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Host T Cells Are the Main Producers of IL-17 within the Central Nervous System during Initiation of Experimental Autoimmune Encephalomyelitis Induced by Adoptive Transfer of Th1 Cell Lines

Jason R. Lees,* Yoichiro Iwakura,† and John H. Russell2*

Experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis, has long been thought to be mediated by Th1 CD4+ T cells. Using adoptive transfer techniques, transfer of CNS specific Th1 T cells was sufficient to induce EAE in naive mice. However, recent studies found a vital role for IL-17 in induction of EAE. These studies suggested that a fraction of IL-17-producing T cells that contaminate Th1 polarized cell lines are largely responsible for initiation of EAE. In this study, we tracked the appearance and cytokine production capacity of adoptively transferred cells within the CNS of mice throughout EAE disease. IL-17-producing, adoptively transferred cells were not enriched over the low percentages present in vitro. Thus, there was no selective recruitment and/or preferential proliferation of adoptively transferred IL-17-producing cells during the induction of EAE. Instead a large number of CNS infiltrating host T cells in mice with EAE were capable of producing IL-17 following ex vivo stimulation. The IL-17-producing T cells contained both αβ and γδ TCR+ T cells with a CD4+CD8− or CD4−CD8+ phenotype. These cells concentrated within the CNS within 3 days of adoptive transfer, and appeared to play a role in EAE induction as adoptive transfer of Th1 lines derived from wild-type mice into IL-17-deficient mice induced reduced EAE clinical outcomes. This study demonstrates that an encephalitogenic Th1 cell line induces recruitment of host IL-17-producing T cells to the CNS during the initiation of EAE and that these cells contribute to the incidence and severity of disease. The Journal of Immunology, 2008, 180: 8066–8072.

The invasion of CD4+ T cells into the CNS is thought to play a significant role in the pathology of the animal model experimental autoimmune encephalomyelitis (EAE), as well as the human disease multiple sclerosis (MS). Previous studies in MS and EAE suggested that the cytokine profile of infiltrating CD4+ T cells is vital in determining the extent of disease pathology. Several groups previously demonstrated that T cells present within both EAE and MS lesions produced Th1 cytokines, particularly IFN-γ (1, 2). Further work demonstrated that skewing of these Th1 infiltrating cells to a Th2 type cytokine profile was sufficient to ameliorate EAE clinical symptoms (3–6). A role for Th1 cells in EAE seemed clearly defined when adoptive transfer of Th1 polarized myelin Ag-specific T cells was shown to be sufficient to induce EAE (4, 6, 7). Finally, studies done with mice deficient in T-bet, a regulatory element required for Th1 responses, demonstrated the necessity of T-bet expression to the induction of EAE (8, 9).

Despite clear indications of encephalitogenic potential in myelin peptide-specific Th1 polarized T cell lines, the actual role of the iconic Th1 cytokine IFN-γ in EAE is controversial. Significant evidence indicates that IFN-γ could have either ameliorating or exacerbating effects on EAE clinical disease severity, with the effects observed largely depending on the clinical outcome tested and model system used (10–18). Studies performed in IFN-γ-deficient mice revealed a significant exacerbation of disease. These effects were seen both as increases in EAE disease severity in classic EAE model systems and susceptibility to EAE in normally resistant mouse strains (13, 14, 18). As such, while there still remains some doubt as to the ultimate contribution(s) of IFN-γ to EAE initiation and clinical severity, it is very clear that IFN-γ is not absolutely required for disease induction, leading some to question whether Th1 cells are truly involved in EAE induction (19).

Recently, a great deal of excitement was generated by work that suggested a vital role for IL-17 in the induction of several autoimmune diseases, including EAE (19–21). Initial studies demonstrated a profound defect in the initiation of EAE in IL23 gene-knockout (KO) mice, associated with the loss of IL-17-producing, but not IFN-γ-producing, T cells (19). These findings were recapitated in IL1 receptor-deficient mice, with resistance to EAE induction again associated with a lack of IL-17-producing T cells (22). Examination of IL-17-deficient models revealed delays in EAE induction as well as significant decreases in EAE clinical scores (23, 24). Additionally, studies have demonstrated the capacity of T cells skewed to produce IL-17, termed Th17 cells, to induce EAE upon adoptive transfer (19). Indeed, following adoptive transfer, Th17 cells were found to induce significantly greater
encephalitogenic responses on a cell-to-cell basis when compared with Th1 cells in the same experiment (19). Further examination of the T cells found within the CNS after active induction of EAE revealed both IL-17 and IFN-γ producing cell populations. At least one study demonstrated cell populations that produced both cytokines simultaneously, with the dual nature of the cytokine production only inhibited by long term in vitro culture (25).

