Th1 and Th17 Cells Regulate Innate Immune Responses and Bacterial Clearance during Central Nervous System Infection

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Brain abscesses arise following parenchymal infection with pyogenic bacteria and are typified by inflammation and edema, which frequently results in a multitude of long-term health problems. The impact of adaptive immunity in shaping continued innate responses during late-stage brain abscess formation is not known but is important, because robust innate immunity is required for effective bacterial clearance. To address this issue, brain abscesses were induced in TCR αβ knockout (KO) mice, because CD4+ and NKT cells represented the most numerous T cell infiltrates. TCR αβ KO mice exhibited impaired bacterial clearance during later stages of infection, which was associated with alterations in neutrophil and macrophage recruitment, as well as perturbations in cytokine/chemokine expression. Adoptive transfer of either Th1 or Th17 cells into TCR αβ KO mice restored bacterial burdens and innate immune cell infiltrates to levels detected in wild-type animals. Interestingly, adoptively transferred Th17 cells demonstrated plasticity within the CNS compartment and induced distinct cytokine secretion profiles in abscess-associated microglia and macrophages compared with Th1 transfer. Collectively, these studies identified an amplification loop for Th1 and Th17 cells in shaping established innate responses during CNS infection to maximize bacterial clearance and differentially regulate microglial and macrophage secretory profiles.

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lating ongoing innate immune responses during infection. We demonstrate that TCR αβ+ cells regulate bacterial burdens, neutrophil and macrophage influx, and shape the cytokine/chemokine milieu during brain abscess development. In mice that lacked αβ T cells, γδ T cell infiltrates were elevated, which may represent a compensatory response to facilitate bacterial clearance. Adoptive transfer of either purified Th1 or Th17 cells into TCR αβ knockout (KO) mice was capable of restoring bacterial burdens and alterations in neutrophil and macrophage influx/activation to levels observed in wild-type (WT) animals, emphasizing the link between adaptive and innate immunity during CNS bacterial infection. Collectively, these results suggested that manipulating Th1 and Th17 cells could expedite *S. aureus* clearance from the CNS parenchyma and limit the extent of tissue damage.

Materials and Methods

**Mouse strains**

TCR αβ KO (C57BL/6 background; CD45.2), C1d KO, and B6 SJL mice congenic for the CD45 allele (CD45.1) on a C57BL/6 background were purchased from The Jackson Laboratory (Bar Harbor, ME). For the majority of studies, age- and sex-matched C57BL/6 mice were obtained from Charles River (Frederick, MD) as WT controls. To exclude potential variation arising from strain differences between C57BL/6 and C57BL/6J mice, several adoptive transfer experiments were also performed with age-matched C57BL/6J animals purchased from The Jackson Laboratory. Both approaches produced nearly identical results, allowing the conclusion that strain differences in the source of C57BL/6 mice did not impact the results obtained.

**Bacterial strain and generation of experimental brain abscesses**

*S. aureus* strain USA300, a community-acquired MRSA clinical isolate recovered from a patient with a fatal brain abscess (22), was encapsulated with live USA300 (1–2 × 10^5^ CFU) by stereotaxic injection into the striatum and monitored daily for clinical signs of disease, including hunched posture, ruffled fur, lethargy, and weight loss. The animal-use protocol was approved by the University of Nebraska Medical Center Institutional Animal Care and Use Committee and is in accord with the University of Nebraska Medical Center regulations for the use of rodents.

**Recovery of brain abscess-associated cells and FACS analysis**

FACS analysis was used to characterize the relative percentages of abscess-associated T cell subsets and their infiltration kinetics, in addition to the effects of manipulating the T cell compartment on innate immune cell influx into brain abscesses. Briefly, mice were perfused to eliminate leukocytes from the vasculature, whereupon the entire infected hemisphere was collected to recover abscess-associated cells. Brain tissues were minced in HBSS supplemented with 10% FBS (HyClone, Logan, UT) and filtered through a 70-μm nylon mesh cell strainer, whereupon an aliquot of tissue homogenate from each animal was removed to quantitate bacterial burdens. Bacterial strain and generation of experimental brain abscess...
100 μl PBS, into the retro-orbital sinus 1 d prior to brain abscess induction, whereas control animals received an equivalent volume of sterile PBS. Based on intracellular cytokine staining results, the numbers of Th1 or Th17 cells injected into recipients was adjusted so that 10^6 cells of each cytokine specificity were injected (which increased the total number of adoptively transferred cells by ~10–15%).

