nature medicine

Crucial role of interleukin-7 in T helper type 17 survival and expansion in autoimmune disease

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Interleukin-7 receptor (IL-7R) is genetically associated with susceptibility to multiple sclerosis. Here we describe that IL-7 is essential for survival and expansion of pathogenic T helper type 17 (T_H17) cells in experimental autoimmune encephalomyelitis (EAE). IL-7 directly expanded effector T_H17 cells in EAE and human T_H17 cells from subjects with multiple sclerosis, whereas it was not required for T_H17 differentiation. IL-7R antagonism rendered differentiated T_H17 cells susceptible to apoptosis through the inhibition of Janus kinase–signal transducer and activator of transcription-5 (JAK-STAT5) pathway and altered expression of the prosurvival protein Bcl-2 and the proapoptotic protein Bax, leading to decreased severity of EAE. In contrast, T_H1 and regulatory T (T_{reg}) cells were less susceptible to or not affected by IL-7R antagonism *in vivo*. The selectivity was attributable to minimal expression of IL-7Rα in T_{reg} cells and correlated with a high level of *Socs1* (encoding suppressor of cytokine signaling-1) expression in T_H1 cells. The study reveals a unique, previously undescribed role of IL-7–IL-7R in T_H17 cell survival and expansion and has implications in the treatment of autoimmune disease.

Multiple sclerosis, a chronic inflammatory and demyelinating disease of the central nervous system (CNS)[1,2], is generally considered as an autoimmune pathology in which T_H1 and T_H17 cells have a key role[2–4]. In particular, T_H17 cells[5–9] were recently identified as a distinct lineage of $CD4^+$ effector T cells and are associated with various models of autoimmune disorders, such as experimental autoimmune encephalomyelitis (EAE)[10,11]. It is crucial to understand *in vivo* differentiation and maintenance of T_H17 cells and the underlying mechanisms in the context of an autoimmune process[12,13]. There is evidence suggesting that the development of T_H17 cells is driven by the proinflammatory cytokine milieu characteristic of the autoimmune process. Several cytokines, such as transforming growth factor-β (TGF-β), IL-6, IL-1β and IL-21, have been shown to regulate and induce T_H17 cell differentiation, contributing crucially to the clinical outcome of autoimmune disease[14–18]. In this regard, IL-23 has been implicated to have a major role in the terminal differentiation of T_H17 cells potentially through its effect on reexpression of IL-7R on T_H17 cells[19–21]. However, the mechanism underlying the *in vivo* maintenance of differentiated T_H17 cells remains poorly understood.

Recently, a single nucleotide polymorphism in the *Il7r* gene was determined to be associated with susceptibility to multiple sclerosis[22–25]. It was the first time a gene aside from those encoding the human leukocyte antigens was linked to multiple sclerosis susceptibility. Furthermore, in subjects with multiple sclerosis, the expression levels of *Il7r* and *Il7* messenger RNA in cerebrospinal fluid are higher when compared to those in controls[23]. IL-7 belongs to the IL-2 cytokine family and exerts signaling activities through its receptor, comprised of a specific α chain and a common γ chain shared by receptors for other cytokines[26,27]. IL-7–IL-7R signaling is essential for central T cell development and homeostasis of the peripheral T cell pool[28–33]. However, the mechanism potentially linking the role of IL-7–IL-7R to multiple sclerosis is currently unknown.

We undertook this study to investigate the potential role of IL-7–IL-7R signaling in the development of pathogenic T_H1 and T_H17 cells in EAE and their counterparts in subjects with multiple sclerosis. In particular, we examined the role of IL-7 and IL-7R antagonism as compared to that of IL-6 and IL-23. The results described here show that IL-7 is a key cytokine required for pathogenic T_H17 cells to survive and undergo *in vivo* expansion through the JAK-STAT5 pathway, whereas T_H17 differentiation is largely controlled by IL-6 through JAK-STAT3. This study provides compelling evidence for a previously undescribed role of IL-7–IL-7R in pathogenic T_H17 cell development and function in EAE and lends strong rationale for IL-7R antagonism as a potential treatment for multiple sclerosis and perhaps other autoimmune conditions.

RESULTS

Amelioration of EAE by IL-7R or IL-7 antagonism

We first examined the role of IL-7–IL-7R signaling in the development and function of T_H1 and T_H17 cells in EAE, a mouse model induced by myelin oligodendrocyte glycoprotein peptide ($MOG_{35–55}$) with adjuvant to mimic CNS pathology in multiple sclerosis. We initiated IL-7Rα–specific antibody treatment at the onset of EAE when committed T_H1 and T_H17 cells underwent *in vivo* expansion.

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Received 29 May 2009; accepted 18 November 2009; published online 10 January 2010; doi:10.1038/nm.2077

