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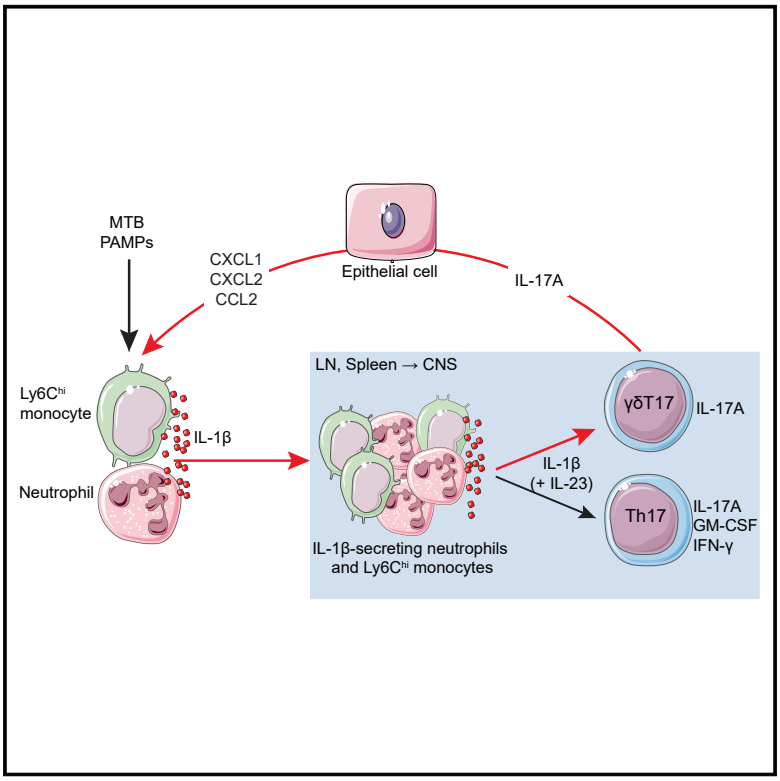
Immunity

Interleukin-17A Serves a Priming Role in Autoimmunity by Recruiting IL-1β-Producing Myeloid Cells that Promote Pathogenic T Cells

Graphical Abstract



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In Brief

Experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis, is an autoimmune disease in which the cytokine IL-17A is thought to mediate CNS tissue damage. McGinley et al. report a new role for IL-17A in mobilizing innate immune cells that secrete IL-1β, an inflammatory cytokine that primes encephalitogenic T cells.

Highlights

- IL-17A is required for priming but not effector function of Th17 cells in EAE
- IL-17A-defective mice are highly resistant to induction of EAE—rescued by IL-1β
- IL-17A acts in a positive feedback loop to induce IL-1β production early in EAE
- IL-17A-induced chemokines mobilize IL-1β-secreting neutrophils and monocytes

McGinley et al., 2020, Immunity 52, 342–356
February 18, 2020
https://doi.org/10.1016/j.immuni.2020.01.002

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Interleukin-17A Serves a Priming Role in Autoimmunity by Recruiting IL-1β-Producing Myeloid Cells that Promote Pathogenic T Cells

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https://doi.org/10.1016/j.immuni.2020.01.002

SUMMARY

Interleukin-17A (IL-17A) is a major mediator of tissue inflammation in many autoimmune diseases. Anti-IL-17A is an effective treatment for psoriasis and is showing promise in clinical trials in multiple sclerosis. In this study, we find that IL-17A-defective mice or mice treated with anti-IL-17A at induction of experimental autoimmune encephalomyelitis (EAE) are resistant to disease and have defective priming of IL-17-secreting γδ T (γδT17) cells and Th17 cells. However, T cells from *Il17a*$^{-/-}$ mice induce EAE in wild-type mice following *in vitro* culture with autoantigen, IL-1β, and IL-23. Furthermore, treatment with IL-1β or IL-17A at induction of EAE restores disease in *Il17a*$^{-/-}$ mice. Importantly, mobilization of IL-1β-producing neutrophils and inflammatory monocytes and activation of γδT17 cells is reduced in *Il17a*$^{-/-}$ mice. Our findings demonstrate that a key function of IL-17A in central nervous system (CNS) autoimmunity is to recruit IL-1β-secreting myeloid cells that prime pathogenic γδT17 and Th17 cells.

INTRODUCTION

Interleukin-17A (IL-17A), IL-17A-producing T helper-17 (Th17) cells, and IL-17A-producing γδ T (γδT17) cells are key mediators of inflammatory pathology and hence therapeutic targets in the treatment of many autoimmune diseases, including psoriasis, rheumatoid arthritis, and multiple sclerosis (MS). Antibodies that block IL-17 or its receptor are highly effective in the treatment of psoriasis, and promising results have emerged from an early clinical trial with the anti-IL-17A antibody, secukinumab, in patients with relapsing-remitting (RR) MS (Havrdová et al., 2016).

Studies in the mouse model for MS, experimental autoimmune encephalomyelitis (EAE), have suggested that IL-17A has a variety of proinflammatory effects in CNS autoimmunity. Th17 cells produce IL-17A and express RORγt; a deficiency in RORγt confers resistance to EAE (Ivanov et al., 2006). The development of Th17 cells from naive CD4^{+} T cells is directed by IL-6 in combination with transforming growth factor-β (TGF-β) (Bettelli et al., 2006; Mangan et al., 2006), and this process is enhanced by the presence of IL-1β (Veldhoen et al., 2006). IL-23 is required for their expansion and terminal differentiation into mature effector Th17 cells (McGeachy et al., 2009). Our group demonstrated a vital role for IL-1β in CNS autoimmunity (Sutton et al., 2006). IL-23 in synergy with IL-1β induces the secretion of IL-17A from Th17 cells and γδ T cells (Sutton et al., 2009).

IL-17A plays a crucial role in host defense against fungal and bacterial infection at mucosal surfaces through the induction of chemokines that recruit neutrophils and, with IL-22, the induction of anti-microbial peptides (Kumar et al., 2013; Mills, 2008). The function of IL-17A in autoimmunity is considered to involve activation of proinflammatory cytokines, chemokines, and matrix metalloproteinases in disease tissue, in particular in synergy with other cytokines, such as tumor necrosis factor (TNF) and IL-22 (Hirata et al., 2010; Park et al., 2005). IL-17A has been shown to drive the production of IL-1β, IL-6, TNF, and granulocyte macrophage-colony stimulating factor (GM-CSF) from fibroblasts (Fossiez et al., 1996) and epithelial cells (Ishigame et al., 2009), but also from macrophages (Jovanovic et al., 1998) and dendritic cells (DCs) (Sutton et al., 2009). Furthermore, IL-17-induced IL-1β production from T cells via activation of the ASC-NLRP3 inflammasome promotes Th17 cell proliferation and survival in the CNS (Martin et al., 2016). Finally, IL-17A induces chemokine expression, such as CCL2 (MCP-1) and CXCL2 (MIP-2) as well as CCL20, which recruits CCR6-expressing IL-17A-producing cells (Hirata et al., 2010), and can promote the expansion and survival of recruited neutrophils and monocytes through the induction of G-CSF and GM-CSF (Parsonage et al., 2008; Schwarzenberger et al., 2000).

Despite the overwhelming evidence that Th17 cells and γδT17 cells play pathogenic roles in many autoimmune diseases, the role of IL-17A in EAE is still controversial and poorly understood. It has been reported that mice defective in IL-17A are resistant to

EAE (Komiyama et al., 2006; Yang et al., 2008), but others have reported that absence of IL-17A has a minimal effect on the course of disease (Haak et al., 2009). Furthermore, overexpression of IL-17A in $CD4^+$ and $CD8^+$ T cells, or neutralization of IL-17A after the induction of EAE, has only a minor impact on the severity of disease (Haak et al., 2009; Hofstetter et al., 2005).

In this study, we demonstrate that IL-17A production by autoantigen-specific $CD4^+$ T cells is not essential for the development of EAE. Instead, we show that early IL-17A, derived mostly from $V\gamma4^+$ $\gamma\delta$ T cells, promotes chemokine induction that drives recruitment of IL-1β-producing neutrophils and $Ly6C^{hi}$ inflammatory monocytes to the draining lymph nodes (LNs), which in turn promotes the development of pathogenic $\gamma\delta$ T cells, Th17 cells, and autoimmunity. Our findings demonstrate that a key function of IL-17A in CNS autoimmunity is to mobilize IL-1β-secreting myeloid cells that prime pathogenic T cells.

RESULTS

IL-17A Is Required for Induction of Effector $CD4^+$ and $\gamma\delta$ T Cell Responses in EAE

The precise function of IL-17A in CNS autoimmunity is still unclear. Here, we found that *Il17a*$^{-/-}$ mice were resistant to the induction of EAE. The onset of clinical signs of MOG-induced EAE was delayed in *Il17a*$^{-/-}$ compared with C57BL/6 wild-type (WT) mice (Figure 1A). By day 20 of EAE, 100% of WT mice had developed severe disease, whereas only 35% of *Il17a*$^{-/-}$ mice showed only mild clinical signs. *Il17a*$^{-/-}$ mice had significantly reduced numbers of infiltrating $CD4^+$ and $\gamma\delta$ T cells in the spinal cord on day 12 after the induction of EAE (Figure 1B). Furthermore, there was also a significant reduction in numbers of IL-17A, GM-CSF, and interferon (IFN)-γ-secreting $CD4^+$ and $\gamma\delta$ T cells in the spinal cords of *Il17a*$^{-/-}$ compared with WT mice (Figure 1C and 1D). These results suggest that resistance to EAE in *Il17a*$^{-/-}$ mice is due to a failure of encephalitogenic T cells to infiltrate the CNS.

To analyze the role of IL-17A at different stages of EAE, we investigated the effect of neutralizing IL-17A at induction or effector stages of disease. Treatment with anti-IL-17A during the effector phase of EAE on days 10, 13, 15, and 18 (Figure 1E) or days 7, 10, 12, and 15 (Figure S1) did not affect disease severity. In contrast, mice treated with anti-IL-17A during the induction phase of EAE (days −1 and 2) had delayed and significantly attenuated clinical signs of disease (Figure 1F). Furthermore, mice treated with anti-IL-17A during the induction phase of EAE had reduced numbers of IL-17A, GM-CSF, and IFN-γ-secreting $CD4^+$ T cells in the spleen on day 8 of EAE (Figure 1G) and importantly, reduced induction of pathogenic polyfunctional $IL\text{-}17A^+IFN\text{-}\gamma^+$ and $IL\text{-}17A^+GM\text{-}CSF^+$ $CD4^+$ T cells (Figure 1H). These results suggest that IL-17A plays an essential early role in EAE by activation of encephalitogenic $CD4^+$ T cells.

IL-17A Primes Th17 Cells during Induction of EAE

To examine the possible role of IL-17A in the priming of Th17 cells, we examined Th17 cell responses under homeostatic conditions and after immunization with MOG and CFA (to induce EAE) or with a model antigen and adjuvant in *Il17a*$^{-/-}$ versus WT mice. Peripheral T cell activation was defective in *Il17a*$^{-/-}$ mice after the induction of EAE. The frequencies of IL-17A and IFN-γ-producing $CD4^+$ T cells and IL-17A, GM-CSF, and TNF-producing $\gamma\delta$ T cells were significantly reduced in the LN of *Il17a*$^{-/-}$ compared with WT mice on day 5 of EAE (Figure 2A). Furthermore, expression of RORγt was significantly reduced in $CD4^+$ and $\gamma\delta$ T cells from *Il17a*$^{-/-}$ mice (Figure 2B). In addition, mRNA expression of *Rorc* and *Tbx21*, master transcription factors for Th17 and Th1 cells, and *Itga4*, the alpha subunit of the integrin VLA-4 that drives T cell infiltration into the CNS in EAE, was significantly reduced in LN $CD4^+$ T cells from *Il17a*$^{-/-}$ compared with WT mice on day 5 of EAE (Figure 2C). By day 7 of EAE, the absolute numbers of $CD4^+$ and $\gamma\delta$ T cells were significantly reduced in the draining LN of *Il17a*$^{-/-}$ compared with WT mice (Figure S2A). Furthermore, the absolute numbers of $Ki67^+$ $CD4^+$ and $\gamma\delta$ T cells, and $ROR\gamma t^+$ $\gamma\delta$ T cells, but not $CD4^+$ T cells, were significantly reduced in *Il17a*$^{-/-}$ mice (Figure S2B). In addition, peripheral MOG-specific T cell responses were significantly lower in *Il17a*$^{-/-}$ mice 7 days after induction of EAE. Stimulation of LN and spleen cells with MOG induced significant proliferation of $CD4^+$ T cells from WT, but not *Il17a*$^{-/-}$ mice (Figure 2D). MOG-specific production of GM-CSF and IFN-γ was significantly reduced, whereas IL-17F production was enhanced in *Il17a*$^{-/-}$ mice (Figure 2E). These findings suggest a key role for IL-17A in the induction of autoantigen-specific T cells in EAE, but also suggest that IL-17F may compensate for or be regulated by IL-17A.

We next assessed if the defective MOG-specific Th17 cell response in *Il17a*$^{-/-}$ mice reflected a general defect in priming in these mice. IL-17F, GM-CSF, and IFN-γ production were not significantly reduced in *Il17a*$^{-/-}$ mice immunized with KLH in combination with c-di-GMP and LP1569 (Figure 2F), an adjuvant combination that drives potent Th1 and Th17 cell responses (Allen et al., 2018). This suggests that *Il17a*$^{-/-}$ mice do not have a general defect in T cell priming.

Under resting conditions WT and *Il17a*$^{-/-}$ mice had similar numbers of $CD4^+$ T cells (Figure 2G) and $V\gamma4^-$ ($V\gamma1.1^+$ and $V\gamma6^+$) $\gamma\delta$ T cells (Figure 2H) in the LNs. However, there was a significant reduction in the numbers of $V\gamma4^+$ $\gamma\delta$ T cells in the LNs of *Il17a*$^{-/-}$ mice (Figure 2I). The number of $CD27^-$ $V\gamma4^+$, but not $CD27^+$ $V\gamma4^+$, $\gamma\delta$ T cells was reduced in *Il17a*$^{-/-}$ mice and in *Il1r1*$^{-/-}$ mice (Figure 2J). $CD27^-$ $V\gamma4^+$ $\gamma\delta$ T cells produce IL-17A in response to IL-1β and IL-23, whereas $CD27^+$ $\gamma\delta$ T cells are predominantly IFN-γ-producing (Ribot et al., 2009). Furthermore, $V\gamma4^+$ $\gamma\delta$ T cells from *Il17a*$^{-/-}$ mice had reduced expression of CD44, CD25, Ki67, IL-17RA, and RORγt (Figures 2K and S2C). Collectively, these findings suggest that the defective induction of MOG-specific Th17 cells in *Il17a*$^{-/-}$ mice does not reflect an intrinsic defect in $CD4^+$ T cells in these mice, but may be related to a defect in activation of IL-17A-secreting $\gamma\delta$ T cells, which promote $CD4^+$ T cell responses.