**MILLIPLEX multianalyte bead arrays**

To evaluate proinflammatory mediator expression profiles in brain abscess homogenates and microglia and macrophages recovered from brain abscesses of WT and TCR δKO mice, a custom-designed mouse cytokine/chemokine microbead array was used, according to the manufacturer’s instructions (MILLIPLEX; Millipore, Billerica, MA). This microbead array allows for the simultaneous detection of 19 individual inflammatory molecules in a single 75-μl brain homogenate, including IL-1α, IL-1β, TNF-α, IFN-γ, IL-6, IL-9, IL-10, IL-12p70, IL-12p40, IL-15, IL-17, CXCL1/keratinocyte chemotactant, CXCL2/macrophage inflammatory protein (MIP)-2, CXCL9/monokine induced by IFN-γ, CXCL10/IFN-γ–induced protein 10, CCL2/MCP-1, CCL3/MIP-1α, CCL4/MIP-1β, and CCL5/RANTES. Results were analyzed using a Bio-Plex workstation (Bio-Rad) and adjusted based on the amount of total protein extracted from brain tissue homogenates or per 10^4 cells for normalization.

**Statistical analysis**

Significant differences between experimental groups were determined by one-way ANOVA, followed by the Holm–Sidak method for pair-wise comparisons using SigmaStat (SPSS Science, Chicago, IL).

**Results**

**Brain abscesses are typified by CD4+ and NKT cell infiltrates**

The current study used an *S. aureus* USA300 isolate with important clinical origins (i.e., recovered from an otherwise healthy patient who died of a brain abscess) (22), which is more reminiscent of strains causing natural CNS infections. To investigate the impact of adaptive immune cells on ongoing innate responses during brain abscess development, the infiltration kinetics of various T cell populations was first established. Although we recently examined the impact of TLR2 on T cell infiltration kinetics, these studies were performed with either an *S. aureus* laboratory-adapted strain (20), or limited time points were examined (21). In this article, we report that CD4+ T cell infiltrates were first detectable on day 3 and progressively increased until day 14 after *S. aureus* exposure, with peak levels averaging 20–35% of the total leukocyte infiltrate (Fig. 1). In contrast, fewer CD8+ or γδ T cells were detected over the course of infection.

The influx of NK1.1+ (NKT) cells was more short-lived compared with the CD4+ population, with cells first detected at day 3 postinfection and peaking around day 7 (Fig. 1). Because NK1.1 is expressed on both NK and NKT cells, a NK cell-specific Ab (i.e., NKp46) was used to discriminate between these populations. This staining approach revealed that the majority of NK1.1+ cells

**FIGURE 2.** IL-17–producing CD4+ T cells progressively increase during brain abscess evolution. Abscess-associated CD3+CD4+ T cells were isolated from C57BL/6 mice at the indicated day postinfection by FACS, immediately stimulated ex vivo with PMA + ionomycin for 3 h, and stained for IL-17 or IFN-γ to demonstrate cytokine profiles. A, Representative dot plots depicting cytokine staining at day 7 postinfection. Note that total CD4+ T cells were sorted and immediately processed for intracellular cytokine staining; therefore, CD4 staining was still present in the isotype controls for IL-17 and IFN-γ. B, Changes in the percentages of IL-17– and IFN-γ–producing CD4+ T cells over the course of infection. Results represent the mean ± SEM combined from three independent experiments. *p < 0.05, IL-17+ versus IFN-γ+ cells.
in the infected brain were NKp46−, indicating that they were NKT cells (Supplemental Fig. 1A). Furthermore, CD1d tetramers were also used to confirm the presence of invariant NKT cells infiltrating brain abscesses (Supplemental Fig. 1B). An NKp46+ NK1.1+ population was apparent in the spleens of the same mice, demonstrating that NK cells could be detected, although they did not represent a major brain abscess infiltrate (Supplemental Fig. 1A). Collectively, these findings demonstrated that cell populations known to express the αβ TCR represent the main adaptive infiltrates during brain abscess development.