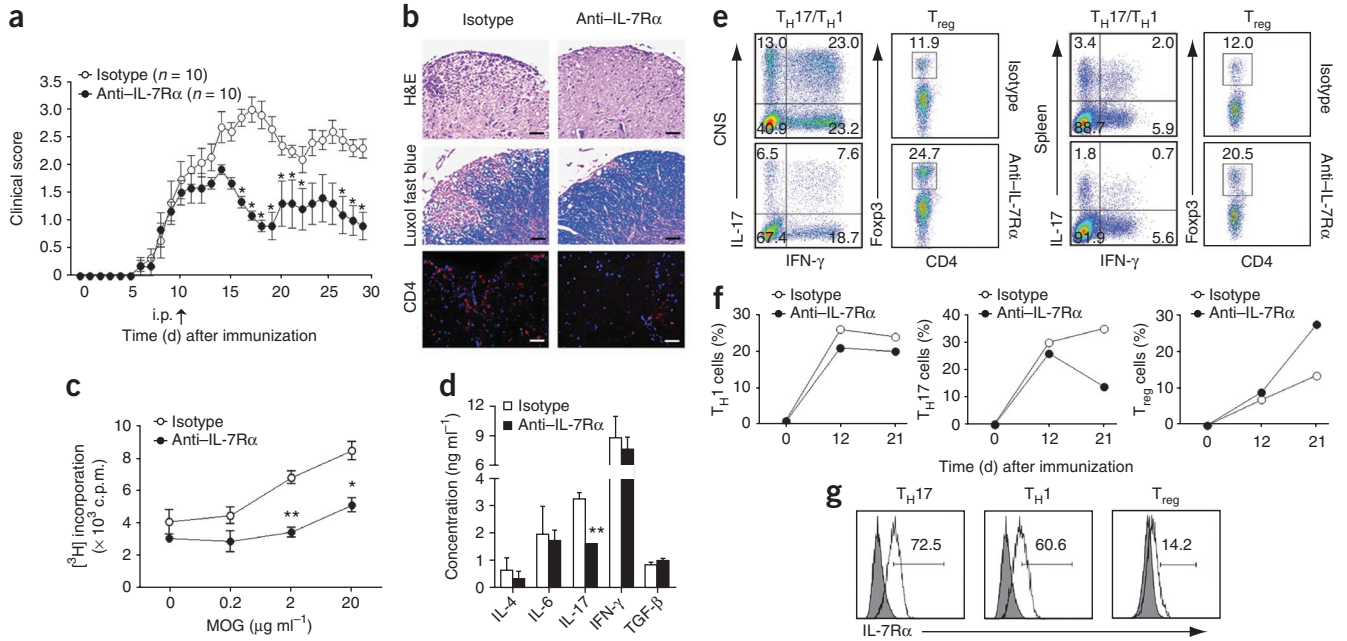

Figure 1 Amelioration of EAE by IL-7R antagonism through selective reduction of T_H17 cells. (**a**) Clinical scores (mean ± s.e.m.) of EAE mice treated with IL-7Rα–specific antibody (anti–IL-7Rα) or isotype control given every other day from day 10 after immunization, as indicated by the arrow. (**b**) Spinal cord sections obtained from treated or control EAE mice at day 21 after immunization were analyzed for the degree of demyelination by Luxol fast blue and for inflammation by H&E and for infiltrating CD4 T cells (red). Scale bars, 50 μm. Data are representative of two independent experiments. (**c**) Proliferation of splenocytes from treated or control EAE mice as in **a**, in response to $MOG_{35–55}$ at the indicated concentrations, as measured by [^{3}H]thymidine incorporation (mean ± s.e.m. of triplicates). (**d**) Concentration of cytokines (mean ± s.d. of triplicates) in the supernatants from splenocyte culture challenged with MOG peptide. (**e**) The percentage of T_H17, T_H1 and T_{reg} cells, as analyzed in CD4$^+$ T cells derived from the CNS (left) or spleen (right) after MOG re-stimulation followed by intracellular staining. (**f**) Dynamic distribution of T_H1, T_H17 and T_{reg} cells in affected CNS of treated or control mice in the course of EAE. (**g**) IL-7Rα surface expression, as examined in gated T_H1, T_H17 and T_{reg} cells derived from EAE mice at day 21 after immunization. IL-7Rα (open), isotype control (shaded). Data are representative of three independent experiments. $^*P < 0.05$ and $^{**}P < 0.01$.

The treatment resulted in a marked reduction of disease severity accompanied by decreased CNS inflammation and demyelination (**Fig. 1a**,**b**). We obtained similar results with IL-7–neutralizing antibody in EAE mice using the same treatment protocol (**Supplementary Fig. 1**). Furthermore, MOG-stimulated T cell reactivity was significantly lower in splenocytes derived from treated EAE mice (**Fig. 1c**). Notably, the treatment effect correlated with a selective reduction in the production of IL-17 in MOG-stimulated T cells (**Fig. 1d**) and in the percentage of effector or infiltrating T_H17 cells in control versus treated EAE mice (spinal cord: 15.0% ± 3.87% versus 7.41% ± 1.94%, $P < 0.01$; spleen: 4.22% ± 1.03% versus 1.87% ± 0.22%, $P < 0.05$) (**Fig. 1e**). The effect on T_H1 cells in treated EAE mice was not significant (spinal cord: 24.3% ± 5.17% versus 20.8% ± 3.22%, $P > 0.05$; spleen: 6.43% ± 2.04% versus 6.88% ± 1.43%, $P > 0.05$). The absolute numbers of CNS-infiltrating T_H17 cells were nearly eightfold lower in treated mice compared to those of control mice ($1.38 \times 10^4 \pm 0.86 \times 10^4$ cells versus $10.2 \times 10^4 \pm 2.83 \times 10^4$ cells, $P < 0.01$). In contrast, the percentage of T_{reg} cells increased in both the CNS and the periphery over the course of EAE in mice treated with IL-7Rα–specific antibody (**Fig. 1e**,**f**).

It should be noted that IL-7Rα–specific antibody treatment remained efficacious in EAE mice predepleted for T_{reg} cells with a CD25-specific antibody[34,35] (data not shown). In parallel, T_H17 cells expressed high amounts of IL-7Rα (71.1% ± 3.97%), whereas the expression level in T_{reg} cells was much lower (17.98% ± 4.07%) (**Fig. 1g**). Although CD4$^+$ T cell infiltration in the spinal cord was markedly reduced, the absolute number and overall composition of peripheral CD4$^+$ and CD8$^+$ T cells and B220$^+$ B cells were not substantially altered (**Supplementary Fig. 2** and **Supplementary Table 1**). In addition, IL-7R antagonism had no effect on *in vivo* T_H1 and T_H2 responses induced by immunization with MOG peptide in T_H1- or T_H2-prone adjuvant (**Supplementary Fig. 3**). These results indicate that pathogenic T_H17 cells are susceptible to IL-7R antagonism.

Distinct roles of IL-7 in T_H17 differentiation and expansion

Proinflammatory cytokines such as IL-6, IL-1β and IL-21, are essential for T_H17 differentiation and the initiation of autoimmune inflammation in EAE[7,14,16,36]. We first investigated whether IL-7–IL-7R signaling was associated with T_H17 differentiation in purified naive CD4$^+$ T cells. We examined the effect of IL-7 by stimulating these cells in the presence or absence of TGF-β. IL-7 did not markedly promote T_H17 differentiation in the presence of TGF-β when compared to IL-6 (**Fig. 2a–c** and **Supplementary Fig. 4a**,**b**). Furthermore, when administered before onset of EAE, IL-7Rα–specific antibody treatment did not affect disease severity, even though it slightly delayed the onset as compared to that in mice treated with control IgG (**Fig. 2d**). The data collectively suggest that IL-7–IL-7R signaling is not required for T_H17 differentiation.

We further addressed whether IL-7–IL-7R signaling is required for maintenance or expansion of committed T_H17 cells. To this end, we exposed splenocytes containing differentiated T_H17 and T_H1 cells derived from EAE mice to exogenous IL-7. Compared to control medium, addition of IL-7 to the medium significantly expanded T_H17 cells (1.72% ± 0.64% versus 10.0% ± 1.38%, $P < 0.01$) and, to a lesser extent, T_H1 cells (3.00% ± 1.75% versus 8.12% ± 3.18%, $P < 0.05$), but not T_{reg} cells (**Fig. 3a**). Consistent with this, in an

