LETTERS

Reciprocal developmental pathways for the generation of pathogenic effector T_H17 and regulatory T cells

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On activation, T cells undergo distinct developmental pathways, attaining specialized properties and effector functions. T-helper (T_H) cells are traditionally thought to differentiate into T_H1 and T_H2 cell subsets. T_H1 cells are necessary to clear intracellular pathogens and T_H2 cells are important for clearing extracellular organisms[1,2]. Recently, a subset of interleukin (IL)-17-producing T (T_H17) cells distinct from T_H1 or T_H2 cells has been described and shown to have a crucial role in the induction of autoimmune tissue injury[3–5]. In contrast, $CD4^+CD25^+Foxp3^+$ regulatory T (T_{reg}) cells inhibit autoimmunity and protect against tissue injury[6]. Transforming growth factor-β (TGF-β) is a critical differentiation factor for the generation of T_{reg} cells[7]. Here we show, using mice with a reporter introduced into the endogenous *Foxp3* locus, that IL-6, an acute phase protein induced during inflammation[8,9], completely inhibits the generation of $Foxp3^+$ T_{reg} cells induced by TGF-β. We also demonstrate that IL-23 is not the differentiation factor for the generation of T_H17 cells. Instead, IL-6 and TGF-β together induce the differentiation of pathogenic T_H17 cells from naive T cells. Our data demonstrate a dichotomy in the generation of pathogenic (T_H17) T cells that induce autoimmunity and regulatory ($Foxp3^+$) T cells that inhibit autoimmune tissue injury.

$CD4^+CD25^+$ T_{reg} cells express the forkhead/winged helix transcription factor Foxp3 (refs 10–12). Using a gene-targeting approach, we generated a mouse in which we introduced a bicistronic enhanced green fluorescent protein (EGFP) reporter into the endogenous *Foxp3* locus (Foxp3–GFP 'knockin' mice), allowing us to track faithfully Foxp3-expressing regulatory cells *in vivo* and study the factors that influence Foxp3 expression and T_{reg} generation. Consistent with previous observations[7], activation of $CD4^+Foxp3^-$ T cells from the Foxp3–GFP knockin mice in the presence of TGF-β induced Foxp3 expression in 50% of T cells (Fig. 1a, b). Next, we assessed the ability of pro-inflammatory cytokines to modulate Foxp3 induction via TGF-β. Notably, TGF-β-mediated conversion of $CD4^+CD25^-$ cells into $Foxp3^+GFP^+$ T_{reg} cells was strongly inhibited by IL-6 (Fig. 1a, b). To understand the mechanism by which IL-6 mediated the inhibition of Foxp3 expression, we tested the cytokine production of T cells that were activated in the presence TGF-β plus IL-6, and found that these cells produced large amounts of IL-17 (Fig. 1c).

Recent studies have shown that IL-23 can differentiate T cells into T_H17 cells (refs 3–5). To address the role of IL-23 compared with TGF-β plus IL-6 in the differentiation of naive T cells into T_H17 cells, we used T cells from a myelin (myelin oligodendrocyte glycoprotein, MOG)-specific T-cell receptor (TCR) transgenic mouse (hereafter called 2D2)[13]. *In vitro* activation of unsorted splenic T cells from the transgenic mice in the presence MOG35–55 peptide and recombinant IL-23 resulted in the generation of 6.5% of IL-17-producing $CD4^+$ T cells (Fig. 2a). Because the IL-23 receptor is expressed only on activated/memory T cells[14,15], we wanted to investigate further whether IL-23 could guide differentiation of naive T cells into T_H17 cells. To address this question, naive 2D2 transgenic T cells ($CD4^+CD62L^{hi}$)