**IL-17–producing CD3αCD4+ T cell infiltrates predominate during brain abscess evolution**

To characterize the cytokine-expression profiles of infiltrating T cell populations over the course of infection, intracellular cytokine staining was performed. Abscess-associated CD3αCD4+ cells were found to produce both IFN-γ and IL-17 (Fig. 2); however, the percentages of CD3αCD4+ IL-17–expressing cells progressively increased over time, whereas CD4+ IFN-γ–producing cells remained relatively constant (Fig. 2). Because both IL-17 and IFN-γ can elicit inflammatory mediator release from several innate immune cell populations, this suggested a link between adaptive and innate immune responses during late-stage CNS parenchymal infection.

**TCR αβ+ cells impact bacterial clearance during brain abscess development**

Because the majority of brain abscess-associated T cell infiltrates are known to express the αβ TCR (i.e., CD4+, NKT, and CD8α cells), we next examined the functional importance of these

![Graph A](image1.png)

**FIGURE 3.** TCR αβ+ cells contribute to bacterial clearance. Brain abscesses were induced in TCR αβ KO and WT mice, whereupon percent survival (A) and bacterial burdens (B) were determined at the indicated time points postinfection. Results represent the mean ± SEM combined from three independent experiments. *p < 0.05, TCR αβ KO versus WT mice.

![Graph B](image2.png)

**FIGURE 4.** TCR αβ+ cells impact chemokine production during brain abscess development. Brain abscesses were induced in TCR αβ KO and WT mice (n = 4–5/group), whereupon abscess homogenates were collected at the indicated time points postinfection, and CXCL2, CCL2, CCL5, and CXCL9 expression was examined using a multi-analyte bead array with results normalized to total protein to correct for differences in tissue sampling size. Results represent the mean ± SEM combined from three independent experiments. *p < 0.05, TCR αβ KO versus WT mice.
populations on a more global scale using TCR αβ KO mice. TCR αβ KO mice were more sensitive to CNS S. aureus infection as revealed by reduced survival rates, which correlated with significantly elevated bacterial burdens compared with WT animals at days 7 and 14 postinfection (Fig. 3A, 3B, respectively). Importantly, αβ+ T cells did not impact S. aureus titers during early infection (i.e., day 3), which was expected because T cell influx was minimal at this time point. These results demonstrated that innate immune mechanisms are effective at controlling bacterial burdens during early infection; however, assistance from TCR αβ+ cells is required to maintain ongoing antibacterial responses at later time points following infection.

**TCR αβ+ cells impact chemokine expression and innate immune cell influx during later stages of brain abscess formation**

To investigate the impact of TCR αβ+ cells on the local cytokine and chemokine milieu, inflammatory mediator expression was quantitated in brain abscess homogenates using multiplex micro bead arrays. Interestingly, the neutrophil chemoattractant CXCL2 was elevated in lesions of TCR αβ KO mice, whereas CCL5 and CXCL9 were significantly decreased (Fig. 4).

Because TCR αβ KO mice demonstrated impaired CNS bacterial clearance and alterations in chemokine expression, we next determined whether this could be explained by differences in the numbers and/or activation status of infiltrating innate immune cell populations. To examine this possibility, FACS analysis was used to quantitate neutrophils (Ly-6G+, F4/80−, CD45hi), macrophages (F4/80+, CD45lo), microglia (F4/80+, CD45lo-intermediate), and MHC class II+ macrophages quantified by FACS. Results represent the mean ± SEM combined from three independent experiments. *p < 0.05, TCR αβ KO versus WT mice.

![Graph showing the percentage of neutrophils, macrophages, and MHC class II+ macrophages over time](http://www.jimmunol.org/)

**FIGURE 5.** TCR αβ+ cells regulate neutrophil and macrophage infiltrates during late-stage brain abscess development. Abscess-associated cells were collected from TCR αβ KO and WT mice (n = 4–5/group), whereupon the percentages of neutrophils (F4/80+, CD45hi, Ly6G+), macrophages (F4/80+, CD45hi), and MHC class II+ macrophages were quantified by FACS. Results represent the mean ± SEM combined from three independent experiments.

