

IL-25 regulates Th17 function in autoimmune inflammation

Melanie A. Kleinschek,¹ Alexander M. Owyang,¹ Barbara Joyce-Shaikh,¹ Claire L. Langrish,¹ Yi Chen,¹ Daniel M. Gorman,¹ Wendy M. Blumenschein,² Terrill McClanahan,² Frank Brombacher,³ Stephen D. Hurst,¹ Robert A. Kastelein,¹ and Daniel J. Cua¹

¹Discovery Research and ²Experimental Pathology and Pharmacology, Schering-Plough Biopharma, Palo Alto, CA 94304

³University of Cape Town, Rondebosch 7701, South Africa

Interleukin (IL)-25 is a member of the IL-17 family of cytokines. However, unlike the other members of this family, IL-25 promotes T helper (Th) 2 responses. We now show that IL-25 also regulates the development of autoimmune inflammation mediated by IL-17-producing T cells. We have generated IL-25-deficient (*il25*^{-/-}) mice and found that they are highly susceptible to experimental autoimmune encephalomyelitis (EAE). The accelerated disease in the *il25*^{-/-} mice is associated with an increase of IL-23 in the periphery and a subsequent increase in the number of inflammatory IL-17-, IFN γ -, and TNF-producing T cells that invade the central nervous system. Neutralization of IL-17 but not IFN γ in *il25*^{-/-} mice prevented EAE, suggesting that IL-17 is a major disease-promoting factor. IL-25 treatment at several time points during a relapse-remitting model or chronic model of EAE completely suppressed disease. IL-25 treatment induced elevated production of IL-13, which is required for suppression of Th17 responses by direct inhibition of IL-23, IL-1 β , and IL-6 expression in activated dendritic cells. Thus, IL-25 and IL-17, being members of the same cytokine family, play opposing roles in the pathogenesis of organ-specific autoimmunity.

CORRESPONDENCE

Daniel J. Cua:
daniel.cua@spcorp.com

Abbreviations used: CNS, central nervous system; DLN, draining lymph node; EAE, experimental autoimmune encephalomyelitis; HR, homologous recombination; MOG, myelin oligodendrocyte glycoprotein; PTX, pertussis toxin; rAdV, replication-deficient adenoviral vector.

IL-25 (IL-17E) is a recently identified member of the IL-17 family of cytokines (1). It has been implicated in the initiation of type 2 immunity by driving the expression of IL-4, IL-5, and IL-13 (1, 2). In vitro, IL-25 is found in activated Th2 cells, bone marrow-derived mast cells, and alveolar macrophages (1, 3, 4). Recently, we identified that IL-25 is produced by a population of CD4⁺ cells in mouse caecal patches (5). Expression of this cytokine is critical for immunity against helminth infections (5, 6). IL-25 protein administration or transgenic overexpression of IL-25 in mice resulted in Th2-like responses associated multiorgan inflammation characterized by elevated expression of IL-4, IL-5, and IL-13, inflammatory cell infiltration, and epithelial cell hyperplasia (1, 2, 7, 8).

M.A. Kleinschek and A.M. Owyang contributed equally to this paper.

C.L. Langrish's present address is Roche Bioscience, Palo Alto, CA 94304.

S.D. Hurst's present address is Genentech, South San Francisco, CA 94080.

The online version of this article contains supplemental material.

These findings suggest that IL-25 may be an important mediator of type 2 immune pathologies.

Besides IL-25, there are five additional members that belong to the IL-17 family of cytokines. Although IL-25 promotes allergic responses, IL-17A had been characterized to promote chronic inflammatory responses (9, 10). We have recently reported that IL-17A is produced by a population of IL-23-dependent pathogenic T cells required for the induction of autoimmune inflammation (11–13). This finding has led to the proposal that these IL-17-producing cells (called Th17) represent a novel subset of CD4⁺ Th cells (14–17). Several recent reports have demonstrated that TGF- β and IL-6 are required for lineage commitment of IL-17-producing T cells (18–20), and this novel differentiation pathway does not share transcription regulatory elements with Th1 and Th2 responses (21, 22). Indeed, we have identified an orphan nuclear receptor—retinoid-related orphan receptor γ t (ROR γ t)—as the critical transcription regulator that controls TGF- β - and IL-6-induced Th17 differentiation (23).

These remarkable findings provide definitive evidence that Th17 cells are indeed a bona fide T cell subset that is independently regulated from Th1 and Th2.

Experimental autoimmune encephalomyelitis (EAE) is an autoimmune inflammatory disease of the central nervous system (CNS) that serves as a disease model for human multiple sclerosis. EAE can be induced by passive transfer of myelin antigen-specific Th17 or Th1, but not Th2 cells (13, 24, 25). In the inflamed CNS, high levels of proinflammatory cytokines, such as IL-17, IL-18, IFN γ , TNF, and IP10 are expressed with very low levels of Th2 cytokines such as IL-4 and -13 (26, 27). Studies have documented that redirecting the Th cell balance toward a type 2 response can suppress autoimmunity (28–30). It is well accepted that type 2 responses provide multiple signals that inhibit IFN γ -producing Th1 cells (31, 32). Recent *in vitro* studies have suggested that Th17 cells are also negatively regulated by IL-4 signaling pathways (21, 22). Nevertheless, very little is known about the *in vivo* regulation of Th17 cells. By generating IL-25-deficient mice, we investigated whether this potent Th2-promoting cytokine plays a physiological role in regulating the development and function of Th1 and/or Th17 encephalitogenic T cells.

RESULTS

IL-25 is locally expressed in the CNS and protects from EAE

IL-25 is a potent cytokine that drives the production of type 2 cytokines such as IL-4, IL-5, and IL-13 (1). During EAE priming, a broad range of cytokines is induced in C57BL/6 mice, including the proinflammatory cytokines IFN γ , IL-17, and IL-18, as well as the type 2 cytokines IL-4 and IL-13. To determine whether IL-25 plays a regulatory role during autoimmune inflammation, we analyzed the expression of this cytokine during the course of EAE. We observed that IL-25 mRNA is expressed in normal spinal cord as well as inflamed CNS (Fig. 1 A). Analysis of CNS-resident cells indicated that IL-25 is predominantly expressed by microglia in the normal CNS (Fig. 1 B). Remarkably, microglia expressed ninefold more IL-25 mRNA during active EAE. In contrast, inflammatory macrophages or infiltrating T cells do not express detectible levels of IL-25 (unpublished data).

We generated IL-25-deficient mice (Fig. S1, available at <http://www.jem.org/cgi/content/full/jem.20061738/DC1>) to determine whether this cytokine has a role in regulating clinical EAE. These mice have no developmental defects, and they generate litters of normal size. Males and females are born in equivalent numbers. Analysis of IL-25-deficient spleens showed normal numbers and percentages of B cells, T cells, and granulocytes. IL-25-deficient thymi had the same number of cells and the same percentages of CD4 and CD8 double-negative, single-positive, and double-positive thymocytes, when compared with C57BL/6 mice.

After myelin oligodendrocyte glycoprotein (MOG) peptide/CFA immunization, IL-25-deficient (*il25*^{-/-}) mice showed accelerated EAE onset and enhanced disease severity compared with WT C57BL/6 mice (Fig. 1 C). *il25*^{-/-} mice

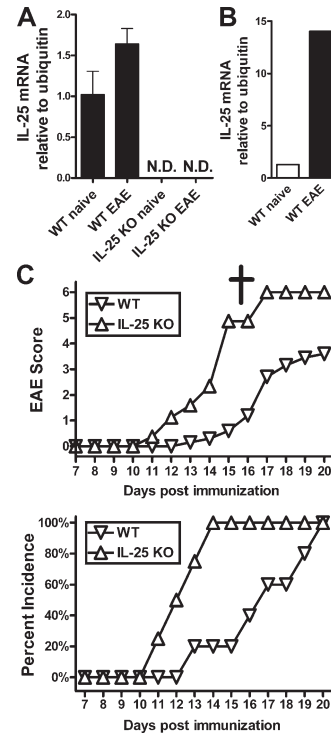


Figure 1. *il25*^{-/-} mice are highly susceptible to EAE. (A) Taqman PCR analysis of IL-25 mRNA levels in spinal cords from C57BL/6 WT normal and diseased versus *il25*-LacZ-KI normal and diseased ($n = 6$ mice per group). Data shown are representative of at least two experiments. ND, not detectable. (B) IL-25 mRNA expression in CNS-resident microglia. Brain leukocytes were isolated from bone marrow chimeras expressing the CD45.2 allotype on radiation-resistant host cells and the CD45.1 allotype on donor leukocytes. CD11b⁺CD45.2^{lo}CD45.1^{neg} resident microglia were purified from >100 irradiation bone marrow chimeric naive or diseased (day 14) mice. Data from quantitative real-time PCR analysis for IL-25 expression were normalized to ubiquitin. (C) Clinical score (top) and percentage of incidence of disease (bottom) of C57BL/6 (WT) and *il25*^{-/-} mice immunized with MOG/CFA s.c. on day 0 and PTX i.v. on days 0 and 2. The dagger indicates that by day 17, all *il25*^{-/-} mice had died from EAE-associated complications. Data shown are 7–9 mice per group and are representative of at least three experiments.

consistently developed EAE 2–3 d earlier than WT mice, and all died (100% mortality at day 17) when immunized at doses optimized to give WT mice a nonlethal disease. In addition, incidence of disease reached 100% 6 d earlier in the *il25*^{-/-} group (Fig. 1 C). Administration of IL-25 to MOG-immunized *il25*^{-/-} completely protected the mice from EAE (Fig. S1 C), confirming that no other defects are responsible for the enhanced susceptibility of *il25*^{-/-} mice to EAE. These results indicate that IL-25 may play an important role in suppressing the inflammatory responses that drive EAE pathogenesis.