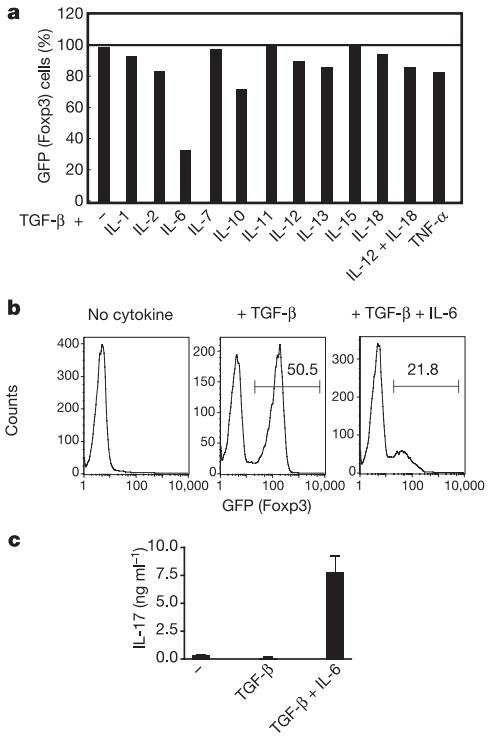

Figure 1 | Inhibition of T_{reg} development by different cytokines. a–c, FACS-sorted $CD4^+GFP^-$ ($Foxp3^-$) cells from Foxp3–GFP knockin mice were stimulated with anti-CD3 and anti-CD28 antibodies for 3 days (**a**, **b**) or anti-CD3 plus antigen-presenting cells for 48 h in the presence of different cytokines (**c**). **a**, Percentage of GFP-expressing cells induced by various cytokines in the presence of TGF-β (the percentage of GFP^+ cells induced in the presence of TGF-β was kept at 100%). **b**, GFP expression in $CD4^+$ T cells. **c**, Amount of IL-17 produced in triplicate wells ±s.d.

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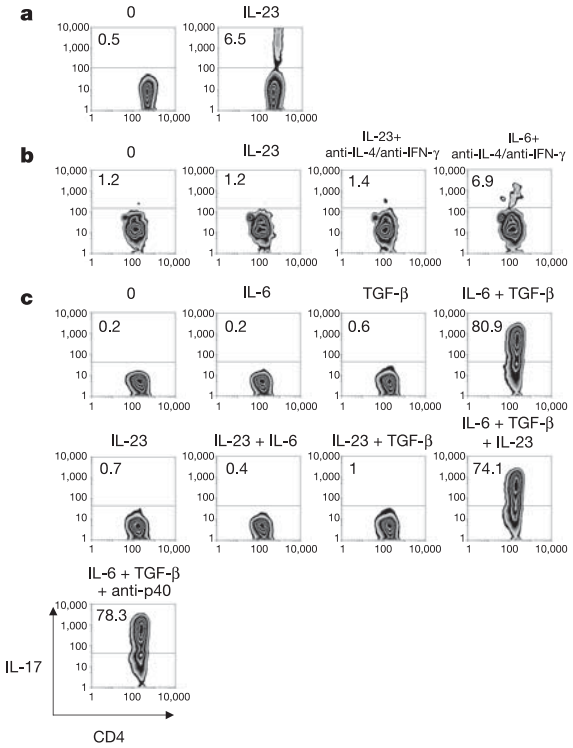

Figure 2 | Cytokines required for the generation of T_H17 cells. **a**, Splenocytes from 2D2 TCR transgenic mice were stimulated with MOG35–55 peptide in neutral conditions or with IL-23. **b**, **c**, FACS-sorted naive $CD4^+CD62L^{hi}$ T cells from 2D2 TCR transgenic mice were stimulated with soluble anti-CD3, C57BL/6 irradiated spleen cells and the indicated cytokines plus neutralizing antibodies. Five days after the activation, cells were re-stimulated with PMA/ionomycin and subjected to intracellular cytokine staining. The contour plots represent IL-17 expression in $CD4^+$ cells.