In contrast, fewer macrophages infiltrated abscesses of TCR αβ KO mice at days 7 and 10 postinfection (Fig. 5), which correlated with decreases in CCL5 and CXCL9 expression (Fig. 4). In addition, those macrophages that infiltrated abscesses of TCR αβ KO mice were less activated, as revealed by diminished MHC class II and inflammatory cytokine expression (Fig. 5, data not shown). No significant changes in the relative percentages or absolute numbers of microglia were observed between abscesses of TCR αβ KO and WT mice (data not shown). Both neutrophil and macrophage infiltrates were equivalent between TCR αβ KO and WT mice at day 3 postinfection (Fig. 5), which was expected because T cell influx was minimal at this time point.

Although initial analysis of abscess-associated T cell populations did not reveal a significant γδ T cell infiltrate, we elected to evaluate this subset in TCR αβ KO mice because it represented the sole remaining T cell type. Interestingly, γδ T cells were significantly elevated in TCR αβ KO animals at days 10 and 14 postinfection (Fig. 6). This may represent a compensatory response to combat elevated bacterial burdens, because γδ T cells use their TCR as a pattern recognition receptor to identify microbial peptide Ags and elicit IL-17 and IFN-γ release (29). Indeed, the time frame during which elevated γδ T cell influx was observed coincided with the decline in bacterial burdens in TCR αβ KO mice, although titers remained significantly elevated compared with WT animals (Fig. 3). Collectively, these results indicated that TCR αβ+ cells play an important role in regulating innate immune cell influx during CNS infection.

**FIGURE 6.** Loss of TCR αβ cells leads to exaggerated γδ T cell influx in brain abscesses. Abscess-associated cells were isolated from TCR αβ KO and WT mice (n = 4–5/group), whereupon the percentages of γδ T cells were identified by FACS. Results represent the mean ± SEM combined from three independent experiments. *p < 0.05, TCR αβ KO versus WT mice.
Adoptive transfer of Th1 or Th17 cells facilitates bacterial clearance and restores innate immune responses in TCR αβ KO mice

To identify which αβ TCR population was most pivotal for maintaining innate immunity during brain abscess development, we performed initial adoptive transfer studies with total CD4+ T cells into TCR αβ KO mice, because they represented the main abscess-associated T cell infiltrate. For these experiments, CD4+ T cells isolated from B6/SJL congenic mice (CD45.1) were depleted of NKT cells and adoptively transferred into TCR αβ KO animals (CD45.2) to facilitate their identification. Infiltration of adoptively transferred CD4+ T cells into brain abscesses of TCR αβ KO mice was demonstrated by the presence of CD45.1+ cells in the parenchyma (Supplemental Fig. 2). Importantly, CD4+ T cell adoptive transfer was capable of reducing S. aureus burdens at both days 7 and 14 postinfection compared with TCR αβ KO mice that did not receive T cells, with titers in the former approaching those observed in WT animals (Fig. 7). Similar restorative responses were also observed with regard to the ability of CD4+ T cell transfer to decrease neutrophil and enhance macrophage influx to levels observed in WT mice (Fig. 7). Together, these data indicated that CD4+ T cells are a major driving force to maintain ongoing innate immune responses during CNS infection, whereas CD8+ and NKT cells play a relatively minor role in comparison.