IL-25 inhibits the Th17 response within the CNS

We have recently described an IL-23-dependent, IL-17-producing T cell population that is highly pathogenic in autoimmune inflammatory diseases (13). Here, we found the

same Th17 cells as well as IFN γ -producing T cells in the CNS of *il25*^{-/-} mice at an earlier time point and in greater numbers than in the WT mice (Fig. 2). 2 d before expected disease onset, the CNS of *il25*^{-/-} mice contained fivefold more IL-17⁺ cells compared with the WT (Fig. 2 A). In contrast, the total numbers of IFN γ -producing T cells were similar in the *il25*^{-/-} and WT control mice. Also, the total number of inflammatory macrophages was comparable between *il25*^{-/-} and WT mice (Fig. S2, available at <http://www.jem.org/cgi/content/full/jem.20061738/DC1>). At the peak of disease, the *il25*^{-/-} mice continue to have greater numbers of IL-17-producing T cells as well as IFN γ and TNF producers (Fig. 2 B).

To examine whether the disease exacerbation observed in the *il25*^{-/-} mice was mediated by Th17 and/or Th1 cells, we injected anti-IL-17 or anti-IFN γ antagonist mAbs into EAE-primed mice. Blockade of IL-17 dramatically inhibited EAE, indicating that Th17 cells play a major role in EAE pathogenesis in *il25*^{-/-} mice (Fig. 2 C). In contrast, anti-IFN γ treatment had no effect, consistent with studies demonstrating that Th1 cells only play a minor role in EAE (13). Thus, the absence of IL-25 leads to a more rapid and intense infiltration of pathogenic Th17 cells into the target organ, which drives an accelerated and exacerbated disease phenotype.

IL-25 is crucial for Th2 cytokine expression in the periphery

During EAE priming, myelin antigen-specific T cells are generated in lymph nodes draining the sites of immunization. In these lymphoid organs, T cell differentiation is dependent on the cytokines produced by antigen-presenting cells.

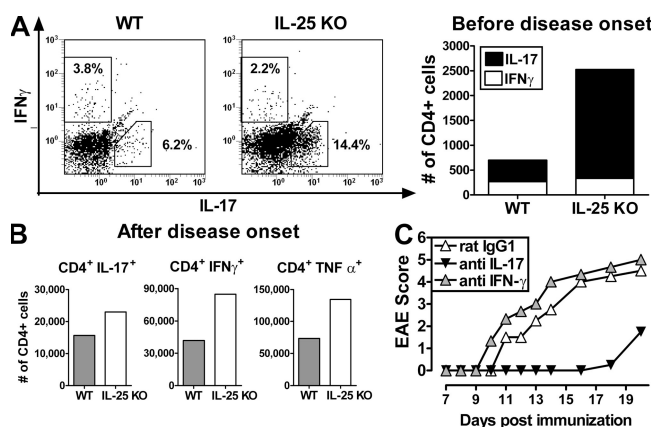


Figure 2. Increased numbers of CNS-infiltrating inflammatory Th17 cells in *il25*^{-/-} mice. (A) Intracellular cytokine staining of FITC-gated CD4⁺ T cells isolated from the CNS of mice at day 7 after immunization (left, flow cytometric analysis; right, calculated number of cytokine-producing cells). (B) Number of cytokine-producing CD4⁺ cells in CNS taken at peak of disease. For A and B, cells from four mice were pooled for intracellular cytokine analysis. Data are representative of two experiments. (C) Disease score of *il25*^{-/-} mice immunized with MOG/CFA ($n = 4$ mice per group) and treated with antibodies against IL-17 or IFN γ or with rat IgG1 control antibody. Data shown are representative of two experiments.

In draining lymph node (DLN) cells from *il25*^{-/-} mice, we found a twofold higher expression of IL-23p19 mRNA than in WT controls at day 7 after immunization (Fig. 3 A). Interestingly, the expression of IL-12p35 was not regulated by IL-25. To determine whether IL-25 affected T cell priming and T cell subset differentiation, we cultured DLN cells at day 7 after immunization in the presence of MOG peptide to assess myelin antigen-specific T cell responses. The Th2 cytokines IL-4, -5, and -13 were significantly reduced in DLN cultures from *il25*^{-/-} mice. IL-17 and IFN γ levels were slightly higher in *il25*^{-/-} DLN cultures; however, the difference did not reach statistical significance (Fig. 3 B). In addition to Th2 responses, regulatory T cells play a crucial role in limiting chronic inflammation. We found that *il25*^{-/-}

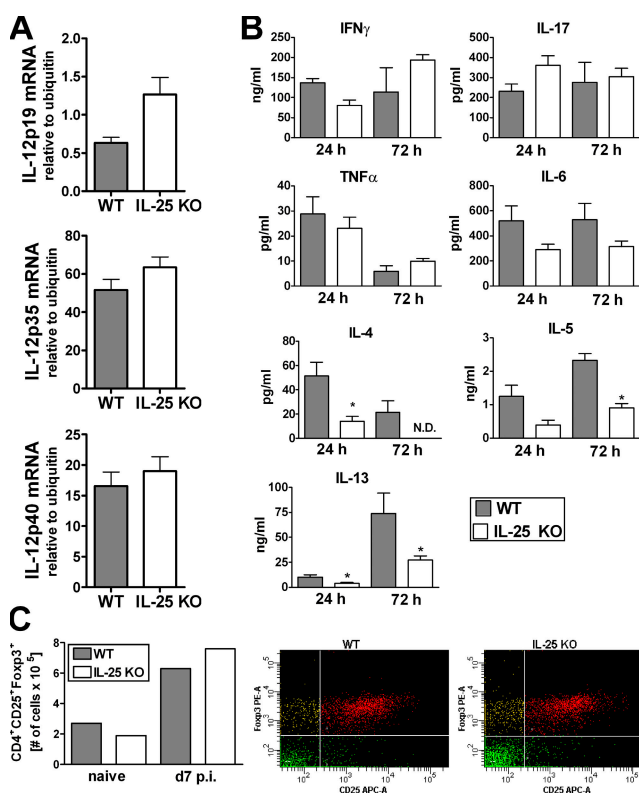


Figure 3. Reduced Th2 cytokine production but normal regulatory T cell response in *il25*^{-/-} DLN cells. (A) mRNA expression in MOG-stimulated DLN cells taken at day 7 after immunization. Expression levels are normalized to ubiquitin. $n = 5$. Obtained DLN samples were pooled in three different pools for mRNA processing. (B) In vitro cytokine recall response. DLN cells from MOG-immunized *il25*^{-/-} and WT mice were isolated at day 7 after immunization. Cells were stimulated with MOG peptide for 3 d and then restimulated with anti-CD3/anti-CD28 for 2 additional days. Supernatants were from 24- or 72-h cultures as indicated. Data are representative of three experiments. All cytokines were measured by cytometric bead array except for IL-13 and IL-17, which were detected by ELISA as described in Materials and methods. ND, not detectable. *, $P < 0.05$ (unpaired two-tailed t test). (C) Generation of regulatory T cells in *il25*^{-/-} and WT mice before (pooled sample of two mice per group) and 7 d after (pooled sample of four mice per group) immunization with MOG/CFA. DLN cells were stained for CD4, CD25, and Foxp3.

mice generated Foxp3⁺ regulatory T cells in numbers similar to WT controls during the course of EAE (Fig. 3 C). These data suggest that IL-25 plays a role in driving IL-4, IL-5, and IL-13 production but does not affect regulatory T cell development.