The finding that a loss of a Th17 T cell population correlated with resistance to active induction of EAE was surprising given the apparent antagonism between Th17 and Th1 lineage development and the number of studies that have demonstrated EAE induction following adoptive transfer of myelin Ag-specific T cells cultured under Th1 biasing conditions. Initial studies demonstrated extremely small numbers of IL-17-producing T cells present within the CNS following adoptive transfer of myelin Ag-specific T cells cultured and the number of studies that have demonstrated EAE induction has led some to suggest that the small numbers of IL-17-producing cells present within a “Th1 line” play a vital role in a line’s encephalitogenicity (19, 22). These findings are particularly interesting in light of previous studies that demonstrated the entry of very small numbers of adoptively transferred cells into the CNS shortly following transfer, with the majority of the transferred cells infiltrating at later time points (26–28). These small numbers of early entry “pioneer” cells have been suggested to play a vital role in EAE induction by conditioning the blood-brain barrier and CNS to allow the later entry of large numbers of encephalitogenic T cells (29).

In this study, we tested the hypothesis that the IL-17-producing T cells present within the adoptively transferred T cell population comprised the “pioneer” population of adoptively transferred cells and, therefore, would preferentially accumulate in the CNS relatively early following adoptive transfer. To address this hypothesis, we examined the cytokine production capacities of T cells present within the CNS at various time points following adoptive transfer. Localization of IL-17 and IFN-γ producing cell populations following adoptive transfer of Th1 polarized encephalitogenic cell lines demonstrated no significant accumulation of IL-17-producing transferred cells early after adoptive transfer or at any other time point tested. Instead, this study found that a host IL-17-producing T cell population was recruited to the CNS following invasion of encephalitogenic Th1 cells.

Materials and Methods

Mice

C57BL/6J and congenic B6.PL-Thy1.2/CyJ mice were purchased from The Jackson Laboratory. IL-17-deficient mice have been previously described (30). IL-17-deficient mice were bred and maintained in house in the Washington University Animal Care Facility. All experiments were approved by the Washington University Animal Care Committee and all mice were housed in an Association for Assessment and Accreditation of Laboratory Animal Care approved facility.

T cell lines

To generate myelin oligodendrocyte glycoprotein (MOG)- and OVA-specific cell lines, CD90.1 congenic B6.PL-Thy1.2/CyJ mice were immunized s.c. with either 50 μg of MOG35–55 peptide (Sigma Genosys) or 900 μg of whole OVA (Sigma-Aldrich), respectively, emulsified in IFA and supplemented with 500 μg/ml Mycobacterium tuberculosis. CD4+ cells were isolated from the spleen as previously described (31). Cells were then stimulated at a concentration of 1 × 106 cells/ml in the presence of 5 × 106 cells/ml irradiated C57BL/6 spleenocytes, 10 μg/ml MOG35–55 peptide, 10 units/ml IL-12, and 10 units/ml IL-2, in 10% FCS containing RPMI 1640 complete media. Cells were isolated after 7 days by Histopaque 1.077 (Sigma-Aldrich), then restimulated under the conditions outlined above, with the only exception being an absence of IL-12. Cells underwent two rounds of stimulation before being stored at −70°C in 10% DMSO containing FCS. Before usage, cells were thawed and restimulated for 7 days with MOG peptide and IL-2. Immediately before injection, cells were re-stimulated for 4 days with MOG peptide and IL-2, separated by Histopaque 1.077, then washed and resuspended in HBSS. To induce EAE, 2.5–5 × 106 cells in 300 μl of HBSS were transferred to 6–8-wk-old mice by retro-orbital injection.