To establish the contribution of Th1 versus Th17 cells in regulating ongoing innate immune responses during CNS abscess development, naive CD4+ T cells were exposed to cytokine cocktails that skew toward a Th1 or Th17 phenotype (26, 27). Successful establishment of Th1 or Th17 polarization was confirmed by intracellular cytokine staining for IFN-γ and IL-17 prior to adoptive transfer, although some IFN-γ/IL-17 double-positive cells were also observed (Supplemental Fig. 3). Infiltration of adoptively transferred Th1 or Th17 cells into brain abscesses of TCR αβ KO mice was demonstrated by tracking CD45.1+ expression and the stability of each Th subtype upon recruitment into brain abscesses was evaluated by intracellular cytokine staining. The majority of adoptively transferred Th1 and Th17 cells homed to the infected brain, whereas fewer cells were distributed in the draining deep and superficial cervical lymph nodes (data not shown). Interestingly, adoptive transfer of either Th1 or Th17 cells was capable of reducing S. aureus burdens at both days postinfection. Brain abscesses were induced in WT, TCR αβ KO, and TCR αβ KO mice that received an adoptive transfer of either 10^6 in vitro skewed Th1 or Th17 cells 24 h prior to S. aureus infection (n = 4–5/group). Animals were euthanized at the indicated time points postinfection, whereupon bacterial burdens were quantitated and normalized to tissue wet weight (g), and neutrophil and macrophage infiltrates were determined by FACS. Results represent the mean ± SEM combined from three independent experiments. *p < 0.05, PBS-injected TCR αβ KO mice versus TCR αβ KO animals receiving adoptively transferred T cells.
7 and 14 postinfection compared with TCR αβ KO mice that did not receive T cells (Fig. 8). In addition, Th1 and Th17 adoptive transfer restored neutrophil numbers, as well as macrophage infiltrates and MHC class II expression, often to a greater extent than that observed in WT mice (Fig. 9, data not shown). Together, these findings suggested that both Th1 and Th17 cells play an important role in eliciting maximal innate immune responses to facilitate bacterial clearance during later stages of brain abscess development.

**Th17 cells demonstrate plasticity following CNS infection**

Interestingly, Th17 adoptive transfer was highly effective at promoting bacterial clearance in TCR αβ KO mice, particularly at day 14 postinfection (Fig. 8). This may be attributed to the more plastic nature of Th17 cells and their ability to acquire IFN-γ production when exposed to high levels of IL-12 and IFN-γ (30, 31). Indeed, this possibility was supported in our studies in which a significant proportion of adoptively transferred Th17 cells, recovered from brain abscesses at days 7 and 14 postinfection, exhibited IFN-γ production (Fig. 10, Supplemental Fig. 3). In contrast, the percentages of infiltrating Th1 cells remained similar to those originally transferred (Fig. 10, Supplemental Fig. 3), which supports their relatively stable phenotype, as described in the literature (32). Although a population of IFN-γ/IL-17 double-positive cells was observed during both Th1- and Th17-polarizing conditions in vitro, the frequency of double-positive CD4 T cells recovered from the infected brain following Th17 transfer was not significantly different (Supplemental Fig. 3). This finding suggested that the apparent plasticity of Th17 cells during CNS infection is not due to increased numbers of IFN-γ/IL-17 double-positive cells. The low levels of IL-17+ cells detected in brain abscesses of TCR αβ KO mice following Th1 transfer (Fig. 10) is likely due to the fact that a small percentage of adoptively transferred cells also produced IL-17 following in vitro skewing (Supplemental Fig. 3).

**Th1 and Th17 cells differentially influence inflammatory mediator secretion profiles in microglia and macrophages**

Although adoptive transfer of either Th1 or Th17 cells was capable of restoring defects in macrophage recruitment into brain abscesses of TCR αβ KO mice, it was not known whether these Th subsets would lead to differential secretory profiles of infiltrating macro-

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**FIGURE 9.** Th1 and Th17 cells regulate neutrophil and macrophage infiltrates during late-stage brain abscess development. Abscess-associated cells were collected from WT, TCR αβ KO, and TCR αβ KO mice that received an adoptive transfer of either $10^6$ in vitro skewed Th1 or Th17 cells 24 h prior to *S. aureus* infection ($n = 4–5$/group). The absolute numbers of neutrophils (Ly-6G+, F4/80−, CD45hi) and macrophages (F4/80+, CD45hi) were quantitated by FACS. Results represent the mean ± SEM combined from three independent experiments. *p < 0.05, TCR αβ KO versus WT or TCR αβ KO mice receiving Th1 or Th17 cells.
To address this question, microglia and macrophages were isolated from brain abscesses of WT, TCR αβ KO, and TCR αβ KO mice receiving adoptively transferred Th1 or Th17 cells by FACS, whereupon cells were incubated in vitro for a 24-h period without bacterial restimulation, in an attempt to capture cellular activation states that were established in vivo. Interestingly, abscess-associated macrophages appeared to be most affected by TCR αβ loss, as reflected by reductions in CXCL1, CXCL2, and TNF-α expression, whereas microglial secretory activity was more refractory to TCR αβ cell loss (Fig. 11). Interestingly, Th1 adoptive transfer significantly augmented macrophage activation, as revealed by CXCL1, CXCL2, and TNF-α expression, whereas microglial secretory activity was more refractory to TCR αβ cell loss (Fig. 11). Interestingly, Th1 adoptive transfer significantly augmented macrophage activation, as revealed by CXCL1, CXCL2, CCL5, and TNF-α release equal to or exceeding WT levels, whereas Th17 cells had little effect (Fig. 11). In contrast, Th17 cells increased microglial cytokine/chemokine release, but Th1 cells had minimal impact (Fig. 11). Significant proinflammatory mediator release was only detected in macrophages and microglia recovered from brain abscesses at day 7, but not day 14, following infection (Fig. 11, data not shown, respectively), which may be a consequence of waning cell activation associated with declining bacterial burdens or, alternatively, mediator levels falling below the limit of detection. Collectively, these results indicated that Th1 and Th17 cells target distinct APC populations in the context of established CNS infection (Fig. 12).