IL-17A Production by Encephalitogenic Th17 Cells Is Not Essential for Development of EAE

To examine the role of IL-17A in the induction versus effector stage of EAE, we used the transfer model where MOG-specific T cells are generated in donor mice (and partly *in vitro*), and the effector phase of disease occurs in recipient mice. MOG-specific T cells from WT and *Il17a*$^{-/-}$ donor mice were expanded

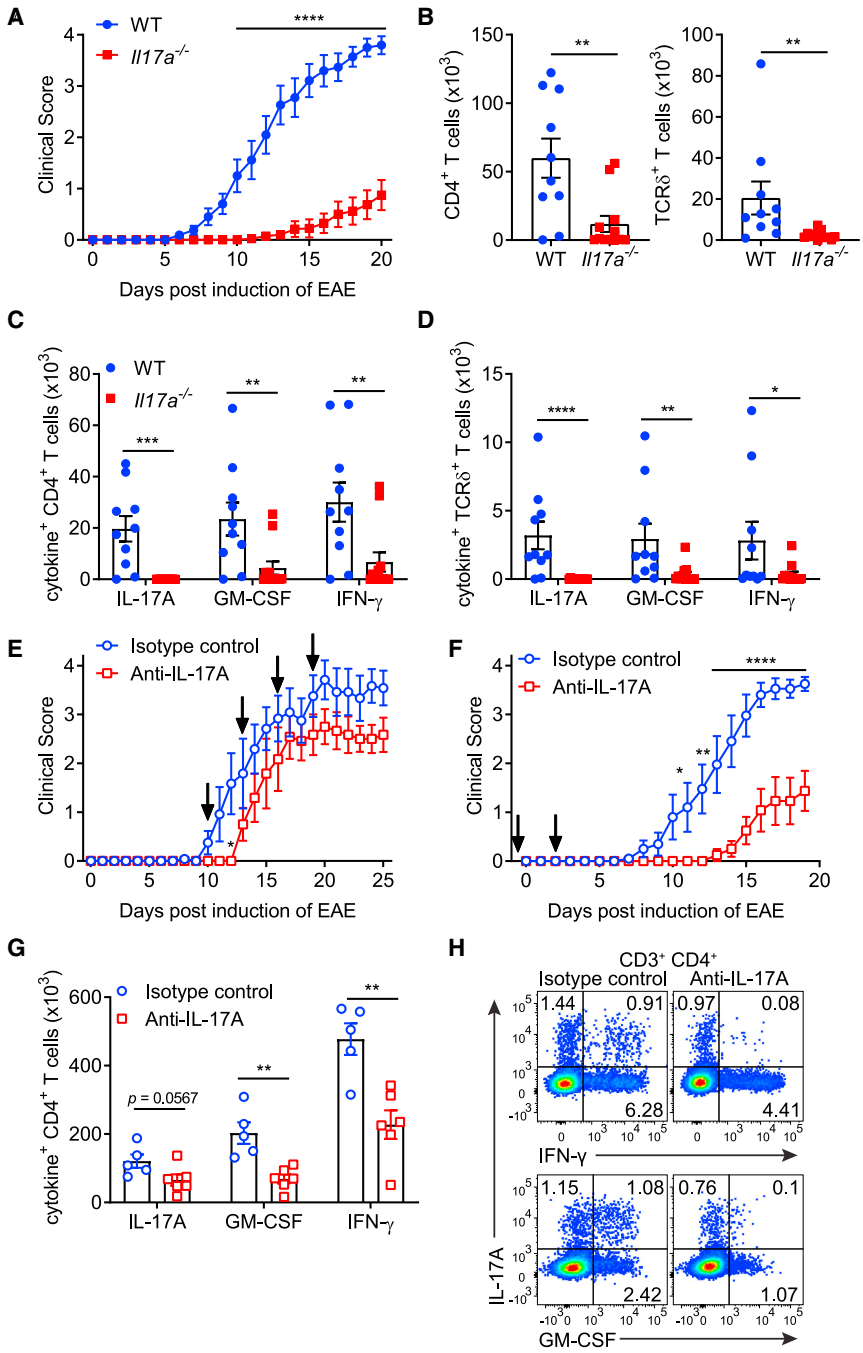


Figure 1. IL-17A Is Required for Induction of EAE and Associated Th17 and γδ T Cell Responses in the CNS

(A) EAE scores of WT and *Il17a*$^{-/-}$ mice (n = 20 or 21 combined from 4 experiments).

(B–D) Flow cytometric analysis of infiltrating CD4$^+$ and γδ T cells in the spinal cords of WT and *Il17a*$^{-/-}$ mice 12 days post induction of EAE (n = 10 or 12 combined from 2 experiments). (B) Absolute numbers of spinal cord-infiltrating CD4$^+$ and TCRδ$^+$ T cells. (C and D) Absolute numbers of IL-17A$^+$ GM-CSF$^+$ and IFN-γ$^+$ (C) CD4$^+$ and (D) TCRδ$^+$ T cells.

(E) EAE scores of WT mice injected i.p. with 200 μg of anti-IL-17A or isotype control antibody on days 10, 13, 16, and 19 of EAE (n = 6).

(F) EAE scores of WT mice injected i.p. with 200 μg of anti-IL-17A or isotype control antibody on days −1 and 2 of EAE (n = 10 or 12 combined from 2 experiments).

(G and H) Absolute numbers of IL-17A$^+$, GM-CSF$^+$, and IFN-γ$^+$ CD4$^+$ T cells in the spleens of mice on day 8 of EAE treated on days −1 and 2 with anti-IL-17A or isotype control antibody (n = 5 or 6) (G), with representative flow cytometry plots (H).

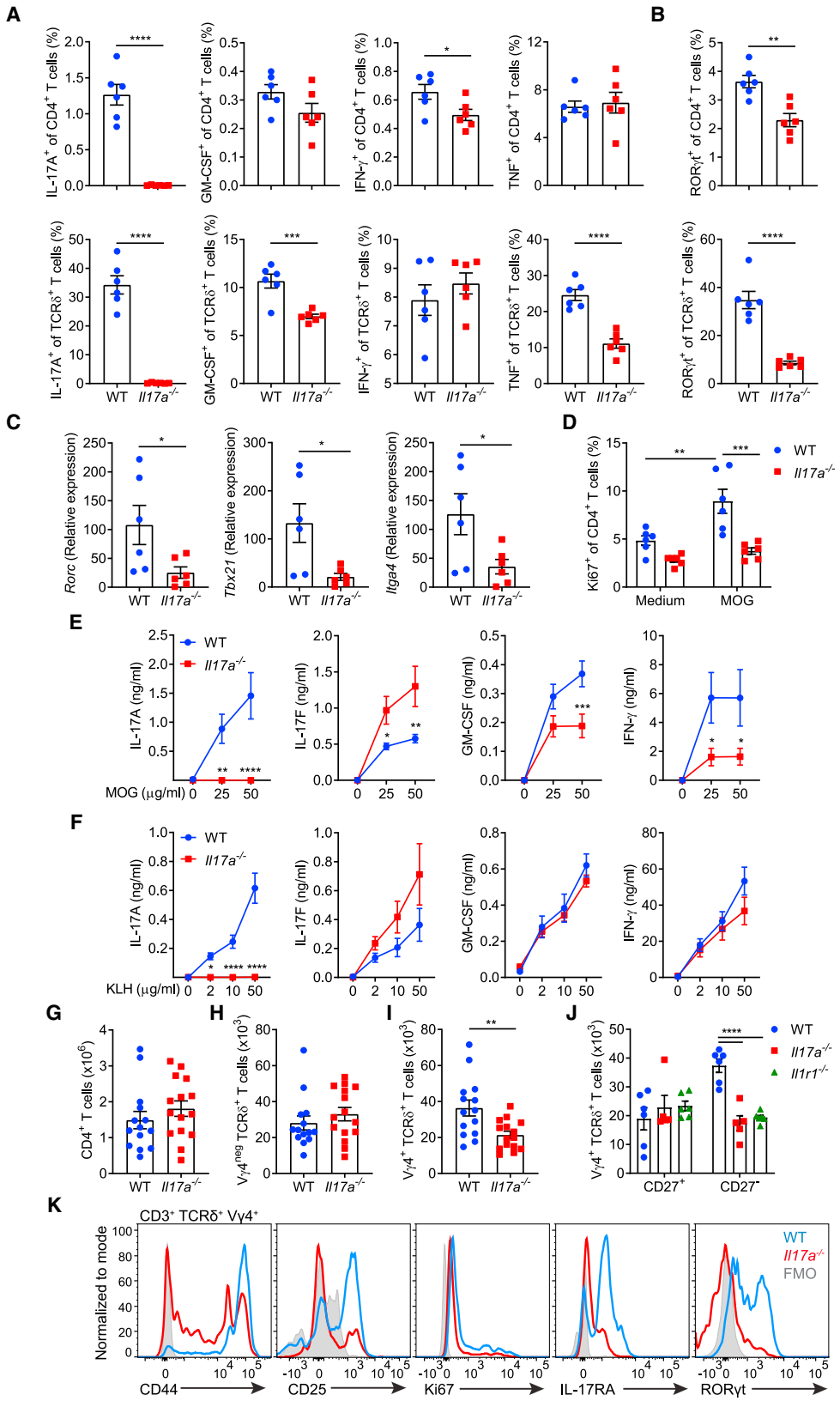
Data in (A–G) are mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 by two-way ANOVA with Sidak's post hoc analysis (A, E, and F), Mann-Whitney U test (B–D), or unpaired t test (G).

See also Figure S1.

in vitro with MOG alone or with MOG, IL-1β, and IL-23 and transferred into naive WT recipient mice. We found comparable concentrations of GM-CSF and IFN-γ in culture supernatants of MOG, IL-1β, and IL-23-stimulated spleen and LN cells from MOG-immunized *Il17a*$^{-/-}$ and WT mice (Figure 3A). Culture supernatants of *Il17a*$^{-/-}$ cells had an elevated concentration of MOG-specific IL-17F (Figure 3A), suggesting that IL-17F may compensate in the absence of IL-17A. There were no differences in expression of CD11a, CD49d, CD69, RORγt, IL-23R, or IL-1R1 on CD4$^+$ T cells from MOG-immunized WT and *Il17a*$^{-/-}$ mice after 24 h of culture with MOG or MOG, IL-1β, and IL-23 (Figure S3A). However, expression of CD69, RORγt, and IL-23R were lower on γδ T cells from *Il17a*$^{-/-}$ mice (Figure S3B).

We found that IL-17A production by encephalitogenic Th17 cells is not essential for the induction of CNS autoimmunity. T cells from *Il17a*$^{-/-}$ or WT mice stimulated with MOG, IL-1β, and IL-23 induced EAE with severe clinical signs (Figure 3B). No clinical signs of EAE were observed in mice injected with WT or *Il17a*$^{-/-}$ cells stimulated with MOG alone, highlighting the requirement for polarizing cytokines during the culture phase of passive EAE (Figure 3B). These results indicate that *in vitro* autoantigen re-stimulation in the presence of IL-1β and IL-23 can overcome the need for IL-17A during the induction phase of disease, suggesting that IL-17A may act in a positive feedback loop to promote production of IL-1β and/or IL-23.

An examination of infiltrating CD4$^+$ T cells in the brains of recipient mice revealed that while mice injected with MOG, IL-1β, and IL-23-stimulated *Il17a*$^{-/-}$ T cells had significantly reduced numbers of IL-17A-secreting CD4$^+$ T cells, they had significantly increased numbers of IL-17F, GM-CSF, and IFN-γ-producing CD4$^+$ T cells in the brain compared with mice injected with the equivalent WT cells (Figure 3C). This suggests that there may be redundancy between these cytokines in the



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effector phase of disease. To test this hypothesis, we used *Il17ra*$^{-/-}$ mice, which are unresponsive to IL-17A and IL-17F, as recipients in the passive transfer model of EAE. The severity of EAE was reduced in *Il17ra*$^{-/-}$ mice that acted as recipients for cells from *Il17a*$^{-/-}$ mice (Figure 3D). Treatment of recipient mice with anti-GM-CSF, following transfer of IL-1β and IL-23-stimulated MOG-specific *Il17a*$^{-/-}$ cells, had a modest effect on the course of disease (Figure 3E). These findings suggest that IL-17F and GM-CSF contribute to the pathogenicity of Th17 cells, but neither of these cytokines alone mediates all aspects of disease induced by T cells from *Il17a*$^{-/-}$ mice. It has also been demonstrated that Th1 cells can induce EAE by adoptive transfer. However, neutralization of IFN-γ in WT recipient mice, following transfer of IL-1β- and IL-23-stimulated MOG-specific *Il17a*$^{-/-}$ cells, resulted in a small attenuation of clinical scores early in disease, but had no effect from day 9 onward (Figure 3F). This is consistent with the finding that IFN-γ has a pathogenic role early but not late in disease (Dungan et al., 2014) and also suggests that IFN-γ does not compensate for the lack of IL-17A production in disease induced by cells from *Il17a*$^{-/-}$ mice.

Early IL-17 in EAE Produced by Vγ4$^+$ γδ T Cells Is Dependent on IL-17A

Our findings suggest that IL-17A promotes Th17 cell responses at the induction phase of EAE. We therefore examined the cellular source of IL-17A early after induction of EAE. IL-17A mRNA was detectable in the LN at 3–6 h and IL-17A protein at 72 h after induction of EAE (Figures 4A and 4B). Unlike Th17 cells, γδT17 cells can be activated during the early stages of inflammatory responses by IL-1β and IL-23 in the absence of T cell receptor (TCR) engagement (Sutton et al., 2009). Here, we demonstrate that CD4$^+$ and Vγ4$^+$ γδ T cells are the major IL-17-producing cells in the LN at 6 and 72 h post induction of EAE (Figure 4C). Expression of the early activation marker, CD69, was significantly increased on CD4$^+$ and Vγ4$^+$ γδ T cells 6 h after the induction of EAE (Figure 4D). Furthermore, Vγ4$^+$ γδ T cells infiltrate the brains of mice with EAE before CD4$^+$ T cells; significant numbers of Vγ4$^+$ γδ T cells were detected in the brains of mice at the onset and peak of clinical signs of EAE, whereas increased numbers of CD4$^+$ T cells were only observed at the peak of disease (Figure 4E). Depletion of Vγ4$^+$ γδ T cells during EAE delayed and attenuated disease (Figure 4F), emphasizing the key pathogenic function of these γδ T cells.

It has been reported that Vγ4$^+$ γδT17 cells are generated *de novo* in the LNs after the induction of EAE, from where they traffic directly to the CNS (Papotto et al., 2017). The frequency of proliferating Vγ4$^+$ γδ T cells was significantly lower in the LN of *Il17a*$^{-/-}$ compared with WT mice 24 h after induction of EAE (Figure 4G), and by day 3 of EAE there were significantly reduced numbers of Vγ4$^+$ γδ T cells in the draining LNs of *Il17a*$^{-/-}$ mice (Figure 4H). In contrast, the numbers of CD3$^+$, CD4$^+$, and Vγ4$^-$ γδ T cells in the LNs were similar in *Il17a*$^{-/-}$ and WT mice (Figure S4A). *Il17a*$^{-/-}$ mice had significantly reduced numbers of TNF and GM-CSF-secreting Vγ4$^+$ γδ T cells in draining LNs on day 3 of EAE (Figure 4I and J). In contrast, we found no differences in cytokine production by Vγ4$^-$ γδ T cells (Figure S4B). These results demonstrate a critical role for IL-17A in activating pathogenic Vγ4$^+$ γδ T cells early in EAE.

IL-17A Is Required for Early IL-1β Production in the Development of EAE

We have demonstrated that resistance to EAE in *Il17a*$^{-/-}$ mice was associated with a significant reduction in effector CD4$^+$ and γδ T cell responses, but also found that T cells from MOG-immunized *Il17a*$^{-/-}$ mice can induce EAE passively following *in vitro* re-stimulation with MOG, IL-1β, and IL-23, which activates γδT17 cells and expands MOG-specific Th17 cells. Therefore, it is possible that *Il17a*$^{-/-}$ mice may be defective in IL-1β and/or IL-23 production required to activate γδ T cells and to expand Th17 cells *in vivo* after the induction of EAE. We found significantly reduced expression of *Il1b*, but not *Il23a*, in the draining LNs of *Il17a*$^{-/-}$ compared with WT mice 72 h after the induction of EAE (Figure 5A). Conversely, intraperitoneal (i.p.) administration of IL-17A induced significant expression of *Il1b*, but not *Il23a*, in the inguinal LNs of WT mice within 3 h (Figure 5B). Furthermore, expression of *Il1b* mRNA and IL-1β protein, but not *Il23a*, was significantly reduced in the brains of *Il17a*$^{-/-}$ compared with WT mice on day 10 of EAE (Figures 5C and 5D). These findings demonstrate a defect in IL-1β production in *Il17a*$^{-/-}$ mice, suggesting that IL-17A may promote IL-1β production during development of EAE, but do not rule out a role for IL-23. To address this hypothesis, we administered IL-1β and IL-23 to *Il17a*$^{-/-}$ mice during the induction phase of EAE and found that it restored their susceptibility to disease (Figure 5E). Furthermore, administration of IL-1β and IL-23 to naive mice enhanced production of IL-17A and GM-CSF by Vγ4$^+$ γδ T cells within 4 h (Figure S5A). Administration of IL-1β alone at the induction of

Figure 2. Defective Th17 Responses in *Il17a*$^{-/-}$ Mice Is Not Due to an Intrinsic Priming Defect

(A–C) WT and *Il17a*$^{-/-}$ mice were immunized with MOG and CFA and on day 5 LN cells were analyzed by flow cytometry (n = 6). (A) Frequency of IL-17A$^+$, GM-CSF$^+$, IFN-γ$^+$, and TNF$^+$ CD4$^+$ and γδ T cells. (B) Frequency of RORγt$^+$ CD4$^+$ and γδ T cells. (C) mRNA expression of *Rorc*, *Tbx21*, and *Itga4* in CD4$^+$ T cells purified by magnetic-activated cell sorting (MACS).

