulation promotes T cell responses

We next determined the effect of TCR-mediated downregulation of T β RI on T cell responses. Since the majority of T cells are hyperactivated in T cell-specific T β R-deficient mice, we used naïve CD4⁺ T cells from *Tgfbri*^{f/f} *Esr1*-cre mice to address this question. Similar CD4⁺ T cell frequencies were detected in the lymphoid organs of *Tgfbri*^{f/f} *Esr1*-cre mice compared to wild-type mice, and T β RI was deleted in *Tgfbri*^{f/f} *Esr1*-cre mice upon

completion of tamoxifen treatment (data not shown). Naïve CD4⁺ T cells from tamoxifen-treated *Tgfbri^{f/f}Esr1-cre* mice (Tam) and untreated control *Tgfbri^{f/f}Esr1-cre* mice (Ctrl) were cultured with different concentrations of soluble anti-CD3 and APCs, and levels of T cell proliferation and IFN γ production were measured. In response to TCR stimulation, greater cell proliferation was detected in T β RI-deficient T cells than those in control T cells. Furthermore, we detected a greater difference between T β RI-deficient and control T cells when the cells were stimulated with a low concentration of anti-CD3 compared to a high concentration (Figure S5A). When T cells were stimulated with plate-bound anti-CD3, which provides stronger TCR stimulation than soluble anti-CD3, a difference in cell proliferation was only detected when the cells were stimulated with a low, but not a high concentration of anti-CD3 between T β RI-deficient and control T cells (Figure S5B). This was most likely due to depletion of T β RI in control T cells by strong TCR stimulation. We also detected a similar pattern in IFN γ production: weak anti-CD3 stimulation led to a more dramatic difference in IFN γ production between T β RI-deficient and control T cells, compared to strong anti-CD3 stimulation (Figure S5C). In addition, T β RI-deficient T cells were more resistant to suppression by Treg cells (Figure S5D), consistent with findings that TGF β signaling in responder cells is required for Treg cell-mediated immunosuppression (Fahlen et al., 2005). These results demonstrate an increase in the magnitude of the T cell response to TCR stimulation in the absence of T β RI expression.

We next investigated whether T cell responses would be affected if we override the inherent T β R downregulation by overexpressing T β R in naïve T cells. We generated retrogenic mice using bone marrow cells infected with retrovirus for T β RI^{GFP}, or T β RII^{Cherry} or controls (Ctrl^{GFP}, Ctrl^{Cherry}). Naïve CD4⁺ T cells expressing T β RI^{GFP} or T β RII^{Cherry} were isolated from the retrogenic mice based on their respective fluorescence. Elevated levels of T β RI or T β RII expression were detected in T β RI^{GFP+} and T β RII^{Cherry+} T cells, respectively, compared with their controls (Figure 5A and S6A). TGF β was not able to upregulate transgenic expression of T β RI, as T β RI^{GFP} expression was driven by a CMV promoter that was subject to different regulatory mechanisms compared to the endogenous T β RI. T β RI^{GFP+}CD4⁺ T cells showed a significant decrease in IL-2 expression and increase in Foxp3 expression in response to TCR stimulation, even in the absence of exogenous TGF β (Figure 5A), and these changes were further enhanced by TGF β . Consistently, T β RI^{GFP+}CD4⁺ T cells exhibited less proliferation compared to Ctrl^{GFP+}CD4⁺ T cells when stimulated with anti-CD3 and APCs (Figure 5B). In contrast, IL-2 was expressed at a similar level between T β RII^{Cherry} and Ctrl^{Cherry} T cells upon stimulation (Figure S6A).

We also tested the response of these transgenic T cells *in vivo* using a colitis model based on T cell responses to commensal bacteria in the gut. *Rag1^{-/-}* mice that received naïve T β RI^{GFP+}CD4⁺ T cells were better able to maintain their body weight than mice that received naïve Ctrl^{GFP+}CD4⁺ T cells, which showed dramatic weight loss (Figure 5C), and the severity of colitis in mice was significantly lower in the former group (Figure 5D and 5E). Significantly fewer effector CD4⁺ T cells were detected in mice that received T β RI^{GFP+}CD4⁺ T cells compared to those in the controls (Figure 5F). More Treg cells were also found in mice that received T β RI^{GFP+}CD4⁺ T cells (Figure 5G), suggesting an enhancement of TGF β signaling in these T cells. In contrast, naïve T β RII^{Cherry+}CD4⁺ T

cells induced weight loss and colitis to a similar degree as naïve Ctrl^{Cherry+}CD4⁺ T cells (Figure S6B–S6D), although less effector T cells were observed in the colon of mice that received TβRII^{Cherry+}CD4⁺ T cells (Figure S6E). There was also no difference in the frequencies of Treg cells between mice that received TβRII^{Cherry+} or Ctrl^{Cherry+} T cells (Figure S6F). These data further suggest that it is the level of TβRI, but not TβRII, that determines TGFβ signaling in T cells. Taken together, these depletion and overexpression experiments establish that downregulation of TβRI allows for efficient T cell responses.

Restoration of TβRI in activated T cells reduces T cell proliferation

We also investigated the effect of restoration of TβRI expression on activated T cells. Our hypothesis was that if TCR-mediated TβRI downregulation was indeed required for optimal T cell responses, restoration of TβRI in activated T cells would restrain this. To address this question, activated CD4⁺ T cells were infected with retrovirus for either TβRI^{GFP} or TβRII^{Cherry}, followed by TCR re-stimulation. As expected, activated T cells that expressed either TβRI^{GFP} or TβRII^{Cherry} maintained high levels of TβRI or TβRII, respectively, upon TCR re-stimulation (Figure 6A and S7A). However, expression of TβRI^{GFP} but not TβRII^{Cherry} in activated T cells downregulated the expression of IL-2 when the T cells were re-stimulated (Figure 6A and S7A), suggesting an inhibitory role of TβRI but not TβRII even in activated T cells.

We next examined the effect of TβRI^{GFP} and TβRII^{Cherry} on activated T cells *in vivo* by expressing TβRI^{GFP} or TβRII^{Cherry} in activated DO11.10 transgenic T cells (TβRI^{GFP+}-DO11.10, TβRII^{Cherry+}-DO11.10) (Figure 6B). TβRI^{GFP+}-DO11.10 T cells were less responsive to challenge with OVA peptide compared to Ctrl^{GFP+}-DO11.10 T cells (Figure 6C). Conversely, TβRII^{Cherry} had no effect on the proliferative response of activated DO11.10 T cells when challenged with OVA peptide (Figure S7B).

When TβRI^{GFP+}- and TβRII^{Cherry+}-DO11.10 T cells were tested in a mouse model of airway inflammation (Figure 6D), significantly fewer TβRI^{GFP+}-DO11.10 T cells were found in the lungs and the draining lymph nodes compared to Ctrl^{GFP+}-DO11.10 T cells (Figure 6E). This was consistent with reduced Ki-67 expression in TβRI^{GFP+}-DO11.10 T cells (Figure 6F). Although none of the mice developed prominent pathological changes in their lungs upon completion of intratracheal challenge with OVA protein (data not shown), which was likely due to very low frequencies of both TβRI^{GFP+}- and Ctrl^{GFP+}-DO11.10 T cells in the lung tissue, significantly fewer TβRI^{GFP+}-DO11.10 T cells were found in the lungs (Figure 6E). Moreover, we found that restoration of TβRI in activated T cells led to reduced NFκB activity, as indicated by a decreased level of phosphorylated p65 compared to the control T cells (Figure 6G), which may account for reduced T cell responses in TβRI^{GFP+} activated T cells. In contrast, expression of TβRII^{Cherry} did not affect the frequency and proliferation of activated DO11.10 T cells (Figure S7C and S7D). These results altogether demonstrate that restoration of TβRI in activated T cells reduced T cell responses to antigens.

T β RI expression is reduced in naïve T cells of autoimmune patients

Since strong TCR stimulation is required for downregulation of T β RI, we hypothesized that there would be a difference in the level of T β RI between healthy individuals and patients with autoimmunity. We tested this hypothesis in recently diagnosed and untreated SLE patients. We detected significantly lower T β RI expression in naïve CD4⁺CD45RA⁺ T cells from SLE patients compared to those from healthy individuals (Figure 7A). Consistently, upon anti-CD3 stimulation, naïve T cells from SLE patients further reduced their T β RI (Figure 7A) and exhibited significantly more proliferation than healthy control T cells (Figure 7B). There was an increase in the proliferation of naïve T cells from healthy individuals when anti-CD3 stimulation was increased from 1 to 10 μ g/ml. However, naïve T cells from SLE patients already reached the plateau for proliferation at a low concentration of anti-CD3 (1 μ g/ml) and failed to proliferate further when simulated with a high concentration (10 μ g/ml) (Figure 7B), indicating a significant decrease in TCR signaling threshold in SLE naïve T cells. Thus, the data suggest that intrinsic differences such as T β RI expression in naïve T cells between healthy individual and autoimmunity patients may contribute to increased T cell responses.

Discussion

A mounting body of evidence suggests that the quiescent state of naïve T cells is actively maintained by extrinsic and intrinsic mechanisms. We here revealed active TGF β signaling in naïve T cells, suggesting that T cells are controlled by TGF β signaling in their naïve state before they receive sufficient TCR stimulation. This notion is supported by the fact that mice with T-cell specific deletion of T β R exhibit systemic T cell activation and responses (Li et al., 2006; Liu et al., 2008; Marie et al., 2006). It was unclear as to how T cells achieve T cell activation despite the omnipresence of TGF β in tissues and blood. We showed that strong TCR stimulation induced downregulation of T β RI, which reduced sensitivity of T cells to TGF β . Furthermore, TCR-mediated downregulation of T β RI is essential for T cells to induce optimal T cell responses, as evidenced by the data from T β RI-overexpressing retrogenic mice, as well as restoration of T β RI expression in activated T cells. Therefore, our results demonstrate that in addition to the two signals that are required for T cell activation, TCR-mediated downregulation of T β RI acts as “third criterion” for T cells to achieve the full array of immune responses.