Discussion
Although it is well established that innate immunity is required for optimal establishment of adaptive immune responses, comparatively fewer studies have examined the reciprocal relationship. The latter has received greater attention recently with the identification of Th17 cells regulating innate immune mechanisms (33–35). However, to the best of our knowledge, there is little information describing the ability of adaptive immunity to shape ongoing innate immune responses during CNS bacterial infection, which warrants investigation because a robust innate immune response is essential for efficient bacterial clearance (10, 11, 14). The current study revealed cross-talk between the adaptive and innate arms during CNS infection by demonstrating that Th1 and Th17 cells play an important role in expediting bacterial clearance and impacting neutrophil and macrophage recruitment and activation status (Fig. 12). In establishing that T cells positively regulate ongoing innate immune responses during brain abscess development, it may be possible to manipulate their activity to expedite bacterial clearance. This rapid response may equate to a reduction in tissue necrosis and decline in long-term neurologic deficits that often affect patients who recover from brain abscesses (2–4).

Interestingly, our data demonstrated Th17 plasticity within the infected brain, which, to our knowledge, represents the first report of this process during CNS bacterial infection. Specifically, following Th17 transfer, the frequency of Th1 cells associated with brain abscesses was significantly increased compared with the small percentage of IFN-γ–producing cells that were originally injected along with Th17 cells (a 100% pure population of Th17 cells could not be attained with currently available cytokine cocktails). In contrast, the percentages of Th1 cells recovered from brain abscesses nearly equaled those that were originally transferred (Supplemental Fig. 4). These findings are in agreement with recent studies in models of experimental autoimmune encephalo-
lomyelitis in which Th17 cells were found to acquire IFN-γ production, whereas Th1 cells were shown to be a more stable phenotype (30).

Currently, it is not possible to evaluate the impact of S. aureus-specific Th1 or Th17 cells in TCR αβ KO mice because immunodominant S. aureus Ags remain to be defined. Therefore, our
The approach was to expand naive CD4+ cells from mice without prior S. aureus infection and skew cells toward a Th1 or Th17 phenotype in vitro prior to adoptive transfer into TCR αβ KO mice. Because of the strong chemokine gradients generated during infection, we could not assess the impact of S. aureus-reactive versus nonspecific T cell infiltrates that entered the infected CNS. In addition, it is important to note that S. aureus produces numerous superantigens that lead to the clonal activation of T cell subsets bearing specific Vβ TCRs. Indeed, a recent study from our laboratory showed the preferential accumulation of Vβ8.2+ T cells in brain abscesses that are reactive with staphylococcal enterotoxin B (21, 36). Importantly, we also found that T cells are highly activated within brain abscesses and, upon isolation, continue to proliferate at least once in vitro without further restimulation (data not shown), suggesting the presence of potent T cell proliferation signals in vivo. This possibility is also strengthened by the fact that we only transferred a total of 10^6 Th1 or Th17 cells into TCR αβ KO mice, yet significant effects on innate immune mechanisms and bacterial burdens were observed, implying their in vivo expansion. However, it is likely that T cells were also driven to expand via homeostatic proliferation to fill the void in the T cell compartment in TCR αβ KO mice. Studies are currently in progress to evaluate whether adoptive transfer of CD4+ T cells from OVA TCR-transgenic mice impact innate responses during brain abscess development in TCR αβ KO animals to address the requirement for Ag specificity.