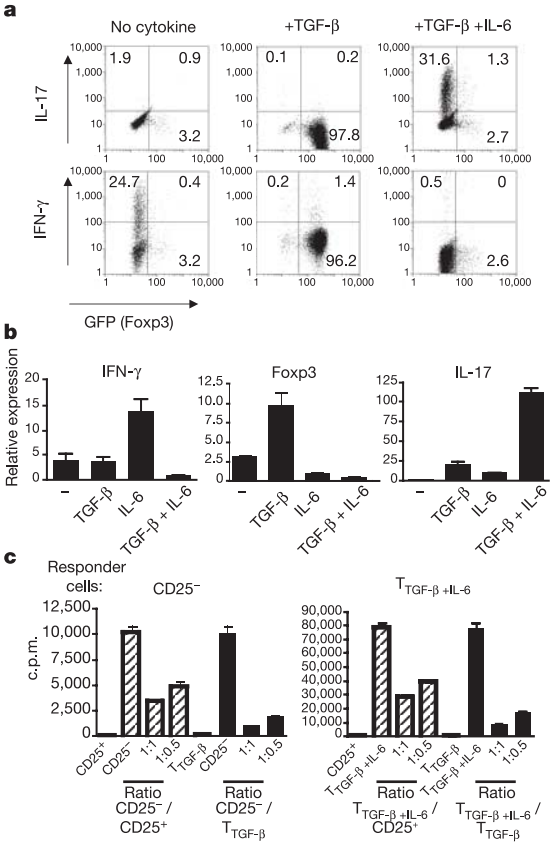

Figure 3 | Reciprocal expression of Foxp3 and IL-17 in T cells during differentiation. FACS-sorted naive $CD4^+CD62L^{hi}GFP^-$ T cells from 2D2 × Foxp3–GFP knockin mice were stimulated with soluble anti-CD3, antigen-presenting cells and the indicated cytokines. **a**, Foxp3 (GFP) and cytokine (IL-17 or IFN-γ) intracellular expression in $CD4^+$ T cells after 5 days. **b**, Expression of IFN-γ, Foxp3 and IL-17 mRNA relative to control actin (in triplicate wells ±s.d.). **c**, Proliferation in triplicate wells (±s.d.) of $CD4^+CD25^-$ cells and $T_{TGF-β+IL-6}$ cells (T cells differentiated in the presence of TGF-β and IL-6) in the presence of antigen-presenting cells, soluble anti-CD3 and different concentrations of $CD4^+CD25^+$ T cells or $T_{TGF-β}$ cells (T cells generated in the presence of TGF-β) measured by $[^3H]$thymidine incorporation.

were stimulated with anti-CD3 in the presence or absence of IL-23. Surprisingly, IL-23 alone, in the presence of neutralizing antibodies to IL-4 and interferon-γ (IFN-γ) (Fig. 2b), or together with other cytokines (IL-6 or TGF-β), did not induce differentiation of IL-17-producing T cells (Fig. 2c). These results suggest that IL-23 may expand the pool of IL-17-producing cells from *in vivo* activated/memory T-cell populations, but that IL-23 is incapable of differentiating IL-17-producing cells from naive T-cell precursors. Although neither TGF-β nor IL-6 alone induced the generation of IL-17-producing T cells from naive 2D2 TCR transgenic T cells, a combination of the two cytokines (IL-6 plus TGF-β), consistent with a recently published report[16], induced most naive T cells (80.9%) to produce IL-17 (Fig. 2c). TGF-β could serve to inhibit IL-4 and IFN-γ production and thus allow IL-6 to induce preferentially the generation of T_H17 cells. However, culture of naive T cells with IL-6 and a cocktail of neutralizing antibodies for IL-4 and IFN-γ modestly increased (by 6.7%) the number of IL-17-producing cells compared with cells cultured with IL-6 alone, suggesting that TGF-β does not just suppress the generation of T_H1 and T_H2 cells but actively participates in the generation of T_H17 cells (Fig. 2b). In addition, IL-17 production induced by IL-6 plus TGF-β was not inhibited by the addition of a neutralizing antibody specific for the p40 chain of IL-23 (Fig. 2c, see also Supplementary Fig. S1).

We have shown that whereas IL-6 suppresses TGF-β-induced Foxp3 expression and T_{reg} generation, the combination of IL-6 and TGF-β promotes the generation of T_H17 cells, suggesting that effector and regulatory T cells may differentiate from the same precursor T cell depending on the balance of cytokines present in the environment. To address this hypothesis, we differentiated naive $CD4^+CD62L^{hi}GFP^-$ T cells from 2D2 × Foxp3–GFP knockin mice under neutral conditions, or in the presence of TGF-β or TGF-β plus IL-6, and monitored the expression of IL-17 (marker of effector T cells) versus Foxp3–GFP (marker of T_{reg} cells). Activation of T cells with anti-CD3 in the absence of exogenous cytokines did not result in the induction of either IL-17 or Foxp3, but instead resulted in the induction of some IFN-γ-producing T cells (Fig. 3a, b). Addition of TGF-β in the culture resulted in the generation of $Foxp3^+$ T cells but not IL-17 or IFN-γ production; however, addition of TGF-β plus IL-6 to T cells during differentiation completely abrogated the expression of Foxp3 and resulted in concomitant expression of IL-17 from these T cells (Fig. 3a, b). No IFN-γ-producing cells were detected under these conditions. Thus, the induction of Foxp3 and IL-17 production in T cells seems to be mutually exclusive. To test the functionality of cells stimulated with TGF-β or TGF-β plus IL-6, we purified $CD4^+CD62L^{hi}GFP^-$ cells from Foxp3–GFP knockin mice and activated them in the presence of either TGF-β or TGF-β plus IL-6. Most TCR-activated $CD4^+$ T cells cultured in the presence of TGF-β became GFP positive, and the cells cultured in the presence of TGF-β plus IL-6 remained GFP negative. T cells generated in the presence of TGF-β and expressing Foxp3 (GFP^+) were anergic and inhibited the proliferative response of $CD4^+CD25^-$ T cells similar to naturally occurring $CD4^+CD25^+$ T_{reg} cells (Fig. 3c), demonstrating that TGF-β does induce *bona fide* T_{reg} cells. In