Exogenous IL-25 inhibits EAE development and progression

We next determined whether exogenous IL-25 can suppress acute as well as relapse–remitting EAE. We used replication-deficient adenoviral vectors (rAdVs) to induce local CNS or systemic IL-25 expression (33). SJL mice were used for these studies because they exhibit a multiple relapse–remitting pattern of clinical disease. Using rAdV gene transfer vectors, we found that intracerebral delivery of IL-25–rAdV 1 d before immunization fully protected SJL mice from EAE (Fig. 4 A). Mice treated with GFP–control–rAdV were not protected. An intracerebral injection of 3×10^9 viral particles (Fig. 4 A) or i.v. injection of 10^{10} particles (not depicted) were equally effective. Injection of IL-25–rAdV 10 d after initial priming could also block EAE (Fig. 4 A). The histological analysis of spinal cords 18 d after immunization shows large numbers of parenchymal and perivascular infiltrates in the control group. IL-25–rAdV–treated mice, in contrast, show minimal to no infiltrate (Fig. 4 B and Table S1, available at <http://www.jem.org/cgi/content/full/jem.20061738/DC1>). We next examined whether IL-25 treatment can inhibit the effector phase of CNS inflammation using a passive transfer model of EAE. Recombinant adenoviral vector–mediated IL-25 expression prevented EAE development when the treatment was given at the time of cell transfer, or even 3 d after cell transfer (Fig. 4 C).

In multiple sclerosis, patients usually suffer from episodes of clinical disease relapse separated by periods of remission. Because IL-25–rAdV could protect from EAE when administered at either the priming stage (day 1 treatment) or during the effector stage (day 10 treatment), we tested whether IL-25 could be used therapeutically to modify clinical disease. Remarkably, we found that IL-25–rAdV completely blocked EAE relapse when the treatment was given after the first episode of paralysis (Fig. 4 D). In contrast, control–rAdV–treated mice suffered two rounds of disease relapse over a period of 51 d. Collectively, these results suggest that besides its ability to prevent disease, IL-25 may regulate effector T cells and reduce their pathogenic functions.

IL-25 induced Th2 and suppressed Th17 responses

IL-25–deficient mice exhibited increased Th17 responses that promoted severe EAE pathology (Fig. 2). This result led us to examine the *in vivo* mechanisms of action of IL-25 treatment during disease induction. Daily s.c. injection with mouse IL (mIL)–25 protected the cytokine–treated mice from EAE (Fig. 5 A) as compared with saline–treated C57BL/6 mice as well as *il25*^{−/−} mice. Given that the severe disease observed in *il25*^{−/−} mice appeared to be driven by IL-17 rather than IFN γ (Fig. 2 C), we examined whether IL-25 could inhibit EAE in *ifng*^{−/−} mice. Indeed, IL-25 treatment protected

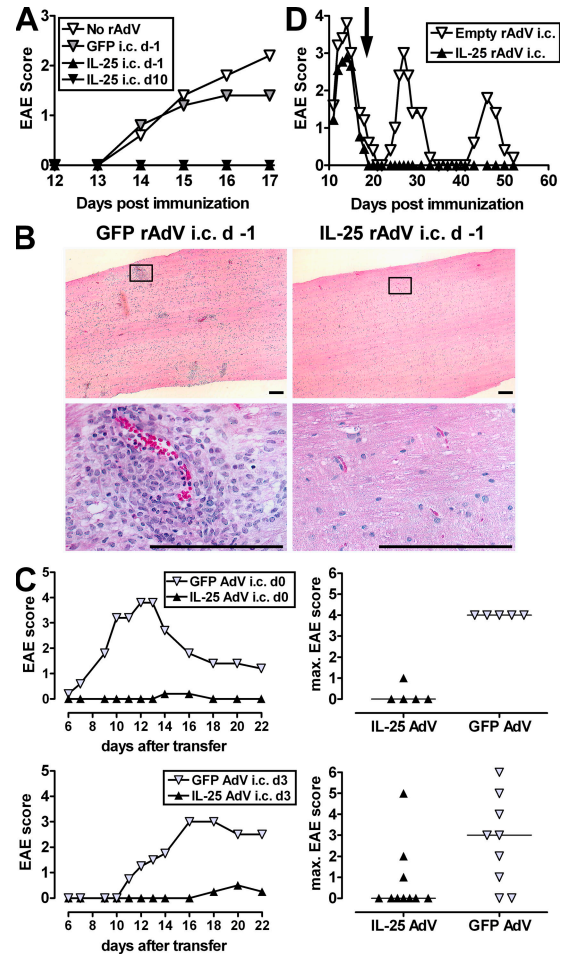


Figure 4. IL-25 inhibits the effector phase of EAE and suppresses clinical disease relapse. (A) Clinical score of SJL mice ($n = 5$ per group) injected with 3×10^9 particles of indicated rAdV by intracerebral ventricular injection 1 d before or 10 d after immunization. (B) Histopathological analysis of spinal cords from SJL mice at day 18 after immunization. Mice were treated with GFP–rAdV (left) or IL-25–rAdV (right) at day 1. (bottom) 10-fold magnification of boxed area in top panels. Bars, 100 μ m. Results of histological analysis are summarized in Table S1 (available at <http://www.jem.org/cgi/content/full/jem.20061738/DC1>). (C) Clinical score of mice ($n = 4$ –5 per group) that received 2×10^7 activated PLP-specific lymphocytes. Recipient mice were treated with 3×10^9 particles of indicated rAdV at day 0 (top) or day 3 (bottom) after PLP-cell adoptive transfer. (left) Kinetics of mean disease score; (right) maximum disease score per individual mouse during 16 d of observation. (D) Clinical score of EAE-primed mice ($n = 7$ –10 per group) injected with 3×10^9 particles of indicated rAdV by an intracerebral ventricular route during disease remission (20 d after immunization, arrow). Mice were observed for two disease-relapse cycles. Results are representative of at least two experiments.

ifng^{−/−} mice from EAE, suggesting that IL-25 regulation of the Th17 pathway is independent of IFN γ (Fig. 5 B).

To further characterize the immune responses regulated by IL-25, we profiled the *ex vivo* gene expression pattern of DLN cells 7 d after MOG/CFA priming. We observed a marked down-regulation of IL-17 in IL-25–treated C57BL/6

as well as *ifng*^{-/-} mice (Fig. 5 C). Both genotypes showed an increased expression of IL-13 after IL-25 treatment. Interestingly, IL-25 did not regulate the expression of total IFN γ mRNA levels in response to priming with MOG peptide and CFA. It is notable that most of the IFN γ response in the DLNs is directed against the mycobacterial component of the adjuvant (only 1–2% MOG-specific T cell response and up to 10–30% anti-mycobacterial response). Thus, it is important to determine the myelin-specific T cell response. The in vitro MOG-specific cytokine recall analysis showed that DLN cells from IL-25-treated mice produced less IL-17 and IFN γ as compared with saline-treated controls (Fig. 5 D). Type 2 cytokines such as IL-4, IL-5, and IL-13 were markedly increased in IL-25-treated animals. Intracellular cytokine staining of DLN cell cultures revealed lower relative and absolute numbers of IL-17-producing CD4⁺ T cells in IL-25-treated *ifng*^{-/-} as well as C57BL/6 mice (Fig. 5 E). These findings established that treatment with recombinant IL-25 can suppress autoimmune inflammation by inducing Th2 while suppressing Th1 and Th17 responses.

IL-13 is required for IL-25-mediated protection from EAE and Th17 suppression

Previous studies as well as data shown here have demonstrated that IL-25 strongly induced expression of IL-4 and -13, which are potent EAE inhibitors (27, 34). Therefore, we determined whether IL-25 suppression of EAE required the presence of these type 2 cytokines. Daily injection of mIL-25 protein protected *Il4*^{-/-} mice from EAE (Fig. 6 A). However, IL-25 could not provide protection to *Il13*^{-/-} mice (Fig. 6 B). The requirement for IL-13 signaling was confirmed by using *il4ra*^{-/-} mice, which cannot respond to either IL-4 or IL-13. As shown in Fig. 6 C, IL-25 protein failed to protect the *il4ra*^{-/-} mice as well. These findings show that IL-13 plays an important role in IL-25-mediated suppression of EAE.

Following the observation that IL-25 regulates EAE through suppression of Th17 responses, we used an in vitro cell culture system to further investigate the underlying mechanism. Naive DO11.10 CD4⁺ T cells were cocultured with LPS-activated CD11c⁺ DCs in the presence of neutralizing antibodies against IFN γ and IL-4. Addition of IL-23 to these cultures induced 10% of IL-17-producing T cells (Fig. 6 D), whereas untreated cultures only contained 0.3% of IL-17 producers (not depicted). The frequency of Th17 cells induced by IL-23 was reduced by half when the cells were grown in the presence of IL-25, showing that IL-25 blocked the in vitro Th17 response, independent of IL-4 (Fig. 6 D). We also observed that IL-25 dramatically enhanced IL-13 production, even in the presence of IL-23, anti-IL-4, and anti-IFN γ (Fig. 6 E).