When naïve CD4⁺ T cells were TCR-stimulated, a significant decrease in T β RI expression was observed in both mRNA and protein levels, which was associated with reduction in TGF β signaling in activated T cells. Despite a rapid decrease in T β RII mRNA during T cell activation, the amount of T β RII protein surprisingly remained relatively unchanged in activated T cells. Although we found that TCR signals accelerated decay of T β RII mRNA, this did not lead to a decrease in the protein level. One possibility of this non-correlation may be the long half-life of T β RII protein in T cells, but this remains to be elucidated. Alternatively, it is possible that we detected the decrease in T β RII mRNA during a short window of time when it was transiently downregulated. Indeed, significant downregulation of T β RI and T β RII mRNA was seen in T cells that were stimulated *in vivo* for 24 hours by strong antigen stimulation. However, only the reduction of T β RI, but not T β RII mRNA was

detected seven days after stimulation, suggesting that the change in T β RII mRNA is not sustained.

Our data also strongly suggest that T β RI and T β RII might be fundamentally different with regard to their correlation with TGF β signaling in T cells. Previous studies showed that both T β RI and T β RII were important in TGF β -mediated T cell tolerance since mice with T cell-specific deletion of either T β RI or T β RII die from lethal autoimmune disease (Li et al., 2006; Liu et al., 2008; Marie et al., 2006). This was expected, as T β RII is required to bind to TGF β ligands and then activates T β RI. However, multiple lines of evidence show that T β RI plays a more important role than T β RII in TCR-mediated downregulation of TGF β signaling: 1) T β RI protein, but not T β RII protein, was reduced upon TCR stimulation; 2) No difference in colitis severity was seen between mice that received T β RII^{Cherry} overexpressing naïve T cells and those that received control T cells, whereas naïve T cells overexpressing T β RI^{GFP} were unable to induce severe colitis; 3) Mice that received T β RI^{GFP+} T cells displayed increased Treg cell frequencies, but not mice that received T β RII^{Cherry+} T cells; 4) Restoration of T β RI alone in activated T cells was sufficient to reduce T cell proliferation upon antigen challenge whereas T β RII-overexpression had no effect. Although mechanistically elusive, these results do suggest that T β RI serves as the accurate reflection of TGF β signaling that controls the development of T cell responses. This conclusion however, does not eliminate a role for T β RII in TGF β signaling; only, that the changes in levels of T β RII do not reflect and control TGF β signaling in T cells in response to TCR stimulation. A recent study on the conversion of natural killer (NK) cells into type 1 innate lymphoid cells (ILC1s) has indicated that T β RI can achieve TGF β signaling independently of T β RII, in these cells (Cortez et al., 2017). However, the non-redundant role of T β RII in TGF β signaling in T cells has been shown by other groups (Li et al., 2006; Marie et al., 2006). Thus, it is likely that T β R and its downstream targets may function differently among different immune cell subsets.

We found that T β RI expression is tightly regulated by the strength of TCR stimulation. T cells that were stimulated with weak or low dose TCR stimuli showed no reduction and, very likely, higher T β RI expression compared to naïve T cells. In marked contrast, T cells that were activated with strong or high dose TCR stimuli exhibited rapid and profound reduction of T β RI compared to naïve T cells. This finding has important implications in understanding the quiescence program and general lack of autoimmunity in healthy individuals, despite the existence of self-reactive T cells that regularly encounter self-antigens. Moreover, our findings may help reconcile how T cell quiescence is maintained while T cell activation and proliferation are allowed during encounter with pathogens, even in the presence of TGF β . Low levels of active TGF β in blood and tissues are normally sufficient to prevent unwanted T cell response to self-antigens, despite the fact that the majority of TGF β is present in a latent form. This is because, following establishment of central and peripheral tolerance, T cells that are normally present in the body recognize self-antigens with low affinity. “Tickling” by the low affinity self-antigens is not sufficient to induce T β RI downregulation, and allows for ongoing TGF β signaling. On the other hand, engagement of TCR with high affinity antigens or foreign antigens is able to induce T β RI downregulation and promote T cell responses. This was demonstrated when N4 peptide induced T cell activation and T β RI downregulation in OT-I T cells, whereas E1 peptide was

not able to induce T β RI downregulation, even at a high peptide dose. It was previously shown that E1 peptide induced positive selection of OT-I thymocytes (Daniels et al., 2006), and we show in our experiments that this peptide did not induce T cell activation and T β RI downregulation in peripheral OT-I T cells, which reflects the interaction between naïve T cells and self-antigens. Thus, in addition to the amount of endogenous TGF β in the environment, the strength of TCR stimulation also determines the inhibitory effects of TGF β on T cells. Given that TGF β signaling in T cells is essential for Treg cell generation, our data may also explain the phenomenon that weak TCR stimulation favors the generation of Treg cells (Gottschalk et al., 2010). We found higher T β RI expression in T cells, greater Treg cell frequencies and lower Th1 and Th17 frequencies in mice that received low-dose antigen stimulation compared to those that received high dose.

The findings in this study may provide an explanation as to why activated T cells are more resistant to suppression by Treg cells compared to naïve T cells (Korn et al., 2007; Tu et al., 2013). It can be envisioned that T β RI downregulation in activated T cells contributes to their resistance to Treg cells. Supporting this notion, we have shown that T cells that lack T β RI are more resistant to Treg cell suppression. Furthermore, our findings may provide insight as to why Treg cells are much more effective in preventing the development of autoimmunity than in treating ongoing diseases (Tu et al., 2013). Since pathogenic T cells in ongoing autoimmunity are already activated by autoantigens, reduced T β RI expression and TGF β signaling in these T cells may render Treg cells ineffective.

Our finding that TGF β prevents TCR-mediated T β RI downregulation in T cells has important implications in our understanding of the development and pathogenesis of autoimmunity and cancer. The preservation and even upregulation of T β RI by TGF β present in blood and tissues may be critical in enforcing quiescence in naïve T cells and preventing T cells from abnormal activation in response to TCR stimulation, thus avoiding autoimmunity. This may also account for insufficient anti-tumor T cell responses in tumor patients, despite the presence of tumor antigen-specific T cells within tumors. Since TGF β is produced in large amounts in the tumor microenvironment, TGF β may inhibit anti-tumor immunity by upregulation of T β RI in T cells, thereby increasing their threshold for T cell activation. This conclusion is supported by previous studies that demonstrate anti-tumor immunity and tumor clearance after reduction of TGF β signaling in T cells (Gorelik and Flavell, 2001; Thomas and Massague, 2005). Furthermore, poor immunogenicity of tumor antigens may further impede anti-tumor responses since weak antigen stimulation does not induce T β RI downregulation, especially in the presence of substantial amounts of TGF β . Therapeutic strategies to deliver strong antigen stimulation to downregulate T β RI expression in T cells may be critical in achieving better anti-tumor immunity. We have recently shown that TGF β signaling to Treg cells is required for Treg cell activity (Konkel et al., 2017), which suggests that Treg cells rely heavily on TGF β for the maintenance of their phenotype. This may be facilitated by the high expression of T β RI in Treg cells. Furthermore, Treg cells express higher levels of membrane-bound TGF β compared to non-Treg cells (Nakamura et al., 2001), and it is possible that membrane-bound TGF β is required to keep Treg cells active and increase sensitivity of Treg cells to TGF β .

The surprising finding that IL-6 was able to reduce the positive effect of TGF β on T β RI expression in T cells provides insights into our understanding of the mechanisms at play in anti-infection immunity and the development of autoimmunity. Since IL-6 is secreted by innate immune cells during infection, it is possible that IL-6 induced by bacterial or viral pathogens suppresses upregulation of T β RI by TGF β and therefore reduces the threshold of TCR activation. Furthermore, this may help explain why autoimmunity can occur in individuals following infection: IL-6 induced by infection may lower the ability of TGF β to suppress self-reactive T cells by reducing T β RI expression on T cells and facilitate the differentiation of autoimmune pro-inflammatory T cells. Our findings also raise the possibility that, in addition to promoting expression of IL-23 receptor (Zhou et al., 2008), IL-6 facilitates Th17 cell generation by restraining T β RI expression. Although mechanistically elusive, it is likely that the NF κ B pathway is also involved: we showed herein that NF κ B negatively regulates T β RI and IL-6 was previously reported to induce NF κ B activation (Wang et al., 2003).

Indeed, TCR-mediated NF κ B activation provides a molecular mechanism that links TCR signaling to the regulation of TGF β signaling. We showed that NF κ B and NFAT inhibitors were both able to suppress the upregulation of IL-2 transcription in stimulated T cells. Nevertheless, only NF κ B inhibitor was able to prevent T β RI downregulation. This finding suggests that the maintenance of T β RI expression by NF κ B inhibitor was not the result of inhibition of T cell activation, but due to specific inhibition of the NF κ B pathway. This is in line with a previous report showing that strong TCR stimulation dampens Treg cell generation through the TCR-CARD11-NF κ B pathway (Molinero et al., 2011). We have observed direct binding of CARD11 and T β RI proteins in activated T cells (data not shown), suggesting a possibility for post-translational regulation of T β RI by NF κ B. It should be noted that, in addition to the TCR-NF κ B-T β RI axis, T cells might employ other mechanisms to regulate TGF β signaling during T cell activation, such as the TCR-protein kinase C θ pathway (Giroux et al., 2010). Although SMAD7 has been shown in other cell types to be an inhibitor of TGF β signaling, it did not play a role in the regulation of T β RI expression during T cell activation.

In contrast to previous reports that mainly focused on the inhibitory role of TGF β signaling in T cell activation, we here reveal that the cross-talk between TGF β and TCR is not unidirectional. TCR signals, through NF κ B, reciprocally reduce TGF β signaling by downregulating T β RI expression. We thus propose a model in which TGF β signaling is regulated by TCR signals, which in turn controls the switch between T cell quiescence and activation: when T cells encounter weak TCR stimuli such as self-antigens or tumor antigens, T β RI expression allows for TGF β signaling which inhibits T cell activation and/or promotes Treg cell generation, further reinforcing T cell quiescence and tolerance. However, when T cells encounter strong TCR stimuli such as pathogens, an adequate T cell response is generated due to reduced sensitivity of activated T cells to TGF β as a result of T β RI downregulation. In addition, proinflammatory cytokines such as IL-6 may interfere with the balance of T cell quiescence and activation enforced by TGF β . This may promote anti-infection immunity or trigger the onset of autoimmunity. In sum, this model may provide deeper insights into many fundamental immunology questions, including how naïve T cells

are kept quiescent and how T cell activation occurs despite the perpetual presence of inhibitory signals.