(D and E) Spleen and LN cells from WT and *Il17a*$^{-/-}$ mice on day 7 of EAE were stimulated for 72 h with medium or MOG peptide. (D) Frequency of Ki67$^+$ CD4$^+$ T cells (n = 6). (E) IL-17A, IL-17F, GM-CSF, and IFN-γ concentrations in supernatants by ELISA (n = 12, combined from two experiments).

(F) IL-17A, IL-17F, GM-CSF, and IFN-γ production by ELISA on KLH-stimulated LN cells from WT and *Il17a*$^{-/-}$ mice immunized in the footpad with KLH, c-di-GMP, and LP1569 (n = 10 or 11, combined from two experiments).

(G–I) Absolute numbers of (G) CD3$^+$ CD4$^+$, (H) CD3$^+$ TCRδ$^+$ Vγ4$^-$, and (I) CD3$^+$ TCRδ$^+$ Vγ4$^+$ T cells in the LN of naive WT and *Il17a*$^{-/-}$ mice (n = 14 or 15, combined from three experiments).

(J) Absolute numbers of CD27$^+$ and CD27$^-$ Vγ4$^+$ γδ T cells in the LN of naive WT, *Il17a*$^{-/-}$, and *Il1r1*$^{-/-}$ mice (n = 6).

(K) Representative flow cytometry plots of CD44, CD25, Ki67, IL-17RA, and RORγt expression on Vγ4$^+$ γδ T cells from the LN of naive WT and *Il17a*$^{-/-}$ mice (n = 9)

Data in (A–J) are mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001 ****p < 0.0001 by unpaired t test (A–C and I), two-way ANOVA with Tukey's (D), or Sidak's (E and F) post hoc analysis, or one-way ANOVA with Dunnett's post hoc analysis (J).

See also Figure S2.

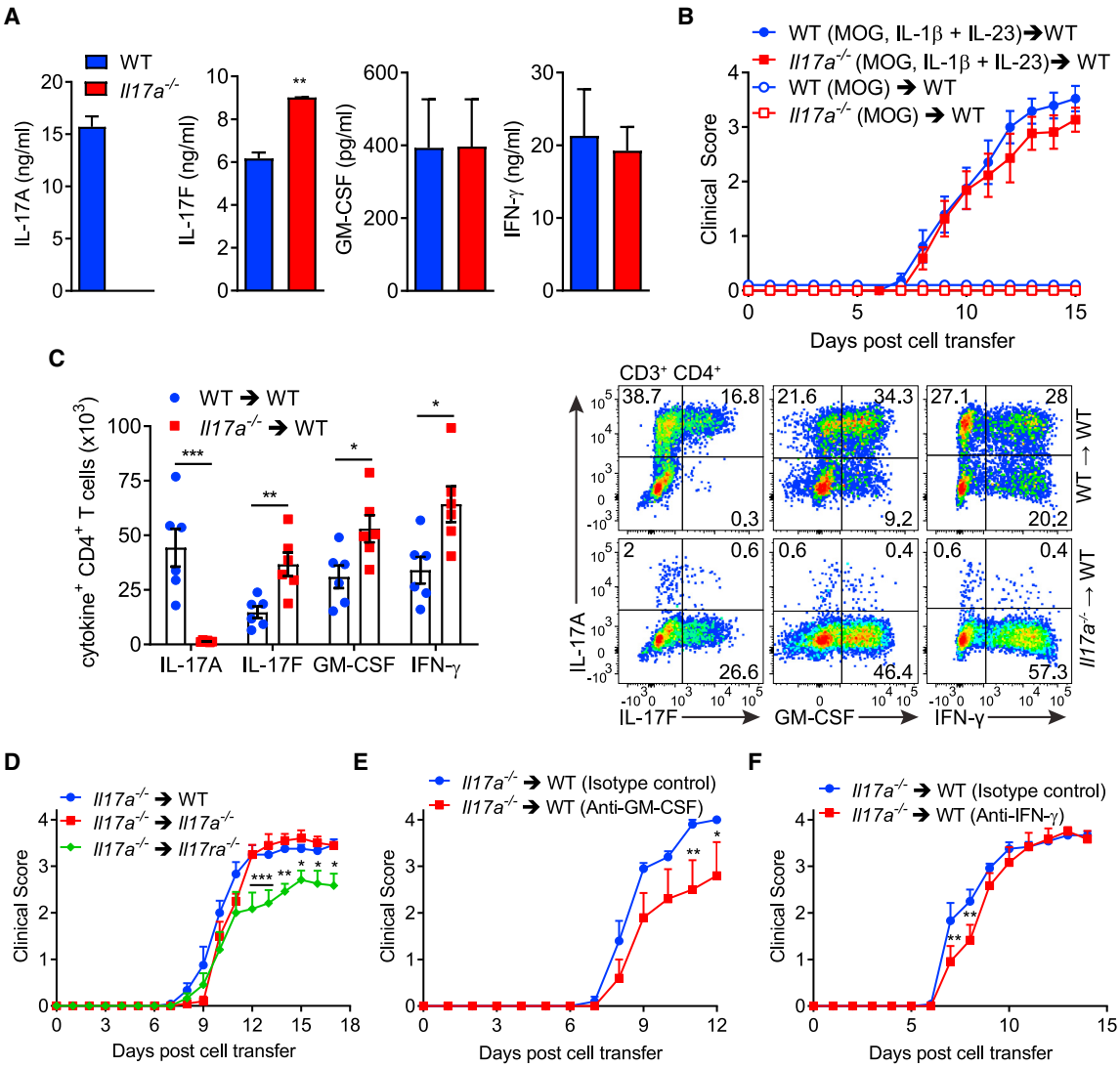


Figure 3. T Cells from *Il17a*$^{-/-}$ Mice Can Transfer EAE Following *In Vitro* Culture with MOG, IL-1β, and IL-23

(A–C) LN and spleen cells from WT or *Il17a*$^{-/-}$ mice 10 days after immunization with MOG and CFA were stimulated for 72 h *in vitro* with MOG (100 μg/mL), or with MOG, IL-1β, and IL-23 (both 10 ng/mL). (A) IL-17A, IL-17F, GM-CSF, and IFN-γ in supernatants by ELISA. (B) EAE scores of WT recipient mice post cell transfer (n = 11 or 12). (C) Absolute numbers of IL-17A$^+$, IL-17F$^+$, GM-CSF$^+$, and IFN-γ$^+$ CD4$^+$ T cells in the brains of recipient mice on day 14 post cell transfer with representative flow cytometry plots (n = 6).

(D) EAE scores of recipient WT, *Il17a*$^{-/-}$, or *Il17ra*$^{-/-}$ mice after adoptive transfer of LN and spleen cells from MOG-immunized *Il17a*$^{-/-}$ mice stimulated for 72 h with MOG, IL-23, and IL-1β (n = 5 or 6).

(E and F) EAE scores of recipient WT mice treated with isotype control antibodies or (E) anti-GM-CSF (300 μg) on days 0, 3, 6, and 9 or (F) anti-IFN-γ (500 μg) on days 0 and 7 after adoptive transfer of LN and spleen cells from MOG-immunized *Il17a*$^{-/-}$ mice that were stimulated for 72 h with MOG, IL-23, and IL-1β (n = 5 or 6).

Data in (A–F) are mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001 by unpaired t test (A and C) or two-way ANOVA with Sidak's post hoc analysis (D–F).

See also Figure S3.

EAE also restored susceptibility to disease in *Il17a*$^{-/-}$ mice (Figure 5F) and mildly exacerbated EAE in WT mice (Figure 5G). Moreover, administration of IL-17A to *Il17a*$^{-/-}$ mice at the induction of active EAE restored susceptibility to disease (Figure 5F), which argues against a T cell development defect in *Il17a*$^{-/-}$ mice. These findings support the data in Figure 1 that IL-17A plays a key role in the induction rather than effector phase of EAE and provide definitive evidence that failure to produce IL-1β early in disease explains the resistance to EAE in *Il17a*$^{-/-}$ mice.

We provide further evidence of a role for IL-1β in promoting encephalitogenic T cells by neutralization of IL-1β in active and passive EAE models. Administration of anti-IL-1β to WT recipient mice following adoptive transfer of MOG-specific T cells from WT donor mice significantly attenuated the severity of passive EAE (Figure 5H). This was associated with a significant reduction

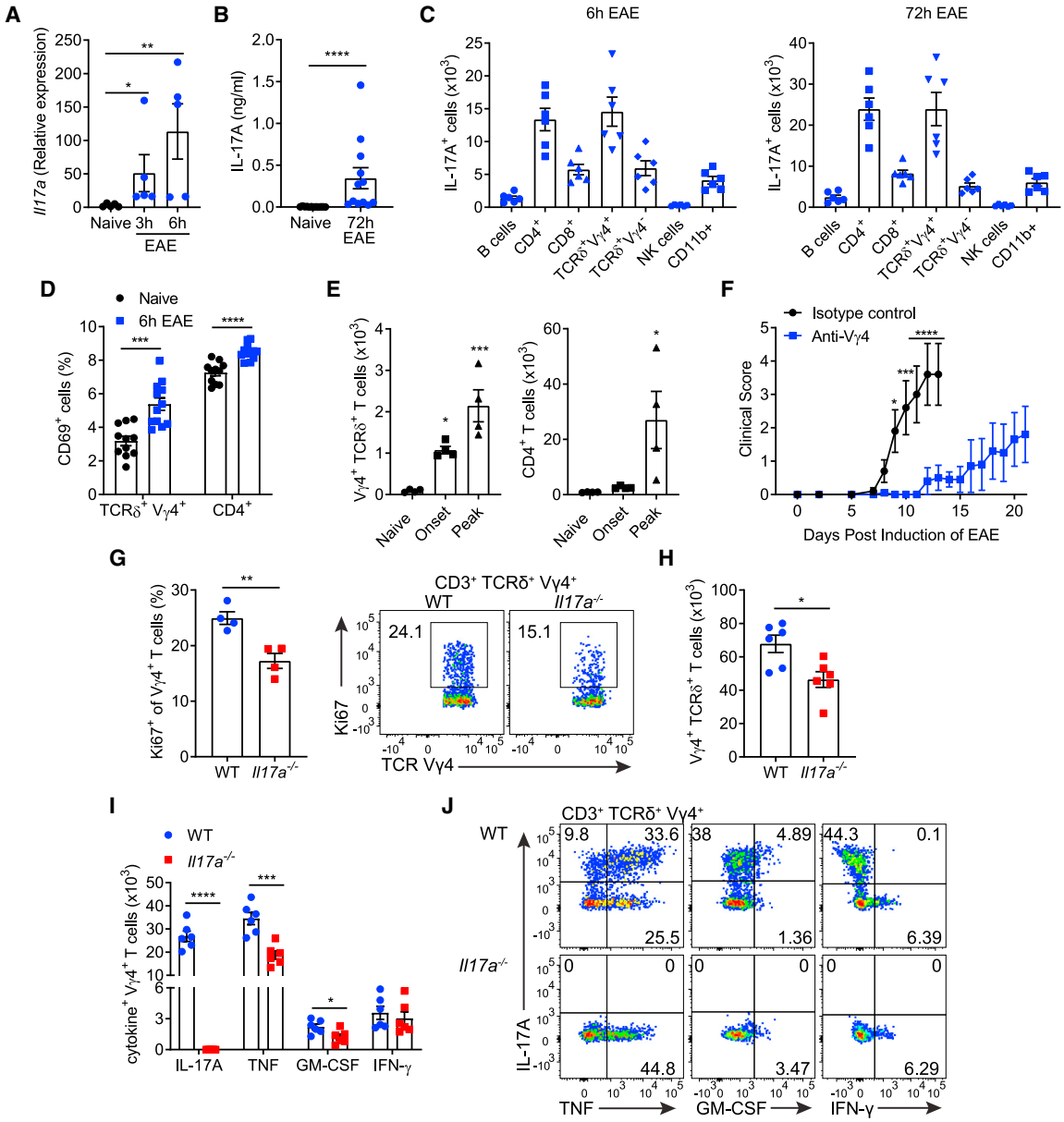


Figure 4. Vγ4$^+$ γδ T Cells Are a Key Source of Early IL-17A in EAE

(A) *Il17a* mRNA expression in the LNs of WT mice 3 and 6 h post induction of active EAE (n = 5).

(B) IL-17A concentration by ELISA on LN cells from naive WT mice or mice with EAE (day 3) after 24 h culture (n = 12, combined from 2 experiments).

(C) Absolute numbers of IL-17A-producing B cells, CD4$^+$, CD8$^+$, Vγ4$^+$ γδ, and Vγ4$^-$ γδ T cells, natural killer (NK) cells and CD11b$^+$ cells in the LN 6 and 72 h after the induction of active EAE (n = 6).

(D) Frequency of CD69$^+$ Vγ4$^+$ γδ and CD4$^+$ T cells in the LN of naive mice or mice 6 h post induction of EAE (n = 11 or 12, combined from two experiments).

(E) Absolute numbers of infiltrating Vγ4$^+$ γδ and CD4$^+$ T cells in the brains of naive mice or mice on day 8 (onset) or day 14 (peak) of EAE (n = 4).

(F) EAE scores of WT mice treated with anti-Vγ4 antibody (250 μg) or an isotype control antibody on day −1 and every third day after induction of active EAE (n = 5)

(G) Frequency of Ki67$^+$ Vγ4$^+$ γδ T cells in LNs of *Il17a*$^{-/-}$ and WT mice 24 h after induction of EAE, with representative flow cytometry plots (n = 4).

(H–J) Absolute numbers of (H) CD3$^+$TCRδ$^+$Vγ4$^+$ cells and (I) IL-17A$^+$, TNF$^+$, GM-CSF$^+$, and IFN-γ$^+$ CD3$^+$TCRδ$^+$Vγ4$^+$ cells in LNs of mice on day 3 of EAE with (J) representative flow cytometry plots (n = 6).

Data in (A–I) are mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001 ****p < 0.0001 by Kruskal-Wallis test (A), Mann-Whitney *U* test (B), unpaired t test (D and G–I), one-way ANOVA (E), or two-way ANOVA with Sidak's post hoc analysis (F).