Flow cytometric analysis

Single-cell suspensions of mononuclear cells present with the CNS were produced as previously reported (32). Briefly, mice were perfused with 35 ml of ice-cold PBS via cardiac puncture. The brain and spinal cords of mice were removed and mechanically disrupted through a 40-μm filter. The resultant slurry was incubated for 1 h at room temperature with continual agitation in the presence of 100 μg/ml of type 1 collagenase, 10 μg/ml of the protease inhibitor tosyl-L-lysine chloromethyl ketone, and 1 μg/ml of DNase. Single mononuclear cell suspensions were then separated from the slurry via a Percoll density gradient involving centrifugation and collection of cells accumulated at the interface of stacked 70 and 37% percoll solutions.

In all flow cytometric experiments, single-cell suspensions were stained for surface markers on ice for 20 min with continual agitation. Abs against CD3, CD4, CD8, CD25, CD44, CD45.2, CD62L, CD69, CD90.1, CD90.2, TCR β, and TCR γδ were all obtained from eBioscience. Staining with anti-CD90.1 and anti-CD90.2 Abs was used to distinguish adoptively transferred and host T cells, respectively.

Intracellular cytokine staining

Intracellular staining was performed, with some modification, as previously described (33). In brief, 1 × 106 cells were restimulated on plates precoated with anti-CD3 (10 μg/ml PBS) (eBioscience) or by incubation with 50 ng/ml PMA and 1 μM ionomycin (Sigma-Aldrich), with concurrent blockade of cytokine secretion by treatment with 1 μg/ml brefeldin A (Sigma-Aldrich) for 2–4 h at 37°C. Cells were stained for cell surface molecules then fixed with 5% buffered formalin phosphate (5% paraformaldehyde in PBS) for 20 min at room temperature, washed with PBS, and permeabilized with PBS supplemented with 0.5% BSA, 0.1% sodium azide, and 0.1% saponin for 10 min at room temperature. After washing, the cells were stained with 5 μg/ml IL-17-PE or allophycocyanin and/or IFN-γ-FITC or isotype controls for 5 min at room temperature with continual agitation. After washing four times, cells were immediately collected using a FACSCalibur (BD Immunocytometry Systems) and were analyzed using CellQuest software (BD Immunocytometry Systems).

Soluble factor activation

Single-cell suspensions of mononuclear cells present with the CNS were produced as described above. The transwell system was established by insertion of a 0.4-μm cell culture insert into the well of a 6-well plate. A total of 1 × 106 cells were placed in the upper chamber or lower chamber of the transwell as noted. The lower chambers were precoated with 10 μg/ml anti-CD3 in PBS or left uncoated. All lower chambers were blocked with 2% BSA containing PBS for 2 h at 37°C before addition of test cells. Following addition of cells to the transwell system the plate was incubated for 4 h at 37°C with concurrent blockade of cytokine secretion by treatment with 1 μg/ml brefeldin A, as described above. Cells were recovered from individual chambers and prepared for flow cytometric evaluation of intracellular cytokine production as outlined above.

Statistics

All statistics were performed using GraphPad Prism 4 software. Individual tests used are stated in figure legends. A value of p < 0.05 was considered significant.

Results

Encephalitogenic and host T cell cytokine production and entry into the CNS

Given the recent information regarding the necessity for IL-17 production in initiation of EAE, we decided to examine the capacity of previously characterized encephalitogenic MOG35–55-specific cell lines produced under Th1 polarizing conditions to produce IL-17. Cell lines were examined for IL-17 production 4 days after Ag-specific stimulation, immediately before adoptive transfer. As previously reported, following stimulation with either activating plate-bound anti-CD3 Ab (data not shown) or PMA and ionomycin, very few cells were found to produce IL-17 (Fig. 1A).
In contrast, large numbers of the encephalitogenic cells were capable of producing IFN-γ following stimulation (Fig. 1A).