STAR Methods

Experimental Model and Subject Details

Mice—C57BL/6, CD45.1 (C57BL/6) and *Rag1*^{-/-} mice were purchased from The Jackson Laboratory. DO11.10x*Rag2*^{-/-}, 5CC7.CD45.1x*Rag2*^{-/-}, BALB/c and B10.A mice were obtained from Taconic. *Tgfb2*^{f/f}*Esr1*-cre and *Smad3*^{-/-} mice were previously described (Nakatsukasa et al., 2015; Yang et al., 1999). *Tgfb1*^{f/f}*Esr1*-cre mice were generated in house by crossing *Esr1*-cre mice with *Tgfb1*^{f/f} mice. *Smad7*^{f/f}*Cd4*-cre mice were generated by crossing *Cd4*-cre mice with *Smad7*^{f/f} mice obtained from M. Diaz (National Institutes of Health). *Tgfb1*^{f/f}*Esr1*-cre and *Tgfb2*^{f/f}*Esr1*-cre mice were treated with tamoxifen (1 µg/mouse) per day for 5 days to delete TβRI or TβRII, respectively. Lymphoid tissue from *Card11*^{-/-} and *Nfkb1* N-Tg mice were obtained from M. Alegre (University of Chicago). Lymphoid tissues from *Nfkb1*^{-/-} and *Nfkb2*^{-/-} mice were obtained from U. Siebenlist (National Institutes of Health). Lymphoid tissues from *Ret*^{-/-} mice were obtained from R. Sen (National Institutes of Health). Lymphoid tissues from OT-Ix*Rag1*^{-/-} mice were obtained from G. Altan-Bonnet (National Institutes of Health). All animal studies were approved by the Animal Care and Use Committees of National Institute of Dental and Craniofacial Research, National Institutes of Health. 6 to 10 weeks old, male and female sex-matched mice were used for all experiments.

Sources of human T cells—Human peripheral blood mononuclear cells (PBMC) were provided by healthy volunteers and obtained from the NIH Department of Transfusion Medicine through their approved protocol. PBMC from SLE patients and healthy controls were harvested at The Affiliated Drum Tower Hospital of Nanjing University Medical School, Nanjing Medical University (Nanjing, China) through their approved protocol. Donors from 20 to 58 years old, of both genders, were recruited in this study. All samples were provided on a de-identified basis. A signed informed consent was obtained from all donors.

Method Details

Real-time PCR—Total RNA was derived from cells using RNeasy Mini Kit (Qiagen) and reversed transcribed using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Quantitative real-time PCR was performed according to the protocol of TaqMan Gene Expression Master Mix (Applied Biosystems) with the following TagMan primers: *Hprt*, Mm00446968_m1; *Tgfb1*, Mm00436964_m1, Hs00610320_m1, Rn00688966_m1; *Tgfb2*, Mm03024091_m1, Hs00234253_m1; *Ii2*, Mm00434256_m1; *18s*, Mm03928990_g1; *Gapdh*, Hs02758991_m1; and *Foxp3*, Mm00475162_m1.

Flow cytometry analysis—Intranuclear staining was carried out using the Fixation/Permeabilization buffer solution (eBioscience) according to manufacturer's instruction. For intracellular cytokine staining, cells were stimulated with PMA (50 ng/ml), Ionomycin (250 ng/ml) and Golgi-Plug (1:1000 dilution, BD PharMingen) at 37 °C for 3 hr, followed by

fixation with the Fixation/Permeabilization buffer solution (BD Biosciences) according to manufacturer's instruction. The staining procedures for MOG_{38–49}-specific tetramer were previously described (Kasagi et al., 2014). Stained cells were analyzed on a LSRFortessa (BD Biosciences) and data was analyzed with FlowJo software.

***In vitro* T cell activation**—Naïve mouse (CD4⁺CD62L⁺CD44⁻) and human (CD4⁺CD45RA⁺) T cells were purified by magnetic cell sorting (Miltenyi Biotec) and stimulated with indicated concentration of plate-bound anti-CD3 and anti-CD28, with or without addition of various recombinant cytokines at 37 °C for the indicated durations. In some experiments, cells were treated with IKK inhibitor VII (1 μM), Cyclosporin A (100 ng/ml) or caffeic acid phenethyl ester (20 μM) for 1 hr before stimulation with anti-CD3 and anti-CD28. Naïve 5CC7 T cells were stimulated with irradiated APCs plus 102S, MCC or K5 peptide at 10 ng/ml for 24 hr. Naïve OT-I T cells were stimulated with irradiated APCs plus N4, T4 or E1 peptide at either 0.1 or 10 ng/ml for 24 hr.

***In vivo* T cell activation**—DO11.10x*Rag2*^{-/-} mice were treated with either PBS or indicated doses of OVA_{323–339} peptide for 24 hr before T cells were analyzed. In adoptive transfer experiments, naïve 5CC7.CD45.1 T cells (2 × 10⁶) were transferred into B10.A mice and the mice were immunized the next day with indicated doses of MCC_{88–103} peptide. 5CC7.CD45.1 T cells were purified by flow cytometric cell sorting based on their CD45.1 expression 7 days after the immunization. In some experiments, the recipient B10.A mice were immunized with MCC_{88–103} peptide plus CFA (IFA supplemented with 300 μg/mouse *M. Tuberculosis*) at tail base and 5CC7 T cells in the draining inguinal lymph nodes were harvested for analysis. For the generation of MOG-specific T cells, C57BL/6 mice were immunized with MOG_{35–55} peptide (200 μg/mouse) plus either IFA or CFA at tail base. 7 days after pMOG immunization, MOG-specific T cells in the draining inguinal lymph nodes were identified using MOG_{38–49}-specific tetramer and purified by flow cytometric cell sorting for analysis.

Isolation of nascent RNA—0.5 mM 5-ethynyl uridine (5-EU) was added into the T cell culture and was incorporated into the cells for 30 min. Total RNA was isolated using RNeasy Mini Kit (Qiagen) and 5-EU-labeled nascent RNA was captured using Click-iT Nascent RNA Capture Kit (Life Technologies) according to manufacturer's instruction. The captured nascent RNA was then compared with total RNA in real-time PCR.

Analysis of RNA decay during T cell stimulation—Naïve CD4⁺ T cells were stimulated with plate-bound anti-CD3 and anti-CD28 for indicated period of time. Cell were washed with PBS and incubated with actinomycin D (10 μg/ml). Total RNA was isolated at discrete time points over a 4 hr period using TRIzol reagent (Invitrogen) to determine mRNA half-life.

ChIP assay—Naïve and activated CD4⁺ T cells (1 × 10⁷) were prepared for ChIP assay using HighCell# ChIP Kit (Diagenode), according to manufacturer's instruction. Cell lysates were sonicated, followed by precipitation with anti-p50, anti-p52, anti-p65, anti-RelB, anti-cRel, control rabbit IgG or control goat IgG. ChIP DNA was analyzed by real-time PCR

using Power SYBR Green PCR Master Mix with the following primers: forward (5'-TCTGGAGCTCATTTTGGCGT) and reverse (5'-AGCGGGAGCAGTCATAGGT A).

T cell proliferation and Treg cell suppression assays—Freshly isolated naïve T cells were labeled with CellTrace CFSE according to manufacturer's instruction. CFSE-labeled mouse T cells were stimulated with indicated concentration of anti-CD3 and irradiated APCs or plate-bound anti-CD3 and anti-CD28 (1 µg/ml). CFSE-labeled human T cells were stimulated with indicated concentration of plate-bound anti-CD3 and anti-CD28 (1 µg/ml). After 3 days of culture, dilution of CFSE fluorescence was used to determine cell division. In Treg cell suppression assay, CD4⁺CD25⁺ Treg cells were purified by magnetic cell sorting (Miltenyi Biotec) and cultured with CFSE-labeled CD4⁺ T cells (Teff), at indicated Treg:Teff ratios, together with irradiated APCs and anti-CD3 (1 µg/ml) for 3 days. Treg cell suppression was determined by the percentage of T cells that were inhibited by Treg cells compared with T cells cultured alone.

TβR retroviral constructs—Expression plasmids for *Tgfb1* and *Tgfb2* were previously described (Yu et al., 2002). cDNA encoding full-length *Tgfr1* and *Tgfr2* were subcloned into pMSCV-IRES-GFP and pMSCV-IRES-Cherry retroviral vectors, respectively. Hek293T cells were transfected with pCL-Eco packaging plasmid and retroviral plasmids using Turbofect (ThermoFisher). Retroviral supernatant was harvest 24–48 hr after transfection.

Generation of TβR retrogenic mice—TβRI^{GFP}, TβRII^{Cherry} and corresponding control (Ctrl^{GFP}, Ctrl^{Cherry}) retrogenic mice were generated via retroviral-transduced bone marrow reconstitution. Briefly, bone marrow was harvested from 8-week old donor C57BL/6 mice, 72 hr after administration of 5-fluorouracil (0.2 mg per gram of body weight). Bone marrow cells were cultured in C-DMEM supplemented with 20% FBS, mIL-3 (20 ng/ml), hIL-6 (50 ng/ml) and mSCF (50 ng/ml). Bone marrow cells were spininfected with retroviral supernatant and polybrene (5 µg/ml) at 2500 rpm for 2 hr at 32°C. Cells were harvested 48 hr later and sorted for GFP or Cherry expression, and injected into sublethally-irradiated *Rag1*^{-/-} mice together with wild-type bone marrow cells at 1:1 ratio. GFP⁺ or Cherry⁺ naïve CD4⁺ T cells were harvested from these mice by flow cytometric cell sorting 8–10 weeks after transplantation.