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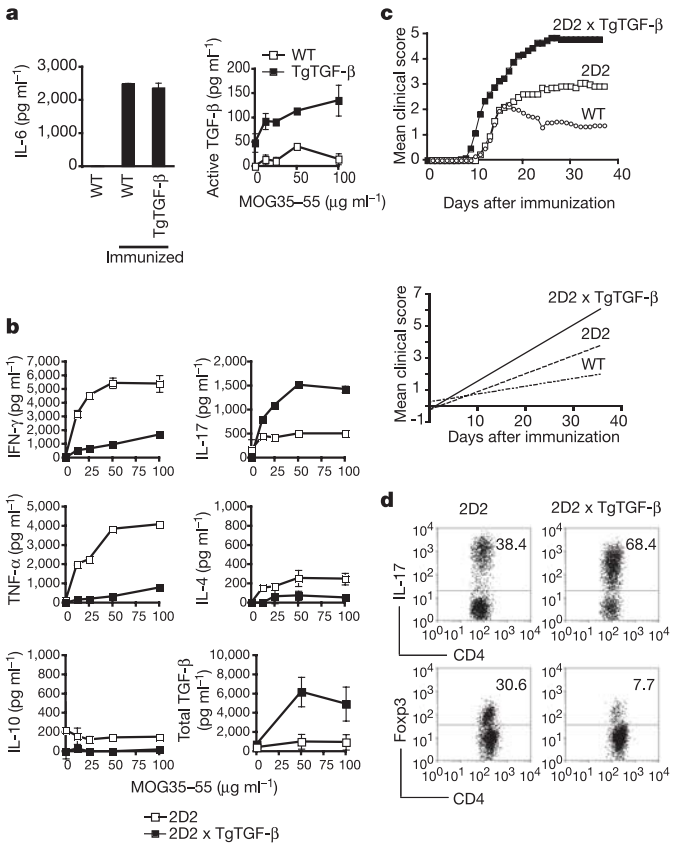

Figure 4 | Enhanced IL-17 production by T cells and CNS autoimmunity in TGF-β transgenic mice. a, **b**, Splenocytes from naive C57BL/6 wild-type (WT) mice (**a**, left panel), wild-type and TgTGF-β mice immunized with MOG35–55 peptide (**a**, right panel), and 2D2 and 2D2 × TgTGF-β mice immunized with MOG35–55 peptide (**b**), were stimulated with lipopolysaccharide (for IL-6) or MOG35–55 peptide (for other cytokines). Data represent mean cytokine produced in each group ($n = 3$) ± s.d. **c**, Wild-type C57BL/6 ($n = 13$), 2D2 ($n = 11$) and 2D2 × TgTGF-β ($n = 13$) mice were immunized with MOG35–55 in CFA and monitored for clinical signs of EAE (top panel). Linear regression analysis of the individual disease curves reveals a highly significant difference ($P < 0.0001$) between each of the EAE groups (bottom panel). **d**, Frequency of IL-17- and Foxp3-expressing $CD4^+$ T cells isolated from the CNS of 2D2 × TgTGF-β or 2D2 mice at the peak of disease.

contrast, T cells differentiated in the presence of TGF-β and IL-6 (and that produce IL-17) were neither anergic nor immunosuppressive because they showed greater proliferation compared with the $CD4^+CD25^-$ T cells. Proliferation of T cells differentiated *in vitro* in the presence of TGF-β and IL-6 could be suppressed both by naturally occurring $CD4^+CD25^+$ T_{reg} cells and by *in vitro* TGF-β-converted $Foxp3^+$ T cells (Fig. 3c).

