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

Monica M. Holley and Tammy Kielian

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 εβ knockout (KO) mice, because CD4+ and NKT cells represented the most numerous T cell infiltrates. TCR εβ 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 εβ 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. The Journal of Immunology, 2012, 188: 1360–1370.

Brain abscess formation is consequent of chronic bacterial infections of the middle ear, sinuses, or teeth or from bacterial dissemination from systemic sites (1). Once pyogenic bacteria invade the brain parenchyma, a localized area of cerebritis ensues, which develops into a purulent lesion encapsulated by a well-vascularized fibrotic wall. Long-term morbidity issues often arise in patients recovering from brain abscesses as a result of the extensive parenchymal damage typically associated with infection, which can manifest as seizures, cognitive deficits, and/or hemiparesis (2–4). Diagnosing and treating brain abscesses can be complicated by delays in appropriate treatment due to initial nonspecific clinical presentation and may conclude in the rupture of untreated lesions within the ventricular space engendering up to 80% mortality (1, 3). Currently, brain abscess therapy encompasses long-term systemic antibiotics (6–8 wk duration) and surgery or guided needle aspiration to allow for drainage and a reduction in intracranial pressure (3). It is estimated that 1 in every 10,000 hospital admissions of an infectious disease nature in the United States results from a brain abscess, with Staphylococcus aureus representing one of the most frequent causes (1, 5). Although only a few CNS infections have been attributed to methicillin-resistant S. aureus (MRSA) to date, the emergence of community-acquired MRSA strains causing brain abscesses is increasing, with infections occurring in otherwise healthy individuals (6, 7). Therefore, alternative approaches, such as immune modulation, could enhance treatment options once more is known about the antibacterial immune responses that ensue during brain abscess development and mechanisms responsible for maximal pathogen clearance. Indeed, immune-based approaches represent an attractive therapeutic alternative to antibiotics by minimizing direct mutational pressures on bacteria and decreasing the likelihood of developing resistant strains.

Previous studies from our laboratory and other investigators established the genesis of a rapid innate immune response during CNS abscess formation (8–11). For example, microglial and astrocyte activation is immediately evident within hours postinfection, followed by neutrophil accumulation within 12–24 h, which continues throughout abscess development (11–13). Macrophages accumulate along the abscess margins and are readily detected at day 3 postinfection, and prior work from our laboratory established that rapid pathogen recognition within the CNS compartment is essential for establishing a protective antimicrobial response (14).

Despite this information, the mechanisms responsible for maintaining a robust innate immune response to ensure pathogen clearance during late-stage CNS infection remain relatively undefined. One possibility is that components of adaptive immunity, in particular various T cell populations, provide critical signals to perpetuate ongoing innate antibacterial responses. This possibility is supported by recent studies in which Th17 cells impact innate immune responses via indirect effects on neutrophil recruitment (15–17), as well as NKT cells that span innate and adaptive immunity (18, 19). Although some information is available regarding the kinetics of T cell entry into brain abscesses (10), these studies were either performed with a laboratory-adapted S. aureus strain (20), or limited time points were examined (21). More importantly, the functional impact of T cell populations on brain abscess progression, the main subtypes involved, and their cross-talk with ongoing innate antibacterial responses have not been investigated, and all represent novel aspects of the current study.

The objective of the current report was to assess the functional importance of major abscess-associated T cell subsets in modu-
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), CD1d 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 in agarose beads prior to injection, as previously described (11). Mice were infected with 2 μl live USA300 (1–2 × 10^6 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 protocol was approved by the University of Nebraska Medical Center Institutional Animal Care and Use Committee and is in accord with the National Institutes of Health (NIH) guidelines 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. Next, the resulting slurry was digested for 30 min at 37˚C of tissue homogenate from each animal was removed to quantitate bacterial burdens. Next, the resulting slurry was digested for 30 min at 37˚C of tissue homogenate from each animal was removed to quantitate bacterial burdens. Next, the resulting slurry was digested for 30 min at 37˚C of tissue homogenate from each animal was removed to quantitate bacterial burdens. Next, the resulting slurry was digested for 30 min at 37˚C of tissue homogenate from each animal was removed to quantitate bacterial burdens. Next, the resulting slurry was digested for 30 min at 37˚C of tissue homogenate from each animal was removed to quantitate bacterial burdens. Next, the resulting slurry was digested for 30 min at 37˚C of tissue homogenate from each animal was removed to quantitate bacterial burdens. Next, the resulting slurry was digested for 30 min at 37˚C of tissue homogenate from each animal was removed to quantitate bacterial burdens. Next, the resulting slurry was digested for 30 min at 37˚C of tissue homogenate from each animal was removed to quantitate bacterial burdens. Next, the resulting slurry was digested for 30 min at 37˚C.
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 αβ KO 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 chemoattractant, 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 10^6 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 multiple comparisons using SigmaStat (SPSS Science, Chicago, IL).

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**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...
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 CD3+CD4+ 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 CD3+CD4+ cells were found to produce both IFN-γ and IL-17 (Fig. 2); however, the percentages of CD3+CD4+ 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 CD8+ cells), we next examined the functional importance of these
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 were quantified by FACS. Results represent the mean ± SEM combined from three independent experiments. *p < 0.05, TCR αβ KO versus WT mice.