Adoptive transfer model of colitis—GFP⁺CD4⁺CD45RB^{high}CD25⁻ or Cherry⁺CD4⁺CD45RB^{high}CD25⁻ T cells were harvested from TβRI^{GFP}, TβRII^{Cherry} and their corresponding control mice by flow cytometric cell sorting. The sorted T cells (3×10^5) were transferred into *Rag1*^{-/-} mice. Recipient mice were weighed twice weekly for 5–7 weeks, after which they were euthanized. The presence and severity of colitis in recipient mice were determined by their body weight loss and histological examination of colon tissue. The histopathological assessment of colitis was as followed: score 0, normal colon tissue; score 1, very mild, scattered mononuclear cell infiltration; score 2, mild submucosal mononuclear cell infiltration, sometimes accompanied by focal aggregates of mononuclear cells into the mucosal area, with minimal epithelial hyperplasia and mucin depletion from goblet cells; score 3, moderate mononuclear cell infiltration, accompanied by moderate epithelial hyperplasia and mucin depletion; score 4, marked mononuclear cell infiltration

throughout the glandular mucosa and often transmural, with ulceration and marked epithelial hyperplasia and mucin depletion; score 5, as for score 4 except loss of intestinal glands. Lamina propria lymphocytes were isolated from colon tissue as previously described (Zanvit et al., 2015).

***In vivo* challenge of T β R-overexpressing activated T cells**—Naïve CD4⁺ T cells from DO11.10x*Rag2*^{-/-} mice were activated overnight with plate bound anti-CD3 and anti-CD28. Cells were spininfected with retroviral supernatant and polybrene at 2500 rpm for 90 min at 32°C. Activated DO11.10 T cells that expressed T β RI^{GFP}, T β RII^{Cherry} or control (Ctrl^{GFP}, Ctrl^{Cherry}) were harvested 48 hr later and labeled with CellTrace Violet according to manufacturer's instruction. The labeled cells (1×10^6) were transferred into BALB/c mice and the recipient mice were immunized the next day with OVA_{323–339} peptide plus CFA at tail base and the draining inguinal lymph nodes were harvested for analysis 2 days later. In the model of airway inflammation, BALB/c mice that received activated DO11.10 T cells (0.5×10^6) expressing T β RI^{GFP}, T β RII^{Cherry} or control (Ctrl^{GFP}, Ctrl^{Cherry}) were challenged intratracheally with OVA protein (100 μ g) once a day for 4 days before the mice were sacrificed for analysis. T cells were isolated from lung tissue by digesting lung tissue in DMEM medium supplemented with DNase (2 mg/ml) and collagenase (4 mg/ml) for 40 min at 37°C.

Quantification and Statistical Analysis

Statistical analysis—Statistical analysis was performed using either unpaired two-tailed Student's *t*-tests or two-way ANOVA in GraphPad Prism.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Drs. M. Diaz, U. Siebenlist, R. Sen and G. Altan-Bonnet, NIH for providing gene mutated mice and reagents. We thank the FACS core of NIDCR for their technical assistance. This research was supported by the Intramural Research Program of NIDCR, NIH and by the National Science Foundation of China Project #81120108021 and #81720108020 (L.S.) and the Jiangsu Province Major Research and Development Program, China #BE2015062 (L.S.). C.P.Z. C was supported in part by the A*STAR International Fellowship, Singapore. S.A. P was supported in part by the Korean Biomedical Scientist Fellowship Program.

References

- Boothby MR, Mora AL, Scherer DC, Brockman JA, Ballard DW. Perturbation of the T lymphocyte lineage in transgenic mice expressing a constitutive repressor of nuclear factor (NF)-kappaB. *J Exp Med.* 1997; 185:1897–1907. [PubMed: 9166419]
- Chang M, Jin W, Chang JH, Xiao Y, Brittain GC, Yu J, Zhou X, Wang YH, Cheng X, Li P, et al. The ubiquitin ligase Peli1 negatively regulates T cell activation and prevents autoimmunity. *Nat Immunol.* 2011; 12:1002–1009. [PubMed: 21874024]
- Chen W, Jin W, Hardegen N, Lei KJ, Li L, Marinos N, McGrady G, Wahl SM. Conversion of peripheral CD4⁺CD25⁻ naive T cells to CD4⁺CD25⁺ regulatory T cells by TGF-beta induction of transcription factor Foxp3. *J Exp Med.* 2003; 198:1875–1886. [PubMed: 14676299]
- Chikuma S, Suita N, Okazaki IM, Shibayama S, Honjo T. TRIM28 prevents autoinflammatory T cell development in vivo. *Nat Immunol.* 2012; 13:596–603. [PubMed: 22544392]

- Cortez VS, Ulland TK, Cervantes-Barragan L, Bando JK, Robinette ML, Wang Q, White AJ, Gilfillan S, Cella M, Colonna M. SMAD4 impedes the conversion of NK cells into ILC1-like cells by curtailing non-canonical TGF-beta signaling. *Nat Immunol.* 2017; 18:995–1003. [PubMed: 28759002]
- Daniels MA, Teixeira E, Gill J, Hausmann B, Roubaty D, Holmberg K, Werlen G, Hollander GA, Gascoigne NR, Palmer E. Thymic selection threshold defined by compartmentalization of Ras/MAPK signalling. *Nature.* 2006; 444:724–729. [PubMed: 17086201]
- Derynck R, Zhang YE. Smad-dependent and Smad-independent pathways in TGF-beta family signalling. *Nature.* 2003; 425:577–584. [PubMed: 14534577]
- Egawa T, Albrecht B, Favier B, Sunshine MJ, Mirchandani K, O'Brien W, Thome M, Littman DR. Requirement for CARMA1 in antigen receptor-induced NF-kappa B activation and lymphocyte proliferation. *Curr Biol.* 2003; 13:1252–1258. [PubMed: 12867038]
- Egen JG, Allison JP. Cytotoxic T lymphocyte antigen-4 accumulation in the immunological synapse is regulated by TCR signal strength. *Immunity.* 2002; 16:23–35. [PubMed: 11825563]
- Fahlen L, Read S, Gorelik L, Hurst SD, Coffman RL, Flavell RA, Powrie F. T cells that cannot respond to TGF-beta escape control by CD4(+)CD25(+) regulatory T cells. *J Exp Med.* 2005; 201:737–746. [PubMed: 15753207]
- Feng X, Wang H, Takata H, Day TJ, Willen J, Hu H. Transcription factor Foxp1 exerts essential cell-intrinsic regulation of the quiescence of naive T cells. *Nat Immunol.* 2011; 12:544–550. [PubMed: 21532575]
- Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat Immunol.* 2003; 4:330–336. [PubMed: 12612578]
- Giroux M, Delisle JS, O'Brien A, Hebert MJ, Perreault C. T cell activation leads to protein kinase C theta-dependent inhibition of TGF-beta signaling. *J Immunol.* 2010; 185:1568–1576. [PubMed: 20592275]
- Gorelik L, Flavell RA. Immune-mediated eradication of tumors through the blockade of transforming growth factor-beta signaling in T cells. *Nat Med.* 2001; 7:1118–1122. [PubMed: 11590434]
- Gottschalk RA, Corse E, Allison JP. TCR ligand density and affinity determine peripheral induction of Foxp3 in vivo. *J Exp Med.* 2010; 207:1701–1711. [PubMed: 20660617]
- Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *Science.* 2003; 299:1057–1061. [PubMed: 12522256]
- Josefowicz SZ, Lu LF, Rudensky AY. Regulatory T cells: mechanisms of differentiation and function. *Annu Rev Immunol.* 2012; 30:531–564. [PubMed: 22224781]
- Kasagi S, Zhang P, Che L, Abbatiello B, Maruyama T, Nakatsukasa H, Zanvit P, Jin W, Konkel JE, Chen W. In vivo-generated antigen-specific regulatory T cells treat autoimmunity without compromising antibacterial immune response. *Sci Transl Med.* 2014; 6:241ra278.
- Konkel JE, Zhang D, Zanvit P, Chia C, Zangarle-Murray T, Jin W, Wang S, Chen W. Transforming Growth Factor-beta Signaling in Regulatory T Cells Controls T Helper-17 Cells and Tissue-Specific Immune Responses. *Immunity.* 2017; 46:660–674. [PubMed: 28423340]
- Korn T, Reddy J, Gao W, Bettelli E, Awasthi A, Petersen TR, Backstrom BT, Sobel RA, Wucherpfennig KW, Strom TB, et al. Myelin-specific regulatory T cells accumulate in the CNS but fail to control autoimmune inflammation. *Nat Med.* 2007; 13:423–431. [PubMed: 17384649]
- Krogsgaard M, Prado N, Adams EJ, He XL, Chow DC, Wilson DB, Garcia KC, Davis MM. Evidence that structural rearrangements and/or flexibility during TCR binding can contribute to T cell activation. *Mol Cell.* 2003; 12:1367–1378. [PubMed: 14690592]
- Li MO, Flavell RA. TGF-beta: a master of all T cell trades. *Cell.* 2008; 134:392–404. [PubMed: 18692464]
- Li MO, Sanjabi S, Flavell RA. Transforming growth factor-beta controls development, homeostasis, and tolerance of T cells by regulatory T cell-dependent and -independent mechanisms. *Immunity.* 2006; 25:455–471. [PubMed: 16973386]
- Liu Y, Zhang P, Li J, Kulkarni AB, Perruche S, Chen W. A critical function for TGF-beta signaling in the development of natural CD4+CD25+Foxp3+ regulatory T cells. *Nat Immunol.* 2008; 9:632–640. [PubMed: 18438410]