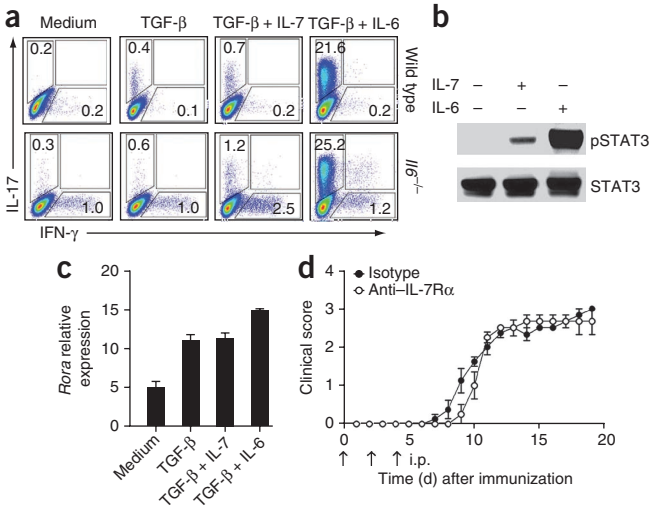

Figure 2 The role of IL-7 in T_H17 differentiation. (**a**) FACS-sorted naïve T cells of the $CD4^+CD25^-CD62L^{hi}CD44^{lo}$ phenotype were obtained from wild-type or *Il6*$^{-/-}$ mice and activated in the presence of CD3- and CD28-specific antibodies plus the indicated cytokines. The percentage of T_H17 or T_H1 cells was measured by intracellular staining. Data are representative of three independent experiments. (**b**) Expression of total and phosphorylated STAT3 (pSTAT3), as determined by immunoblotting in purified naive $CD4^+$ T cells treated *in vitro* with IL-7 or IL-6. (**c**) Quantitative RT-PCR analysis of *Rora* mRNA expression (mean ± s.d.) in T_H17 cell differentiation culture in the presence of TGF-β or in combination with IL-7 or IL-6. (**d**) Clinical score (mean ± s.e.m.) of MOG-immunized mice ($n = 5$) treated with IL-7Rα–specific antibody or isotype control given on days 0, 2 and 4 as marked by the arrows. Data are representative of three separate experiments.

in vivo system, administration of IL-7 at the onset of EAE exacerbated disease severity compared to that in control EAE mice (**Fig. 3b**). Disease exacerbation by IL-7 correlated significantly with expansion of effector T_H17 cells in the lymph nodes (4.55% ± 0.29% versus 2.66% ± 0.32%, $P < 0.01$) and increased infiltration of T_H17 cells (12.3% ± 3.6% versus 6.5% ± 1.7%, $P < 0.01$) but not T_H1 cells (10.1% ± 2.6% versus 7.8 ± 1.4%, $P > 0.05$) into the spinal cord compared to control mice (**Fig. 3b**). The effect was independent of IL-6, as IL-7 could expand T_H17 cells in *Il6*$^{-/-}$ mice challenged with MOG peptide (**Supplementary Fig. 4c,d**). There was markedly less proliferation of donor T_H17 cells but not T_H1 cells in EAE mice that received IL-7Rα–specific antibody treatment, as evidenced by Ki-67 staining (**Fig. 3c**). We ruled out the potential involvement of IL-15 (refs. 37,38) (data not shown) or thymic stromal lymphopoietin, a related cytokine that binds a receptor consisting of IL-7Rα and thymic stromal lymphopoietin receptor[39,40], as they did not promote T_H17 cell expansion (**Supplementary Fig. 5a–c**).

We further investigated the properties of IL-7 in T_H17 cell expansion in $CD4^+$ T cells derived from MOG-specific 2D2 T cell receptor–transgenic mice[41] and compared with the expansion induced by IL-23 and IL-6. IL-7 markedly induced *in vitro* expansion (31.2% ± 2.90% versus 3.46% ± 0.34%, $P < 0.01$) and proliferation of T_H17 cells,

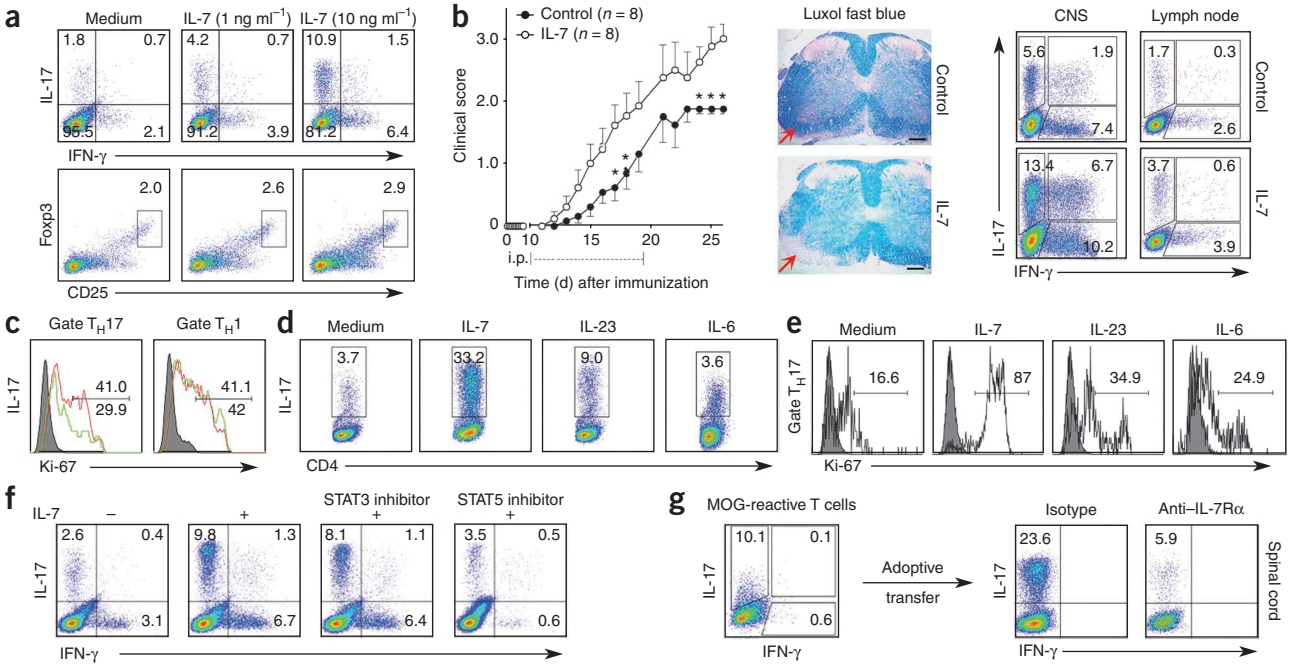

Figure 3 Requirement for IL-7 in T_H17 cell expansion through JAK-STAT5. (**a**) Flow cytometric analysis of T_H17, T_H1 and T_{reg} subsets in gated $CD4^+$ T cells from 3-d splenocyte culture in the presence of IL-7. (**b**) Clinical score (left), spinal cord histopathology (middle, scale bars, 100 μm) and percentage of T_H17 and T_H1 cells (right) in EAE mice treated with IL-7. Clinical score data are expressed as mean ± s.e.m. $^*P < 0.05$. (**c**) Levels of Ki-67 (open histogram) in gated T_H17 and T_H1 cells from splenocytes obtained from EAE mice treated with IL-7Rα–specific antibody (green) or isotype control (red). Shaded histograms represent Ki-67 isotype staining. (**d**,**e**) Purified $CD4^+$ T cells from MOG-immunized 2D2 mice were cultured with the indicated cytokines for 72 h and analyzed for the percentage of T_H17 cells (**d**) and of Ki-67$^+$ cells in the gated T_H17 cells (**e**). (**f**) Percentage of T_H17 and T_H1 cells in splenocytes from EAE mice pre-incubated with the indicated specific inhibitors before IL-7 treatment for 72 h. (**g**) MOG-stimulated T cell blasts from *Ifng*$^{-/-}$ EAE mice were transferred into irradiated *Egfp*-transgenic mice (2×10^7 cells per mouse), which were treated with IL-7Rα–specific antibody or isotype control from the day of transfer. Shown is the percentage of T_H17 and T_H1 cells in CNS-infiltrating mononuclear cells. Data are representative of at least two independent experiments.