It is important to note similarities and differences between our model system and other recent studies examining CD4+ T cells and bacterial infections, because it emphasizes the importance of the site of infection and the immune cells that can most readily access various tissues. For example, McLoughlin and colleagues (37, 38) reported that abscesses did not form during S. aureus skin and soft tissue infections in TCR αβ KO mice, which were associated with efficient neutrophil recruitment and reduction in bacterial burdens. In contrast, we showed that TCR αβ KO mice displayed impaired S. aureus clearance within the CNS, typified by enhanced neutrophil accumulation, likely in an attempt to contain the infection. It is important to note that the findings from McLoughlin and colleagues may be attributed to compensatory activity by epidermal γδ T cells, which can also produce IFN-γ and IL-17; however, this possibility was not examined by the investigators. Indeed, we observed in the current study that γδ T cell influx was significantly elevated at later stages of brain abscess development in TCR αβ KO mice. The functional impact of these cells in controlling infection in the absence of other T cell populations remains uncertain. However, it is clear from our studies that CD4+ Th1 and Th17 cells are critical in shaping the intensity and duration of ongoing innate responses during late-stage CNS infection. Although NKT cells can express CD4, their possible involvement was minimized during the sorting process by only collecting CD4+ NK1.1+ cells. However, NKT cells were reported to express several combinations of surface markers, often with transient expression patterns; therefore, a subset of NKT cells may still have been included in our adoptive transfer studies (18, 39). Because of this, a definitive role for NKT cells in regulating inflammation during brain abscess development is currently being explored in our laboratory using CD1d KO mice that lack all NKT subsets (40, 41).

Another intriguing finding was that Th1 and Th17 cells induced differential inflammatory secretion profiles in abscess-associated macrophages versus microglia. For example, adoptive transfer of Th1 cells led to enhanced chemokine and TNF-α production in macrophages, whereas microglia were not affected. In contrast, Th17 transfer led to increased mediator release from microglia but had minimal effects on macrophages. These findings indicated the existence of novel cross-talk between each Th subset and mononuclear phagocyte target; however, the specific modes of action responsible for these differences remain to be defined. Another interesting observation was that infiltrating macrophages were more sensitive to the loss of TCR αβ+ cells, because inflammatory mediator release was dramatically reduced in macrophages recovered from abscesses of TCR αβ KO mice. In contrast, the extent of microglial activation, as measured by proinflammatory mediator release, was similar in microglia recovered from brain abscesses of WT and TCR αβ KO animals. Macrophage activation may be especially critical, because the chemokines CXCL9 and CCL5 have also been shown to exhibit direct microbicidal activity (42–45) and are significantly attenuated in TCR αβ KO mice. Therefore, the reduced expression of these kinocidins may represent one mechanism responsible for the increased bacterial burdens observed in TCR αβ KO animals. Additionally, the marked decrease in MHC class II expression in macrophages recovered from brain abscesses of TCR αβ KO mice is likely due to diminished IFN-γ levels, a major cytokine product of Th1 cells, which is known to upregulate MHC class II expression. To our knowledge, this is the first report demonstrating differential effects of Th1 and Th17 cells on microglia versus macrophages during CNS infection, discerning the fact that specialized responses are triggered during inflammation. It will be interesting to determine whether these differences are localized to specific microdomains within the abscess environment; however, this question lies beyond the scope of the current report.

In summary, this study demonstrated the important role that adaptive immunity plays in shaping established innate immune responses during CNS infection. Specifically, we found that Th1 and Th17 cells facilitate bacterial clearance and neutrophil and macrophage infiltration/activation during the later stages of brain abscess formation. Importantly, Th17 cells infiltrating brain abscesses displayed plasticity and acquired the ability to produce IFN-γ. In addition, another novel aspect of our work was the finding that Th1 and Th17 cells provide distinct signals that culminate in unique secretory profiles of resident microglia and infiltrating macrophages. Collectively, this information could be used to heighten antimicrobial activity to expedite bacterial clearance from the CNS during infections concomitant with conventional antibiotic therapy.

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Disclosures

The authors have no financial conflicts of interest.

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