See also Figure S4.

in brain-infiltrating IL-17A, GM-CSF, and IFN-γ-secreting CD4$^+$ T cells (Figure 5I). Furthermore, treatment with anti-IL-1β only at the induction of active EAE (days −1 and +2) significantly delayed onset and reduced the severity of disease (Figure 5J). Finally, we found that mice treated with anti-IL-1β at induction of EAE have significantly reduced MOG-specific production of

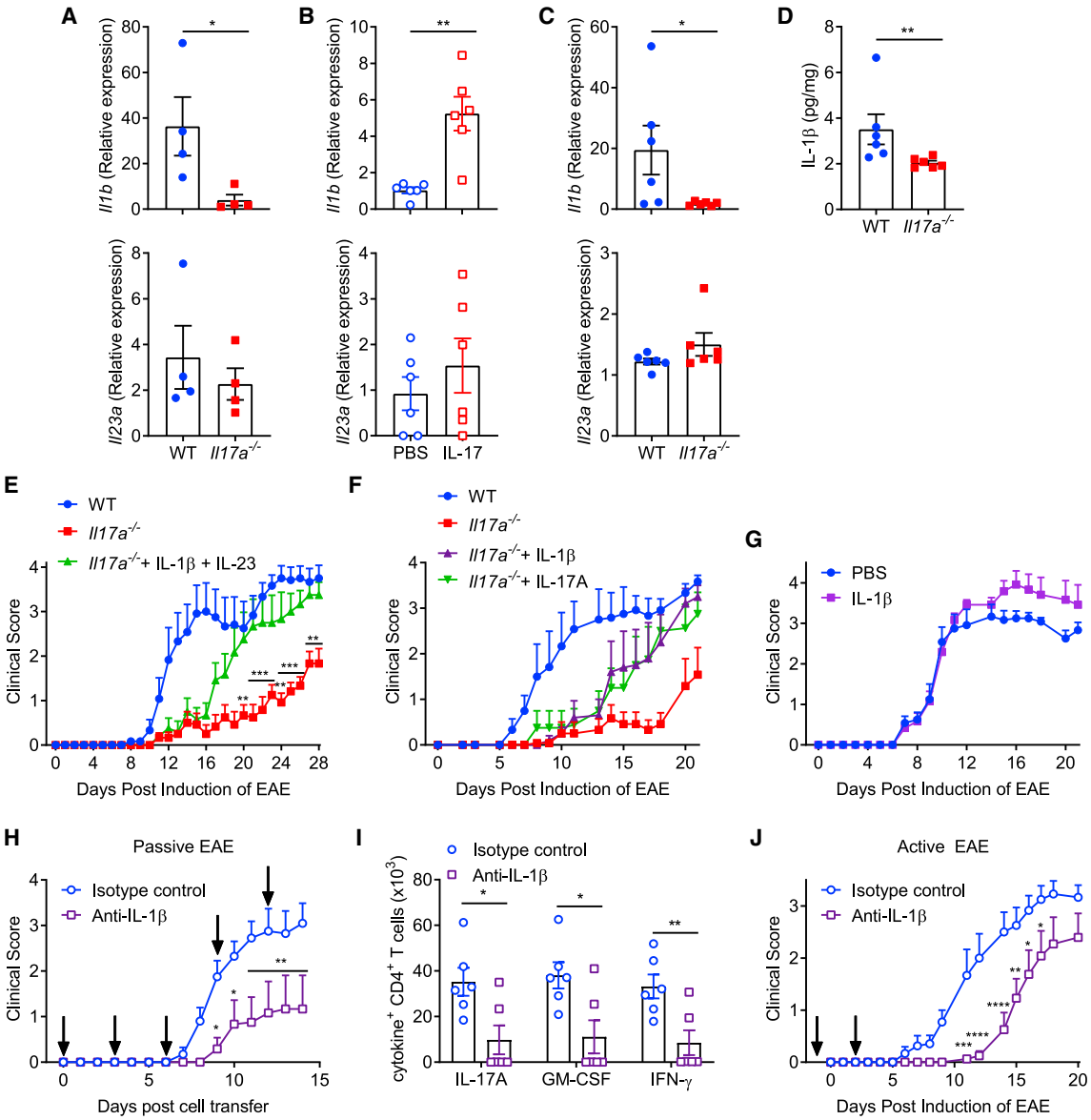


Figure 5. IL-17A Promotes Early IL-1β Production in the Development of EAE

(A–C) *Il1b* and *Il23a* mRNA expression in (A) LNs 72 h post induction of EAE (n = 4), (B) LNs of WT mice 3 hr post i.p. injection of IL-17A (500 ng/mouse) (n = 6), and (C) brains on day 10 of EAE in WT and *Il17a*$^{-/-}$ mice (n = 6) determined by qRT-PCR normalized to 18S rRNA and relative to *Il17a*$^{-/-}$ or PBS.

(D) IL-1β quantification by ELISA in the brains of WT and *Il17a*$^{-/-}$ mice on day 10 of EAE (n = 6)

(E) EAE scores of WT and *Il17a*$^{-/-}$ mice injected with PBS or IL-1β and IL-23 (200 ng of each) on days 0, 2, and 4 of EAE (n = 6).

(F) EAE scores of WT and *Il17a*$^{-/-}$ mice injected with PBS, IL-1β, or IL-17A (500 ng of each) 6 and 72 h post induction of EAE (n = 5 or 6).

(G) EAE scores of WT mice injected with PBS or IL-1β on days 0, 2, and 4 of EAE (n = 6).

(H and I) Passive EAE was induced in WT mice by transfer of T cells from MOG-immunized WT mice that were stimulated for 72 h with MOG (100 μg/mL), IL-1β (1 ng/mL), and IL-23 (10 ng/mL) (n = 6). Recipient mice were treated with anti-IL-1β or isotype control antibody (200 μg/mouse) on days 0, 3, 6, 9, and 12 after cell transfer. (H) EAE scores. (I) Absolute numbers of brain-infiltrating IL-17A^{+}, GM-CSF^{+}, or IFN-γ^{+} CD4^{+} T cells on day 14 of passive EAE.

(J) EAE scores of WT mice injected i.p. with 200 μg of anti-IL-1β or isotype control antibody (200 μg/mouse) on days −1 and 2 of active EAE (n = 12, combined from 2 experiments).

Data in (A–J) are mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 by Mann-Whitney *U* test (A–D and I), or two-way ANOVA with Sidak's post hoc analysis (E, H, and J).

See also Figure S5.

IL-17A and GM-CSF by spleen and LN cells on day 7 of EAE (Figure S5B). Collectively, our findings demonstrate that IL-17A, probably from γδ T cells, plays a vital role early in the development of EAE by activating a positive feedback loop that promotes IL-1β production required to activate encephalitogenic T cells.

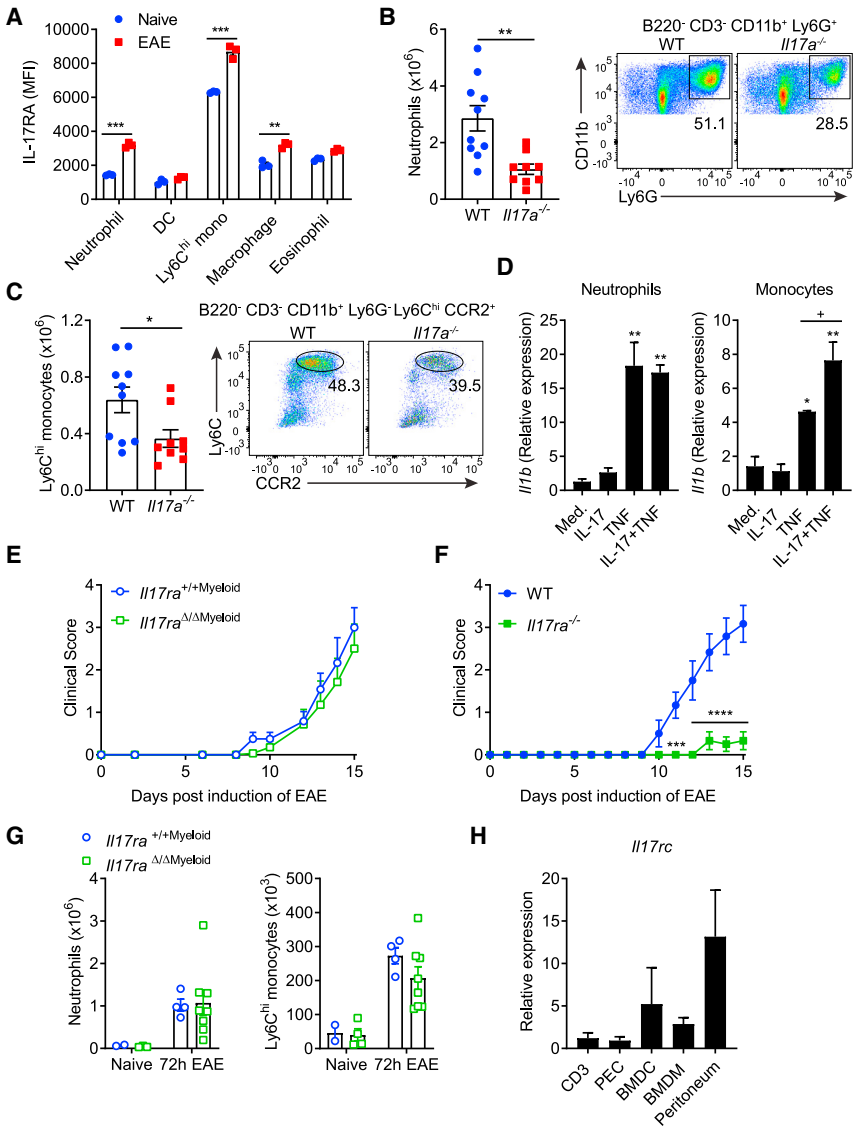


Figure 6. IL-17A Mobilizes but Does Not Directly Activate Neutrophils and Inflammatory Monocytes during EAE

(A) IL-17RA expression by flow cytometry on innate populations in the spleens of naive mice or on day 3 of EAE. Results are the median fluorescence intensity (MFI) (n = 3).

(B and C) Absolute numbers of neutrophils and (C) Ly6C^{hi} monocytes in the spleens of WT and *Il17a*$^{-/-}$ mice on day 3 of EAE, with representative flow cytometry plots (numbers are % of total CD11b^{+}) (n = 9 or 10 combined from 2 experiments).

(D) *Il1b* mRNA expression in neutrophils and monocytes from the bone marrow of naive C57BL/6 mice following stimulation with IL-17A (10 ng/mL) and/or TNF (10 ng/mL) evaluated by qRT-PCR normalized to 18S rRNA and relative to unstimulated cells. *p < 0.05, **p < 0.01 versus unstimulated, $^{+}$p < 0.05 versus TNF by one-way ANOVA.

(E) EAE scores of *Il17ra*$^{+/+Myeloid}$ and *Il17ra*$^{\Delta/\Delta Myeloid}$ (n = 6 or 7).

(F) EAE scores of WT and *Il17ra*$^{-/-}$ mice (n = 6).

(G) Absolute numbers of neutrophils and Ly6C^{hi} monocytes in the spleens of *Il17ra*$^{+/+Myeloid}$ and *Il17ra*$^{\Delta/\Delta Myeloid}$ mice before and 72 h after induction of EAE.

(H) *Il17rc* expression in T cells, peritoneal exudate cells (PEC), BMDC, BMDM, and peritoneal epithelial tissue evaluated by qRT-PCR normalized to 18S rRNA and relative to CD3^{+} T cells.

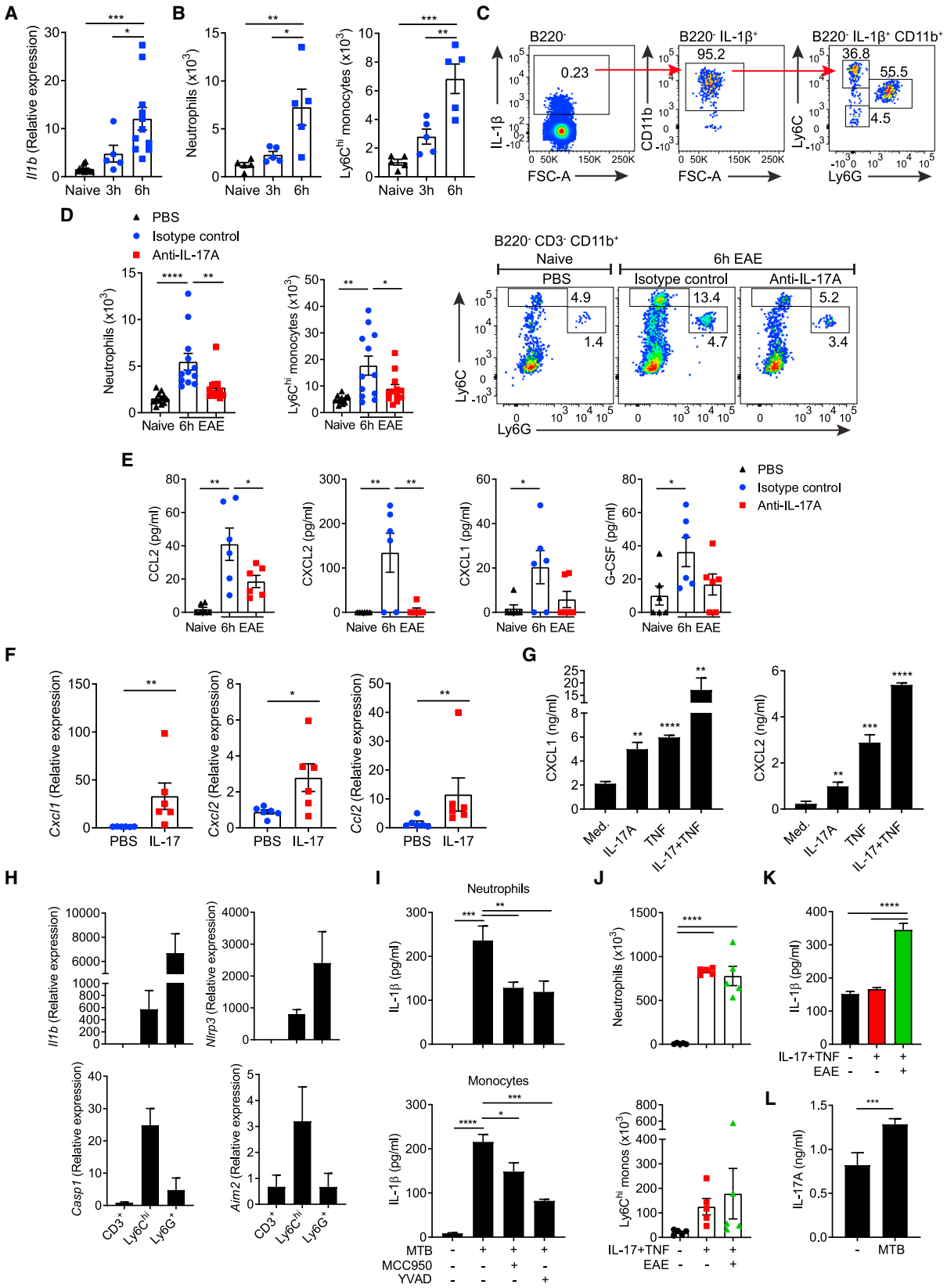
Data are mean ± SEM (A–C and E–G) or mean ± SD (D and H). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, by unpaired t test (A–C), or two-way ANOVA with Sidak's post hoc analysis (F).

See also Figure S6.

IL-17A Mobilizes but Does Not Act Directly on Neutrophils and Inflammatory Monocytes

Our findings demonstrate that IL-17A promotes early IL-1β production, which activates γδ T cells and Th17 cells in the induction of EAE. We examined the cellular targets for IL-17A and the possible source of IL-1β early in EAE. We found very high IL-17RA expression on Ly6C^{hi} inflammatory monocytes, which was significantly enhanced on day 3 of EAE (Figure 6A). Expression of IL-17RA was also significantly enhanced on neutrophils and macrophages early in EAE (Figure 6A). We found no significant difference in IL-17RA expression between *Il17a*$^{-/-}$ and WT mice on neutrophils, DCs, monocytes, macrophages, and eosinophils (Figure S6A). However, *Il17a*$^{-/-}$ mice had significantly reduced numbers of neutrophils and inflammatory Ly6C^{hi} monocytes, but not DCs, in the spleen 72 h after the induction of EAE (Figures 6B, 6C, and S6B). These findings suggest that neutrophils and monocytes may be a source of IL-1β early in EAE and that IL-17A may stimulate IL-1β production. However, IL-17A did not induce expression of *Il1b* mRNA in purified neutrophils or monocytes (Figure 6D). Stimulation with IL-17A in combination with TNF did promote expression of *Il1b* mRNA (Figure 6D), but not IL-1β protein (data not shown).