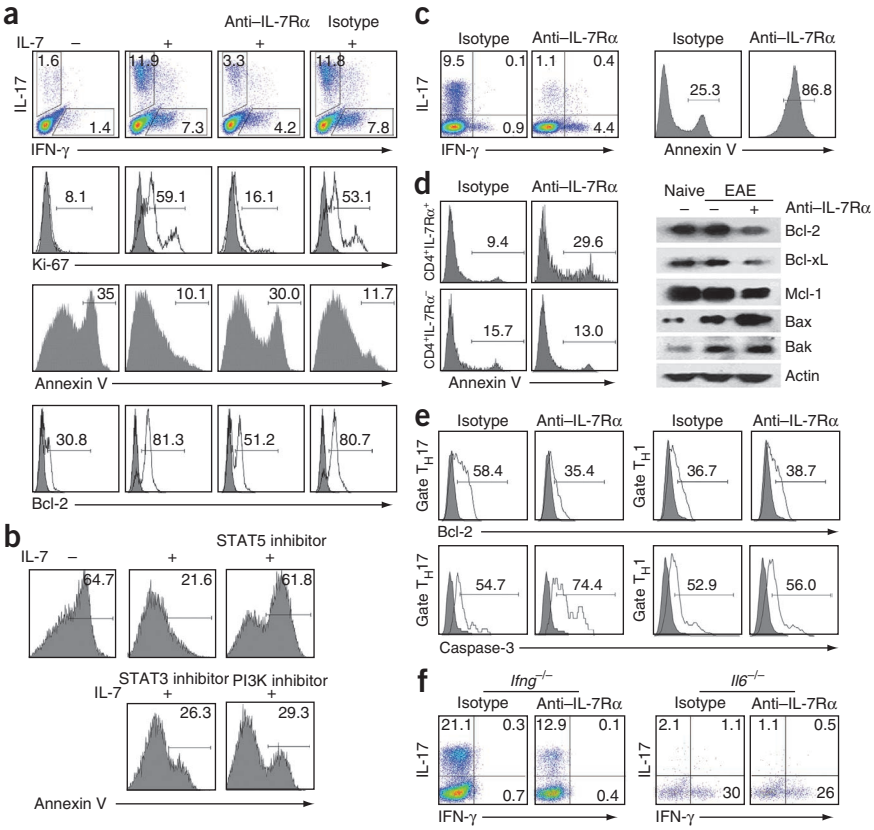

Figure 4 Mechanism underlying the survival of differentiated T_H17 cells mediated by IL-7. (**a**,**b**) Splenocytes from EAE mice were preincubated with the indicated antibodies (**a**) or specific inhibitors (**b**) and subsequently treated with IL-7 for 72 h. (**a**) Ki-67 (open histogram), Bcl-2 (open histogram) and annexin V in $CD4^+$ T cells. (**b**) Annexin V staining for apoptotic cells. (**c**) Percentage of T_H17 and T_H1 (left) and annexin V^+ (right) cells in donor $CD4^+$ T cells obtained from irradiated recipients treated with IL-7Rα–specific antibody or isotype control. (**d**) *Ex vivo* analysis of apoptosis in splenic $CD4^+$ T cells from treated or control EAE mice in IL-7Rα^+ and IL-7Rα^- subsets by annexin V staining (left) and by immunoblotting of pro- and antiapoptotic proteins (right). (**e**) Bcl-2 or active caspase-3 (open histogram) levels in gated T_H17 and T_H1 cells from the same cell preparations as in **d**. (**f**) Splenocytes from MOG-immunized *Ifng*$^{-/-}$ or *Il6*$^{-/-}$ mice (day 14 after infection) were re-stimulated with MOG peptide for 72 h. The resulting T cell blasts were adoptively transferred into irradiated *Egfp*-transgenic mice (2×10^7 cells per mouse) that subsequently received IL-7Rα–specific antibody or isotype control (200 μg per mouse). Donor T_H17 and T_H1 cells from recipient peripheral blood mononuclear cells were analyzed by flow cytometry. Data are representative of at least two independent experiments.

as indicated by increased Ki-67 staining (90.5% ± 6.42% versus 15.2% ± 5.60%, $P < 0.01$), whereas IL-23 had a lesser effect (**Fig. 3d**,**e**). In contrast, *in vitro* expansion of T_H17 cells was not affected by IL-6 (**Fig. 3d**). IL-7–mediated T_H17 and T_H1 cell expansion was abolished by a STAT5 inhibitor but not a STAT3 inhibitor (**Fig. 3f**). We also examined the *in vivo* fate of differentiated T_H17 cells by adoptively transferring MOG-stimulated T cells derived from mice deficient in interferon-γ (IFN-γ; *Ifng*$^{-/-}$ mice) and enriched for T_H17. The results revealed that *in vivo* expansion of T_H17 cells was severely impaired, as was evident in spinal cord of recipient mice treated with IL-7Rα–specific antibody (**Fig. 3g**). We further compared the effect of IL-7R antagonism on *in vivo* expansion of T_H17 cells with the effect of IL-23p19–specific antibody[42] in separate adoptive transfer experiments in wild-type mice. The results indicated that neutralization with IL-23p19–specific antibody had no effect on the percentage of proliferating donor T_H17 cells in treated recipient mice compared to controls, as indicated by Ki-67 protein abundance (**Supplementary Fig. 6a**,**b**). Thus, our data show that survival and expansion of pathogenic T_H17 is controlled by IL-7 signaling through regulation of STAT5 phosphorylation.

IL-7 as a survival signal for committed T_H17 cells

We wanted to further investigate the mechanism underlying the role of IL-7 as a survival signal for committed T_H17 cells to expand. It seemed that differentiated T_H17 cells derived from EAE mice underwent apoptosis that could be rescued by IL-7. IL-7 promoted the proliferation of T_H17 cells, as evidenced by Ki-67 staining (62.6% ± 2.72% versus 11.2% ± 2.3%, $P < 0.01$) and significantly increased the expression of Bcl-2 protein (75.7% ± 3.9% versus 29.7% ± 2.2%, $P < 0.01$), which was accompanied by a lower percentage of annexin V^+ apoptotic cells (10.1% ± 0.30% versus 38.2% ± 4.91%, $P < 0.01$) (**Fig. 4a**). These effects of IL-7 could be neutralized by pre-incubation with IL-7Rα–specific antibody but not isotype control antibody (**Fig. 4a**). The protective effect of IL-7 was clearly mediated through the JAK-STAT5 pathway, as it could be blocked by a STAT5-specific inhibitor (66.8% ± 17.1% versus 22.3% ± 1.82%, $P < 0.05$) but not by a STAT3 inhibitor or a phosphoinositide 3-kinase inhibitor (**Fig. 4b**). Furthermore, when we adoptively transferred MOG-stimulated T cells containing T_H17 and T_H1 cells into naive mice, treatment of the recipient mice with IL-7Rα–specific antibody caused a marked reduction in the percentage of donor T_H17 cells in the blood (2.64% ± 1.77% versus 12.2% ± 6.81%, $P < 0.05$) accompanied by an increased percentage of apoptotic cells (**Fig. 4c**).