- Marie JC, Liggitt D, Rudensky AY. Cellular mechanisms of fatal early-onset autoimmunity in mice with the T cell-specific targeting of transforming growth factor-beta receptor. *Immunity*. 2006; 25:441–454. [PubMed: 16973387]
- Molinero LL, Miller ML, Evaristo C, Alegre ML. High TCR stimuli prevent induced regulatory T cell differentiation in a NF-kappaB-dependent manner. *J Immunol*. 2011; 186:4609–4617. [PubMed: 21411734]
- Nakamura K, Kitani A, Strober W. Cell contact-dependent immunosuppression by CD4(+)CD25(+) regulatory T cells is mediated by cell surface-bound transforming growth factor beta. *J Exp Med*. 2001; 194:629–644. [PubMed: 11535631]
- Nakao A, Afrakhte M, Moren A, Nakayama T, Christian JL, Heuchel R, Itoh S, Kawabata M, Heldin NE, Heldin CH, ten Dijke P. Identification of Smad7, a TGFbeta-inducible antagonist of TGF-beta signalling. *Nature*. 1997; 389:631–635. [PubMed: 9335507]
- Nakatsukasa H, Zhang D, Maruyama T, Chen H, Cui K, Ishikawa M, Deng L, Zanvit P, Tu E, Jin W, et al. The DNA-binding inhibitor Id3 regulates IL-9 production in CD4(+) T cells. *Nat Immunol*. 2015; 16:1077–1084. [PubMed: 26322481]
- Natarajan K, Singh S, Burke TR Jr, Grunberger D, Aggarwal BB. Caffeic acid phenethyl ester is a potent and specific inhibitor of activation of nuclear transcription factor NF-kappa B. *Proc Natl Acad Sci U S A*. 1996; 93:9090–9095. [PubMed: 8799159]
- Ruegamer JJ, Ho SN, Augustine JA, Schlager JW, Bell MP, McKean DJ, Abraham RT. Regulatory effects of transforming growth factor-beta on IL-2- and IL-4-dependent T cell-cycle progression. *J Immunol*. 1990; 144:1767–1776. [PubMed: 2407783]
- Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. *Cell*. 2008; 133:775–787. [PubMed: 18510923]
- Shi Y, Massague J. Mechanisms of TGF-beta signaling from cell membrane to the nucleus. *Cell*. 2003; 113:685–700. [PubMed: 12809600]
- Shull MM, Ormsby I, Kier AB, Pawlowski S, Diebold RJ, Yin M, Allen R, Sidman C, Proetzel G, Calvin D, et al. Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. *Nature*. 1992; 359:693–699. [PubMed: 1436033]
- Smith-Garvin JE, Koretzky GA, Jordan MS. T cell activation. *Annu Rev Immunol*. 2009; 27:591–619. [PubMed: 19132916]
- Stephen TL, Payne KK, Chaurio RA, Allegranza MJ, Zhu H, Perez-Sanz J, Perales-Puchalt A, Nguyen JM, Vara-Ailor AE, Eruslanov EB, et al. SATB1 Expression Governs Epigenetic Repression of PD-1 in Tumor-Reactive T Cells. *Immunity*. 2017; 46:51–64. [PubMed: 28099864]
- Takada K, Jameson SC. Naive T cell homeostasis: from awareness of space to a sense of place. *Nat Rev Immunol*. 2009; 9:823–832. [PubMed: 19935802]
- Thomas DA, Massague J. TGF-beta directly targets cytotoxic T cell functions during tumor evasion of immune surveillance. *Cancer Cell*. 2005; 8:369–380. [PubMed: 16286245]
- Tu E, Bourges D, Gleeson PA, Ang DK, van Driel IR. Pathogenic T cells persist after reversal of autoimmune disease by immunosuppression with regulatory T cells. *Eur J Immunol*. 2013; 43:1286–1296. [PubMed: 23420509]
- Tu E, Chia PZ, Chen W. TGFbeta in T cell biology and tumor immunity: Angel or devil? *Cytokine Growth Factor Rev*. 2014; 25:423–435. [PubMed: 25156420]
- Tzachanis D, Freeman GJ, Hirano N, van Puijenbroek AA, Delfs MW, Berezovskaya A, Nadler LM, Boussiotis VA. Tob is a negative regulator of activation that is expressed in anergic and quiescent T cells. *Nat Immunol*. 2001; 2:1174–1182. [PubMed: 11694881]
- Wang L, Walia B, Evans J, Gewirtz AT, Merlin D, Sitaraman SV. IL-6 induces NF-kappa B activation in the intestinal epithelia. *J Immunol*. 2003; 171:3194–3201. [PubMed: 12960348]
- Waterhouse P, Penninger JM, Timms E, Wakeham A, Shahinian A, Lee KP, Thompson CB, Griesser H, Mak TW. Lymphoproliferative disorders with early lethality in mice deficient in Ctla-4. *Science*. 1995; 270:985–988. [PubMed: 7481803]
- Yang K, Neale G, Green DR, He W, Chi H. The tumor suppressor Tsc1 enforces quiescence of naive T cells to promote immune homeostasis and function. *Nat Immunol*. 2011; 12:888–897. [PubMed: 21765414]

- Yang X, Letterio JJ, Lechleider RJ, Chen L, Hayman R, Gu H, Roberts AB, Deng C. Targeted disruption of SMAD3 results in impaired mucosal immunity and diminished T cell responsiveness to TGF-beta. *EMBO J.* 1999; 18:1280–1291. [PubMed: 10064594]
- Yu L, Hebert MC, Zhang YE. TGF-beta receptor-activated p38 MAP kinase mediates Smad-independent TGF-beta responses. *EMBO J.* 2002; 21:3749–3759. [PubMed: 12110587]
- Zanvit P, Konkel JE, Jiao X, Kasagi S, Zhang D, Wu R, Chia C, Ajami NJ, Smith DP, Petrosino JF, et al. Antibiotics in neonatal life increase murine susceptibility to experimental psoriasis. *Nat Commun.* 2015; 6:8424. [PubMed: 26416167]
- Zhou L, Lopes JE, Chong MM, Ivanov II, Min R, Victora GD, Shen Y, Du J, Rubtsov YP, Rudensky AY, et al. TGF-beta-induced Foxp3 inhibits T(H)17 cell differentiation by antagonizing RORgamma function. *Nature.* 2008; 453:236–240. [PubMed: 18368049]

Highlights

1. Naïve T cells show active TGF β signaling and T β R expression
2. Strong TCR stimulation decreases T β RI and TGF β signaling by CARD11 and NF κ B
3. Overexpression of T β RI suppresses T cell response and autoimmunity
4. T β RI expression is reduced in naïve T cells of SLE patients

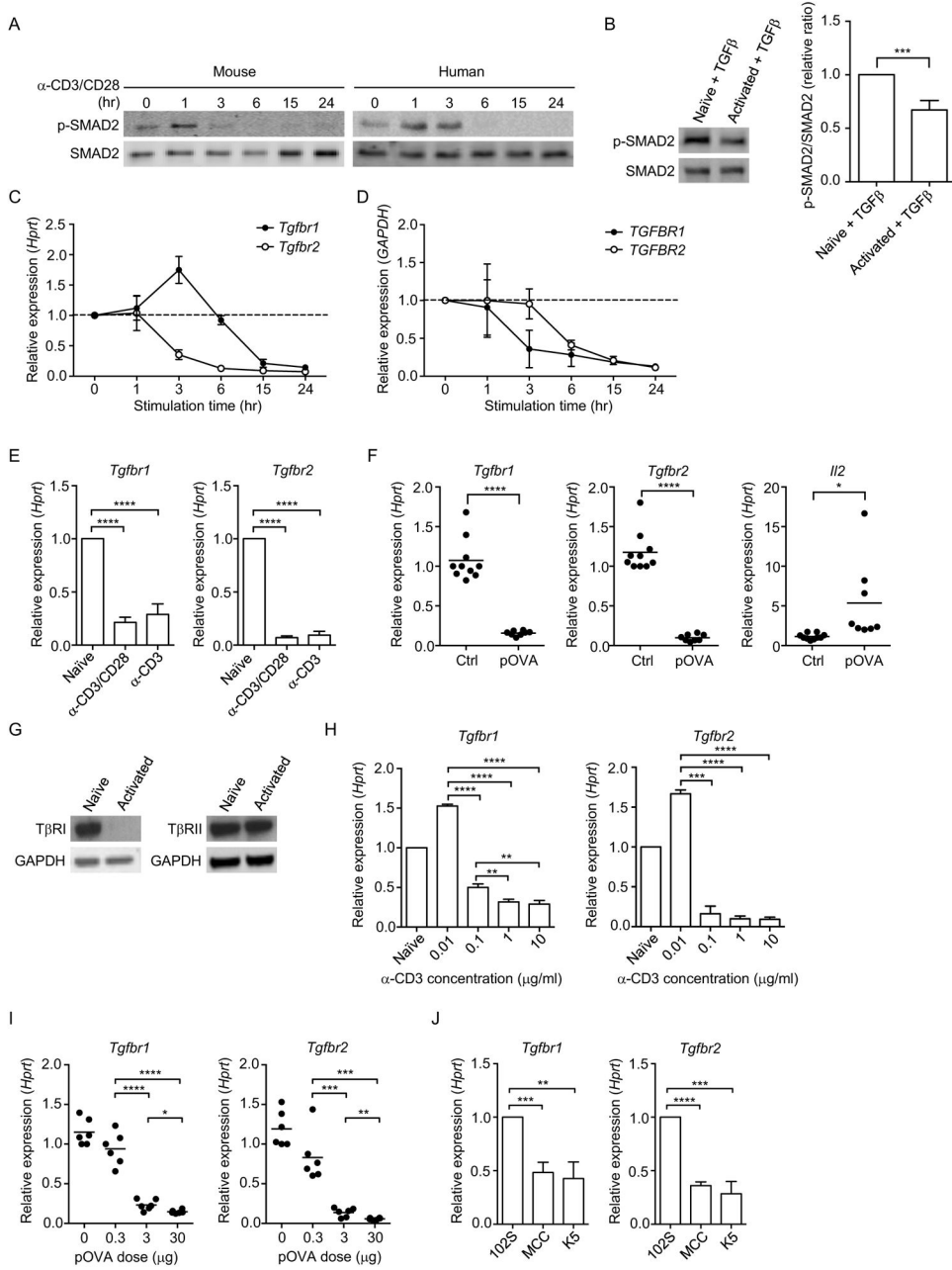


Figure 1. T cell activation induces TβR downregulation

(A) Western blot of p-SMAD2 and SMAD2 in naïve CD4⁺ T cells stimulated for indicated time periods with anti-CD3 and anti-CD28. (B) Western blot of p-SMAD2 and SMAD2 in naïve (Naïve + TGFβ) or previously-activated CD4⁺ T cells (Activated + TGFβ) stimulated for 24 hr with anti-CD3 and anti-CD28 plus TGFβ. (C, D) *Tgfb1* and *Tgfb2* mRNA in naïve (C) mouse and (D) human CD4⁺ T cells stimulated for indicated time with anti-CD3 and anti-CD28. (E) *Tgfb1* and *Tgfb2* mRNA in naïve CD4⁺ T cells stimulated for 24 hr with anti-CD3 plus anti-CD28 or anti-CD3 alone. (F) *IL2*, *Tgfb1* and *Tgfb2* mRNA in T cells from DO11.10x*Rag2*^{-/-} mice treated with PBS (Ctrl) or OVA peptide (pOVA). (G)