To further examine the possible role of IL-17A in activating myeloid cells during the induction of EAE, we utilized mice where IL-17RA was genetically ablated in myeloid cells, generated by crossing *Il17ra*-flox (El Malki et al., 2013) and *Lyz2*-cre mice, producing offspring, designated *Il17ra*$^{+/+Myeloid}$ and *Il17ra*$^{\Delta/\Delta Myeloid}$ (Gomes et al., 2016). IL-17RA was not expressed on CD11b^{+}, Ly6C^{hi}, or Ly6G^{+} cells in *Il17ra*$^{\Delta/\Delta Myeloid}$ mice, but was expressed on these cells from *Il17ra*$^{+/+Myeloid}$ mice, whereas expression of IL-17RA on CD11b^{-} cells was similar in *Il17ra*$^{\Delta/\Delta Myeloid}$ and *Il17ra*$^{+/+Myeloid}$ mice (Figure S6C). *Il17ra*$^{\Delta/\Delta Myeloid}$ mice were susceptible to the induction of EAE (Figure 6E). In contrast, *Il17ra*$^{-/-}$ mice, in which every cell is devoid of IL-17RA, were resistant to induction of EAE (Figure 6F). However, unlike *Il17a*$^{-/-}$ mice, recruitment of neutrophils and Ly6C^{hi} monocytes to the spleen (Figure 6G) and LN (Figure S6D) after the induction of EAE was not inhibited in mice with a myeloid-specific deletion of IL-17RA. These findings suggest that IL-17A signaling in myeloid cells is not essential for development of EAE, and IL-17A may act on other cell types, such as epithelial cells, which have high expression of IL-17RC (Figure 6H), to recruit myeloid cells that secrete IL-1β in response to another stimulus.



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IL-17A Mobilizes IL-1β-Producing Neutrophils and Ly6C^{hi} Inflammatory Monocytes Early in EAE

We examined the hypothesis that rather than inducing IL-1β production directly from myeloid cells, the function of IL-17A was to recruit IL-1β-producing myeloid cells during development of EAE. We found significantly increased expression of *Il1b* mRNA in LN 6 h after the induction of EAE (Figure 7A), and this corresponded with infiltration of neutrophils and inflammatory monocytes (Figure 7B). Furthermore, inflammatory monocytes and neutrophils were the predominant sources of IL-1β in LN 6 h after induction of EAE (Figure 7C). Importantly, recruitment of neutrophils and inflammatory monocytes to the LN 6 h after the induction of EAE was significantly reduced in mice treated with anti-IL-17A (Figure 7D) or in *Il17a*$^{-/-}$ mice (Figure S7A), indicating that early recruitment of these inflammatory innate populations is IL-17-dependent. In contrast, treatment with anti-IL-17A did not affect the absolute numbers of DCs in the LN of mice 6 h after the induction of EAE (Figure S7B). In the CNS, inflammatory monocytes were the dominant source of IL-1β on day 15 of EAE, with a smaller contribution from neutrophils (Figure S7C). Furthermore, numbers of neutrophils and inflammatory monocytes (Figure S7D) and importantly IL-1β-secreting neutrophils (Figure S7E) and inflammatory monocytes (Figure S7F) in the CNS during EAE was significantly reduced in *Il17a*$^{-/-}$ mice.

Expansion and recruitment of neutrophils is largely mediated by G-CSF, CXCL1, and CXCL2, whereas CCR2^{+} Ly6C^{hi} inflammatory monocytes are recruited via CCL2. We found that secretion of CXCL1, CXCL2, CCL2, and G-CSF was enhanced in the LN of mice 6 h after induction of EAE and was significantly reduced in mice treated with anti-IL-17A (Figure 7E). Furthermore, expression of *Ccl2*, *Cxcl1*, *Cxcl2*, and *Csf3* was significantly lower in the brains of *Il17a*$^{-/-}$ compared with WT mice on day 10 of EAE (Figure S7G).

Having shown that epithelial cells have high expression of IL-17RC, the limiting factor in IL-17 signaling, we examined the possibility that IL-17 drives chemokine production by epithelial cells. We found increased expression of *Ccl2*, *Cxcl1*, and *Cxcl2* in peritoneum tissue (Figure 7F) and LN cells (Figure S7H) of mice injected i.p. with IL-17A. Furthermore, mesothelial cells from squamous epithelium of the peritoneum of naive mice secreted CXCL1 and CXCL2 when stimulated with IL-17A, and this was enhanced by addition of TNF (Figure 7G). These findings uncover a key role for IL-17A in promoting production of chemokines, probably from epithelial cells, which recruit IL-1β-producing neutrophils and inflammatory monocytes, to activate IL-17 production from γδ T cells.

We next examined the stimulus for IL-1β production by myeloid cells early after induction of EAE, focusing on the possible effect of killed *Mycobacterium tuberculosis* (MTB), a component of CFA used for induction of EAE. In comparison to T cells, both neutrophils and Ly6C^{hi} monocytes had very high expression of *Il1b* and *Nlrp3* and expressed *Casp1* mRNA under homeostatic conditions (Figure 7H). Ly6C^{hi} monocytes from resting mice also had increased expression of *Aim2* mRNA (Figure 7H). Furthermore, neutrophils and inflammatory monocytes secreted mature IL-1β protein in response to MTB, and this was significantly reduced by co-incubation with MCC950, a small molecule inhibitor of the NLRP3 inflammasome (Coll et al., 2015), or ac-YVAD-cmk, an inhibitor of caspase-1 (Figure 7I). In contrast, MTB failed to induce production of IL-23 by neutrophils or monocytes (data not shown). However, DCs did secrete IL-23 as well as IL-1β when stimulated with MTB (Figure S7I).

We next demonstrated that injection of IL-17A with TNF recruited neutrophils and inflammatory monocytes into the peritoneal cavity of *Il17a*$^{-/-}$ mice (Figure 7J). IL-17A alone also recruited neutrophils, but this, like other activities of IL-17A by itself, was more pronounced and consistent when given with TNF (data not shown). Injection of IL-17A and TNF did not stimulate IL-1β protein production, but co-injection with MOG and CFA (as in induction of EAE) induced significant mature IL-1β protein production in the peritoneal cavity of *Il17a*$^{-/-}$ mice (Figure 7K). Finally, we demonstrated that IL-17A production by IL-23-stimulated γδ T cells was significantly enhanced by co-culture with MTB-stimulated IL-1β-producing neutrophils and monocytes (Figures 7L and S7J). These findings demonstrate that a key function of IL-17A in the induction of EAE is to recruit neutrophils and Ly6C^{hi}

Figure 7. IL-17A Mobilizes IL-1β-Secreting Neutrophils and Inflammatory Monocytes

(A) *Il1b* mRNA expression in the LN of WT mice 3 and 6 h post induction of EAE versus naive (n = 5 or 11, combined from two experiments).

(B) Absolute numbers of neutrophils and Ly6C^{hi} monocytes in the LN of WT mice 3 and 6 h post induction of EAE versus naive (n = 5).

(C) IL-1β-producing cells in the LN of WT mice 6 h post induction of EAE.

(D and E) WT mice were injected with PBS, anti-IL-17A or isotype control antibodies (200 μg/mouse) 12 h before the induction of EAE. (D) Absolute numbers of neutrophils and Ly6C^{hi} monocytes in the LN 6 h after the induction of EAE, with representative flow cytometry plots (n = 12, combined from two experiments). (E) CCL2, CXCL2, CXCL1 and G-CSF concentrations by ELISA on LN cell supernatants after overnight culture (n = 6, representative of two experiments).

(F) *Cxcl1*, *Cxcl2*, and *Ccl2* mRNA expression in peritoneal mesothelial tissue of WT mice 3 h after i.p. injection of PBS or IL-17A (500 ng/mouse), evaluated by qRT-PCR normalized to 18S rRNA and relative to PBS group (n = 5).

(G) CXCL1 and CXCL2 production by ELISA on mesothelial cells from the peritoneal tissue of naive C57BL/6 mice stimulated with IL-17A (500 ng/mL) and/or TNF (10 ng/mL).

(H) *Il1b*, *Nlrp3*, *Casp1*, and *Aim2* mRNA expression by flow cytometry sorted CD3^{+} T cells, Ly6C^{hi} monocytes, and Ly6G^{+} neutrophils from LN of naive C57BL/6 mice evaluated by qRT-PCR normalized to 18S rRNA and relative to CD3^{+} T cells.

(I) IL-1β production by ELISA on neutrophils and monocytes from the bone marrow of naive C57BL/6 mice following stimulation with MTB (50 μg/mL) ± MCC950 (1 μM) or YVAD (50 μM).

(J and K) *Il17a*$^{-/-}$ mice were injected i.p. with PBS or IL-17A (500 ng) + TNF (100 ng) with or without immunization with MOG and CFA (n = 5). (J) Absolute numbers of neutrophils and Ly6C^{hi} monocytes in PEC after 6 h. (K) IL-1β production by ELISA in supernatant of PEC after overnight culture.

(L) IL-17A production by ELISA on purified γδ T cells (LN), neutrophils, and monocytes (bone marrow) from naive C57BL/6 mice stimulated with IL-23 (10 ng/mL) ± MTB (50 μg/mL) for 18 h.

Data are mean ± SEM (A, B, D–F, and J) or mean ± SD (G–I, K, and L) *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 by one-way ANOVA (A, B, D, E, G, and I–K), Mann Whitney *U* test (F), or unpaired t test (L).

See also Figure S7.

inflammatory monocytes that are activated by MTB to produce mature IL-1β, which can synergize with IL-23 to promote activation of IL-17A-producing encephalitogenic T cells.

DISCUSSION

The significant finding of this study is that early IL-17A derived from γδ T cells is essential for recruitment of IL-1β-producing neutrophils and Ly6C^{hi} inflammatory monocytes that, together with IL-23, drive encephalitogenic T cells that mediate pathology in CNS autoimmunity. Prior to our study, the consensus view on the pathogenic role of IL-17A in autoimmune disease was that it promotes the production of inflammatory mediators from epithelial cells, such as keratinocytes, or fibroblasts in the diseased tissue (Cua and Tato, 2010; Mills, 2011). However, our findings suggest that the key function of IL-17A early in EAE is to mobilize innate immune cells that prime pathogenic T cells in the LNs. Furthermore, once Th17 cells are induced the role of IL-17A in the effector function of these cells becomes redundant. Therefore, in addition to acting as an effector cytokine that mediates the pathogenic activity of T cells in the CNS (Kang et al., 2010, 2013), our study suggests that IL-17A plays a crucial feedback role in the recruitment of IL-1β-producing myeloid cells required for further activation and expansion of γδT17 cells and subsequently Th17 cells in autoimmunity.

There is overwhelming evidence that Th17 cells and γδT17 cells play a pathogenic role in numerous autoimmune diseases, including psoriasis, MS, and rheumatoid arthritis (Jones et al., 2012; Mills, 2011; Miossec and Kolls, 2012). Although IL-17A is an important drug target in many human autoimmune diseases, and anti-IL-17 is highly effective in treating psoriasis, it is still not clear whether IL-17A mediates all the pathogenic activity of Th17 cells and γδT17 cells. Indeed, studies in the EAE model have generated conflicting data, with some suggesting that *Il17a*$^{-/-}$ mice have reduced disease (Komiyama et al., 2006; Yang et al., 2008) and another showing that overexpression of IL-17A in CD4$^+$ and CD8$^+$ T cells does not enhance disease (Haak et al., 2009). Our study provides unequivocal evidence that IL-17A is pathogenic in EAE. We demonstrate that IL-17-defective mice are highly resistant to EAE, but these mice also had reduced GM-CSF and IFN-γ production. Treatment of mice with anti-IL-17A also significantly attenuated EAE, but was only effective when administered at induction of disease, where it suppressed the generation of polyfunctional Th17 cells. Anti-IL-17A had little effect on the clinical course of disease when administered at the onset of clinical signs of EAE. Administration of IL-17A rescued the susceptibility of *Il17a*$^{-/-}$ mice to induction of EAE, which argues against a development defect in these mice. Furthermore, we found no evidence of a general CD4$^+$ T cell priming defect in *Il17a*$^{-/-}$ mice. However, we did see an intrinsic defect in CD27$^-$ Vγ4$^+$ γδT17 cells in *Il17a*$^{-/-}$ mice. The frequency of CD27$^-$ Vγ4$^+$ γδ T cells was also reduced in *Ilr1*$^{-/-}$ mice, suggesting that the defective priming of IL-17-secreting CD27$^-$ Vγ4$^+$ γδ T cells may be mediated through IL-1.

Transfer of T cells from MOG-immunized *Il17a*$^{-/-}$ mice, following culture with MOG, IL-1β, and IL-23 to specifically expand Th17 cells, induced EAE similar to that seen following transfer of T cells from WT mice. Autoantigen-specific Th1 cells are capable of inducing EAE, albeit with a different syndrome to Th17 cells (O'Connor et al., 2008). Furthermore, Th17 cells can acquire T-bet and the ability to produce IFN-γ, especially under the influence of IL-12 (Annunziato et al., 2007). GM-CSF, produced by Th1 and Th17 cells, was also shown to be a key pathogenic cytokine in EAE (Codarri et al., 2011; El-Behi et al., 2011). However, we found that neutralization of GM-CSF or IFN-γ in mice that were the recipients of T cells from MOG-immunized *Il17a*$^{-/-}$ mice only modestly reduced disease severity. Furthermore, T cells from *Il17a*$^{-/-}$ mice induced disease in mice lacking IL-17RA, which is also the receptor for IL-17F. It has been reported that IL-17F acts as a paracrine regulator of IL-17A production; IL-17F binding to IL-17RA suppresses IL-17A from T cells (Nagata et al., 2008). Furthermore, deletion of IL-17RA specifically in gut epithelium leads to enhanced Th17 responses during EAE (Kumar et al., 2016). Our findings suggest that Th17 cells do not need to secrete IL-17A to be pathogenic in EAE and this may not be explained simply by redundancy with Th1 or other Th17 cell-derived cytokines. It has been reported that while encephalitogenic T cells express IL-17A and IL-17F, these cytokines only marginally contribute to the development of autoimmune CNS disease; overexpression of IL-17A in CD4$^+$ and CD8$^+$ T cells did not enhance disease and treatment of *Il17f*$^{-/-}$ mice with anti-IL-17A from day 4 of EAE did not impact on the course of disease (Haak et al., 2009). We found that treatment of WT mice with anti-IL-17A at induction, but not at the effector stage of disease, inhibited development of EAE. Our study suggests a major function of IL-17A is to mobilize IL-1β-producing neutrophils and inflammatory monocytes early in EAE, which are essential for the activation of encephalitogenic γδ T cells and Th17 cells. Activation of T cells from MOG-immunized *Il17a*$^{-/-}$ mice *in vitro* with MOG, IL-1β, and IL-23 can overcome the requirement for IL-17A. Furthermore, treatment of *Il17a*$^{-/-}$ mice with IL-1β and IL-23 or IL-1β alone at induction of EAE conferred disease susceptibility.

We have previously reported that *Il1r1*$^{-/-}$ mice are highly resistant to EAE (Sutton et al., 2006). IL-1β synergizes with IL-23 to promote activation of IL-17A, IL-17F, GM-CSF, and IL-21 production by γδ T cells and memory Th17 cells (Lalor et al., 2011; Lukens et al., 2012; Sutton et al., 2009). The present study shows that CD4$^+$ and γδ T cell responses are significantly attenuated in *Il17a*$^{-/-}$ mice, and these mice have substantially reduced IL-1β production in the LN during the induction phase of EAE. Furthermore, treatment of *Il17a*$^{-/-}$ mice with IL-1β or IL-17A at the time of EAE induction restored their susceptibility to disease. Importantly, neutralization of IL-1β early in EAE or in recipient mice that received IL-17-secreting encephalitogenic T cells significantly attenuated EAE, suggesting that IL-1β is required early in EAE for the induction of γδT17 cells and for expansion of Th17 cells. This is consistent with the demonstration that IL-1β in the absence of IL-23 can promote expansion of Th17 cells (Chung et al., 2009). Collectively, these findings demonstrate that IL-17A functions early in the course of EAE to recruit innate immune cells that secrete inflammatory cytokines, especially IL-1β, that activate γδ T cells and eventually Th17 cells.