We next determined the role of IL-13 in regulating Th17 cells. We observed a reduction of the IL-23-induced IL-17 production after addition of IL-25 (Fig. 6 F). This inhibitory effect of IL-25 on IL-17 production was completely abolished by the addition of neutralizing antibodies against IL-13

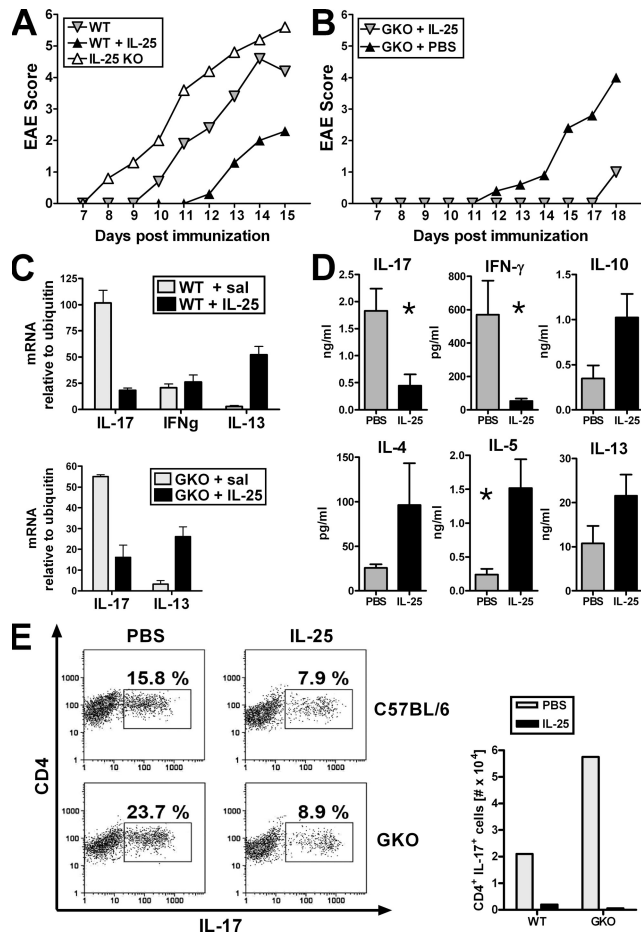


Figure 5. IL-25 induces Th2 and suppresses Th17 independent of IFN γ . (A) Clinical score of immunized WT and *ifng*^{-/-} mice compared with immunized WT mice injected daily s.c. with mIL-25 protein from day 1 to the end of the experiment. Results are representative for at least four experiments with five to six mice per group. (B) Clinical score of *ifng*^{-/-} (GKO) mice ($n = 5$ per group) injected daily with PBS or mIL-25 (s.c.) from days 1–12. Results are representative of two experiments. (C) DLN cells from MOG-immunized WT or *ifng*^{-/-} mice treated with IL-25 or saline were analyzed for cytokine gene expression at day 7 after immunization (five mice per group; DLN cells were processed in three different pools for mRNA analysis). Cytokine gene expression was normalized to ubiquitin. (D) In vitro cytokine recall response. DLN cells from MOG-immunized WT mice treated with PBS or mIL-25 daily s.c. were isolated at day 7 after immunization. Cells were stimulated with MOG peptide for 3 d and restimulated with anti-CD3/anti-CD28 for 2 additional days. Cytokine levels in supernatants were measured by CBA or ELISA. Cells from individual animals were cultured separately, and data are mean cytokine levels \pm SD of three to five mice per treatment group. For IFN γ detection, nine mice per treatment group are shown. Data are representative of at least two experiments. *, $P < 0.05$. (E) DLN cells from MOG-immunized WT and *ifng*^{-/-} (GKO) mice treated with IL-25 or saline (pool of four mice per group) were stimulated in vitro with MOG for 3 d followed by incubation with IL-2 for another 2 d. Frequencies (FACS plots) and absolute numbers (bar graph) of MOG-specific IL-17-producing CD4⁺ cells were assessed. Data shown are representative of two experiments.

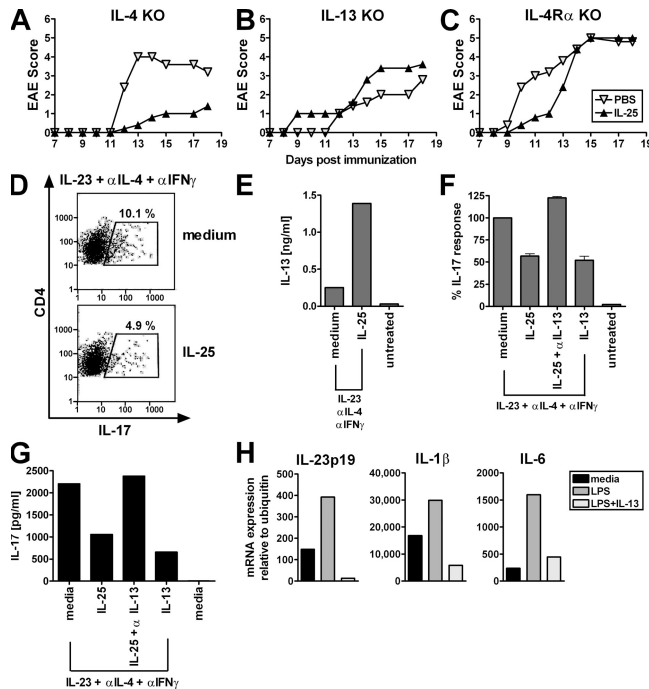


Figure 6. IL-13 is required for Th17 suppression in IL-25-mediated protection from EAE. Clinical score of *il4*^{-/-} (A), *il13*^{-/-} (B), or *il4ra*^{-/-} mice (C), with five mice per group injected daily with PBS or mIL-25 (s.c.) from days 1–12. Results are representative of two experiments. (D–F) Naive T cells and LPS-activated CD11c⁺ DCs were obtained from DO11.10 × RAG^{-/-} mice (pool of five mice) and incubated for 3 d with IL-23 in the presence of OVA peptide. IFN γ and IL-4 were neutralized in these cultures. (D) The effect of IL-25 addition on the number of IL-23-induced IL-17 producers was assessed by intracellular staining for IL-17. (E) IL-13 expression in these cultures was determined by LUMINEX. (F) The modulation of IL-17 protein production in IL-23-driven cultures by IL-25, IL-25 plus anti-IL-13, and IL-13 is shown in percentage of IL-23-induced IL-17 production without addition of exogenous factors (mean IL-17 production is 202.3 pg/ml, assessed by LUMINEX). Data shown are representative of at least two experiments (D and E) or pooled data from two similar experiments (F). (G) Purified MOG_{33–55} TCR transgenic T cells and LPS-activated CD11c⁺ DCs were incubated for 4 d with IL-23 in the presence of MOG peptide and neutralizing antibodies against IFN γ and IL-4. IL-17 production after incubation with IL-25, IL-25 plus anti-IL-13, and IL-13 was measured by LUMINEX. (H) DCs were purified from spleens and superficial lymph nodes and activated with LPS with or without addition of IL-13. After 24 h, mRNA expression relative to ubiquitin was measured for IL-23p19, IL-1 β , and IL-6 by quantitative real-time PCR. Shown is an experiment using DCs from C57BL/6 mice representative of two C57BL/6 and two BALB/c experiments with a similar outcome ($n = 3$ –5 mice pooled per experiment).

(Fig. 6 F). Moreover, blockade of IL-13 led to enhanced IL-17 production. Addition of recombinant IL-13 to IL-23-driven cultures reduced IL-17 production similar to the effects of adding recombinant IL-25 (Fig. 6 F). We confirmed that IL-13 is a suppressor of IL-17 production using cells from MOG_{33–55} TCR transgenic mice (Fig. 6 G). Because the IL-13 receptor is not expressed on T cells (35), we examined the effects of IL-13 on DC function. Remarkably, IL-13

suppressed LPS-mediated IL-23, IL-1, and IL-6 expression, which are required for Th17 differentiation and function (Fig. 6 H). Collectively, these data demonstrate that IL-25 mediates EAE protection as well as Th17 suppression via IL-13. In turn, IL-13 directly suppresses DC production of Th17-promoting factors.