We further analyzed the underlying molecular events involving anti- and proapoptotic proteins in $CD4^+$ T cells derived from EAE mice treated with IL-7Rα–specific antibody or an isotype control. The higher percentage of annexin V^+ T cells in the $CD4^+$IL-7Rα^+ T cell population correlated with a lower expression of Bcl-2, Bcl-xL and Mcl-1 and elevated amounts of the proapoptotic proteins Bax and Bak in EAE mice treated with IL-7Rα–specific antibody (**Fig. 4d**). To further elaborate on the survival of T_H17 over T_H1 cells, we determined Bcl-2 or active caspase-3 protein amounts in splenic T_H17 or T_H1 cells derived from EAE mice treated with IL-7Rα–specific antibody. The data confirmed that T_H17 cells were more dependent on IL-7 signaling for survival (**Fig. 4e**), which is consistent with the differential requirements of IL-7 for *in vivo* T_H17 cell survival compared to survival of T_H1 cells. Specifically, we adoptively transferred MOG-stimulated donor T cells enriched for T_H17 (from *Ifng*$^{-/-}$ EAE mice) or T_H1 (from MOG-immunized *Il6*$^{-/-}$ mice) cells into *EGFP*-transgenic mice (used to track the cells) treated with IL-7Rα–specific antibody or an isotype control. The results showed that IL-7R antagonism selectively decreased percentage of donor

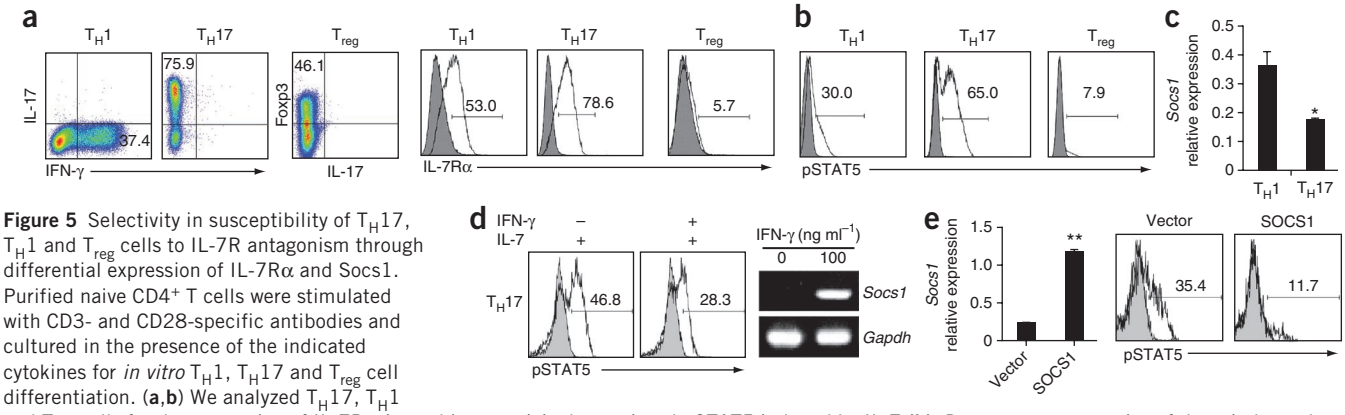

Figure 5 Selectivity in susceptibility of T_H17, T_H1 and T_{reg} cells to IL-7R antagonism through differential expression of IL-7Rα and Socs1. Purified naive CD4$^+$ T cells were stimulated with CD3- and CD28-specific antibodies and cultured in the presence of the indicated cytokines for *in vitro* T_H1, T_H17 and T_{reg} cell differentiation. (**a**,**b**) We analyzed T_H17, T_H1 and T_{reg} cells for the expression of IL-7Rα (open histogram) (**a**, bottom) and pSTAT5 induced by IL-7 (**b**). Data are representative of three independent experiments. (**c**) *Socs1* mRNA levels in *in vitro*–differentiated T_H1 and T_H17 cells, as determined by real-time RT-PCR. (**d**) Differentiated T_H17 cells were cultured in the absence or presence of IFN-γ (100 ng ml^{-1}) and subsequently stimulated with IL-7 to determine pSTAT5 levels by flow cytometry (left) and *Socs1* mRNA levels by RT-PCR (right). *Gapdh*, glyceraldehyde 3-phosphate dehydrogenase. (**e**) Activated CD4$^+$ T cells were infected with retroviruses expressing GFP (vector) or SOCS1-GFP (SOCS1) and cultured under T_H17-polarizing conditions for 4 d. *Socs1* mRNA levels (left) and IL-7–induced pSTAT5 levels (right) were determined. Data are representative of three separate experiments. $^*P < 0.05$ and $^{**}P < 0.01$.

T_H17 but not T_H1 cells in recipient mice (**Fig. 4f**). Thus, IL-7 is a key survival signal for differentiated T_H17 cells that is mediated by regulating the levels of anti- and proapoptotic proteins through the JAK-STAT5 pathway.

Selectivity of IL-7R antagonism for T_H17 cells

We were interested in delineating the underlying mechanism for the observed selectivity seen in T_H17 over T_H1 or T_{reg} cells with respect to the requirement for IL-7 to survive and expand and susceptibility to IL-7R antagonism. IL-7Rα was highly expressed in *in vitro*–differentiated T_H17 cells and, to a lesser degree, in T_H1 cells, whereas it was minimally expressed in T_{reg} cells (74.9% ± 10.9%, 55.6% ± 7.32% and 6.82% ± 2.71%, respectively, **Fig. 5a**). When exposed to exogenous IL-7, T_H17 cells showed a marked responsiveness, as measured by high levels of STAT5 phosphorylation as compared to T_H1 and T_{reg} cells (54.4% ± 14.0%, 25.4% ± 7.26% and 9.16% ± 1.77%, respectively, **Fig. 5b**).

