Update on T cells in the virally infected brain: Friends and foes

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Update on T cells in the virally infected brain: friends and foes

Shenjian Ai and Robyn S. Klein

Purpose of review
The present review will outline neuroprotective and neurotoxic effects of central nervous system (CNS) infiltrating T cells during viral infections. Evidence demonstrating differential roles for antiviral effector and resident memory T-cell subsets in virologic control and immunopathology in the CNS will be discussed. Potential therapeutic targets emanating from a growing understanding of T-cell-initiated neuropathology that impacts learning and memory will also be delineated.

Recent findings
The critical role for T cells in preventing and clearing CNS infections became incontrovertible during the era of acquired immunodeficiency syndrome. Recent studies have further defined differential roles of T-cell subsets, including resident memory T cells (Trm), in antiviral immunity and, unexpectedly, in postinfectious cognitive dysfunction. Mechanisms of T-cell-mediated effects include differential innate immune signaling within neural cells that are virus-specific.

Summary
T-cell cytokines that are essential for cell-mediated virologic control during neurotropic viral infections have recently been identified as potential targets to prevent post-infection memory disorders. Further identification of T-cell subsets, their antigen specificity, and postinfection localization of Trm will enhance the efficacy of immunotherapies through minimization of immunopathology.

Keywords
cognition, glia, neuroimmune, T cells, viral encephalitis

INTRODUCTION
The central nervous system (CNS) has evolved multiple mechanisms to preserve and repair its complex structural and functional organization during neurologic diseases. After viral infections, innate and adaptive immune responses variably contribute to viral clearance and recovery, depending on the virus and its targets. Cell-mediated immunity in the brain primarily involves noncytolytic mechanisms of viral clearance that preserve neurons and supporting cells that express neurotropic factors (reviewed in [1]). T cells are critically involved in virologic control, gaining parenchymal access via local restimulation after T-cell receptor (TCR) recognition of viral antigens within perivascular spaces. Interactions with activated microglia also maintain T-cell effector functions within the CNS parenchyma. T cells may also undergo differentiation into regulatory, memory and tissue resident subsets, which, depending on their location, impact various aspects of brain function via cytokine-mediated effects on neural cells types including microglia, astrocytes and neural stem cells. These neuroimmune interactions may trigger processes that promote or prevent repair of acute injury sustained during the height of antiviral inflammatory responses, leading to recovery or progressive neurologic diseases, including dementia.

INFLAMMATION OF THE CENTRAL NERVOUS SYSTEM UPON VIRAL INFECTIONS
The CNS is a site of high anatomic and cellular complexity. The major cell types within the CNS...
KEY POINTS

- Virologic control within the CNS requires type I IFN-mediated establishment of chemokine gradients that recruit virus-specific T cells.
- Virus-mediated induction of type I IFN expression within the CNS is required for the differentiation and proliferation of infiltrating T cells into Tregs.
- Treg expression of TGF-β promotes CNS residency of CD8 T cells via upregulation of CD103.
- PD1 pathways inhibit CD8 T-cell activation, and promote their retention within the CNS as Tm.
- Trm-derived IFN-γ induces microglial activation resulting in elimination of synapses within the hippocampal circuitry involved in spatial learning.

include neurons, astrocytes, oligodendrocytes, ependymal cells, and microglia, all of which are potential viral targets and initiate innate immune signals to recruit antiviral mononuclear cells into the CNS for virologic control [2]. Three anatomic distinct barriers: acellular barrier, blood–brain barrier (BBB) and blood–cerebrospinal fluid barrier (BCSFB), surround the CNS and provide both immune specialization for the CNS and entry sites for immune cells upon pathogen invasion [3]. Infiltration of immune cells from the periphery is driven by a variety of factors including upregulation of adhesion molecules and disruption of endothelial and epithelial brain barriers [4], and the generation of appropriate chemoattractant gradients created by virally infected cells both at barriers and within the CNS [5,6]. These localizing cues promote not only the recruitment of mononuclear cells into perivascular spaces, but their parenchymal entry and interaction with resident cells within the CNS.