DISCUSSION

In organ-specific autoimmunity, the balance of cytokines is a key determinant of resistance or susceptibility. In EAE, disease susceptibility is thought to correlate with the expression of proinflammatory cytokines such as IL-17, IFN γ , TNF, IL-6, and IL-1 β . On the other hand, Th2 cytokines such as IL-4 and IL-13 have been shown to be important for preventing or ameliorating disease (26, 27, 36). In the current study, we show that IL-25, a Th2-promoting cytokine, can protect mice from EAE, and loss of IL-25 results in enhanced proinflammatory cytokine production and accelerated CNS pathology. This accelerated disease is also associated with an increased number of IL-17⁺, IFN γ ⁺ and TNF-producing T cells that invade the CNS. Even though IFN γ -producing cells can induce EAE and their presence within the CNS likely contributed to acute inflammation, neutralization of IFN γ in *il25*^{-/-} mice did not lead to disease amelioration. In contrast, anti-IL-17 mAb treatment significantly inhibited EAE in *il25*^{-/-} mice, suggesting that the IL-17 immune pathway plays an important role in the disease process. We also demonstrate that IL-25 treatment protects mice from EAE, and this protection clearly depends on the suppression of IL-17-producing T cells. Thus, the presence of IL-25 supports a cytokine environment that limits chronic inflammatory responses.

IL-25 is constitutively expressed in the CNS (37). Interestingly, EAE-resistant BALB/c mice constitutively expressed higher CNS levels of IL-25 mRNA compared with moderately susceptible C57BL/6 (unpublished data). In addition, we found that resident microglia is a primary cellular source of IL-25 in a normal CNS. Importantly, there is a ninefold induction of IL-25 expression in microglia during CNS inflammation. This IL-25 expression pattern supports the hypothesis that microglia have protective roles during chronic CNS inflammation. The observation of tissue-specific expression of IL-25 is consistent with our recent study in a helminth infection model, where we showed that locally expressed IL-25 is crucial for the ability of mice to clear the gut parasite *Trichuris muris* (5). Moreover, IL-25 is also constitutively expressed in the lungs (1). Again, highly specialized tissue-resident cells, in this case, alveolar macrophages, were shown to be the source of IL-25 (4). Thus, IL-25 is expressed in organ systems where regulation of inflammation is of critical importance. In healthy digestive and respiratory tracts, an anti-inflammatory environment has to be maintained because of the constant exposure to commensal microbes. In immune-privileged sites, such as the eyes or the CNS, inflammation must be constrained because of the detrimental consequences of tissue swelling responses in these organs.

During EAE priming, IL-25 induces IL-4, IL-5, and IL-13 in the periphery. Although IL-4 and IL-13 have been reported to be protective in EAE, there is little evidence that IL-5 plays a regulatory role in EAE (38). In contrast, the role of IL-4 in suppressing organ-specific autoimmunity is well documented (29, 33, 39–41). IL-4 is required for the differentiation of Th cells toward the Th2 pathway (42, 43). In EAE, deviation of the immune system toward a Th2 response correlates with disease resistance (44, 45), and delivery of IL-4 *in vivo* can modulate the immune response to ameliorate disease (29, 46). Surprisingly, IL-25 treatment still protected *il4*^{-/-} mice from EAE, suggesting that the levels of IL-4 induced by IL-25 treatment are not sufficient for EAE suppression. In contrast to IL-4, we found that IL-13 is necessary for IL-25-mediated EAE suppression. Both IL-4R α - (required for IL-13 signaling) and IL-13-deficient mice were not protected by IL-25 treatment, suggesting that IL-25 is acting via IL-13 to suppress disease. On its own, IL-13 protects rats from EAE, through its ability to deactivate inflammatory macrophages, as well as suppressing the production of nitric oxide, IL-1 β , and TNF (27).

Here, we demonstrate a novel role for IL-13 in inhibiting Th17 differentiation and function. However, IL-13 can only partially inhibit IL-17 production. In contrast, IL-4 can completely block IL-17 response, consistent with two recent reports (21, 22). These results suggest that IL-13 and IL-4 may use different mechanisms to inhibit Th17 cells. Indeed, although T cells are shown to express the shared IL-4/IL-13 receptor chain IL-4R α , the specific IL-13 receptor is not expressed on T cells (35). Therefore, IL-13 might act by inhibiting DC function (27). Indeed, we observed that IL-13 blocked Th17-promoting factors such as IL-1 β , IL-6, and IL-23.

It is intriguing that in our Th17 cultures (DC-derived IL-6, IL-1 β , TGF- β plus exogenous IL-23, anti-IL-4, and anti-IFN γ), IL-25 still induced IL-13 production. We have observed that IL-25 led to IL-13 production in CD4⁺ T cell populations *in vitro* (unpublished data). However, it is still unclear which T cell populations are responsive to IL-25 *in vivo*. It is also possible that IL-25 acts on NKT cells, which play an important role in the early IL-4 production during an immune response (47, 48). NKT cells secrete IL-4, IL-13, and IFN γ upon activation and have an important immunoregulatory role (49, 50). Interestingly, activation of NKT cells by the synthetic ligand α -galactosylceramide can induce IL-4 and IL-13, which protects mice from autoimmune inflammation (51–53). Another possible target of IL-25 may be antigen-presenting cells such as DCs. The IL-25 receptor, IL-17RB, has been found to be up-regulated in DCs under Th2-inducing conditions (54). Consistent with previous reports (55, 56), we observed that IL-25 could induce IL-13 mRNA expression in cultured DCs (unpublished data). Further studies are needed to understand how these IL-13-expressing cells regulate Th17 development.

We have shown that although IL-25 and IL-17 are members of the same cytokine family, they play opposing roles in

the regulation of organ-specific autoimmunity. The type 2 responses promoted by IL-25 drive a novel regulatory mechanism for controlling Th17 responses. Unexpectedly, this regulation relies on IL-13 rather than IL-4, suggesting that IL-13 may be secreted at higher levels in the target organs during autoimmune inflammation. This protective mechanism is important because the lack of IL-25 causes devastating inflammatory responses during EAE. Thus, IL-25 expression by CNS-resident microglia may provide an important mechanism that limits brain and spinal cord inflammation. This unique property suggests that IL-25 may be a potential therapeutic agent for a range of organ-specific inflammatory disorders.

MATERIALS AND METHODS

Animals. *il25*^{-/-} mice were generated in the C57BL/6 background (Fig. S1). A C57BL/6J genomic BAC library (Genome Systems) was screened with a full-length mL-25 cDNA probe, and a positive clone was identified and used to generate a shotgun plasmid library (SeqWright). Subclones were sequenced and assembled to generate 15,747 bp of sequence encompassing the *il25* locus. Using a genomic subclone (F6), a BglII site was introduced in exon1 at the ATG via site-directed mutagenesis. This clone was digested with BglII, and a 5,442-bp 5' region of homology was cloned into the unique BglII site of pbgal-basic (CLONTECH Laboratories, Inc.). A 1,049-bp 3' region of homology was generated by PCR using a genomic subclone (C6) incorporating unique SpeI and NotI restriction sites and cloned into pBS-lox-Neo-lox. This plasmid was digested with SalI, and the lox-Neo-lox-3' region of homology was ligated into the unique SalI site of the 5' arm-pbgal-Basic, yielding the final targeting vector. The NheI-linearized targeting vector was electroporated into C57BL/6 ES cells, and Neo-resistant clones were analyzed for homologous recombination (HR) using a PCR-based screening strategy followed by Southern blot confirmation with 5' and 3' probes. A confirmed HR clone was electroporated with a Cre recombinase expression-containing plasmid, and several Neo-sensitive clones were screened using Southern blot to identify the Neo-flipped HR *il25-LacZ* ES line. This line was injected into C57BL/6 blastocysts, and *il25-LacZ-KI*^{-/-} progeny were obtained by intercrossing *il25-LacZ-KI*^{+/-} animals. A PCR-based genotyping strategy was developed to track the *il25* WT and knockin alleles. *il25*-targeted allele, sense, 5'-GCTGACTCTCAACATTCTACTCCTCC-3', and antisense, 5'-CCTGCTGCTTCAGGTAGGGCTTTG-3'; WT *il25* allele, sense, 5'-CTACAGACAGGCTCCACATGGACC-3', and antisense, 5'-CCTGCTGCTTCAGGTAGGGCTTTG-3'.

C57BL/6J and *il4*^{-/-} (C57BL/6-*Il4*^{tm1Nnt/J}) mice were obtained from The Jackson Laboratory. *il13*^{-/-}, *ifng*^{-/-}, and DO11.10 \times Rag^{-/-} mice were from the DNAX colonies. Breeding pairs of *il4ra*^{-/-} mice were obtained from F. Brombacher (University of Cape Town, Rondebosch, South Africa) and a colony maintained at DNAX. C57BL/6 MOG₃₃₋₅₅ TCR transgenic mice were obtained from T. Backstrom (Malaghan Institute of Medical Research, Wellington, New Zealand). Animals were maintained in a specific pathogen-free environment and tested negative for pathogens in routine screening. All experiments were performed following the guidelines of the DNAX Institutional Animal Care and Use Committee.