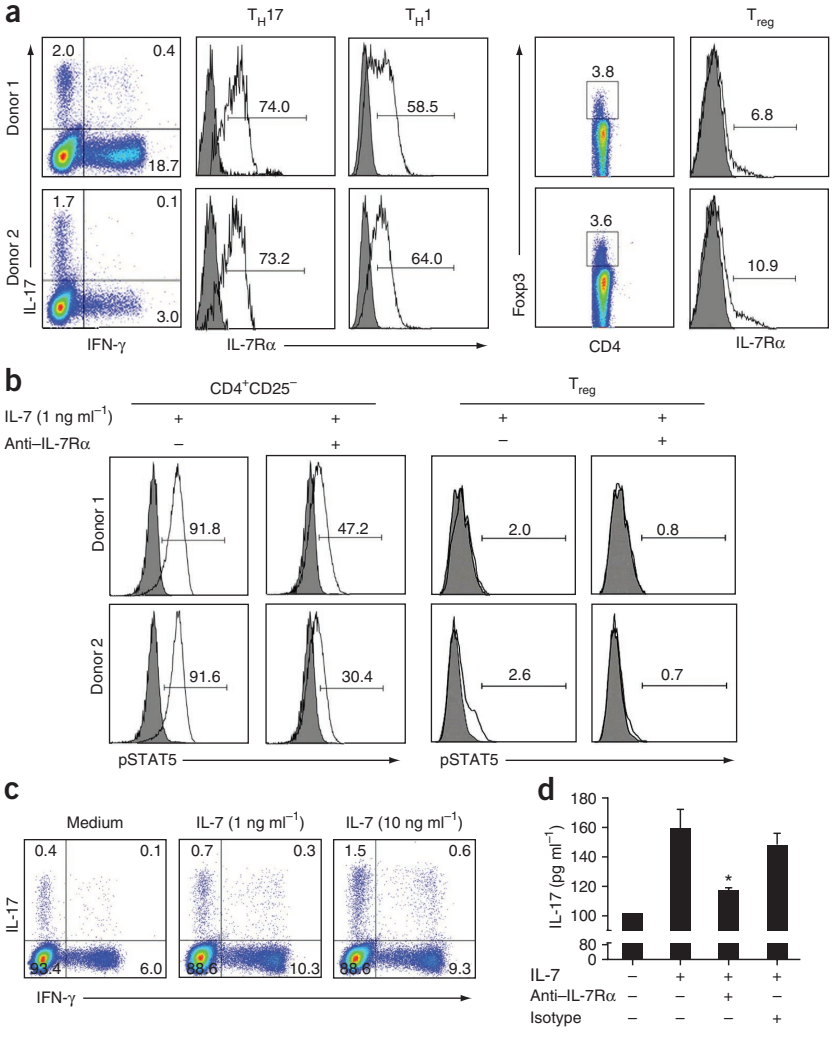

Figure 6 Effects of IL-7 and IL-7Rα antagonism on survival and expansion of T_H17 cells derived from subjects with multiple sclerosis. (**a**) The expression of IL-7Rα in T_H17, T_H1 and T_{reg} cells in two representative peripheral blood mononuclear cell samples by flow cytometry. (**b**) Purified CD4$^+$CD25$^-$ or CD4$^+$CD25^{hi} (T_{reg}) cells were preincubated with IL-7Rα–specific antibody or isotype control and subsequently cultured in the presence of IL-7 and analyzed for pSTAT5 levels by flow cytometry. The open or shaded areas indicate IL-7 treatment or control, respectively. (**c**) Purified CD4$^+$ T cells of the memory phenotype (CD45RO$^+$CD45RA$^-$) were treated with IL-7 and analyzed for the percentage of T_H17 and T_H1 cells by intracellular staining. (**d**) Purified CD4$^+$ T cells were further differentiated, and the resulting FACS-sorted CCR6^{hi} populations enriched for differentiated T_H17 cells were preincubated with IL-7Rα–specific antibody or isotype control and subjected to IL-7 treatment. Supernatants were measured for the production of IL-17 by ELISA. Data are representative of and reproducible from at least ten individual specimens. $^*P < 0.05$.

We further found that the discrepancy between T_H17 and T_H1 in the response to IL-7 was associated with the expression of *Socs1*, a known IFN-γ–inducible repressor gene for JAK-STAT1 signaling[43], which was low in T_H17 cells (**Fig. 5c**). Addition of IFN-γ to differentiated T_H17 cell culture upregulated *Socs1* expression and rendered T_H17 cells less sensitive to IL-7, as determined by reduced STAT5 phosphorylation (**Fig. 5d**). Overexpression of *Socs1* in T_H17 cells led to reduced STAT5 phosphorylation induced by IL-7 (**Fig. 5e**). Collectively, the data show that the selectivity in susceptibility of T_H17 cells to IL-7 is associated with minimal expression of IL-7Rα in T_{reg} cells and intrinsic SOCS-1 activity in T_H1 cells.

Effect of IL-7 on survival and expansion of human T_H17 cells

We further evaluated the role of IL-7 in T_H17 survival and expansion in relation to IL-7R antagonism in peripheral blood mononuclear cells derived from subjects with multiple sclerosis. $CD4^+$ T cells enriched for differentiated T_H17 and T_H1 cells had high expression of IL-7Rα (73.6% ± 0.57% and 61.3% ± 3.89%), whereas the receptor was not detectable or minimally expressed in $CD4^+CD25^+Foxp3^+$ T_{reg} cells (**Fig. 6a**). We could elicit increased STAT5 phosphorylation by IL-7 in the $CD4^+CD25^-$ population, but not in $CD4^+CD25^+$ T_{reg} cells (**Fig. 6b**). Notably, we found IL-7 to directly expand T_H17 cells of the memory phenotype in a dose-dependent manner (**Fig. 6c**). Furthermore, *in vitro* exposure of differentiated T_H17 cells to IL-7 led to significantly increased production of IL-17 ($n = 20$, $P < 0.01$; **Supplementary Fig. 7**), which could be blocked by IL-7R antagonism (**Fig. 6d**). Thus, IL-7–IL-7R signaling had a similar role in human T_H17 development in multiple sclerosis as it does in EAE.

DISCUSSION

In this study, we provide compelling evidence that IL-7–IL-7R signaling is required for survival and expansion of committed T_H17 cells in both mouse and human experimental systems, whereas it has no substantial role in T_H17 differentiation. This is supported by both *in vitro* experiments and *in vivo* data that IL-7 administration or IL-7R antagonism, given before EAE onset, at which time T_H17 cells undergo differentiation, is not sufficient to alter the severity of the disease, whereas the same regimens administered after onset markedly affect the clinical course of EAE. This unique effect of IL-7 is independent of IL-6, as demonstrated here in the *in vivo* and *in vitro* experiments with *Il6*$^{-/-}$ mice. Thus, the findings strongly indicate that the dichotomic process of T_H17 development is controlled chiefly by IL-6 through JAK-STAT3 for T_H17 differentiation[44,45] and by IL-7 through JAK-STAT5 for T_H17 maintenance. STAT5 signaling is involved in both phases of T_H17 development but is driven by different cytokines, that is, T_H17 differentiation is driven through the effect of IL-2 (refs. 46–48) and T_H17 cell survival and expansion is driven by IL-7 as described here. In contrast, the effect of IL-23 on T_H17 cell development involves another mechanism of action through the JAK-STAT3 pathway[49]. It is conceivable that IL-23 acts through stabilization of STAT3 to promote T_H17 differentiation, whereas IL-7 is crucial for T_H17 survival and expansion through STAT5 signaling, which could not be blocked by IL-23p19–specific antibody (data not shown). The requirement for the IL-23 receptor in the reexpression of IL-7Rα in effector and memory T_H17 cells points to a potential connection between the IL-7 and IL-23 pathways[21]. Collectively, the network of cytokines, including IL-6, IL-23 and IL-7, has differential roles at the two distinct phases of T_H17 development (**Supplementary Fig. 8**).