To determine whether the small numbers of IL-17-producing cells observed in vitro accurately reflected the distribution of cells in vivo, we examined the cytokine production capacity of cells recovered from the CNS at various time points following adoptive transfer. Small numbers (86/10^5 total CNS cells) of adoptively transferred cells appeared in the CNS within ~1 day of adoptive transfer (Fig. 1B). These cells were found to principally (64%) contain IFN-γ-producing cells. As such, in this model, IL-17-producing, adoptively transferred cells are not preferentially recruited to the CNS at early time points. Further examination revealed that while IL-17-producing adoptively transferred cells were observed at later times points, the number of IL-17producing adoptively transferred cells never increased over the number of IL-17-producing cells found in vitro, demonstrating a lack of preferential accumulation during EAE initiation, peak, and resolution of acute disease (Fig. 1B).

Although adoptively transferred cells remained largely incapable of producing IL-17, within the CNS a host CD90.2^+ T cell population was found to produce IL-17 after stimulation with either plate bound anti-CD3 (data not shown) or PMA and ionomycin (Fig. 2). At the peak of EAE clinical symptoms, host CNS CD90.2^+ T cell populations were found to produce IL-17 and IFN-γ. Encephalitogenic CD4^+ T cell line invasion are capable of producing IL-17. Transferred and host cells were examined for their capacity to produce IL-17 following 2 h of in vitro stimulation with PMA and ionomycin. Cells were gated based on forward and side scatter profile (A, B, and C) as well as expression of CD90.1 or CD90.2 alleles (D). B, IL-17-producing cells were detected only within host CD90.2^+ cells present in the CNS. C, The gate shown was applied to all groups in D. D, The number of IL-17-producing cells increased throughout EAE induction and peaked at day 10. The data shown are representative of five experiments. Each experiment tested tissues pooled from two mice. E. Data from five experiments was compiled and analyzed by one way ANOVA using Tukey’s multiple comparison test. *p < 0.01 compared with Naive group.

To determine the necessity for antigenic recognition in recruitment of IL17Hc, we also examined the relative capacity of CNS Ag-specific T cells and T cells specific for a non-self Ag to increase numbers of IL17Hc (Fig. 2). These studies demonstrated that...
Despite the capacity of activated OVA-specific T cells to invade the CNS, they were incapable of increasing the host IL-17-producing population, suggesting that antigenic stimulation of the adoptively transferred population is essential to this process.

It was possible that the IL-17-producing T cells might represent contaminating cells from the blood introduced during CNS harvesting. To address this issue we compared the numbers of IL\textsubscript{17}H\textsubscript{c} in both the blood and CNS of control and EAE mice. Although the percentage of IL\textsubscript{17}H\textsubscript{c} was similar in control CNS and the blood from both control and EAE mice, CNS isolates from EAE mice contained ~3-fold greater numbers (Fig. 3). As such, the IL\textsubscript{17}H\textsubscript{c} found in control animals may represent contaminating cells introduced during the CNS harvest, but the IL\textsubscript{17}H\textsubscript{c} in EAE mice represent a significant concentration in cell number over cells found within the blood, and as such must be actively recruited to or produced within the CNS. These findings demonstrate the specific recruitment and/or production of IL-17-producing host cells within the CNS during EAE induction.

We next wished to examine the physiological relevance of host IL-17 production to initiation and pathogenesis of adoptive transfer EAE. A previous study demonstrated reduction in severity of EAE in IL-17-deficient mice (23). We used these mice to determine what role host IL-17 production plays in induction of EAE by Th1 encephalitogenic T cells produced from an IL-17 intact progenitor. Although equivalent total numbers of host T cells were recruited to the CNS of both groups following EAE, the IL-17-producing population was completely absent in IL-17-deficient mice (Fig. 4). As compared with wild-type (WT) controls, adoptive transfer of WT MOG-specific T cells into IL-17-deficient mice resulted in reduced clinical symptoms of EAE (Table I).

As the observed effect of host IL-17 deficiency was small under ordinary disease conditions, we wished to examine what effects lack of host IL-17 might have upon less severe disease induction. To examine this issue, we injected mice with reduced numbers of pathogenic Th1 T cells to induce suboptimal EAE disease (34). Reduction in cell numbers resulted in a significant reduction in mean peak clinical score when compared with mice with optimally induced EAE (1.70 ± 0.82 vs 3.3 ± 1.1, respectively). During suboptimal disease initiation, host deficiency in IL-17 resulted in a significant decrease in disease incidence. (Table II) Together these data demonstrate that host production of IL-17 plays a role in EAE development following adoptive transfer of encephalitogenic Th1 cells.