Western blot of T β RI, T β RII and GAPDH in naïve or CD4⁺ T cells stimulated for 24 hr with anti-CD3 and anti-CD28. (H) *Tgfb1* and *Tgfb2* mRNA in naïve CD4⁺ T cells stimulate for 24 hr with 1 μ g/ml anti-CD28 plus indicated concentration of anti-CD3. (I) *Tgfb1* and *Tgfb2* mRNA in T cells from DO11.10x*Rag2*^{-/-} mice treated with indicated doses of pOVA. (J) *Tgfb1* and *Tgfb2* mRNA in naïve 5CC7 T cells cultured for 24 hr with APCs and indicated peptides. Data are pooled from two (D, I), three (C, F, H, J) or four (B, E) independent experiments or are representative of three (A, G) independent experiments. In (F, I), each circle represents the data from one mouse. Student's *t*-test was used; bars (mean), error bars (SD), **P* < 0.05, ***P* < 0.01, ****P* < 0.001 and *****P* < 0.0001. See also Figure S1.

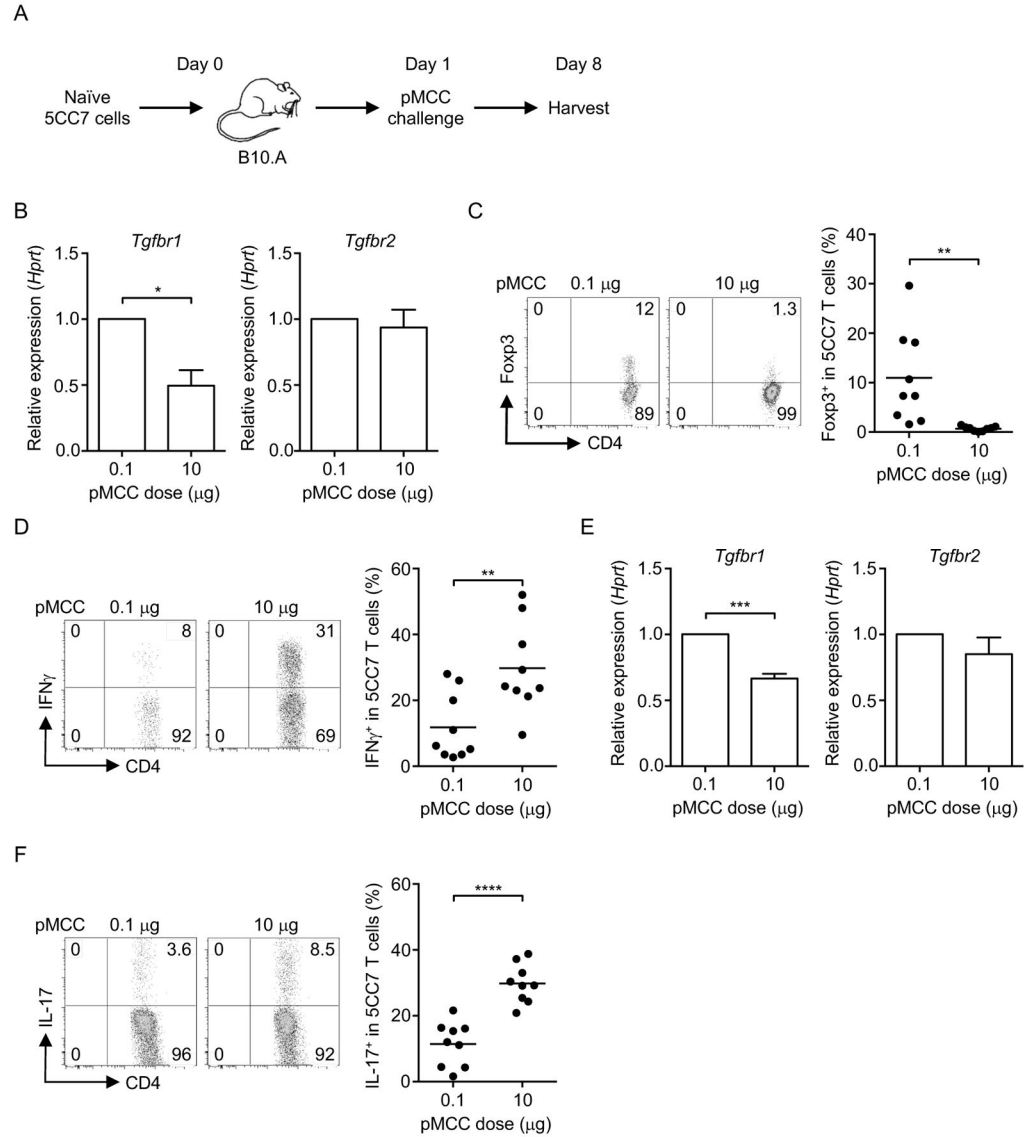


Figure 2. Antigen stimulation strength affects T cell differentiation and TBRI expression
 (A) Experimental workflow of naïve 5CC7 T cells challenged with pMCC. (B) *Tgfb1* and *Tgfb2* mRNA and, (C) Treg cell and (D) Th1 cell frequencies in 5CC7 T cells from mice challenged with indicated doses of pMCC. (E) *Tgfb1* and *Tgfb2* mRNA and, (F) Th17 cell frequency in 5CC7 T cells from mice challenged with indicated doses of pMCC plus CFA. Data are pooled from three independent experiments. In (C, D, F), each circle represents one mouse. Student’s *t*-test was used; bars (mean), error bars (SEM), **P* < 0.05, ***P* < 0.01, ****P* < 0.001 and *****P* < 0.0001.