It is remarkable that the *in vivo* effect of IL-7R antagonism in EAE is highly selective for cells and, to a lesser extent, T_H1 cells predominantly of the effector and memory phenotype and spares T_{reg} cells. The selectivity for T_H17 cells over T_{reg} cells is readily explained by the differential expression of IL-7Rα between the two cell types, rendering T_H17 cells susceptible and T_{reg} cells resistant to IL-7R antagonism[50,51]. This selectivity seems to be key in rebalancing the ratio of pathogenic T_H17 cells and T_{reg} cells[52–54] by IL-7R antagonism in EAE and is attributable to the treatment efficacy. However, the discrepancies in the magnitude of IL-7–induced responsiveness and susceptibility to IL-7R antagonism between T_H17 and T_H1 cells cannot be simply explained by the expression of IL-7Rα, as both subsets highly express IL-7Rα. We show here that the intrinsic expression and activity of SOCS1 is responsible for the discrepancies. That is, SOCS1 naturally expressed in T_H1 cells or experimentally induced in T_H17 cells by IFN-γ is attributable to dampened susceptibility to IL-7 or IL-7R antagonism, as SOCS1 acts as a repressor for the STAT5 activity required for IL-7 signaling[55]. Thus, the specific ability of T_H17 cells to survive involves an intrinsic requirement for IL-7 when activated in the course of EAE.

The previously undescribed mechanism of action of IL-7–IL-7R signaling in T_H17 cell survival and expansion as discussed here provides powerful explanations for the treatment efficacy of IL-7R antagonism in EAE and therapeutic implications for human autoimmune diseases such as multiple sclerosis. IL-7–IL-7R antagonism is likely to have unique therapeutic advantages. On the one hand, the treatment offers the selectivity that distinguishes pathogenic T_H1 and T_H17 cells from T_{reg} and unrelated immune cells. On the other hand, additional therapeutic advantages of IL-7R antagonism involve its selective effect on survival and expansion of effector T_H17 cells versus T_H17 cell differentiation. It is evident that IL-6 or IL-23 antagonism given by a prevention protocol when T_H17 cells undergo differentiation is effective during EAE, whereas the same regimen administered after EAE is established shows no efficacy[42]. As implied here, IL-7 antagonism mainly targeting committed T_H17 cells is likely to be advantageous for multiple sclerosis.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturemedicine/.

Note: Supplementary information is available on the Nature Medicine website.

ACKNOWLEDGMENTS

We thank C. Dong for critical reading of this manuscript.

AUTHOR CONTRIBUTIONS

X.L., S.L. and J.Z.Z. designed and discussed the study; X.L., C.W. and Z.T. performed the majority of the T cell experiments; J.W. performed viral gene expression experiments; Y.Z. performed EAE mouse experiments; B.W., X.Q. and L.L. performed the adoptive transfer experiments; R.L. and H.P. performed immunoblotting experiments; M.S. and A.L. performed histopathology analyses; J.H. performed human *in vitro* experiments; X.L., S.L., T.B.G., L.F., H.L. and J.Z.Z. contributed to the writing of the paper; J.Z.Z. supervised the project.

COMPETING INTERESTS STATEMENT

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturemedicine/.

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ONLINE METHODS

Experimental autoimmune encephalomyelitis induction and treatment. We purchased wild-type and *Egfp*-transgenic C57BL/6 (B6) mice from Shanghai Laboratory Animal Center. *Ifng*$^{-/-}$ and *Il6*$^{-/-}$ mice were from the Jackson Laboratory. We obtained MOG-specific T cell receptor–transgenic B6 mice (2D2) through a licensing agreement with Brigham and Women's Hospital. We induced EAE by complete Freund's adjuvant–$MOG_{35–55}$ peptide immunization and scored the mice daily[56]. For adoptive transfer experiments, we re-stimulated splenocytes from MOG-immunized mice with MOG peptide for 72 h. We transferred T cell blasts (2×10^7 cells per mouse) intravenously into irradiated (400 rad) naive recipients. The various treatment regimens included recombinant mouse IL-7 (rmIL-7, 5 μg per mouse every third day, R&D Systems) or antibodies to IL-7Rα (SB/14; BD Biosciences), IL-7 (AB-407-NA; R&D systems) or IL-23p19 (AF1619; R&D Systems) or isotype control antibody (553926; BD Biosciences) at 200 μg per mouse every other day, starting from day 0 or day 10 after immunization. All mouse protocols were approved by GlaxoSmithKline Institutional Animal Care and Use Committee.

Histology and immunofluorescence. We fixed the spinal cord in 4% paraformaldehyde and paraffin-embedded it. We stained 5-μm sections with Luxol fast blue or H&E. For immunofluorescence staining, we stained spinal cord frozen sections with rat antibody to mouse CD4 (H129.19; BD Biosciences) and CD11b (M1/70; eBioscience) and Cy3-conjugated donkey antibody to rat IgG (705-166-147; Jackson ImmunoResearch Laboratories) as secondary antibody, with DAPI (Sigma) for nuclei staining.

T cell purification. We used FACS (FACSAria, BD Biosciences) to sort $CD44^{lo}CD62L^{hi}CD25^{-}$ naive T cells. We prepared CNS-infiltrating mononuclear cells by Percoll gradient separation[56]. We studied clinically definite human subjects with multiple sclerosis ($n = 20$), and the protocol was approved by the Institutional Review Board at Baylor College of Medicine and GlaxoSmithKline. We obtained informed consent before sample collection and prepared mononuclear cells from the whole blood by Ficoll-Hypaque centrifugation (Amersham Biosciences).

T cell proliferation and cytokine measurement. We stimulated splenocytes (5×10^5 per well) with $MOG_{35–55}$ peptide for 72 h. We determined [^{3}H]-thymidine incorporation during the last 18 h of culture with a β-counter (PerkinElmer). We measured IL-4, IL-6, IL-17 and IFN-γ in culture supernatants using the mouse Flowcytomix Multiplex kit or the ELISA kit (R&D Systems) for TGF-β according to the manufacturers' instructions.