**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. *p < 0.05, TCR αβ KO versus WT mice.

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.
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. 4), 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-

![Graphs showing neutrophil and macrophage counts](image1)

**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^−, CD45^hi) and macrophages (F4/80^+, CD45^hi) 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.
phages or resident microglia in vivo. 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 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 expression, whereas microglial secretory activity was more refractory to TCR αβ cell loss (Fig. 11). Significantly, Th1 adoptive transfer significantly augmented macrophage activation, as revealed by CXCL1, CXCL2, CCL5, and TNF-α expression, whereas microglial secretory activity was more refractory to TCR αβ cell loss (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 encephalitis.
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

**FIGURE 11.** Th1 and Th17 cells elicit distinct inflammatory mediator-secretion profiles in abscess-associated macrophages and microglia. Macrophages (F4/80+, CD45hi) and microglia (F4/80+, CD45lo-intermediate) were isolated by FACS from brain abscesses of WT, TCR αβ KO, or TCR αβ KO mice after adoptive transfer of Th1 or Th17 cells at day 7 postinfection. Abscess-associated macrophages and microglia were incubated for 24 h in vitro without bacterial restimulation, in an attempt to capture cellular activation states that were established in vivo, whereupon inflammatory mediator expression was evaluated by microbead array analysis. Results represent the amount of inflammatory mediator expression normalized per 10⁴ cells. 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 Th1 or Th17 cell transfer.

**FIGURE 12.** Th1 and Th17 cells impact established innate immune responses during later stages of brain abscess development. CD3⁺CD4⁺ Th1 and Th17 cells regulate neutrophil and macrophage infiltrates during later stages of CNS parenchymal infection to expedite bacterial clearance. In addition, Th1 cells target infiltrating macrophages to regulate inflammatory mediator release, whereas Th17 cells appear to preferentially affect microglia. It remains to be determined whether these events are Ag specific or instead result from the intense chemokine gradient generated during infection and nonspecific T cell activation by staphylococcal superantigens.
model system and other recent studies examining CD4+ T cells and requirement for Ag specificity.

In contrast, we showed that TCR αβ T cells from OVA TCR-transgenic mice impact innate responses during tissue infections in TCR 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.

References


Supplemental Fig 1. Brain abscesses are associated with NKT but not NK cell infiltrates. (A) Cells were isolated from brain abscesses or spleens of C57BL/6 mice at day 7 post-infection and stained with NK1.1-FITC, CD4-PECy5, and NKp46-Alexa Fluor 647 for FACS analysis to differentiate between NK (CD4−, NK1.1+, NKp46+) and NKT cells (CD4+, NK1.1+, NKp46−). Results are representative of five independent experiments. In (B), abscess-associated cells were isolated from WT and NKT (CD1d) KO mice at day 5 post-infection, whereupon invariant NKT cells were identified on the basis of CD1d tetramer staining and absence of the NK-specific marker NKp46. Additional evidence to confirm the identity of invariant NKT infiltrates was the lack of CD1d tetramer staining in CD1d KO mice, which lack all NKT populations. Results are representative of two independent experiments.
Supplemental Fig 2. Adoptively transferred CD4$^+$ T cells migrate into brain abscesses. Brain abscesses were induced in WT, TCR $\alpha\beta$ KO (both CD45.2), and TCR $\alpha\beta$ KO mice that received an adoptive transfer of 10$^6$ purified CD4$^+$ T cells (NKT-depleted) from B6/SJL mice (CD45.1) 24 h prior to S. aureus infection (n= 4-5/group). Animals were euthanized at day 7 post-infection, whereupon the extent of CD4$^+$ T cell invasion was determined by FACS. Results are representative of eight independent experiments.
Supplemental Fig 3. Demonstration of IFN-γ/IL-17 double-positive cells following in vitro skewing and plasticity of Th17 cells following CNS extravasation. (A) In vitro skewing of naïve CD4+ T cells led to the induction of IFN-γ/IL-17 double-positive cells
under both Th1 and Th17 polarizing conditions, which remained relatively constant upon recovery from brain abscesses at day 14 after infection. (B) Comparisons in the percentages of IL-17 producing CD4+ T cells following in vitro skewing (top) vs. adoptively transferred cells recovered from brain abscesses at day 14 post-infection (bottom). T cells were exposed to PMA/ionomycin for 3 h to evaluate IL-17 expression by intracellular cytokine staining. Results are representative of three independent experiments.
Supplemental Figure 4. Th1 cells maintain their cytokine expression profile upon migration into brain abscesses \textit{in vivo}. Comparisons in the percentages of IFN-\(\gamma\) producing CD4\(^+\) T cells following \textit{in vitro} skewing (top) versus adoptively transferred cells recovered from brain abscesses at day 14 post-infection (bottom). T cells were exposed to PMA/ionomycin for 3 h to evaluate IFN-\(\gamma\) expression by intracellular cytokine staining. Results are representative of three independent experiments.