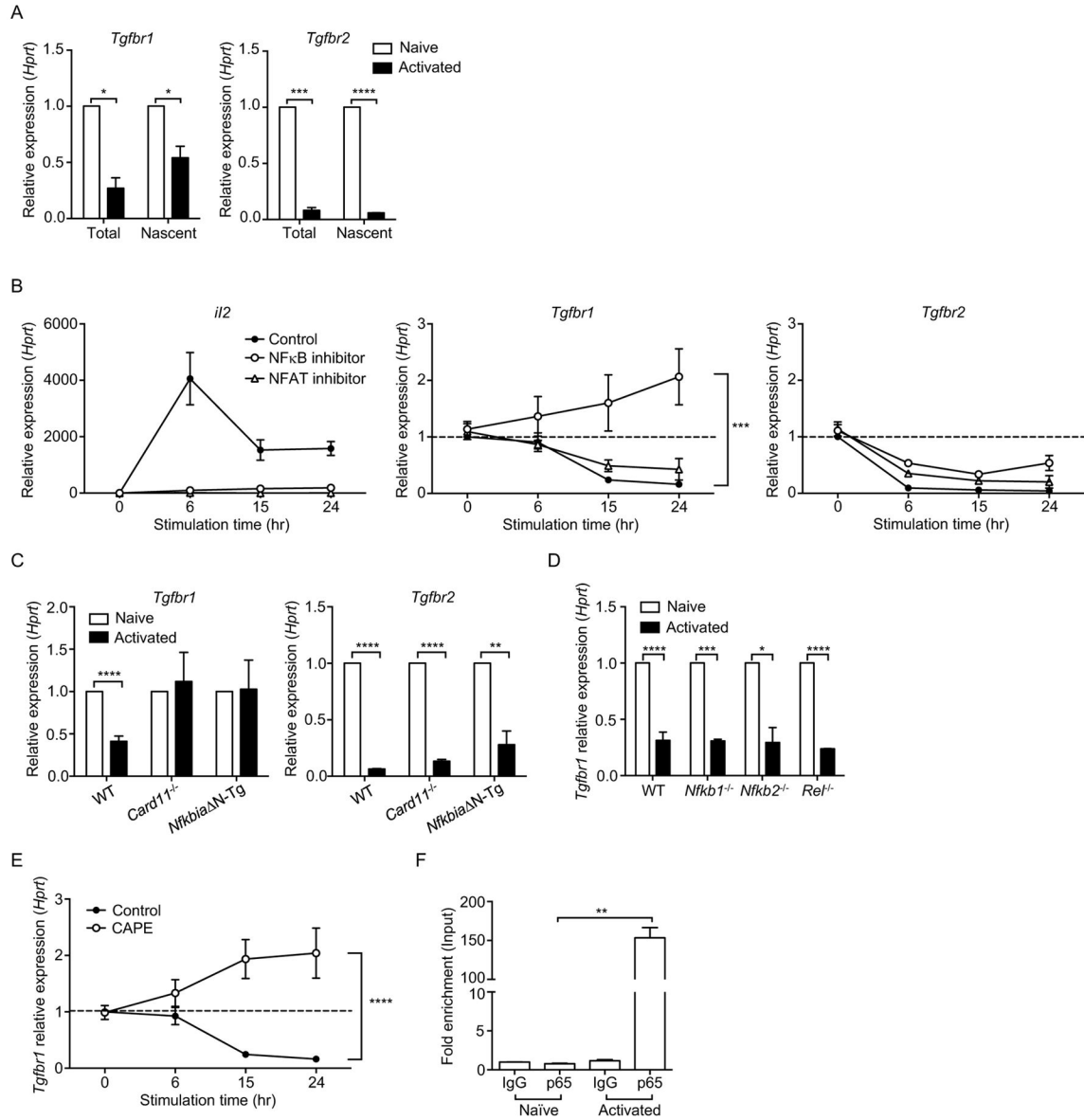


Figure 3. NFκB downregulates TβRI expression during T cell activation

(A) Total and nascent *Tgfb1* and *Tgfb2* mRNA in naïve or CD4⁺ T cells stimulated for 24 hr with anti-CD3 and anti-CD28 (Activated). (B) *Tgfb1*, *Tgfb2* and *IL2* mRNA in naïve CD4⁺ T cells stimulated for indicated time periods with anti-CD3 and anti-CD28 plus DMSO (Control), NFκB or NFAT inhibitor. (C) *Tgfb1* and *Tgfb2* mRNA in wild-type (WT), *Card11*^{-/-} or *Nfkb1a* N-Tg naïve or CD4⁺ T cells stimulated for 24 hr with anti-CD3 and anti-CD28 (Activated). (D) *Tgfb1* mRNA in WT, *Nfkb1*^{-/-}, *Nfkb2*^{-/-} or *Rel*^{-/-} naïve or CD4⁺ T cells stimulated for 24 hr with anti-CD3 and anti-CD28 (Activated). (E) *Tgfb1* mRNA in naïve CD4⁺ T cells stimulated for indicated time periods with anti-CD3 and anti-CD28 plus DMSO (Control) or CAPE. (F) ChIP-coupled real-time PCR analysis of p65 enrichment in the promoter region of *Tgfb1* gene of naïve or T cells stimulated for 24 hr with anti-CD3 and anti-CD28 (Activated). p65 enrichment was assessed using p65 antibody

and presented relative to input and compared with control IgG. Data are pooled from two (A, B, D), three (E) or four (C) independent experiments or are representative of three (F) independent experiments. In (A, C, D, F), Student's *t*-test was used. In (B, E), two-way ANOVA was used; bars (mean), error bars (SEM), **P* < 0.05, ***P* < 0.01, ****P* < 0.001 and *****P* < 0.0001. See also Figure S2 and S3.

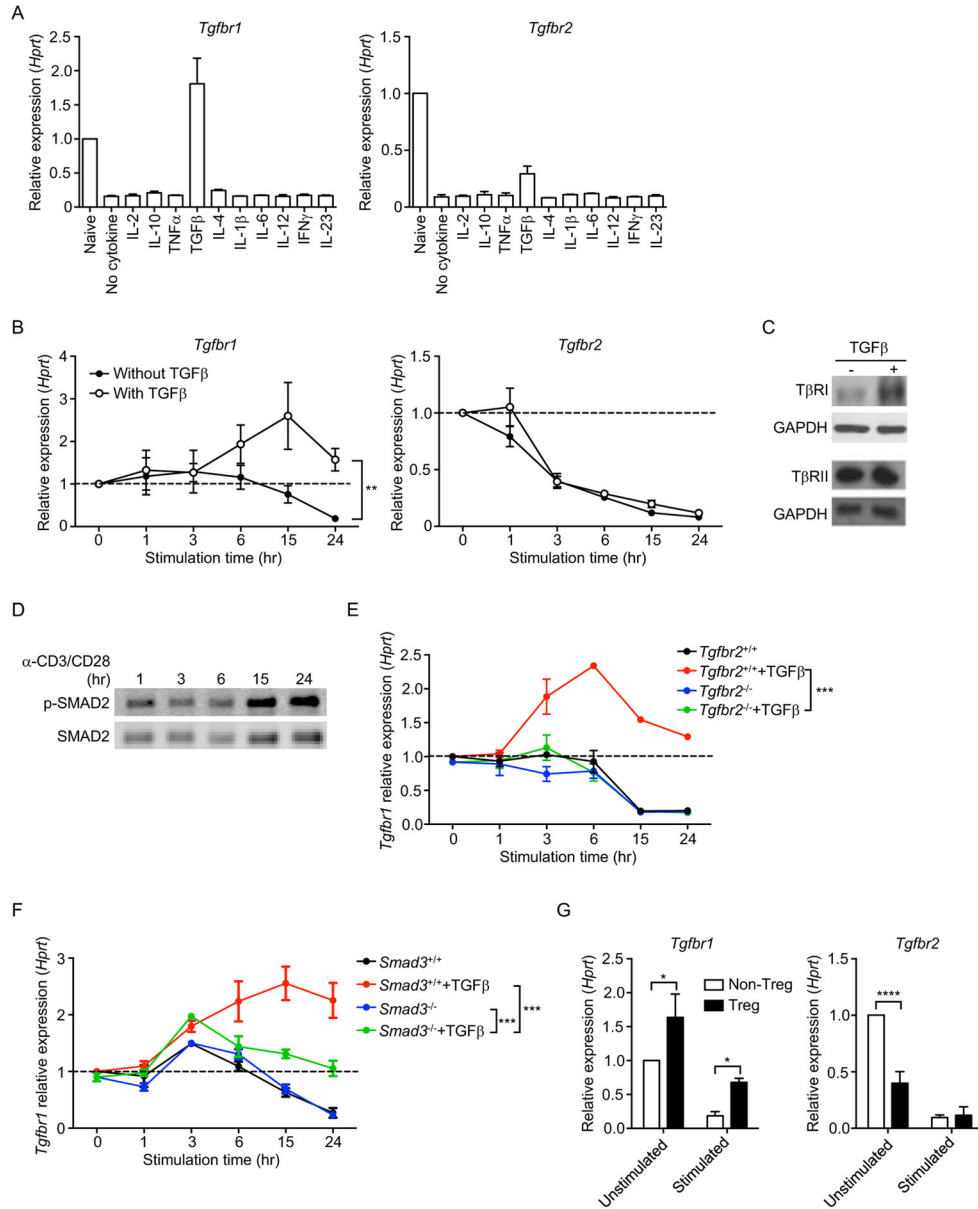


Figure 4. TGFβ upregulates TβRI expression

(A) *Tgfb1* and *Tgfb2* mRNA in naïve or CD4⁺ T cells stimulated for 24 hr with anti-CD3 and anti-CD28 plus different cytokines as indicated. (B) *Tgfb1* and *Tgfb2* mRNA in naïve CD4⁺ T cells stimulated for indicated time periods with anti-CD3 and anti-CD28 in the presence or absence of TGFβ. (C) Western blot of TβRI, TβRII and GAPDH in CD4⁺ T cells stimulated with anti-CD3 and anti-CD28 for 24 hr in the presence or absence of TGFβ. (D) Western blot of p-SMAD2 and SMAD2 in T cells stimulated for indicated time periods with anti-CD3 and anti-CD28 plus TGFβ. (E) *Tgfb1* mRNA in naïve *Tgfb2*^{f/f} *Esr1*-cre CD4⁺ T cells stimulated for indicated time periods with anti-CD3 and anti-CD28 in the presence or absence of TGFβ. *Tgfb2*^{+/+}; untreated *Tgfb2*^{f/f} *Esr1*-cre mice, *Tgfb2*^{-/-}; tamoxifen-treated *Tgfb2*^{f/f} *Esr1*-cre mice. (F) *Tgfb1* mRNA in naïve *Smad3*^{-/-} or *Smad3*^{+/+}

CD4⁺ T cells stimulated for indicated time periods with anti-CD3 and anti-CD28 in the presence or absence of TGFβ. (G) *Tgfb1* and *Tgfb2* mRNA in CD4⁺CD25⁻ non-Treg cells and CD4⁺CD25⁺ Treg cells that were unstimulated or stimulated with anti-CD3 and anti-CD28 for 24 hr. Data are pooled from two (A, E, F, G) or three (B) independent experiments or are representative of three (C, D) independent experiments. In (B, E, F), two-way ANOVA was used. In (G), Student's *t*-test was used; bars (mean), error bars (SD in A, E-G; SEM in B), **P* < 0.05, ***P* < 0.01, ****P* < 0.001 and *****P* < 0.0001. See also Figure S4.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