EAE induction. For studies in gene-deficient mice, we used the C57BL/6-MOG₃₃₋₅₅ model of EAE, as *il25*^{-/-}, *ifng*^{-/-}, *il4*^{-/-}, *il4ra*^{-/-}, and *il13*^{-/-} were bred on a C57BL/6 background. C57BL/6 mice were immunized with 100 μ g MOG₃₃₋₅₅ peptide and CFA containing 1 mg H37Ra s.c. on day 0 and 100 ng pertussis toxin (PTX) i.v. on day 0 and again on day 2. For disease-prevention studies in relapse-remitting EAE, SJL mice were immunized with 50 μ g PLP₁₃₉₋₁₅₁ plus CFA containing 200 μ g H37Ra s.c. on day 0. SJL mice were given 100 ng PTX on day 0 only. For passive transfer EAE, donor SJL mice were immunized with 50 μ g PLP₁₃₉₋₁₅₁ in CFA (no PTX adjuvant was used for the donor mice). 7–8 d after PLP priming, DLN cells were stimulated for 3–4 d with 20 μ g/ml PLP₁₃₉₋₁₅₁, and 2×10^7 viable cells

were transferred into naive recipient SJL mice. Clinical scores were as follows: tail weakness, grade 1; hind limb weakness, grade 2; partial hind limb paralysis and weight loss, grade 3; paraplegia and incontinence, grade 4; quadriplegia and wasting, grade 5; and moribund, grade 6.

IL-25, IL-25-rAdV, and antibody treatment. Recombinant mouse IL-25 protein was administered daily s.c., beginning at 1 d before MOG immunization. For administration into the CNS, rAdV gene transfer vectors were suspended in 10 μ l PBS and injected into the right-lateral cerebral ventricle using a 28-gauge needle, as described previously (33). For peripheral delivery, rAdVs suspended in 100 μ l PBS were injected into the tail vein. Antibodies for neutralization of IFN γ (clone XMG1.2, rIgG1; 200 μ g per mouse) and IL-17 (clone 1D10, rIgG1; 1 mg per mouse) and the corresponding isotype control (rat IgG1; 1 mg per mouse) were administered s.c. once a week starting 1 d before immunization.

Isolation of CNS inflammatory cells. C57BL/6 mice were immunized as described. At day 7 (before disease onset) and at peak disease (day 11 in the *il25*^{-/-} and day 14 in WT), mice were killed, and CNS-infiltrating T cells, macrophages, and resident microglia were isolated by digesting brain and spinal cord homogenate with collagenase and DNase followed by Percoll gradient centrifugation as described previously (57). We determined the number of CD4⁺CD45^{hi} T cells and CD4⁻CD11b⁺CD45^{hi} inflammatory macrophages in the CNS by multiplying the percentage of lineage marker-positive cells by the total number of mononuclear cells isolated from the CNS. For further characterization, intracellular cytokine staining was performed (see the following section). For analysis of IL-25 mRNA expression in the CNS, inflammatory macrophages and resident microglia were isolated from irradiation bone marrow chimeric mice produced by the injection of CD45.1 B6 bone marrow cells into irradiated CD45.2 C57BL/6 mice. The CD11b⁺CD45.1^{hi}CD45.2^{neg} inflammatory macrophages and CD11b⁺CD45.2^{lo}CD45.1^{neg} resident microglia (radiation-resistant cells remaining with the host CD45 allotype) were purified by three-color flow cytometric sorting from >100 irradiation bone marrow chimeric mice (11). Purified and sorted cells from naive or diseased animals were pooled (microglia or inflammatory macrophages) and analyzed by quantitative real-time PCR for IL-25.

Intracellular staining. CNS-infiltrating cells were obtained from MOG/CFA-immunized mice as described in the previous paragraph. Cells were stained with anti-CD4-FITC, fixed, and permeabilized with Cytofix/Cytoperm (BD Biosciences). Intracellular staining with antibodies against IL-17, IFN γ , and TNF (BD Biosciences) was performed and analyzed by flow cytometry. T cells were identified by gating on CD4. The number of cytokine-producing cells was calculated from the percentage of cytokine-positive CD4⁺ T cells. We determined the number of CD4⁺ T cells in the CNS by multiplying the percentage of lineage marker-positive cells by the total number of mononuclear cells isolated from the CNS. For intracellular cytokine staining of DLN cells, DLNs were taken at day 7 after immunization. After 3 d of culture with MOG, cells were incubated with IL-2 for 2 d. Cells were then incubated with PMA/ionomycin in the presence of Golgi-Plug (BD Biosciences) for 5 h, and intracellular staining was performed as described. Absolute cell numbers were obtained by multiplying relative numbers with total number in culture plate well. For intracellular Foxp3-staining, DLNs were taken at day 7 after immunization and processed immediately for CD4, CD25, and Foxp3 staining with the Mouse regulatory T cell staining kit (eBioscience).

Cytokine assays. Mice were immunized as described. DLNs (axillary, brachial, and inguinal) were isolated at day 7, before disease onset. Single-cell suspensions were plated at 6 \times 10⁶ cells/well in 24-well plates in the presence of 100 μ g/ml MOG₃₃₋₅₅ peptide in cRPMI. Supernatants were collected after 3 d of culture. Cells were rested for 4 d in the presence of 1–2 ng/ml IL-2. After 7 d, cells were restimulated with 10 μ g/ml α CD3 (plate bound) plus 5 μ g/ml α CD28, and supernatants were collected at

24 and 72 h. Alternatively, supernatants were collected at 48 h after restimulation. Cytokine production was analyzed by sandwich ELISA (IL-13 and IL-17 kits; R&D Systems) or cytometric bead array (Mouse Th1/Th2 cytokine CBA and Inflammation CBA kits; BD Biosciences). For the CBA assay, 50 μ l of supernatant was incubated with a mixture of beads coated with capture antibodies for IL-2, IL-4, IL-5, IFN γ , or TNF (for Th1/Th2 kit), or IL-6, IL-10, MCP-1, IFN γ , TNF, or IL-12p70 (for Inflammation kit). Addition of the PE-conjugated detection antibodies forms a sandwich complex. After 2 h of incubation and one wash, samples were analyzed by flow cytometry. Standard curves were generated from analysis of titrated cytokine standards (at least 10 serial dilutions) using the BD CBA analysis software, which runs on Excel (Microsoft). IL-13 and IL-17 in samples from in vitro Th17 assays were assessed using a multiplexing bead array (Linc) according to the manufacturer's recommendations. In brief, 25 μ l of supernatants were incubated with assay buffer, and fluorescent beads were conjugated to capture antibodies for the cytokines of interest. After addition of the detection antibody mix and PE-conjugated streptavidin, plates were read on a LUMINEX 100 machine (Luminex). Data were analyzed using Masterplex QT (MiraiBio) and GraphPad Prism (GraphPad).

Histological analysis. Spinal cords were taken at day 18 after immunization. Fixated tissue was embedded in paraffin wax, and 5- μ m-thick longitudinal sections were stained with hematoxylin and eosin. Lesions were counted, and each scored based on the size of the inflammatory infiltrate (score 1, <10 cells; score 3, 10–50 cells; score 5, >50 cells). The total number of lesions was assessed per section, and mean and SD were calculated per treatment group. Cumulative score per section was divided by total number of lesions to obtain a mean lesion score per section. Mean and SD were calculated per treatment group.

Quantitative real-time PCR. DLN were taken at day 7 after immunization from five mice per indicated group and processed in three different pools. Single-cell suspensions were prepared by mashing lymph nodes through a 70- μ m cell strainer (BD Biosciences). CNS leukocytes were prepared and purified as described. DC cultures were prepared as stated in the following section. Total RNA was prepared from frozen cell pellets using RNeasy columns (QIAGEN), and 5 μ g were subjected to treatment with DNase (Ambion) and reverse transcribed using Superscript II (Invitrogen) with oligo (dT)15 primers (Roche) and random hexamers (Promega). Cytokine-specific mRNA was measured by real-time quantitative PCR (Applied Biosystems). Gene expression levels were normalized to ubiquitin.

In vitro cultures. Spleens and lymph nodes were taken from naive DO11.10 \times RAG^{-/-} BALB/c mice, which only produce OVA peptide-specific naive T cells. Cells were also isolated from naive C57BL/6 MOG₃₃₋₅₅ TCR Tg (clone 2D2) mice or from WT C57BL/6 mice (for DC isolations). CD4⁺ as well as CD11c⁺ cells were purified by magnetic bead separation (MACS; Miltenyi Biotec) after collagenase digestion of tissue. For Th17 polarization, CD11c⁺ cells (5 \times 10⁵ cells per well in a 24-well plate) were activated with 0.5 ng/ml LPS and incubated with OVA peptide as well as neutralizing antibodies against IL-4 (BVD4-1D11; 10 μ g/ml) and IFN γ (XMG1.2; 10 μ g/ml) for 2 h before addition of CD4⁺ cells in a 1:1 ratio. 20 ng/ml IL-23 was added to expand Th17 cells. Depending on the experimental setup, 100 ng/ml IL-25, 100 ng/ml IL-3, or 10 μ g/ml anti-IL-13 were added to the cultures. Cultures with no cytokine addition served as a control. After 72 h, cells were incubated with 5 ng/ml IL-2 for an additional 48 h. Supernatants were then sampled for cytokine analysis, and cells were stimulated with PMA/ionomycin for 5 h in the presence of Golgi-Plug (BD Biosciences). Intracellular staining for IL-17 was performed as described. For DC cultures, 10⁶ CD11c⁺ DCs were plated in a 48-well plate, rested overnight, and incubated with or without IL-13 1 h before addition of LPS. Cells were harvested after 24 h and prepared for gene expression analysis.