TGF-β has been shown to be a very important immunosuppressive cytokine that not only directly suppresses effector T-cell function but also induces $CD4^+CD25^+$ T_{reg} cells and T_H3 cells[17,18]. To test the effect of TGF-β on T cells *in vivo*, we have generated a TGF-β transgenic mouse (TgTGF-β) in which TGF-β1 is placed under the IL-2 promoter. *In vitro* activation of 2D2 × TgTGF-β T cells with MOG35–55 peptide results in the production of large amounts of TGF-β, and these cells suppress experimental autoimmune encephalomyelitis (EAE) on adoptive transfer, showing that activation of TGF-β alone in these cells results in the generation of regulatory T cells that suppress EAE (Y.C. and H.L.W., manuscript submitted). To test the combined effect of TGF-β and IL-6 *in vivo*, we immunized TgTGF-β mice with MOG35–55 peptide emulsified in complete Freund's adjuvant (CFA). Spleen cells from immunized wild-type or TgTGF-β mice produced large amounts of IL-6 compared with spleen cells from non-immunized mice (Fig. 4a). T cells from immunized 2D2 TCR transgenic mice produced large amounts of IFN-γ, tumour-necrosis factor-α (TNF-α) and very low levels of IL-4, IL-10 and IL-17 (Fig. 4b). In contrast, T cells from MOG/CFA-immunized 2D2 × TgTGF-β mice produced TGF-β, low levels of IFN-γ and TNF-α, and very high levels of IL-17 (Fig. 4a, b). We then immunized 2D2 and 2D2 × TgTGF-β mice with MOG/CFA for the development of EAE. 2D2 TCR transgenic mice developed more severe disease compared with wild-type mice (Fig. 4c). Notably, the 2D2 × TgTGF-β mice developed the most severe and fulminant disease (Fig. 4c, Table 1 and Supplementary Table S1). Furthermore, analysis of the cytokine profile of the infiltrating $CD4^+$ T cells in the central nervous system (CNS) of 2D2 versus 2D2 × TgTGF-β mice showed that the double-transgenic mice had twice as many infiltrating IL-17-producing $CD4^+$ T cells in the CNS than 2D2 transgenic mice. Consistent with the idea of a reciprocal relationship between T_{reg} cells and T_H17 cells, the 2D2 × TgTGF-β mice had a reduced frequency (75% less) of $Foxp3^+$ T_{reg} cells infiltrating in the CNS compared with the 2D2 transgenic mice (Fig. 4d). These *in vivo* data further show that activation of T cells in the presence of TGF-β and IL-6 results in the predominant generation of IL-17-producing T cells and fewer $Foxp3^+$ T_{reg} cells and that, under these conditions, mice develop exacerbated autoimmune disease.

Our data suggest that there is not only a functional antagonism between T_H17 and T_{reg} cells but that there is a dichotomy in their generation as well. Therefore, T_{reg} cells and T_H17 effectors arise in a mutually exclusive fashion, depending on whether they are activated in the presence of TGF-β or TGF-β plus IL-6. At the steady-state level or in the absence of any inflammatory insult, TGF-β produced in the immune system will suppress the generation of effector T cells and induce $Foxp3^+$ regulatory T cells, and thereby maintain self-tolerance. However, on infection or inflammation, IL-6 produced by the activated innate immune system will suppress the generation of TGF-β-induced T_{reg} cells and induce a pro-inflammatory T-cell response predominated by T_H17 cells. This is consistent with the *in vivo* observation that destructive arthritis in IFN-γ-deficient mice can be treated with anti-IL-17, and neutralization of IL-17 results in the generation of $CD4^+CD25^+$ regulatory T cells[19], further supporting the concept that there is a functional antagonism between T_H17 effectors and $CD4^+CD25^+$ T_{reg} cells. Furthermore, IL-6-deficient mice have been shown to be highly resistant to the development

Table 1 | EAE in 2D2 and 2D2 × TgTGF-β mice

Group	Incidence	Mortality (%)	Mean day of onset (mean ± s.d.)	Mean maximum score (mean ± s.d.)
Wild type	12 of 13 (92.3%)	0	12.2 ± 1.99*	2.83 ± 0.33‡
2D2	8 of 11 (72.7%)	25.0	11.8 ± 1.91†	4.17 ± 0.68§
2D2 × TgTGF-β	13 of 13 (100%)	90.9	10.1 ± 1.50*†	4.91 ± 0.30‡§

Mice were immunized with MOG35–55 peptide emulsified in complete Freund's adjuvant and monitored for the development of EAE. Statistical analysis was performed by comparing three groups using one-way analysis of variance (ANOVA) followed by a Newman–Keuls multiple comparison as a post-hoc test.
*$P < 0.05$.
†$P < 0.05$.
‡$P < 0.001$.
§$P < 0.01$.