A key function of early IL-1β production in EAE is to promote activation of γδT17 cells and to amplify Th17 cells (Sutton et al., 2009). In the present study, we found that Vγ4$^+$ γδ T cells are a major source of IL-17A early in EAE, and these cells infiltrate the CNS of mice before encephalitogenic CD4$^+$ T cells. Furthermore, depletion of Vγ4$^+$ γδ T cells substantially

suppressed induction of Th17 cells and development of EAE. Proliferation and secretion of TNF and GM-CSF by Vγ4$^+$ $\gamma\delta$ T cells was significantly reduced in *Il17a*$^{-/-}$ mice after induction of EAE. These findings demonstrate that a major function of IL-17A is to mobilize innate immune cells that produce IL-1β, which together with IL-23 promotes the activation of $\gamma\delta$T17 cells.

We identified Ly6C^{hi} inflammatory monocytes and neutrophils as important IL-17A-recruited populations following the induction of EAE. We found that Ly6C^{hi} monocytes and neutrophils are key sources of IL-1β in the LN and CNS of mice during EAE and this is consistent with previous studies (Lévesque et al., 2016; Ronchi et al., 2016). We found that recruitment of neutrophils and Ly6C^{hi} monocytes to the LN of *Il17a*$^{-/-}$ mice or mice treated with anti-IL-17A was significantly reduced at 6 h post induction of EAE and this was associated with reduced induction of CXCL1, CXCL2, and CCL2. Reduced recruitment of these inflammatory populations was also observed in the spleen 3 days post induction of EAE and in the CNS on day 15 of EAE. IL-17A has previously been shown to regulate neutrophil expansion via systemic induction of G-CSF (Mei et al., 2012), and the importance of neutrophils in EAE is highlighted by studies where depletion of neutrophils delays and attenuates disease (Aubé et al., 2014; Steinbach et al., 2013). Infiltration of Ly6C^{hi} inflammatory monocytes into the CNS is critical in the pathogenesis of EAE (King et al., 2009; Mildner et al., 2009), and production of IL-17A by $\gamma\delta$ T cells selectively mobilizes a population of small peritoneal macrophages in a model of ovarian cancer (Rei et al., 2014). We found that i.p. administration of IL-17 to naive mice drove expression of CXCL1, CXCL2, and CCL2 not only in the peritoneal mesothelium, but also in the inguinal LNs, where increased expression of pro-IL-1β was also observed. In the context of EAE, we have demonstrated that IL-17A does not act directly on myeloid cells, but rather induces epithelial-derived chemokines that recruit neutrophils and inflammatory monocytes that produce IL-1β in response to MTB, the immunostimulatory component of CFA used to induce EAE.

Therapeutic targeting of the IL-23-IL-17A immune axis has become a major focus for the development of drugs for the treatment of autoimmune diseases. Antibodies that target IL-12p40, IL-23, IL-17RA, or IL-17A have shown remarkable success in the treatment of psoriasis (Campa et al., 2016), surpassing the efficacy of TNF inhibitors (Griffiths et al., 2010). The outcomes have been less clear cut in other autoimmune diseases, such as Crohn's disease, where anti-IL-12p40 showed some success (Feagan et al., 2016), but anti-IL-17A enhanced disease (Hueber et al., 2012). In contrast, anti-IL-12p40 was ineffective in the treatment of RR MS (Segal et al., 2008), but promising results have emerged from an early clinical trial with anti-IL-17A (Havrdová et al., 2016). Consistent with this, we show here that *Il17a*$^{-/-}$ mice are resistant to the induction of EAE, and treatment with anti-IL-17A attenuated disease. However, anti-IL-17A was most effective when given at induction of disease. These findings suggest that blocking IL-17A may be more effective in autoimmune diseases with a relapsing-remitting rather than progressive clinical course. It has also been argued that therapeutic antibodies that target inflammatory mediators at the site of inflammation may not be effective because of difficulty in penetrating epithelial surfaces or the blood-brain barrier in MS. Our findings suggest that anti-IL-17A may not need to get into inflamed tissue to exert its anti-inflammatory effect. Anti-IL-17A may at least in part function by inhibiting the feedback effect of IL-17A on innate immune responses that drive the activation of pathogenic $\gamma\delta$ T cells and Th17 cells in the periphery, thereby breaking the cycle of inflammation that mediates disease flares in autoimmune disorders, such as psoriasis and RR MS.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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Supplemental Information can be found online at https://doi.org/10.1016/j.immuni.2020.01.002.

ACKNOWLEDGMENTS

This work was supported by grants from Science Foundation Ireland (11/PI/1036, 16/IA/4468, and 12/RI/2340).

AUTHOR CONTRIBUTIONS

Conceptualization, K.H.G.M. and A.M.M.; Supervision and Funding Acquisition, K.H.G.M.; Methodology and Investigation, A.M.M., C.E.S., S.C.E., C.M.L., J.D., and A.T.; Writing – Original Draft, A.M.M. and K.H.G.M.; Writing – Review & Editing, C.E.S., S.C.E., N.D., J.A.H., and L.B.; Resources, J.A.H., L.B., and N.D.

DECLARATION OF INTERESTS

K.H.G.M. has acted as a consultant for pharmaceutical companies in the IL-17 area. None of the other authors have any competing interests to declare.

Received: May 3, 2018
Revised: April 19, 2019
Accepted: January 10, 2020
Published: February 4, 2020

REFERENCES

Allen, A.C., Wilk, M.M., Misiak, A., Borkner, L., Murphy, D., and Mills, K.H.G. (2018). Sustained protective immunity against Bordetella pertussis nasal colonization by intranasal immunization with a vaccine-adjuvant combination that induces IL-17-secreting T_{RM} cells. Mucosal Immunol. *11*, 1763–1776.

Annunziato, F., Cosmi, L., Santarlasci, V., Maggi, L., Liotta, F., Mazzinghi, B., Parente, E., Filì, L., Ferri, S., Frosali, F., et al. (2007). Phenotypic and functional features of human Th17 cells. J. Exp. Med. *204*, 1849–1861.

Aubé, B., Lévesque, S.A., Paré, A., Chamma, É., Kébir, H., Gorina, R., Lécuyer, M.-A., Alvarez, J.I., De Koninck, Y., Engelhardt, B., et al. (2014). Neutrophils mediate blood-spinal cord barrier disruption in demyelinating neuroinflammatory diseases. J. Immunol. *193*, 2438–2454.

Bettelli, E., Carrier, Y., Gao, W., Korn, T., Strom, T.B., Oukka, M., Weiner, H.L., and Kuchroo, V.K. (2006). Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature *441*, 235–238.

Bot, J., Whitaker, D., Vivian, J., Lake, R., Yao, V., and McCauley, R. (2003). Culturing mouse peritoneal mesothelial cells. Pathol. Res. Pract. *199*, 341–344.

Campa, M., Mansouri, B., Warren, R., and Menter, A. (2016). A Review of Biologic Therapies Targeting IL-23 and IL-17 for Use in Moderate-to-Severe Plaque Psoriasis. Dermatol. Ther. (Heidelb.) *6*, 1–12.

Chung, Y., Chang, S.H., Martinez, G.J., Yang, X.O., Nurieva, R., Kang, H.S., Ma, L., Watowich, S.S., Jetten, A.M., Tian, Q., and Dong, C. (2009). Critical regulation of early Th17 cell differentiation by interleukin-1 signaling. Immunity *30*, 576–587.

Codarri, L., Gyülvészi, G., Tosevski, V., Hesske, L., Fontana, A., Magnenat, L., Suter, T., and Becher, B. (2011). RORγt drives production of the cytokine GM-CSF in helper T cells, which is essential for the effector phase of autoimmune neuroinflammation. Nat. Immunol. *12*, 560–567.

Coll, R.C., Robertson, A.A., Chae, J.J., Higgins, S.C., Muñoz-Planillo, R., Inserra, M.C., Vetter, I., Dungan, L.S., Monks, B.G., Stutz, A., et al. (2015). A small-molecule inhibitor of the NLRP3 inflammasome for the treatment of inflammatory diseases. Nat. Med. *21*, 248–255.

Cua, D.J., and Tato, C.M. (2010). Innate IL-17-producing cells: the sentinels of the immune system. Nat. Rev. Immunol. *10*, 479–489.

Dungan, L.S., McGuinness, N.C., Boon, L., Lynch, M.A., and Mills, K.H. (2014). Innate IFN-γ promotes development of experimental autoimmune encephalomyelitis: a role for NK cells and M1 macrophages. Eur. J. Immunol. *44*, 2903–2917.

El-Behi, M., Ciric, B., Dai, H., Yan, Y., Cullimore, M., Safavi, F., Zhang, G.X., Dittel, B.N., and Rostami, A. (2011). The encephalitogenicity of T(H)17 cells is dependent on IL-1- and IL-23-induced production of the cytokine GM-CSF. Nat. Immunol. *12*, 568–575.

El Malki, K., Karbach, S.H., Huppert, J., Zayoud, M., Reissig, S., Schüler, R., Nikolaev, A., Karram, K., Münzel, T., Kuhlmann, C.R., et al. (2013). An alternative pathway of imiquimod-induced psoriasis-like skin inflammation in the absence of interleukin-17 receptor a signaling. J. Invest. Dermatol. *133*, 441–451.

Feagan, B.G., Sandborn, W.J., Gasink, C., Jacobstein, D., Lang, Y., Friedman, J.R., Blank, M.A., Johanns, J., Gao, L.-L., Miao, Y., et al.; UNITI–IM-UNITI Study Group (2016). Ustekinumab as Induction and Maintenance Therapy for Crohn's Disease. N. Engl. J. Med. *375*, 1946–1960.

Fossiez, F., Djossou, O., Chomarat, P., Flores-Romo, L., Ait-Yahia, S., Maat, C., Pin, J.J., Garrone, P., Garcia, E., Saeland, S., et al. (1996). T cell interleukin-17 induces stromal cells to produce proinflammatory and hematopoietic cytokines. J. Exp. Med. *183*, 2593–2603.

Gomes, A.L., Teijeiro, A., Burén, S., Tummala, K.S., Yilmaz, M., Waisman, A., Theurillat, J.P., Perna, C., and Djouder, N. (2016). Metabolic Inflammation-Associated IL-17A Causes Non-alcoholic Steatohepatitis and Hepatocellular Carcinoma. Cancer Cell *30*, 161–175.

Griffiths, C.E.M., Strober, B.E., van de Kerkhof, P., Ho, V., Fidelus-Gort, R., Yeilding, N., Guzzo, C., Xia, Y., Zhou, B., Li, S., et al.; ACCEPT Study Group (2010). Comparison of ustekinumab and etanercept for moderate-to-severe psoriasis. N. Engl. J. Med. *362*, 118–128.

Haak, S., Croxford, A.L., Kreymborg, K., Heppner, F.L., Pouly, S., Becher, B., and Waisman, A. (2009). IL-17A and IL-17F do not contribute vitally to autoimmune neuro-inflammation in mice. J. Clin. Invest. *119*, 61–69.

Havrdová, E., Belova, A., Goloborodko, A., Tisserant, A., Wright, A., Wallstroem, E., Garren, H., Maguire, R.P., and Johns, D.R. (2016). Activity of secukinumab, an anti-IL-17A antibody, on brain lesions in RRMS: results from a randomized, proof-of-concept study. J. Neurol. *263*, 1287–1295.

Hirata, T., Osuga, Y., Takamura, M., Kodama, A., Hirota, Y., Koga, K., Yoshino, O., Harada, M., Takemura, Y., Yano, T., and Taketani, Y. (2010). Recruitment of CCR6-expressing Th17 cells by CCL 20 secreted from IL-1 β-, TNF-α-, and IL-17A-stimulated endometriotic stromal cells. Endocrinology *151*, 5468–5476.

Hofstetter, H.H., Ibrahim, S.M., Koczan, D., Kruse, N., Weishaupt, A., Toyka, K.V., and Gold, R. (2005). Therapeutic efficacy of IL-17 neutralization in murine experimental autoimmune encephalomyelitis. Cell. Immunol. *237*, 123–130.

Hueber, W., Sands, B.E., Lewitzky, S., Vandemeulebroecke, M., Reinisch, W., Higgins, P.D.R., Wehkamp, J., Feagan, B.G., Yao, M.D., Karczewski, M., et al.; Secukinumab in Crohn's Disease Study Group (2012). Secukinumab, a human anti-IL-17A monoclonal antibody, for moderate to severe Crohn's disease: unexpected results of a randomised, double-blind placebo-controlled trial. Gut *61*, 1693–1700.

Ishigame, H., Kakuta, S., Nagai, T., Kadoki, M., Nambu, A., Komiyama, Y., Fujikado, N., Tanahashi, Y., Akitsu, A., Kotaki, H., et al. (2009). Differential roles of interleukin-17A and -17F in host defense against mucoepithelial bacterial infection and allergic responses. Immunity *30*, 108–119.

Ivanov, I.I., McKenzie, B.S., Zhou, L., Tadokoro, C.E., Lepelley, A., Lafaille, J.J., Cua, D.J., and Littman, D.R. (2006). The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell *126*, 1121–1133.

Jones, S.A., Sutton, C.E., Cua, D., and Mills, K.H. (2012). Therapeutic potential of targeting IL-17. Nat. Immunol. *13*, 1022–1025.

Jovanovic, D.V., Di Battista, J.A., Martel-Pelletier, J., Jolicoeur, F.C., He, Y., Zhang, M., Mineau, F., and Pelletier, J.-P. (1998). IL-17 stimulates the production and expression of proinflammatory cytokines, IL-β and TNF-α, by human macrophages. J. Immunol. *160*, 3513–3521.

Kang, Z., Altuntas, C.Z., Gulen, M.F., Liu, C., Giltiay, N., Qin, H., Liu, L., Qian, W., Ransohoff, R.M., Bergmann, C., et al. (2010). Astrocyte-restricted ablation of interleukin-17-induced Act1-mediated signaling ameliorates autoimmune encephalomyelitis. Immunity *32*, 414–425.

Kang, Z., Wang, C., Zepp, J., Wu, L., Sun, K., Zhao, J., Chandrasekharan, U., DiCorleto, P.E., Trapp, B.D., Ransohoff, R.M., and Li, X. (2013). Act1 mediates IL-17-induced EAE pathogenesis selectively in NG2+ glial cells. Nat. Neurosci. *16*, 1401–1408.

King, I.L., Dickendesher, T.L., and Segal, B.M. (2009). Circulating Ly-6C+ myeloid precursors migrate to the CNS and play a pathogenic role during autoimmune demyelinating disease. Blood *113*, 3190–3197.

Komiyama, Y., Nakae, S., Matsuki, T., Nambu, A., Ishigame, H., Kakuta, S., Sudo, K., and Iwakura, Y. (2006). IL-17 plays an important role in the development of experimental autoimmune encephalomyelitis. J. Immunol. *177*, 566–573.

Kumar, P., Chen, K., and Kolls, J.K. (2013). Th17 cell based vaccines in mucosal immunity. Curr. Opin. Immunol. *25*, 373–380.

Kumar, P., Monin, L., Castillo, P., Elsegeiny, W., Horne, W., Eddens, T., Vikram, A., Good, M., Schoenborn, A.A., Bibby, K., et al. (2016). Intestinal Interleukin-17 Receptor Signaling Mediates Reciprocal Control of the Gut Microbiota and Autoimmune Inflammation. Immunity *44*, 659–671.

Lalor, S.J., Dungan, L.S., Sutton, C.E., Basdeo, S.A., Fletcher, J.M., and Mills, K.H. (2011). Caspase-1-processed cytokines IL-1β and IL-18 promote IL-17 production by gammadelta and CD4 T cells that mediate autoimmunity. J. Immunol. *186*, 5738–5748.

Lévesque, S.A., Paré, A., Mailhot, B., Bellver-Landete, V., Kébir, H., Lécuyer, M.-A., Alvarez, J.I., Prat, A., de Rivero Vaccari, J.P., Keane, R.W., and Lacroix, S. (2016). Myeloid cell transmigration across the CNS vasculature

triggers IL-1β-driven neuroinflammation during autoimmune encephalomyelitis in mice. J. Exp. Med. *213*, 929–949.

Lukens, J.R., Barr, M.J., Chaplin, D.D., Chi, H., and Kanneganti, T.-D. (2012). Inflammasome-derived IL-1β regulates the production of GM-CSF by CD4(+) T cells and γδ T cells. J. Immunol. *188*, 3107–3115.