Online supplemental material. Fig. S1 contains a schematic representation of the WT *il25* locus, the arms used for generating the targeted allele, and the

Neo-flipped-LacZ knockin allele. Fig. S2 shows similar numbers of CNS-infiltrating inflammatory macrophages and total T cells in WT versus *il25^{-/-}* mice at several time points after MOG-EAE induction. Table S1 summarizes the results of the analysis of spinal cord sections from IL-25-treated versus untreated EAE mice (experiment shown in Fig. 4 B) and confirms the enhanced resistance of IL-25-treated mice. Online supplemental material is available at <http://www.jem.org/cgi/content/full/jem.20061738/DC1>.

We thank Thomas Backstrom and Vijay Kuchroo for providing the MOG-TCR transgenic mice. We also thank Alissa Chackerian, Mandy McGeachy, and Brent McKenzie for helpful discussion.

SP Biopharma (formerly DNAX Research) is supported by the Schering-Plough Corporation. The authors have no other conflicting financial interests.

Submitted: 14 August 2006

Accepted: 7 December 2006

REFERENCES

- Fort, M.M., J. Cheung, D. Yen, J. Li, S.M. Zurawski, S. Lo, S. Menon, T. Clifford, B. Hunte, R. Lesley, et al. 2001. IL-25 induces IL-4, IL-5, and IL-13 and Th2-associated pathologies in vivo. *Immunity*. 15: 985–995.
- Hurst, S.D., T. Muchamuel, D.M. Gorman, J.M. Gilbert, T. Clifford, S. Kwan, S. Menon, B. Seymour, C. Jackson, T.T. Kung, et al. 2002. New IL-17 family members promote Th1 or Th2 responses in the lung: in vivo function of the novel cytokine IL-25. *J. Immunol.* 169:443–453.
- Ikeda, K., H. Nakajima, K. Suzuki, S. Kagami, K. Hirose, A. Suto, Y. Saito, and I. Iwamoto. 2003. Mast cells produce interleukin-25 upon Fc epsilon RI-mediated activation. *Blood*. 101:3594–3596.
- Kang, C.M., A.S. Jang, M.H. Ahn, J.A. Shin, J.H. Kim, Y.S. Choi, T.Y. Rhim, and C.S. Park. 2005. Interleukin-25 and interleukin-13 production by alveolar macrophages in response to particles. *Am. J. Respir. Cell Mol. Biol.* 33:290–296.
- Owyang, A.M., C. Zaph, E.H. Wilson, K.J. Guild, T. McClanahan, H.R. Miller, D.J. Cua, M. Goldschmidt, C.A. Hunter, R.A. Kastelein, and D. Artis. 2006. Interleukin 25 regulates type 2 cytokine-dependent immunity and limits chronic inflammation in the gastrointestinal tract. *J. Exp. Med.* 203:843–849.
- Fallon, P.G., S.J. Ballantyne, N.E. Mangan, J.L. Barlow, A. Dasvarma, D.R. Hewett, A. McGlorm, H.E. Jolin, and A.N. McKenzie. 2006. Identification of an interleukin (IL)-25-dependent cell population that provides IL-4, IL-5, and IL-13 at the onset of helminth expulsion. *J. Exp. Med.* 203:1105–1116.
- Pan, G., D. French, W. Mao, M. Maruoka, P. Risser, J. Lee, J. Foster, S. Aggarwal, K. Nicholes, S. Guillet, et al. 2001. Forced expression of murine IL-17E induces growth retardation, jaundice, a Th2-biased response, and multiorgan inflammation in mice. *J. Immunol.* 167:6559–6567.
- Kim, M.R., R. Manoukian, R. Yeh, S.M. Silbiger, D.M. Danilenko, S. Scully, J. Sun, M.L. DeRose, M. Stolina, D. Chang, et al. 2002. Transgenic overexpression of human IL-17E results in eosinophilia, B-lymphocyte hyperplasia, and altered antibody production. *Blood*. 100:2330–2340.
- Nakae, S., Y. Komiyama, A. Nambu, K. Sudo, M. Iwase, I. Homma, K. Sekikawa, M. Asano, and Y. Iwakura. 2002. Antigen-specific T cell sensitization is impaired in IL-17-deficient mice, causing suppression of allergic cellular and humoral responses. *Immunity*. 17:375–387.
- Kolls, J.K., and A. Linden. 2004. Interleukin-17 family members and inflammation. *Immunity*. 21:467–476.
- Cua, D.J., J. Sherlock, Y. Chen, C.A. Murphy, B. Joyce, B. Seymour, L. Lucian, W. To, S. Kwan, T. Churakova, et al. 2003. Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. *Nature*. 421:744–748.
- Murphy, C.A., C.L. Langrish, Y. Chen, W. Blumenschein, T. McClanahan, R.A. Kastelein, J.D. Sedgwick, and D.J. Cua. 2003. Divergent pro- and antiinflammatory roles for IL-23 and IL-12 in joint autoimmune inflammation. *J. Exp. Med.* 198:1951–1957.
- Langrish, C.L., Y. Chen, W.M. Blumenschein, J. Mattson, B. Basham, J.D. Sedgwick, T. McClanahan, R.A. Kastelein, and D.J. Cua. 2005. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J. Exp. Med.* 201:233–240.
- Cua, D.J., and R.A. Kastelein. 2006. TGF-beta, a 'double agent' in the immune pathology war. *Nat. Immunol.* 7:557–559.
- Weaver, C.T., L.E. Harrington, P.R. Mangan, M. Gavrieli, and K.M. Murphy. 2006. Th17: an effector CD4 T cell lineage with regulatory T cell ties. *Immunity*. 24:677–688.
- Dong, C. 2006. Diversification of T-helper-cell lineages: finding the family root of IL-17-producing cells. *Nat. Rev. Immunol.* 6:329–333.
- McKenzie, B.S., R.A. Kastelein, and D.J. Cua. 2006. Understanding the IL-23-IL-17 immune pathway. *Trends Immunol.* 27:17–23.
- Bettelli, E., Y. Carrier, W. Gao, T. Korn, T.B. Strom, M. Oukka, H.L. Weiner, and V.K. Kuchroo. 2006. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature*. 441:235–238.
- Mangan, P.R., L.E. Harrington, D.B. O'Quinn, W.S. Helms, D.C. Bullard, C.O. Elson, R.D. Hatton, S.M. Wahl, T.R. Schoeb, and C.T. Weaver. 2006. Transforming growth factor-beta induces development of the T(H)17 lineage. *Nature*. 441:231–234.
- Veldhoen, M., R.J. Hocking, C.J. Atkins, R.M. Locksley, and B. Stockinger. 2006. TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. *Immunity*. 24:179–189.
- Harrington, L.E., R.D. Hatton, P.R. Mangan, H. Turner, T.L. Murphy, K.M. Murphy, and C.T. Weaver. 2005. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat. Immunol.* 6:1123–1132.
- Park, H., Z. Li, X.O. Yang, S.H. Chang, R. Nurieva, Y.H. Wang, Y. Wang, L. Hood, Z. Zhu, Q. Tian, and C. Dong. 2005. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat. Immunol.* 6:1133–1141.
- Ivanov, I.I., B.S. McKenzie, L. Zhou, C.E. Tadokoro, A. Lepelletier, J.J. LaFaille, D.J. Cua, and D.R. Littman. 2006. The orphan nuclear receptor RORgamma directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell*. 126:1121–1133.
- Segal, B.M., and E.M. Shevach. 1996. IL-12 unmasks latent autoimmune disease in resistant mice. *J. Exp. Med.* 184:771–775.
- Leonard, J.P., K.E. Waldburger, and S.J. Goldman. 1995. Prevention of experimental autoimmune encephalomyelitis by antibodies against interleukin 12. *J. Exp. Med.* 181:381–386.
- O'Garra, A., L. Steinman, and K. Gijbels. 1997. CD4+ T-cell subsets in autoimmunity. *Curr. Opin. Immunol.* 9:872–883.
- Cash, E., A. Minty, P. Ferrara, D. Caput, D. Fradelizi, and O. Rott. 1994. Macrophage-inactivating IL-13 suppresses experimental autoimmune encephalomyelitis in rats. *J. Immunol.* 153:4258–4267.
- Cua, D.J., R.L. Coffman, and S.A. Stohlman. 1996. Exposure to T helper 2 cytokines in vivo before encounter with antigen selects for T helper subsets via alterations in antigen-presenting cell function. *J. Immunol.* 157:2830–2836.
- Shaw, M.K., J.B. Lorens, A. Dhawan, R. DalCanto, H.Y. Tse, A.B. Tran, C. Bonpane, S.L. Eswaran, S. Broccke, N. Sarvetnick, et al. 1997. Local delivery of interleukin 4 by retrovirus-transduced T lymphocytes ameliorates experimental autoimmune encephalomyelitis. *J. Exp. Med.* 185:1711–1714.
- Schif-Zuck, S., J. Westermann, N. Netzer, Y. Zohar, M. Meiron, G. Wildbaum, and N. Karin. 2005. Targeted overexpression of IL-18 binding protein at the central nervous system overrides flexibility in functional polarization of antigen-specific Th2 cells. *J. Immunol.* 174:4307–4315.
- O'Garra, A., and N. Arai. 2000. The molecular basis of T helper 1 and T helper 2 cell differentiation. *Trends Cell Biol.* 10:542–550.
- Zhou, M., and W. Ouyang. 2003. The function role of GATA-3 in Th1 and Th2 differentiation. *Immunol. Res.* 28:25–37.
- Cua, D.J., B. Hutchins, D.M. LaFace, S.A. Stohlman, and R.L. Coffman. 2001. Central nervous system expression of IL-10 inhibits autoimmune encephalomyelitis. *J. Immunol.* 166:602–608.
- Young, D.A., L.D. Lowe, S.S. Booth, M.J. Whitters, L. Nicholson, V.K. Kuchroo, and M. Collins. 2000. IL-4, IL-10, IL-13, and TGF-beta from an altered peptide ligand-specific Th2 cell clone down-regulate