Further examination of the host IL-17-producing cell from the CNS of mice with EAE revealed the IL\textsubscript{17}H\textsubscript{c} population to be CD\textsuperscript{3}\textsuperscript{+} CD\textsuperscript{45}\textsuperscript{+} T cells (Fig. 5). These cells were mixed in TCR chain usage, with γδTCR\textsuperscript{+} cells comprising ~60% of the total IL\textsubscript{17}H\textsubscript{c} population. Indeed, a large number (~40%) of the total γδTCR\textsuperscript{+} cells T cells found in the CNS during EAE were found to be IL\textsubscript{17}H\textsubscript{c}. The remaining subset of the IL\textsubscript{17}H\textsubscript{c} population was found to be positive for the TCR β-chain (Fig. 5). The majority of IL\textsubscript{17}H\textsubscript{c} were also found to have a CD\textsuperscript{25}\textsuperscript{+} CD\textsuperscript{44}\textsuperscript{+}CD\textsuperscript{62L}– CD\textsuperscript{69}\textsuperscript{+} phenotype, suggesting recent activation of the cells (Fig. 5). All IL\textsubscript{17}H\textsubscript{c} were found to be NK1.1\textsuperscript{+}, suggesting that NK cells play no significant role in host cell IL-17 production (Fig. 5). Additional examination found almost all of the IL-17-producing host T cells to be CD\textsuperscript{8} but a proportion (~13%) of IL\textsubscript{17}H\textsubscript{c} to be CD\textsuperscript{4}\textsuperscript{+}.

![Figure 4](http://www.jimmunol.org/)

**FIGURE 4.** Host T cell recruitment in IL-17-deficient mice. EAE was induced by injection of 5 × 10\textsuperscript{6} MOG-specific CD90.1\textsuperscript{+} cells into either WT or IL-17-deficient mice. Fourteen days later, CNS cells were isolated and examined for expression of CD90.2 and IL-17A. All cells shown were selected for analysis based on lymphocytic forward and side scatter characteristics. Tissue from two mice were pooled in each group. The data shown is representative of two experiments.

![Figure 5](http://www.jimmunol.org/)

**FIGURE 5.** Phenotypic analysis of IL-17-producing host cells. IL-17-producing CNS cells recovered from mice with EAE were examined for phenotypic markers. EAE was induced by injection of 5 × 10\textsuperscript{6} MOG-specific CD90.1\textsuperscript{+} cells. Ten days later, all cells were stimulated for 2 h with PMA and ionomycin then stained for IL-17 production and various cell surface markers. All cells shown were selected for analysis based on lymphocytic forward and side scatter characteristics as well as their expression of CD90.2 (gate shown as dark box at top left plot). The data shown is representative of three experiments. In each experiment, tissues pooled from two mice were used.

<table>
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<th>Mouse Genotype</th>
<th>C57BL/6J</th>
<th>IL-17 KO</th>
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<tr>
<td>Incidence of clinical signs (% disease)</td>
<td>22/25 (88%)</td>
<td>10/10 (100%)</td>
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<td>Mean day of onset of clinical signs of EAE</td>
<td>7 ± 3</td>
<td>9 ± 4</td>
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<tr>
<td>Mean peak clinical scores</td>
<td>3.3 ± 1.1</td>
<td>2.6 ± 0.6*</td>
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* Mean day of onset and mean peak clinical scores are followed by SD. All groups were tested for significance using either a Fisher’s exact test or a t test. Only mean peak clinical scores were found to be significantly different (p < 0.006).

* p < 0.01; NA, not applicable.