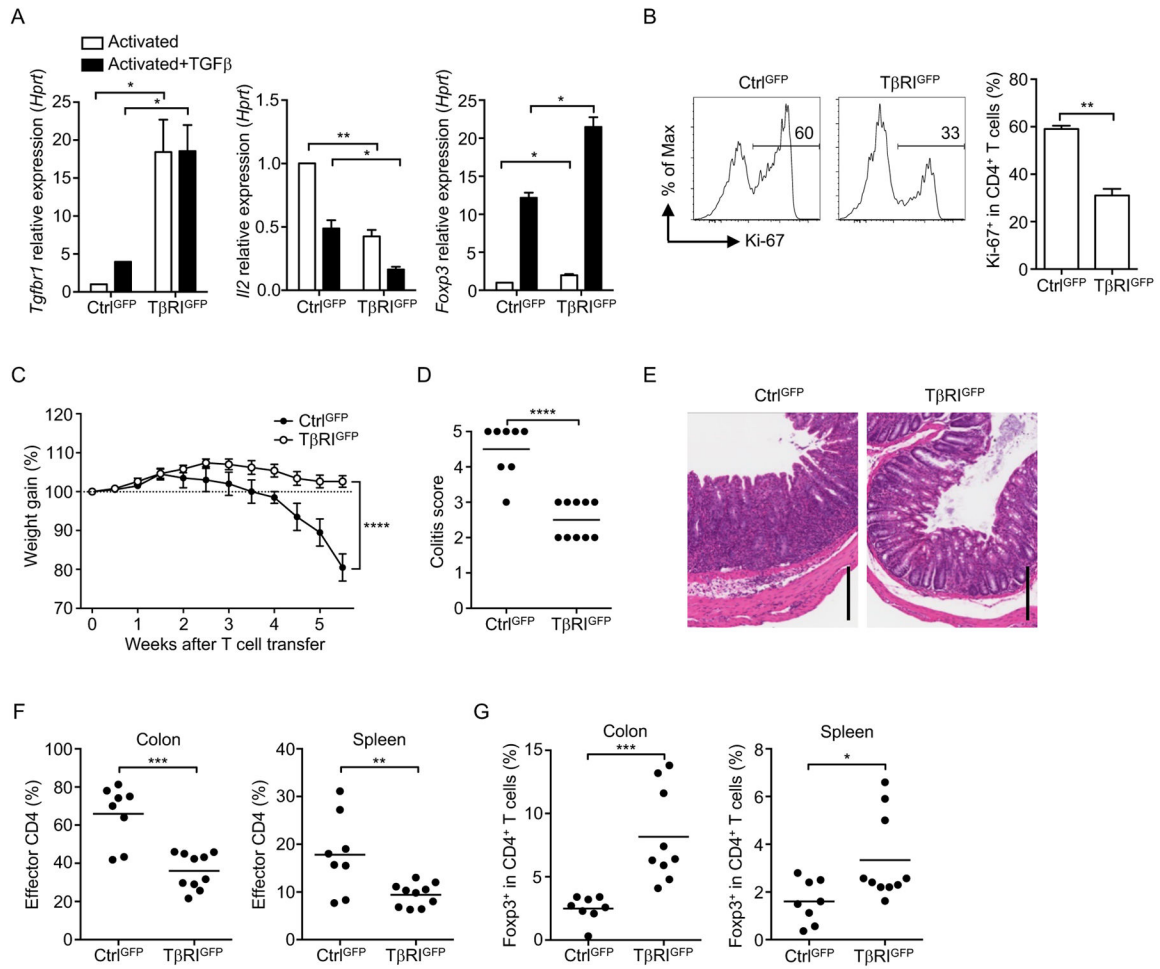


Figure 5. TβRI Overexpression in naïve T cells suppresses T cell proliferation and autoimmunity (A) *Tgfb1*, *Il2* and *Foxp3* mRNA in naïve TβRI^{GFP+} or Ctrl^{GFP+} CD4⁺ T cells stimulated for 24 hr with anti-CD3 and anti-CD28 in the presence or absence of TGFβ. (B) Frequency of Ki-67⁺ in naïve TβRI^{GFP+} or Ctrl^{GFP+} CD4⁺ T cells stimulated for 3 days with anti-CD3 and anti-CD28 plus TGFβ. (C–G) Naïve CD4⁺ T cells from TβRI^{GFP+} or Ctrl^{GFP+} retrogenic mice were transferred into *Rag1*^{-/-} mice. (C) Weight gain of recipient mice after T cell transfer. (D) Quantitation of colon pathology. (E) Representative histology images of colon sections. Scale bars, 200 μm. (F) Frequencies of the donor TβRI^{GFP+} or Ctrl^{GFP+} CD4⁺ T cells in recipient mice. (G) Frequencies of Foxp3⁺ cells in the donor CD4⁺ T cell population. Data are pooled from two (A, B) or three (C–G) independent experiments. In (D, F, G), each circle represents the data from one mouse. In (A, B, D, F, G), Student’s *t*-test was used. In (C), two-way ANOVA was used; bars (mean), error bars (SD in A, B; SEM in C), **P* < 0.05, ***P* < 0.01, ****P* < 0.001 and *****P* < 0.0001. See also Figure S5 and S6.

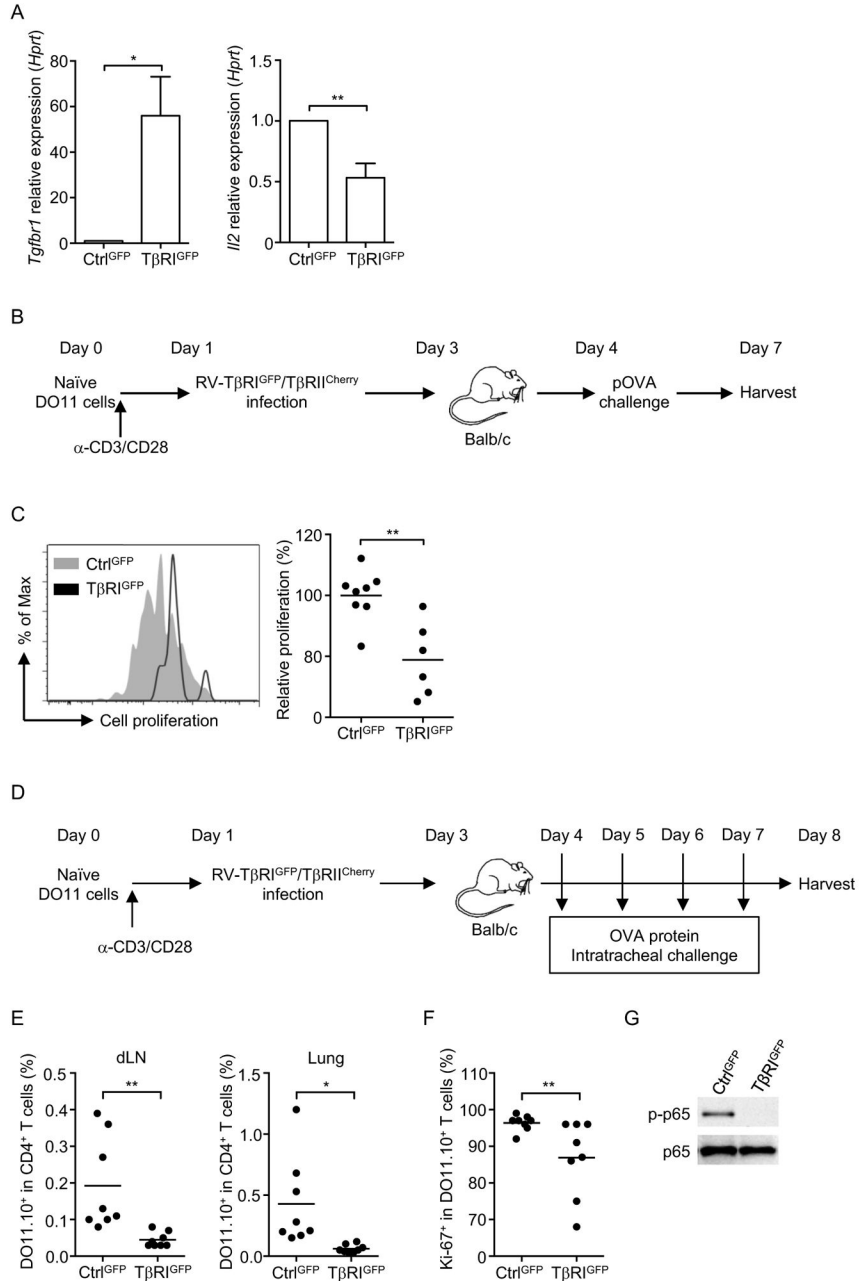


Figure 6. TβRI restoration in activated T cells reduces T cell proliferation
 (A) *Tgfb1*, and *Il2* mRNA in Ctr^{GFP}⁺ or TβRI^{GFP}⁺ activated T cells re-stimulated with anti-CD3 and anti-CD28 for 24 hr. (B) Experimental workflow of TβRI^{GFP}⁺- or TβRI^{Cherry}⁺-DO11.10 T cells challenged with pOVA. (C) Cell proliferation of Ctr^{GFP}⁺- or TβRI^{GFP}⁺-DO11.10 T cells challenged with pOVA. (D) Experimental workflow of TβRI^{GFP}⁺- or TβRI^{Cherry}⁺-DO11.10 T cells challenged with OVA protein in an airway inflammation model. (E) Frequencies of Ctr^{GFP}⁺- or TβRI^{GFP}⁺-DO11.10 T cells in the lungs and draining lymph nodes (dLN) of recipient mice. (F) Frequencies of Ki-67⁺ cells in Ctr^{GFP}⁺- or TβRI^{GFP}⁺-DO11.10 T cells in dLN of recipient. (G) Western blot of

phosphorylated p65 (p-p65) and p65 in Ctrl^{GFP+} or TβRI^{GFP+} activated T cells re-stimulated with anti-CD3 and anti-CD28 for 24 hr. Data are pooled from two (A, E, F) or three (C) independent experiments or are representative of two (G) independent experiments. In (C, E, F), each circle represents one mouse. Student's *t*-test was used; bars (mean), error bars (SEM), **P* < 0.05, ***P* < 0.01. See also Figure S7.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

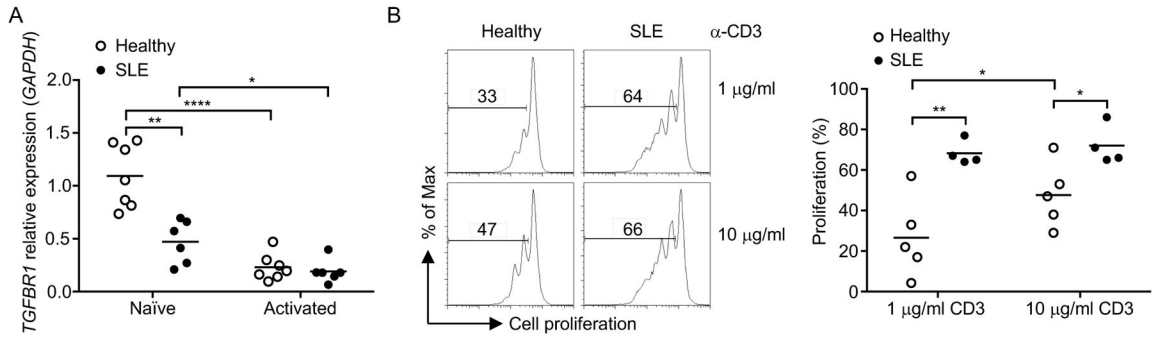


Figure 7. TβRI expression is reduced in naïve T cells of SLE patients

Naïve CD4⁺ T cells were isolated from healthy individuals and SLE patients. (A) *TGFBR1* mRNA in naïve or T cells stimulated for 24 hr with anti-CD3 and anti-CD28 (Activated). (B) Cell proliferation of naïve T cells from healthy individuals or SLE patients stimulated for 3 days with indicated concentration of anti-CD3 and APCs. Each circle represents one individual. Student's *t*-test was used; bars (mean), **P* < 0.05, ***P* < 0.01 and *****P* < 0.0001.