T cell differentiation and expansion. We activated purified naive $CD4^+$ T cells with antibodies to CD3 (145-2C11; BD Biosciences) and CD28 (37.51; BD Biosciences) in the presence of rmIL-12 (419-ML) and IL-4–specific antibody (BVD4-1D11; BD Biosciences) for T_H1 differentiation. For T_H17 differentiation, we used recombinant human TGF-β1 (rhTGF-β1) and rmIL-6 (406-ML) or rmIL-7 (407-ML) (10 ng ml^{-1}; R&D Systems) plus antibodies to IFN-γ (XMG1.2) and IL-4 (10 μg ml^{-1}; BD Biosciences). We used rhTGF-β1 (240-B; 10 ng ml^{-1}) and IL-2 (1150-ML; 40 ng ml^{-1}; R&D Systems) for T_{reg} induction. For human T_H17 differentiation, we used a cocktail containing rhIL-6 (206-IL; 50 ng ml^{-1}), rhIL-1β (201-LB; 10 ng ml^{-1}) and rhIL-23 (1290-IL; 20 ng ml^{-1}) plus antibodies to IFN-γ (16-7317) and IL-4 (16-7048) (10 μg ml^{-1}, eBioscience). In some experiments, we preincubated T cell preparations with antibodies as indicated in **Figures 4a** and **6b**,**d** or specific inhibitors (STAT3, STAT5 or phosphoinositide 3-kinase, Calbiochem) for 1 h before the addition of IL-7.

Flow cytometry. We performed surface staining with antibodies to CD4(GK1.5), CD8(53-6.7), B220(RA3-6B2), CD44(IM7), CD62L(MEL-14), CD25(PC61) (BD Biosciences) and CD127 (25-1274; eBioscience). We performed intracellular staining for IL-17 and IFN-γ after stimulation with phorbol 12-myristate 13-acetate (PMA, 50 ng ml^{-1}) and ionomycin (1 μg ml^{-1}, Sigma) for 5 h in the presence of GolgiPlug (555029; BD Biosciences) according to the manufacturer's protocol. We performed intracellular staining for Foxp3 (FJK-16S; eBioscience), Bcl-2 (3F11; BD Biosciences), active caspase-3 (C92-605), Ki-67 (B56) and phosphorylated STAT3 (4/P-Stat3) and STAT5 (47) (BD Biosciences) according to the manufacturers' instructions. We measured apoptosis using an Annexin V–FITC staining kit (BD Biosciences). We defined and obtained MOG-specific T cells by re-stimulating lymphocyte preparations with MOG-peptide and staining for intracellular cytokines.

Immunoblotting. We isolated splenic $CD4^+$ T cells from naive mice treated with IL-6 or IL-7 at 10 ng ml^{-1} for 30 min or $CD4^+$ T cells from EAE mice. We lysed cells in ice-cold buffer containing protease inhibitors cocktail (Roche). We fractionated the lysates by SDS-PAGE, transferred them to nitrocellulose membranes and analyzed them by immunoblotting with specific antibodies to Bcl-2, Bcl-xL, Mcl-1, Bax and Bak as well as β-actin (Sigma) as a loading control. We used the ECL detection system (Amersham BioSciences) for data analysis.

Retroviral experiments. We cloned mouse *Socs1* complementary DNA into pRetroX-IRES-ZsGreen vector (Clontech). We produced ecotrophic retroviruses from infected EcoPack2-293 cells (Clontech) according to the manufacturer's manual. We first activated $CD4^+$ T cells with T_H1- or T_H17-polarizing media for 24 h. We then infected cells with retrovirus supernatant, followed by centrifugation at 500*g* for 90 min. We maintained the resulting cells in fresh polarizing media (RPMI 1640 with 10% fetal calf serum supplemented with rmIL-12 and IL-4–specific antibody for the T_H1 condition or with rhTGF-β1 and rmIL-6 plus antibodies to IFN-γ and IL-4 for the T_H17 condition) for 5 d before analysis.

Reverse transcription PCR. We extracted total RNA and used it to synthesize first-strand cDNA by Sensiscript RT Kit (Qiagen). We analyzed *Rora*, *Socs1* and *Gapdh* mRNA expression by RT-PCR or real-time PCR (Applied Biosystems). The sequences of the primer pairs were *Rora*: 5′-CCGCTGAGAGGGCTTCAC-3′ and 5′-TGCAGGAGTAGGCCACATTACA-3′; *Socs1*: 5′-CAGGTGGCAGCCGACAATGCGATC-3′ and 5′-CGTAGTGCTCCAGCAGCTCGAAAA-3′; *Gapdh*: 5′-TGCACCACCAACTGCTTAG-3′ and 5′-GGATGCAGGGATGATGTTC-3′.

Statistical analysis. We expressed data as mean ± s.d. or mean ± s.e.m. and tested for statistical significance by the two-tailed Student's *t* test. $P < 0.05$ was considered statistically significant.

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doi:10.1038/nm.2077

Retraction: Crucial role of interleukin-7 in T helper type 17 survival and expansion in autoimmune disease

Xuebin Liu, Stewart Leung, Chunxia Wang, Zhu Tan, Ji Wang, Taylor B Guo, Lei Fang, Yonggang Zhao, Bing Wan, Xia Qin, Limin Lu, Runsheng Li, Heng Pan, Mingjuan Song, Ailian Liu, Jian Hong, Hongtao Lu & Jingwu Z Zhang
***Nat. Med.* 16, 191–197 (2010); published online 10 January 2010; retracted 5 December 2013**

The above manuscript was authored by scientists from the GlaxoSmithKline (GSK) Research and Development Center in Shanghai, China, and a researcher from Baylor Medical College who later became a GSK employee. Following anonymous reports of inaccuracies in this study, GSK conducted an investigation into these allegations.

The investigation established that the data depicted in Figure 6 and in Supplementary Figure 7 were erroneously attributed to experiments at Baylor Medical College with blood cells from patients with multiple sclerosis. In fact, no data from experiments with blood cells from patients with multiple sclerosis and no data from experiments at Baylor Medical College were included in the paper. GSK has therefore concluded that the paper contains erroneous data and requests that it be retracted.

Nature Medicine wishes to acknowledge that the two graphs at the bottom left-hand corner of Figure 2a are identical and that this was an error introduced by the journal during the production of the article. Below are the correct graphs as submitted by the authors (**Fig. 1**).

All authors agreed to the retraction of the paper with the following exceptions and clarifications. Xuebin Liu and Stewart Leung declined to sign the retraction and stand by the conclusions of the paper. Chunxia Wang, Xia Qin and Limin Lu did not respond to *Nature Medicine*'s requests for comment on the retraction. Ji Wang, Lei Fang, Bing Wan, Jian Hong and Hongtao Lu could not be reached by the journal for comment on the retraction. However, Chunxia Wang, Xia Qin, Limin Lu, Ji Wang, Lei Fang, Bing Wan, Jian Hong and Hongtao Lu signed an initial version of the retraction submitted to the journal by GSK.

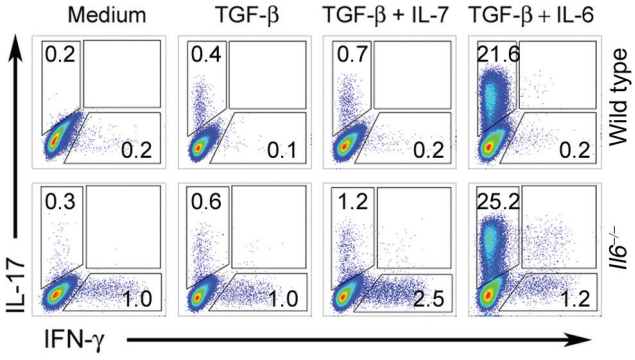

Figure 1