<table>
<thead>
<tr>
<th>Mouse Genotype</th>
<th>C57BL/6J</th>
<th>IL-17 KO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence of clinical signs (% disease)</td>
<td>6/9 (67%)</td>
<td>0/7 (0%)*</td>
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<tr>
<td>Mean day of onset of clinical signs of EAE</td>
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</tr>
<tr>
<td>Mean peak clinical scores</td>
<td>1.70 ± 0.82</td>
<td>NA</td>
</tr>
</tbody>
</table>

* Suboptimal induction was designated based on mean peak clinical scores below a clinical score of 2 in WT groups. Mean day of onset and mean peak clinical scores are followed by SD. Incidence was tested for significance using Fisher’s exact test and found to be significantly different (p < 0.011).

* p < 0.05; NA, not applicable.
Although the transfer of encephalitogenic T cells was clearly required for the increased accumulation of the IL-17Hc population, it was possible that the adoptively transferred Th1 cells could have been producing a factor required for production of IL-17 in already resident CNS T cells. To address this possibility, mononuclear CNS isolates from naive mice were mixed with various numbers of freshly primed Th1 encephalitogenic cells immediately before incubation with plate bound anti-CD3 (Fig. 7). The addition of our encephalitogenic cells to control CNS isolates was not sufficient to drive IL-17 production in the host cells, suggesting that the IL-17Hc belong to a cell subset that is recruited to or preferentially retained within the CNS during Th1 cell invasion.

T cell production of IL-17 has previously been shown to occur through two distinct mechanisms, direct TCR activation and TCR-independent cytokine induction (35, 36). In an effort to further delineate the role of the adoptively transferred cell in host IL-17 expression, we examined the capacity of soluble factors produced by the Th1 cells during anti-CD3 stimulation to drive activation of the IL-17Hc in a transwell system. Exposure to soluble factors from either anti-CD3 stimulated in vitro cultured Th1 cells (data not shown) or anti-CD3 stimulated CNS isolates from mice with EAE was not sufficient to drive IL-17 production in CNS isolates from mice with EAE (Fig. 8). Thus, the IL-17Hc require direct stimulation with anti-CD3, cell-cell contact with Th1 T cells, or some combination thereof to produce IL-17.

Discussion

The data presented here demonstrate the capacity of an adoptively transferred Th1 skewed T cell line to induce EAE despite a marked deficiency in the capacity of the T cell line to produce IL-17. Instead, IL-17 production was observed in a heterogeneous population of T cells including both γδ and αβ TCR expressing cells that were enriched within the CNS during initiation and maintenance of EAE. These cells were found to be either CD4+ or CD4−/CD8−, and over all demonstrated a recently activated phenotype (CD69+/CD25+). These data suggest that host T cells may play an important role in EAE induction by supplying necessary inflammatory cytokines that are not produced in large quantities by initiating encephalitogenic T cells.
The knowledge that induction of EAE via adoptive transfer of Th1 skewed T cell lines results in recruitment of large numbers of host cells and/or activated T cells into the CNS has long been available, as early studies clearly demonstrated the phenomena (37, 38). Despite their presence, the role for CNS invading host T cells in initializing EAE pathogenesis has been trivialized due to findings that demonstrate the capacity of adoptively transferred T cell lines to induce disease in T cell-deficient lymphopenic mice (39, 40). However, recent studies have highlighted the pleiotropic effects that transfer into a lymphopoenic system has on T cells, suggesting that the unique activating nature of the lymphopoenic system could mask a loss of pathogenic capacity associated with lack of host T cell invasion (41, 42). Further, several studies have demonstrated exacerbation of EAE associated with cotransfer of non-CNS Ag-specific cells and encephalitogenic cells (37, 43). These studies demonstrate that host cells could play a role of great importance in EAE initiation and pathogenesis in lymphoid sufficient environments.

In this study, we examined the capacity of both resident host and adoptively transferred T cell populations to produce IFN-γ and/or IL-17 following a short-term stimulation with an anti-CD3 stimulation or a phorbol ester/calcium ionophore combination. The small numbers of IL-17-producing cells we report here within the Th1 skewed cell line come as little surprise given the suggested role for IFN-γ in negative regulation of the Th17 phenotype (44, 45). Indeed, the low incidence of IL-17-producing cells reported here was found without exception in multiple Th1 skewed cell lines specific for distinct Ags (Lees and Russell, unpublished data), suggesting that the methods used to isolate these lines result in low numbers of IL-17-producing cells without regard for antigenic specificity.