- adoptive transfer of experimental autoimmune encephalomyelitis. *J. Immunol.* 164:3563–3572.
35. Graber, P., D. Gretener, S. Herren, J.P. Aubry, G. Elson, J. Poudrier, S. Lecoanet-Henchoz, S. Alouani, C. Losberger, J.Y. Bonnefoy, et al. 1998. The distribution of IL-13 receptor alpha1 expression on B cells, T cells and monocytes and its regulation by IL-13 and IL-4. *Eur. J. Immunol.* 28:4286–4298.
 36. Olsson, T. 1995. Cytokine-producing cells in experimental autoimmune encephalomyelitis and multiple sclerosis. *Neurology.* 45:S11–S15.
 37. Lee, J., W.H. Ho, M. Maruoka, R.T. Corpuz, D.T. Baldwin, J.S. Foster, A.D. Goddard, D.G. Yansura, R.L. Vandlen, W.I. Wood, and A.L. Gurney. 2001. IL-17E, a novel proinflammatory ligand for the IL-17 receptor homolog IL-17Rh1. *J. Biol. Chem.* 276:1660–1664.
 38. Weir, C., C.C. Bernard, and B.T. Backstrom. 2003. IL-5-deficient mice are susceptible to experimental autoimmune encephalomyelitis. *Int. Immunol.* 15:1283–1289.
 39. Falcone, M., A.J. Rajan, B.R. Bloom, and C.F. Brosnan. 1998. A critical role for IL-4 in regulating disease severity in experimental allergic encephalomyelitis as demonstrated in IL-4-deficient C57BL/6 mice and BALB/c mice. *J. Immunol.* 160:4822–4830.
 40. Bettelli, E., M.P. Das, E.D. Howard, H.L. Weiner, R.A. Sobel, and V.K. Kuchroo. 1998. IL-10 is critical in the regulation of autoimmune encephalomyelitis as demonstrated by studies of IL-10- and IL-4-deficient and transgenic mice. *J. Immunol.* 161:3299–3306.
 41. Cua, D.J., H. Groux, D.R. Hinton, S.A. Stohlman, and R.L. Coffman. 1999. Transgenic interleukin 10 prevents induction of experimental autoimmune encephalomyelitis. *J. Exp. Med.* 189:1005–1010.
 42. Le Gros, G., S.Z. Ben-Sasson, R. Seder, F.D. Finkelman, and W.E. Paul. 1990. Generation of interleukin 4 (IL-4)-producing cells in vivo and in vitro: IL-2 and IL-4 are required for in vitro generation of IL-4-producing cells. *J. Exp. Med.* 172:921–929.
 43. Seder, R.A., W.E. Paul, M.M. Davis, and B. Fazekas de St Groth. 1992. The presence of interleukin 4 during in vitro priming determines the lymphokine-producing potential of CD4+ T cells from T cell receptor transgenic mice. *J. Exp. Med.* 176:1091–1098.
 44. Nicholson, L.B., J.M. Greer, R.A. Sobel, M.B. Lees, and V.K. Kuchroo. 1995. An altered peptide ligand mediates immune deviation and prevents autoimmune encephalomyelitis. *Immunity.* 3:397–405.
 45. Cua, D.J., D.R. Hinton, and S.A. Stohlman. 1995. Self-antigen-induced Th2 responses in experimental allergic encephalomyelitis (EAE)-resistant mice. Th2-mediated suppression of autoimmune disease. *J. Immunol.* 155:4052–4059.
 46. Racke, M.K., A. Bonomo, D.E. Scott, B. Cannella, A. Levine, C.S. Raine, E.M. Shevach, and M. Rocken. 1994. Cytokine-induced immune deviation as a therapy for inflammatory autoimmune disease. *J. Exp. Med.* 180:1961–1966.
 47. Yoshimoto, T., and W.E. Paul. 1994. CD4pos, NK1.1pos T cells promptly produce interleukin 4 in response to in vivo challenge with anti-CD3. *J. Exp. Med.* 179:1285–1295.
 48. Stetson, D.B., M. Mohrs, R.L. Reinhardt, J.L. Baron, Z.E. Wang, L. Gapin, M. Kronenberg, and R.M. Locksley. 2003. Constitutive cytokine mRNAs mark natural killer (NK) and NK T cells poised for rapid effector function. *J. Exp. Med.* 198:1069–1076.
 49. Godfrey, D.I., H.R. MacDonald, M. Kronenberg, M.J. Smyth, and L. Van Kaer. 2004. NKT cells: what's in a name? *Nat. Rev. Immunol.* 4:231–237.
 50. Akbari, O., P. Stock, E. Meyer, M. Kronenberg, S. Sidobre, T. Nakayama, M. Taniguchi, M.J. Grusby, R.H. DeKruyff, and D.T. Umetsu. 2003. Essential role of NKT cells producing IL-4 and IL-13 in the development of allergen-induced airway hyperreactivity. *Nat. Med.* 9:582–588.
 51. Singh, A.K., M.T. Wilson, S. Hong, D. Olivares-Villagomez, C. Du, A.K. Stanic, S. Joyce, S. Sriram, Y. Koezuka, and L. Van Kaer. 2001. Natural killer T cell activation protects mice against experimental autoimmune encephalomyelitis. *J. Exp. Med.* 194:1801–1811.
 52. Jahng, A.W., I. Maricic, B. Pedersen, N. Burdin, O. Naidenko, M. Kronenberg, Y. Koezuka, and V. Kumar. 2001. Activation of natural killer T cells potentiates or prevents experimental autoimmune encephalomyelitis. *J. Exp. Med.* 194:1789–1799.
 53. Pal, E., T. Tabira, T. Kawano, M. Taniguchi, S. Miyake, and T. Yamamura. 2001. Costimulation-dependent modulation of experimental autoimmune encephalomyelitis by ligand stimulation of V alpha 14 NK T cells. *J. Immunol.* 166:662–668.
 54. Gratchev, A., J. Kzhyshkowska, K. Duperrier, J. Utikal, F.W. Velten, and S. Goerdts. 2004. The receptor for interleukin-17E is induced by Th2 cytokines in antigen-presenting cells. *Scand. J. Immunol.* 60:233–237.
 55. Wynn, T.A. 2003. IL-13 effector functions. *Annu. Rev. Immunol.* 21:425–456.
 56. Gordon, S. 2003. Alternative activation of macrophages. *Nat. Rev. Immunol.* 3:23–35.
 57. Sedgwick, J.D., S. Schwender, H. Imrich, R. Dorries, G.W. Butcher, and V. ter Meulen. 1991. Isolation and direct characterization of resident microglial cells from the normal and inflamed central nervous system. *Proc. Natl. Acad. Sci. USA.* 88:7438–7442.