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of EAE[20–23]. We have confirmed this observation and further determined that IL-6-deficient mice immunized with the encephalitogenic MOG35–55 peptide have a deficit in IL-17-producing T cells infiltrating the CNS (Supplementary Fig. S2).

Our results support the concept that there is a reciprocal developmental pathway for the generation of pathogenic T_H17 cells and protective T_{reg} cells in the immune system depending on the state of the innate immune system and production of acute phase proteins like IL-6. Once induced, $CD4^+CD25^+Foxp3^+$ cells could also suppress other T-cell subsets (like T_H1 and T_H2 cells), as well as T_H17 cells. T_H17 and T_{reg} subsets may therefore have evolved to induce or regulate tissue inflammation, analogous to the dichotomy of T_H1 and T_H2 T-cell subsets, which primarily mediate immunity against infectious organisms.

METHODS

Mice. Foxp3–GFP knockin, 2D2 MOG TCR and TGF-β transgenic mice were all generated on the C57BL/6 background as described in Supplementary Methods.

***In vitro* T-cell differentiation.** Spleen cells from 2D2 mice were stimulated with 50 μg ml^{-1} MOG35–55 peptide in the presence or absence of IL-23 (20 ng ml^{-1}). $CD4^+$ T cells were purified using anti-CD4 beads (Myltenyi) or further sorted into naive $CD4^+CD62L^{hi}$ cells. $CD4^+$ T cells were stimulated with C57BL/6 irradiated spleen cells and 1 μg ml^{-1} of anti-CD3 (145-2C11) for 3–5 days in the presence of cytokines (human TGF-β1 (3 ng ml^{-1}), mouse IL-6 (20 ng ml^{-1}), IL-23 (20 ng ml^{-1}) (all R&D Systems)) and/or neutralizing antibodies (anti-IL-4 (10 μg ml^{-1}, 11B11), anti-IFN-γ (10 μg ml^{-1}, XMG1.2), anti- IL-12/IL-23 p40 (C17.8)). Cells were supplemented with recombinant IL-2 (50 U ml^{-1}) at day 2 and 4.

Suppression assay. Proliferation was determined by [^{3}H]thymidine incorporation as described in Supplementary Methods.

Cytokine production and intracellular cytokine staining. Cytokine production was determined by enzyme-linked immunosorbent assay as described in Supplementary Methods. For intracellular cytokine staining, T cells were re-stimulated, 5 days after their initial stimulation, with PMA/ionomycin (see Supplementary Methods) and stained according to the manufacturer's directions (BD).

Real-time PCR. The expression of IL-17, IFN-γ and Foxp3 was performed using specific primers and probes (Applied Biosystems). Expression was normalized to the expression of the housekeeping gene actin.

Induction and assessment of EAE. 2D2, 2D2 × TgTGF-β or C57BL/6 mice were injected subcutaneously with 100 μg of MOG35–55 peptide (MEVGWYRSP FSRVVHLYRNGK) emulsified in CFA (Difco) supplemented with 400 μg ml^{-1} *Mycobacterium tuberculosis* and injected twice intravenously with 150 ng of pertussis toxin (List Biological Laboratories). Clinical assessment of EAE was performed daily after disease induction according to the following criteria: 0, no disease; 1, decreased tail tone; 2, hindlimb weakness or partial paralysis; 3, complete hindlimb paralysis; 4, forelimb and hindlimb paralysis; 5, moribund state.