Mangan, P.R., Harrington, L.E., O'Quinn, D.B., Helms, W.S., Bullard, D.C., Elson, C.O., Hatton, R.D., Wahl, S.M., Schoeb, T.R., and Weaver, C.T. (2006). Transforming growth factor-β induces development of the T(H)17 lineage. Nature *441*, 231–234.

Martin, B.N., Wang, C., Zhang, C.J., Kang, Z., Gulen, M.F., Zepp, J.A., Zhao, J., Bian, G., Do, J.S., Min, B., et al. (2016). T cell-intrinsic ASC critically promotes T(H)17-mediated experimental autoimmune encephalomyelitis. Nat. Immunol. *17*, 583–592.

McGeachy, M.J., Chen, Y., Tato, C.M., Laurence, A., Joyce-Shaikh, B., Blumenschein, W.M., McClanahan, T.K., O'Shea, J.J., and Cua, D.J. (2009). The interleukin 23 receptor is essential for the terminal differentiation of interleukin 17-producing effector T helper cells in vivo. Nat. Immunol. *10*, 314–324.

Mei, J., Liu, Y., Dai, N., Hoffmann, C., Hudock, K.M., Zhang, P., Guttentag, S.H., Kolls, J.K., Oliver, P.M., Bushman, F.D., and Worthen, G.S. (2012). Cxcr2 and Cxcl5 regulate the IL-17/G-CSF axis and neutrophil homeostasis in mice. J. Clin. Invest. *122*, 974–986.

Mildner, A., Mack, M., Schmidt, H., Brück, W., Djukic, M., Zabel, M.D., Hille, A., Priller, J., and Prinz, M. (2009). CCR2+Ly-6Chi monocytes are crucial for the effector phase of autoimmunity in the central nervous system. Brain *132*, 2487–2500.

Mills, K.H. (2008). Induction, function and regulation of IL-17-producing T cells. Eur. J. Immunol. *38*, 2636–2649.

Mills, K.H. (2011). TLR-dependent T cell activation in autoimmunity. Nat. Rev. Immunol. *11*, 807–822.

Miossec, P., and Kolls, J.K. (2012). Targeting IL-17 and TH17 cells in chronic inflammation. Nat. Rev. Drug Discov. *11*, 763–776.

Nagata, T., McKinley, L., Peschon, J.J., Alcorn, J.F., Aujla, S.J., and Kolls, J.K. (2008). Requirement of IL-17RA in Con A induced hepatitis and negative regulation of IL-17 production in mouse T cells. J. Immunol. *181*, 7473–7479.

O'Connor, R.A., Prendergast, C.T., Sabatos, C.A., Lau, C.W., Leech, M.D., Wraith, D.C., and Anderton, S.M. (2008). Cutting edge: Th1 cells facilitate the entry of Th17 cells to the central nervous system during experimental autoimmune encephalomyelitis. J. Immunol. *181*, 3750–3754.

Papotto, P.H., Gonçalves-Sousa, N., Schmolka, N., Iseppon, A., Mensurado, S., Stockinger, B., Ribot, J.C., and Silva-Santos, B. (2017). IL-23 drives differentiation of peripheral γδ17 T cells from adult bone marrow-derived precursors. EMBO Rep. *18*, 1957–1967.

Park, H., Li, Z., Yang, X.O., Chang, S.H., Nurieva, R., Wang, Y.-H., Wang, Y., Hood, L., Zhu, Z., Tian, Q., and Dong, C. (2005). A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nat. Immunol. *6*, 1133–1141.

Parsonage, G., Filer, A., Bik, M., Hardie, D., Lax, S., Howlett, K., Church, L.D., Raza, K., Wong, S.-H., Trebilcock, E., et al. (2008). Prolonged, granulocyte-macrophage colony-stimulating factor-dependent, neutrophil survival following rheumatoid synovial fibroblast activation by IL-17 and TNFalpha. Arthritis Res. Ther. *10*, R47.

Rei, M., Gonçalves-Sousa, N., Lança, T., Thompson, R.G., Mensurado, S., Balkwill, F.R., Kulbe, H., Pennington, D.J., and Silva-Santos, B. (2014). Murine CD27(-) Vγ6(+) γδ T cells producing IL-17A promote ovarian cancer growth via mobilization of protumor small peritoneal macrophages. Proc. Natl. Acad. Sci. USA *111*, E3562–E3570.

Ribot, J.C., deBarros, A., Pang, D.J., Neves, J.F., Peperzak, V., Roberts, S.J., Girardi, M., Borst, J., Hayday, A.C., Pennington, D.J., and Silva-Santos, B. (2009). CD27 is a thymic determinant of the balance between interferon-gamma- and interleukin 17-producing gammadelta T cell subsets. Nat. Immunol. *10*, 427–436.

Ronchi, F., Basso, C., Preite, S., Reboldi, A., Baumjohann, D., Perlini, L., Lanzavecchia, A., and Sallusto, F. (2016). Experimental priming of encephalitogenic Th1/Th17 cells requires pertussis toxin-driven IL-1βTh1/Th17 cells requires pertu. Nat. Commun. *7*, 11541.

Schwarzenberger, P., Huang, W., Ye, P., Oliver, P., Manuel, M., Zhang, Z., Bagby, G., Nelson, S., and Kolls, J.K. (2000). Requirement of endogenous stem cell factor and granulocyte-colony-stimulating factor for IL-17-mediated granulopoiesis. J. Immunol. *164*, 4783–4789.

Segal, B.M., Constantinescu, C.S., Raychaudhuri, A., Kim, L., Fidelus-Gort, R., and Kasper, L.H.; Ustekinumab MS Investigators (2008). Repeated subcutaneous injections of IL12/23 p40 neutralising antibody, ustekinumab, in patients with relapsing-remitting multiple sclerosis: a phase II, double-blind, placebo-controlled, randomised, dose-ranging study. Lancet Neurol. *7*, 796–804.

Steinbach, K., Piedavent, M., Bauer, S., Neumann, J.T., and Friese, M.A. (2013). Neutrophils amplify autoimmune central nervous system infiltrates by maturing local APCs. J. Immunol. *191*, 4531–4539.

Sutton, C., Brereton, C., Keogh, B., Mills, K.H., and Lavelle, E.C. (2006). A crucial role for interleukin (IL)-1 in the induction of IL-17-producing T cells that mediate autoimmune encephalomyelitis. J. Exp. Med. *203*, 1685–1691.

Sutton, C.E., Lalor, S.J., Sweeney, C.M., Brereton, C.F., Lavelle, E.C., and Mills, K.H. (2009). Interleukin-1 and IL-23 induce innate IL-17 production from gammadelta T cells, amplifying Th17 responses and autoimmunity. Immunity *31*, 331–341.

Veldhoen, M., Hocking, R.J., Atkins, C.J., Locksley, R.M., and Stockinger, B. (2006). TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. Immunity *24*, 179–189.

Yang, X.O., Chang, S.H., Park, H., Nurieva, R., Shah, B., Acero, L., Wang, Y.-H., Schluns, K.S., Broaddus, R.R., Zhu, Z., and Dong, C. (2008). Regulation of inflammatory responses by IL-17F. J. Exp. Med. *205*, 1063–1075.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-mouse B220 – AF700	eBioscience	Clone: RA3-6B2; Cat#: 56-0452-82; RRID: AB89145
Anti-mouse B220 – BV605	BioLegend	Clone: RA3-6B2; Cat#:103243; RRID: AB_11203907
Anti-mouse CCR2 – PE	BioLegend	Clone: SA203G11; Cat#:150609; RRID: AB_2616981
Anti-mouse CCR2 – PECy7	BioLegend	Clone: SA203G11; Cat#:150611; RRID: AB_2616983
Anti-mouse CD11a – PECy7	BD PharMingen	Clone: 2D7; Cat#:558191; RRID: AB_397055
Anti-mouse CD11b – APCeFluor 780	eBioscience	Clone: M1/70; Cat#:47-0112-82; RRID: AB_1603193
Anti-mouse CD11c – BV605	BioLegend	Clone: N418; Cat#:117333; RRID: AB_11204262
Anti-mouse CD11c – BV785	BioLegend	Clone: N418; Cat#:117336; RRID: AB_2565268
Anti-mouse CD25 - PE	eBioscience	Clone: PC61.5; Cat#:12-0251-82; RRID: AB_4656607
Anti-mouse CD25 – PECy7	eBioscience	Clone: PC61.5; Cat#:25-0251-82; RRID: AB_469608
Anti-mouse CD27 – PECy7	eBioscience	Clone: LG.7F9; Cat#:25-0271-82; RRID: AB_1724035
Anti-mouse CD3 – AF700	eBioscience	Clone: 17A2; Cat#:56-0032-81; RRID: AB_529507
Anti-mouse CD3 – BV650	BioLegend	Clone: 17A2; Cat#:100229; RRID: AB_11204249
Anti-mouse CD3ε - APC	eBioscience	Clone: 145-2C11; Cat#:17-0031-82; RRID: AB_469315
Anti-mouse CD3ε – APC-eFluor 780	eBioscience	Clone: 145-2C11; Cat#:47-0031-82; RRID: AB11149861
Anti-mouse CD3ε – eFluor-450	eBioscience	Clone: 145-2C11; Cat#:48-0031-82; RRID: AB_10735092
Anti-mouse CD3ε – PerCPCy5.5	eBioscience	Clone: 145-2C11; Cat#:45-0031-82; RRID: 1107000
Anti-mouse CD4 – BV421	BioLegend	Clone: GK1.5; Cat#:100437; RRID: AB_10900241
Anti-mouse CD4 – BV785	BioLegend	Clone: RM4-5; Cat#:100552; RRID: AB_2563053
Anti-mouse CD4 – PE-Dazzle	BioLegend	Clone: RM4-5; Cat#:100566; RRID: AB_2563684
Anti-mouse CD44 – BV605	BioLegend	Clone: IM7; Cat#:103047; RRID: AB_2562451
Anti-mouse CD45 – BV711	BioLegend	Clone: 30-F11; Cat#:103147; RRID: AB_2564383
Anti-mouse CD45 – BV785	BioLegend	Clone: 30-F11; Cat#:103149; RRID: AB_2564590
Anti-mouse CD49d – PerCPeFluor 710	eBioscience	Clone: R1-2; Cat#:46-0492-82; RRID: AB_11150051

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Anti-mouse CD69 - FITC	BioLegend	Clone: H1.2F3; Cat#:104506; RRID: AB_313109
Anti-mouse CD8 – PECy7	eBioscience	Clone: 56-6.7; Cat#:25-0081-82; RRID: AB_469584
Anti-mouse F4/80 – PerCPCy5.5	BioLegend	Clone: BM8; Cat#:123128; RRID: AB_893484
Anti-mouse GM-CSF - PE	eBioscience	Clone: MP1-22E9; Cat#:12-7331-82; RRID: AB_466205
Anti-mouse IFN-γ – BV711	BioLegend	Clone: XMG1.2; Cat#:505835; RRID: AB_11219588
Anti-mouse IFN-γ – PECF594	BD Horizon	Clone: XMG1.2; Cat#:562333; RRID: AB_11154588
Anti-mouse IFN-γ – PECy7	eBioscience	Clone: XMG1.2; Cat#:25-7311-82; RRID: AB_469680
Anti-mouse IL-17A - APC	eBioscience	Clone: eBio17B7; Cat#:17-7177-81; RRID: AB_763580
Anti-mouse IL-17A – PerCPCy5.5	eBioscience	Clone: eBio17B7; Cat#:45-7177-82; RRID: AB_925753
Anti-mouse IL-17F – A488	eBioscience	Clone: eBio18F10; Cat#:53-7471-82; RRID: AB_1210529
Anti-mouse IL-17RA - PE	eBioscience	Clone: PAJ-17R; Cat#:12-7182-80; RRID: AB_1582266
Anti-mouse IL-1R1 - PE	BD PharMingen	Clone: 35F5; Cat#:557489; RRID: AB_396727
Anti-mouse IL-23R - APC	BioLegend	Clone: 12B2B64; Cat#:150905; RRID: AB_2687345
Anti-mouse Ki67 – APC-eFluor-780	eBioscience	Clone: SolA15; Cat#: 47-5698-80; RRID: AB_2688065
Anti-mouse Ki67 – e450	eBioscience	Clone: SolA15; Cat#: 48-5698-80; RRID: AB_11149124
Anti-mouse Ki67 – eFluor 660	eBioscience	Clone: SolA15; Cat#: 50-5698-82; RRID: AB_2574235
Anti-mouse Ly6C - FITC	BD PharMingen	Clone: AL-21; Cat#: 553104; RRID: AB_394628
Anti-mouse Ly6G – BV650	BioLegend	Clone: 1A8; Cat#: 127641; RRID: AB_2565881
Anti-mouse Ly6G – Pacific Blue	BioLegend	Clone: 1A8; Cat#: 127612; RRID: AB_2251161
Anti-mouse MHCII – APC	eBioscience	Clone: M5/114.15.2; Cat#: 17-5321-82; RRID: AB_469455
Anti-mouse NK1.1 – PerCPCy5.5	eBioscience	Clone: PK136; Cat#: 45-5941-82; RRID: AB_914361
Anti-mouse pro-IL-1β – PECy7	eBioscience	Clone: NJTEN3; Cat#: 25-7114-80; RRID: AB_2573526
Anti-mouse RORγt – BV650	BD Horizon	Clone: Q31-378; Cat#: 564722; RRID: AB_2738915
Anti-mouse Siglec F – PECF594	BD Horizon	Clone: E50-2440; Cat#: 562757; RRID: AB_2687994
Anti-mouse TCRδ - APC	eBioscience	Clone: GL3; Cat#: 17-5711-82; RRID: AB_842756
Anti-mouse TCRδ – BV605	BioLegend	Clone: GL3; Cat#: 118129; RRID: AB_2563356
Anti-mouse TCRδ - PE	BD PharMingen	Clone: GL3; Cat#: 553178; RRID: AB_394689

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Anti-mouse TCRδ – PerCPeFluor 710	eBioscience	Clone: GL3; Cat#: 45-5711-82; RRID: AB_2016707
Anti-mouse TNF - APC	eBioscience	Clone: MAb11; Cat#: 17-7349-41; RRID: AB_1548821
Anti-mouse Vγ4 - FITC	BioLegend	Clone: UC3-10A6; Cat#: 137704; RRID: AB_10569353
Anti-mouse Vγ4 - PE	BioLegend	Clone: UC3-10A6; Cat#: 137706; RRID: AB_10643577
Anti-mouse Vγ4 – PECy7	eBioscience	Clone: UC3-10A6; Cat#: 25-5828-82; RRID: AB_2573474
*Invivo*mAb anti-mouse IL-17A	BioXCell	Cat# BEO173; RRID: AB_10950102; Clone: 17F3
*Invivo*mAb anti-mouse Vγ2 TCR	BioXCell	Cat#: BEO168; RRID: AB_10950109; Clone: UC3-10A6
*Invivo*mAb anti-mouse/rat IL-1β	BioXCell	Cat#: BEO246; RRID: AB_2687727; Clone: B122
*Invivo*mAb Anti-mouse GM-CSF	John A Hamilton	Clone: 22E9
*Invivo*mAb Anti-mouse IFN-γ	Louis Boon	Clone: XMG1.2
Bacterial and Virus Strains		
Heat-killed *Mycobacterium tuberculosis*	Invivogen	Cat#: tlrl-hkmt
Pertussis toxin	Kaketsucken	N/A
LP1569	EMC Microcollections; Allen et al., 2018	N/A
Chemicals, Peptides, and Recombinant Proteins		
Myelin oligodendrocyte glycoprotein $(MOG)_{35-55}$	Genscript	Cat#: RP10245
Recombinant mouse IL-1β	ImmunoTools	Cat#: 12340015
Recombinant mouse IL-23	Miltenyi Biotec	Cat#: 130-096-677
Recombinant mouse IL-17A	ImmunoTools	Cat#: 12340174
Recombinant mouse TNF	ImmunoTools	Cat#: 12343014
MCC950	Invivogen	Cat#: inh.mcc
Ac-YVAD-cmk	Invivogen	Cat#: inh.yvad
PMA	Sigma-Aldrich	Cat#: P1585
Ionomycin	Sigma-Aldrich	Cat#: I0643
Brefeldin A	Sigma-Aldrich	Cat#: B7651
Keyhole limpet haemocyanin (KLH)	Calbiochem	Cat#: 374817
c-di-GMP	Invivogen	Cat#: tlrl.nacdg
Complete Freund's adjuvant	Chondrex	Cat#: 7023
Critical Commercial Assays		
High capacity cDNA reverse transcription kit	Applied Biosystems, Biosciences	Cat#: 4368814
LIVE/DEAD™ Fixabe Aqua dead cell stain kit	Life Technologies	Cat#: L34957
CountBright™ Absolute Counting Beads	Life Technologies	Cat#: C36950
Foxp3 Transcription Factor staining buffer set	eBioscience	Cat#: 00-5523-00
Neutrophil isolation kit, mouse	Miltenyi Biotec	Cat#: 130-097-658
Monocyte isolation kit, mouse	Miltenyi Biotec	Cat#: 130-100-629
TCRγδ$^+$ T cell isolation kit, mouse	Miltenyi Biotec	Cat#: 130-092-125
MagniSort™ Mouse CD4 T cell enrichment kit	Invitrogen by Thermo Fisher Scientific	Cat#: 8804-6821
Mouse IL-17A Duoset ELISA	R & D	Cat#: DY421