It has been suggested that, despite their low numbers, the IL-17-producing cells present within “Th1” skewed cell lines may play a vital role in the induction of EAE (19, 25). This idea is particularly attractive in light of models that predict a vital role for small numbers of “pioneer” cells that rapidly infiltrate and condition the CNS to allow the full scale T cell infiltration that proceeds EAE clinical manifestation (29). Further, the necessity for cells that constitute a small fraction of the total transferred T cell pool would explain much about the large number of transferred cells required to induce adoptively transferred EAE in many models. As such, the original hypothesis of this study was that the IL-17-producing population within our encephalitogenic T cell lines was acting as a “pioneering” T cell population for the induction of EAE. The data shown here demonstrate that the low numbers of IL-17 cells present within the cell line do not increase in number relative to the classic Th1 cells present within the cell line at any time point examined. These data demonstrate that no specific recruitment, proliferation, or induction of IL-17-producing adoptively transferred myelin-specific T cells occurs throughout EAE induction. As such, these cells are most likely not involved in early conditioning of the CNS for EAE development.

Instead, it is clear from our data that the increase in IL-17-producing cells that occurs within the CNS during adoptively transferred EAE comes almost entirely from host T cells recruited to the CNS following transferred cell invasion. Further, this study demonstrates that host T cell production of IL-17 plays a role in EAE pathogenesis. The IL17-H1 are fairly heterogeneous, but the largest population consisted of γδ TCR+ cells. A high percentage of γδ TCR+ cells present within the CNS were found to produce IL-17 upon stimulation. The γδ TCR+ IL17-H1 reported here share many similarities with recently described γδ TCR+ IL-17-producing cells involved in inflammation in inflammatory arthritis and chronic granulomatous disease (46, 47). These findings are particularly interesting when applied to earlier studies that reported significant amelioration of EAE in γδ T cell-deficient mice (48, 49). The possibility that the defect in EAE induction associated with γδ T cell deficiency may stem from a reduction in IL-17 contribution from these cells is very interesting, but will require more investigation to thoroughly test.

Although EAE conditions are clearly sufficient to allow accumulation of IL-17-producing T cells within the CNS, the mechanisms used in this process remain unknown. Indeed, it remains undetermined whether the increases in IL-17-producing cells observed reflect changes in trafficking pattern, cell retention, cell survival, proliferation, or some combination of these factors. Enhancement or inhibition of any of these factors could result in the accumulation observed.

Active recruitment of the IL-17-producing host cells through production of chemotactic factors could play a role in the accumulation observed. Several groups have demonstrated the modulation of chemokine expression within the CNS during inflammation (50). However, the concentration of IL-17-producing cells in relation to other host T cells infiltrating the CNS during EAE would necessitate chemotactic activation specific to the IL-17-producing cells. Thus, if increases in chemotactic factors do play a role, we would expect expression of chemokine receptors in IL17-H1 not widely observed in other activated T cell populations.

The phenotypic markers of activation observed in experiment 5 suggest that the IL17-H1 may be receiving an antigenic signal within the CNS. It is possible then that the invading adoptively transferred T cells are enhancing presentation of self Ag(s) to IL-17-producing T cells within the CNS. This could then modulate T cell survival and proliferation with resultant changes in cell number.

Ultimately, this study demonstrates the functional capacity of host cells to provide IL-17 during initiation and pathogenesis of adoptively transferred EAE. Further, this work demonstrates the participation of host IL-17 in EAE pathogenesis. Obviously, additional work will need to be performed to determine precisely what role the host IL-17-producing cells play in the induction of adoptively transferred EAE. In particular, it will be interesting to dissect any differences in a requirement for host cell IL-17 production under both normal and lymphopenic conditions. Further work will also be required to determine the antigenic specificities, and activation mechanisms that regulate host IL-17 production. These studies may provide great assistance in determining the exact mechanisms responsible for the IL-17-producing cell accumulation within the CNS itself, and the contributions of different T cell subsets to the initiation and maintenance of autoimmunity in the CNS.

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Disclosures

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References


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