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1. Mosmann, T. R. & Coffman, R. L. T_H1 and T_H2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu. Rev. Immunol.* **7,** 145–173 (1989).
2. Bottomly, K. A functional dichotomy in $CD4^+$ T lymphocytes. *Immunol. Today* **9,** 268–274 (1988).
3. Langrish, C. L. *et al.* IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J. Exp. Med.* **201,** 233–240 (2005).
4. Harrington, L. E. *et al.* Interleukin 17-producing $CD4^+$ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nature Immunol.* **6,** 1123–1132 (2005).
5. Park, H. *et al.* A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nature Immunol.* **6,** 1133–1141 (2005).
6. Sakaguchi, S. Naturally arising $CD4^+$ regulatory T cells for immunologic self-tolerance and negative control of immune responses. *Annu. Rev. Immunol.* **22,** 531–562 (2004).
7. Chen, W. *et al.* Conversion of peripheral $CD4^+CD25^-$ naive T cells to $CD4^+CD25^+$ regulatory T cells by TGF-β induction of transcription factor Foxp3. *J. Exp. Med.* **198,** 1875–1886 (2003).
8. Hirano, T. Interleukin 6 and its receptor: ten years later. *Int. Rev. Immunol.* **16,** 249–284 (1998).
9. Ozato, K., Tsujimura, H. & Tamura, T. Toll-like receptor signaling and regulation of cytokine gene expression in the immune system. *Biotechniques* **33** (Suppl.), S66–S68 (2002).
10. Hori, S., Nomura, T. & Sakaguchi, S. Control of regulatory T cell development by the transcription factor Foxp3. *Science* **299,** 1057–1061 (2003).
11. Fontenot, J. D., Gavin, M. A. & Rudensky, A. Y. Foxp3 programs the development and function of $CD4^+CD25^+$ regulatory T cells. *Nature Immunol.* **4,** 330–336 (2003).
12. Khattri, R., Cox, T., Yasayko, S. A. & Ramsdell, F. An essential role for Scurfin in $CD4^+CD25^+$ T regulatory cells. *Nature Immunol.* **4,** 337–342 (2003).
13. Bettelli, E. *et al.* Myelin oligodendrocyte glycoprotein-specific T cell receptor transgenic mice develop spontaneous autoimmune optic neuritis. *J. Exp. Med.* **197,** 1073–1081 (2003).
14. Aggarwal, S., Ghilardi, N., Xie, M. H., de Sauvage, F. J. & Gurney, A. L. Interleukin-23 promotes a distinct CD4 T cell activation state characterized by the production of interleukin-17. *J. Biol. Chem.* **278,** 1910–1914 (2003).
15. Parham, C. *et al.* A receptor for the heterodimeric cytokine IL-23 is composed of IL-12Rβ1 and a novel cytokine receptor subunit, IL-23R. *J. Immunol.* **168,** 5699–5708 (2002).
16. Veldhoen, M., Hocking, R. J., Atkins, C. J., Locksley, R. M. & Stockinger, B. TGFβ in the context of an inflammatory cytokine milieu supports *de novo* differentiation of IL-17-producing T cells. *Immunity* **24,** 179–189 (2006).
17. Gorelik, L. & Flavell, R. A. Transforming growth factor-β in T-cell biology. *Nature Rev. Immunol.* **2,** 46–53 (2002).
18. Shull, M. M. *et al.* Targeted disruption of the mouse transforming growth factor-β1 gene results in multifocal inflammatory disease. *Nature* **359,** 693–699 (1992).
19. Nardelli, D. T. *et al.* Association of $CD4^+$ $CD25^+$ T cells with prevention of severe destructive arthritis in *Borrelia burgdorferi*-vaccinated and challenged gamma interferon-deficient mice treated with anti-interleukin-17 antibody. *Clin. Diagn. Lab. Immunol.* **11,** 1075–1084 (2004).
20. Samoilova, E. B., Horton, J. L., Hilliard, B., Liu, T. S. & Chen, Y. IL-6-deficient mice are resistant to experimental autoimmune encephalomyelitis: roles of IL-6 in the activation and differentiation of autoreactive T cells. *J. Immunol.* **161,** 6480–6486 (1998).
21. Okuda, Y. *et al.* IL-6 plays a crucial role in the induction phase of myelin oligodendrocyte glucoprotein 35–55 induced experimental autoimmune encephalomyelitis. *J. Neuroimmunol.* **101,** 188–196 (1999).
22. Okuda, Y., Sakoda, S., Saeki, Y., Kishimoto, T. & Yanagihara, T. Enhancement of Th2 response in IL-6-deficient mice immunized with myelin oligodendrocyte glycoprotein. *J. Neuroimmunol.* **105,** 120–123 (2000).
23. Eugster, H. P. *et al.* Superantigen overcomes resistance of IL-6-deficient mice towards MOG-induced EAE by a TNFR1 controlled pathway. *Eur. J. Immunol.* **31,** 2302–2312 (2001).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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