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Mouse IL-17F Duoset ELISA	R & D	Cat#: DY2057
Mouse GM-CSF Duoset ELSIA	R & D	Cat#: DY415
Mouse IL-1β/IL-1F2 Duoset ELISA	R & D	Cat#: DY401
Mouse IL-23 Duoset ELISA	R & D	Cat#: DY1887
Mouse CXCL1/KC Duoset ELISA	R & D	Cat#: DY453
Mouse CXCL2/MIP-2 Duoset ELISA	R & D	Cat#: DY452
Mouse CCL2/JE/MCP-1 Duoset ELSIA	R & D	Cat#: DY479
Mouse G-CSF Duoset ELISA	R & D	Cat#: DY414
Mouse IFN-γ ELISA	BD PharMingen	Cat#: 551216 (capture antibody) Cat#: 554410 (detection antibody)
Experimental Models: Organisms/Strains		
Mouse; C57BL/6	Comparative Medicine Unit, Trinity College Dublin.	N/A
Mouse; *Il17a*$^{-/-}$ (on C57BL/6 background)	Comparative Medicine Unit, Trinity College Dublin.	N/A
Mouse; *Il17ra*$^{-/-}$ (on C57BL/6 background)	Comparative Medicine Unit, Trinity College Dublin.	N/A
Mouse; *Il17ra*$^{+/+Myeloid}$	Nabil Djouder, CNIO, Madrid; Gomes et al., 2016	N/A
Mouse; *Il17ra*$^{\Delta/\Delta Myeloid}$	Nabil Djouder, CNIO, Madrid; Gomes et al., 2016	N/A
Oligonucleotides		
Il23a (Mm00518984_m1)	Applied Biosystems	Cat#: 4331182
Il1b (Mm00434228_m1)	Applied Biosystems	Cat#: 4331182
Il17a (Mm00439618_m1)	Applied Biosystems	Cat#: 4331182
Il17rc (Mm00506606_m1)	Applied Biosystems	Cat#: 4331182
Cxcl1 (Mm04207460_m1)	Applied Biosystems	Cat#: 4331182
Ccl2 (Mm00441242_m1)	Applied Biosystems	Cat#: 4331182
Cxcl2 (Mm00436450_m1)	Applied Biosystems	Cat#: 4331182
Csf3 (Mm00438334_m1)	Applied Biosystems	Cat#: 4331182
Nlrp3 (Mm00840904_m1)	Applied Biosystems	Cat#: 4331182
Casp1 (Mm00438023_m1)	Applied Biosystems	Cat#: 4331182
Aim2 (Mm01295719_m1)	Applied Biosystems	Cat#: 4331182
Rorc (Mm01261022_m1)	Applied Biosystems	Cat#: 4331182
Tbx21 (Mm00450960_m1)	Applied Biosystems	Cat#: 4331182
Itga4 (Mm00439770_m1)	Applied Biosystems	Cat#: 4331182
Euk 18S rRNA	Applied Biosystems	Cat#: 4319413E
Software and Algorithms		
Prism 7	GraphPad Software	https://www.graphpad.com/scientific-software/prism/
FlowJo 10	Becton Dickinson	https://www.flowjo.com/solutions/flowjo

LEAD CONTACT AND MATERIALS AVAILABILITY

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Kingston Mills (kingston.mills@tcd.ie).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Mice

C57BL/6, *Il17a*$^{-/-}$ and *Il17ra*$^{-/-}$ on a C57BL/6 background were bred in house from established colonies. Deletion of IL-17RA in myeloid lineage was obtained by crossing *Il17ra*-flox mouse with *Lyz2*-Cre line and was confirmed by genotyping and flow cytometry

as previously described (Gomes et al., 2016). All mice were maintained according to European Union regulations, and experiments were performed under license (AE19136/P042) from the Irish Health Products Regulation Authority with approval from the Trinity College Dublin BioResources Ethics Committee or the CNIO-ISCIII Ethics Committee and from Community of Madrid (CAM). All mice were housed under specific pathogen-free conditions. All mice within experiments were age and sex matched.

Induction and assessment of EAE

Active EAE was induced by s.c immunization of mice with 100 μg MOG_{35-55} peptide (GenScript) and CFA containing 4 mg/ml (0.4 mg/mouse) of heat killed *M. Tuberculosis* (Chondrex). Mice were injected i.p. with pertussis toxin (PT) (250 ng/mouse) on days 0 and 2 of active EAE. Passive EAE was induced by adoptive transfer of MOG-specific T cells from LN and spleen of donor C57BL/6 or *Il17a*$^{-/-}$ mice immunized with MOG and CFA as previously described (Dungan et al., 2014). Cells were stimulated *in vitro* for 3 days with MOG (100 μg/ml) or MOG, IL-1β (1-10 ng/ml) and IL-23 (10 ng/ml) and 15 × 10^6 viable cells were injected i.p. into recipient mice. Cell supernatants were analyzed by ELISA for the presence of IL-17A, IL-17F, GM-CSF (R&D Systems) and IFN-γ (BD Biosystems). Disease severity was assessed by clinical scores as follows: no clinical signs, 0; limp tail, 1; ataxic gait, 2; hind limb weakness, 3; hind limb paralysis, 4; tetra paralysis/moribund, 5.

In certain experiments mice were injected i.p. with anti-IL-17A (200 μg/mouse; BioXCell; clone 17F3), anti-Vγ4 (250 μg/mouse; BioXCell; clone UC3-10A6), anti-GM-CSF (300 μg/mouse; clone 22E9), anti-IFN-γ (500 μg/mouse; clone XMG1.2), anti-IL-1β (200 μg/mouse; BioXCell; clone B122) or appropriate isotype controls prior to and after induction of active or passive EAE as indicated in figure legends. The concentrations of antibodies used were based on manufacturer's instructions or previous studies by us and others (Codarri et al., 2011). In other experiments mice were injected with recombinant IL-1β (500 ng/mouse; ImmunoTools), IL-1β and IL-23 (200 ng/mouse; Miltenyi Biotec) or IL-17A (500 ng/mouse; ImmunoTools) during the induction phase of disease.

Spleen and LN cells (inguinal, brachial and axillary LN) were isolated on day 5 or 7 of EAE and examined by flow cytometry analysis or stimulated *ex vivo* with medium or MOG_{35-55} antigen (25 - 50 μg/ml) for 3 days. Supernatants were removed and the concentrations of IL-17A, GM-CSF and IFN-γ were determined by ELISA.

METHOD DETAILS

Isolation of mononuclear cells from the CNS

Mice were sacrificed and perfused with 20 mL of PBS before removal of the brain and spinal cord. Organs were lysed in cRPMI using a tissue lyser (QIAGEN). Tissue homogenates were resuspended in 5 mL 40% isotonic Percoll and layered over 5 mL 70% isotonic Percoll before centrifugation at 1300 x g for 20 minutes. Mononuclear cells were removed from the interface of the Percoll gradients, passed through a 70 μm filter and washed in cRPMI. Alternatively, the concentration of IL-1β in brain homogenate was quantified by ELISA, or whole brain homogenate was resuspended in TRIzol for RNA extraction.

Examination of naive and primed T cells in Il17a$^{-/-}$ mice

C57BL/6 WT or *Il17a*$^{-/-}$ mice were immunized in the footpad with KLH (5 μg; Calbiochem), c-di-GMP (10μg; Invivogen) and LP1569 (50 μg; EMC microcollections). After 7 days popliteal LN cells were re-stimulated *ex vivo* with medium or KLH (2 - 50 μg/ml) for 3 days. Supernatants were removed and the concentrations of IL-17A, IL-17F, GM-CSF and IFN-γ were determined by ELISA.

Flow Cytometry

Mononuclear cells isolated from the spleen, LNs (inguinal, brachial and axillary) and CNS were incubated with live/dead stain (LIVE/DEAD Fixable Aqua Dead Cell Stain Kit; Life Technologies; 1:600) and Fcγ block (BD; 1:50). Cells were then washed and surface stained with antibodies specific for CD3ε (clone: 145-2C11), CD4 (RM4-5), CD8 (53-6.7), TCRδ (GL3), TCR-Vγ4 (UC3-10A6), B220 (RA3-6B2), CD11b (M1/70), CD11c (N418), Ly6G (1A8, 1:600), F4/80 (BM8), Ly6C (AL-21), CCR2 (SA203G11), MHCII (M5/11.4.15.2; 1:500), Siglec F (E50-2440), IL-17RA (PAJ-17R) CD44 (IM7), CD25 (PC61.5), CD11a (27D), CD49d (R1-2), CD69 (H1.2F3), IL-23R (12B2B64), IL-1R1 (35F5), NK1.1 (PK136) or CD45 (30-F11). For Intracellular cytokine staining (ICS) cells were stimulated for 5 hr with PMA (10 ng/ml; Sigma), ionomycin (1 μg/ml; Sigma) and brefeldin A (5 μg/ml; Sigma) (IL-17A, IL-17F, GM-CSF, IFN-γ, TNF production) or for 3 hr with monensin (BD GolgiStop; IL-1β production). For intracellular and intranuclear staining, cells were fixed and permeabilized using the Foxp3/Transcription Factor Staining Buffer Kit (eBioscience) according to the manufacturer's protocol. Cells were stained intracellularly with antibodies specific for IL-17A (17B7), IL-17F (18F10), GM-CSF (MP1-22E9), IFN-γ (XMG1.2), TNF (MP6-XT22) or pro-IL-1β (NJTEN3). Cells were stained intranuclearly with antibodies specific for Ki67 (SolA15) and RORγt (Q31-378; 1:100). All antibodies were used at a 1 in 200 dilution, unless otherwise stated. Cells were analyzed using an LSRFortessa flow cytometer (BD) and the data was analyzed with FloJo software. Analysis of the stained populations was performed by gating on single, live cells.

Quantitative reverse transcription-PCR

RNA was extracted from LN cells, brain homogenate, peritoneum tissue, flow cytometry sorted $CD3^+$ T cells, $CD3^-CD11b^+Ly6G^-Ly6C^{hi}$ monocytes and $CD3^-CD11b^+Ly6C^{int}Ly6G^+$ neutrophils from LN or bone marrow-derived monocytes or neutrophils isolated using monocyte or neutrophil isolation kits (Miltenyi Biotec). Extraction was performed using the chloroform/isopropanol method and was reverse transcribed into cDNA using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems).

qRT-PCR was performed using commercially available *Rorc* (Mm01261022), *Tbx21* (Mm00450960), *Itga4* (Mm00439770) *Il1b* (Mm00434228), *Il23a* (Mm01160011), *Il17a* (Mm00439618), *Il17rc* (Mm00506606), *Cxcl1* (Mm04207460), *Cxcl2* (Mm00436450), *Ccl2* (Mm00441242), *Nlrp3* (Mm00840904), *Casp1* (Mm00438023), and *Aim2* (Mm01285719) primers (ABI). qRT-PCR was performed on a PRISM7500 Sequence Detection System (ABI). The amount of each gene was determined by normalization to 18 S rRNA (Mm04277571, ABI) internal control.

Chemokine production by epithelial cells and LN cells

Peritoneal mesothelial cells were isolated from naive C57BL/6 mice as previously described (Bot et al., 2003). Mice were euthanised and peritoneal exudate cells (PEC) were removed by peritoneal lavage with PBS. 10 mL of Trypsin (0.25%) - EDTA 4Na (0.02%) solution (Sigma) was injected into the peritoneum. After 10 min, the trypsin solution was collected and the peritoneal cavities were injected with 10 mL of cRPMI and collected into the same tube to neutralize the trypsin. After 7 days of culture cells were stimulated with IL-17A (500 ng/ml) ± TNF (10 ng/ml) for 18 hr, after which the concentrations of CXCL1 and CXCL2 in the supernatants were determined by ELISA. Mesothelial tissue was also isolated from the peritoneum by touch biopsy to examine expression of *Il17rc* in naive C57BL/6 mice, or expression of *Cxcl1, Cxcl2* and *Ccl2* 3 hr after i.p. injection with PBS or IL-17A (500 ng). Expression of mRNA in peritoneum tissue was quantified by qRT-PCR. In other experiments LN cells from naive and EAE mice (6 hr post induction) treated with anti-IL-17 or isotype control antibodies (200 μg/mouse) were cultured overnight and the concentrations of CXCL1, CXCL2, CCL2 and G-CSF in the supernatants were determined by ELISA.

Inflammasome activation and IL-1β production by neutrophils and monocytes

$CD3^+$ T cells, $CD11b^+$ $Ly6G^+$ neutrophils and $CD11b^+$ $Ly6G^-$ $Ly6C^{hi}$ monocytes were flow cytometry sorted from the LN of naive C57BL/6 mice. Expression of *Il1b, Nlrp3, Casp1* and *Aim2* mRNA was determined by qRT-PCR. Alternatively, neutrophils and monocytes were isolated from the bone marrow of naive mice using neutrophil and monocyte isolation kits (Miltenyi). Cells were stimulated with IL-17A ± TNF (both 10 ng/ml) or MTB (50 μg/ml) ± MCC950 (1 μM) or Ac-YVAD-cmk (50 μM). Mature IL-1β protein in the supernatants was quantified by ELISA after 18 hr culture.

IL-17A in the mobilization IL-1β-producing neutrophils and inflammatory monocytes

$Il17a^{-/-}$ mice were injected with PBS or IL-17A (500 ng) and TNF (100 ng), with or without immunization with MOG and CFA (as for induction of active EAE). Mice were sacrificed after 6 hr and peritoneal exudate cells (PEC) were isolated. Absolute numbers of neutrophils and $Ly6C^{hi}$ monocytes were quantified in the PEC by flow cytometry. PEC from each group were cultured overnight and the concentration of IL-1β in supernatants determined by ELISA.

Co-culture of γδ T cells with MTB-stimulated neutrophils and monocytes

γδ T cells were isolated from the LN and neutrophils and monocytes were isolated from the bone marrow of naive C57BL/6 mice using TCRγδ, neutrophil and monocyte isolation kits (Miltenyi) according to manufacturer's instructions. Cells were co-cultured in the presence of IL-23 (10 ng/ml) ± MTB (50 μg/ml) for 18 hours after which the concentrations of IL-1β and IL-17A in the supernatants were determined by ELISA.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistics

All data was analyzed using Prism 7 (GraphPad Software). Unpaired t tests, Mann-Whitney U tests, one-way and two-way ANOVA and Kruskal-Wallis tests were performed on datasets. Error bars represent standard error of the mean (SEM) or standard deviation (SD). All dots on plots represent biological replicates. Details provided in figure legends.

DATA AND CODE AVAILABILITY

There is no relevant data or code availability.

ADDITIONAL RESOURCES

There are no additional resources.