CENTRAL NERVOUS SYSTEM ANTIVIRAL IMMUNITY: INTERPLAY BETWEEN INTERFERON AND IMMUNE CELLS

Upon invasion of the CNS, viral pattern-associated molecular patterns (PAMPs) bind pattern recognition receptors (PRRs), leading to expression of type I interferons (IFN-I), which includes IFN-α and IFN-β that both bind to IFN-α receptor chain 1 (IFNAR1) [1]. IFNAR is expressed by all cell types, and activates the JAK–STAT signaling pathway upon binding to ligand. One of the major functions of IFN-I is to induce direct antiviral activities in an autocrine, paracrine, or systemic manner [7]. Uninfected microglia and astrocytes can therefore be the sources of IFN-I in the context of viral encephalitis, which will increase IFN-stimulated gene (ISG) expression of themselves and other cells expressing IFNAR1 [7,8]. CXCL10 and CCL2 are prominent chemokines and ISGs produced in the acute settings [5,9–11] to establish an inflammatory milieu in the CNS for the recruitment of infiltrating leukocytes. Although antiviral T-cell recruitment is a critical step in virologic control, recruited and resident myeloid antigen-presenting cells (APC) are required for local T-cell restimulation [12]. Members of the Flaviviridae family of small enveloped viruses with RNA genomes have evolved mechanisms to inhibit IFNAR signaling. For example, Zika virus (ZIKV), a neurotropic flavivirus that induces congenital and adult disorders of the CNS, induces human, but not murine, STAT2 degradation to inhibit IFN-I signaling [13]. Thus, initial investigations of CNS infections with ZIKV utilized either ZIKV-susceptible interferon α/β receptor-deficient (Ifnar1−−) mice or mice with antibody blockade of IFNAR signaling [14**,15**,16]. However, IFN-I is not only vital for antiviral responses, but also required for immunoregulatory control of immune cells. Loss of IFNAR signaling has been shown to impair the proliferation and activation of regulatory T cells (Treg) during acute lymphocytic choriomeningitis virus (LCMV) infection, and effector functions of CD8 and CD4 T cells, resulting in an inefficient viral clearance [17].

EFFICIENCY OF ANTIVIRAL RESPONSES: CHEMOKINE AXES

As mentioned above, ISGs downstream of IFNAR signaling include a variety of cytokines and chemokines that may destabilize BBB junctional proteins and/or recruit antiviral T cells, respectively. T-cell chemoattractants including CCL2, CCL5, CXCL9, and CXCL10 may also be induced by interleukin (IL)-1, TNF, and type II IFN (IFN-II). The expression of CXCR3, whose ligands are CXCL9 and CXCL10 under IFN induction, on T cells permits their entry into the CNS during infection. CXCL10 is expressed at the BBB. During CNS infection with West Nile Virus (WNV), CXCL10 expressed by neurons guides the trafficking of CXCR3+CD8+ T cells into the brain [18]. The CCR2–CCL2 axis, which recruits both myeloid and lymphoid cells, has been heavily studied in the migration of inflammatory monocytes during CNS infection and inflammation. Astrocytes have been shown to produce CCL2 in multiple neurologic disease, such as experimental autoimmune encephalomyelitis (EAE) [25], mechanical injury [26], and WNV infection [23]. Virally infected neurons, however, may also be a source of CCL2 [19], which might recruit
macrophages or activated microglia for phagocytic purposes. CCL2 is also expressed by activated endothelial cells and microglia, the latter of which may continue to express low levels of CCL2 after recovery [2]. A new subset of CD8 T cells expressing both CCR2 and programmed cell death protein 1 (PD-1) has been identified within the CNS of mice that were acutely infected via intravenous (i.v.) injection of Japanese encephalitis virus (JEV) [20]. High levels of CCR2 expression were also detected in hippocampal CD8 T cells after viral recovery of both WNV and ZIKV [21**]. These findings suggest that CCR2 expression may play a role in the maintenance and function of memory T cells in the CNS. The functional consequence of CCR2 expression by T cells in different stages of CNS pathology remains to be explored.

In addition to the T-cell/monocyte-recruiting CCL2, upregulation of proinflammatory chemokine CCL5, also known as RANTES (Regulated on Activation, Normal T-Cell Expressed and Secreted), is also commonly observed in the context of neuroinflammation. Both neurons and astrocytes have shown to be the source of CCL5 in WNV infection [19]. ZIKV and tick-borne encephalitis virus (TBEV), another neurotropic flavivirus endemic to Europe and the far east, also have been reported to induce CCL5 production in human primary astrocytes [22,23*]. Taken together, the interplay between neuronal and immune cells creates an IFN-γ–chemokine network, which is essential to initiating viral containment at acute stage of infection by promoting immune cell infiltration, directly involving lymphocytes and monocytes, in order to maintain the appropriate level of immune cell activities after viral clearance. The upregulation of sustained expression of chemokines could exert neurotoxicity via maintenance of neurotoxic T cells within the CNS parenchyma [21**].

T CELL SUBSETS AND ANTIVIRAL IMMUNITY IN THE CENTRAL NERVOUS SYSTEM

Cell-mediated immunity, especially the infiltration and accumulation of antiviral T cells in the CNS under virological challenge, is critical for virologic control and survival from viral encephalitis. Although both CD8 and CD4 T cells invade the CNS, CD8 T cells constitute the majority of the infiltrating lymphocytes [24**]. CD8 T cells that clear virus from infected neurons may do so largely through noncytolytic mechanisms that may be regulated by peripheral expression of IL-7 [25]. However, the continuous presence of T cells in the CNS after viral clearance has been shown to promote persistent microglial activation leading to synapse elimination with lack of repair [21**]. The CNS entry of T cells is tightly regulated through chemokine gradients that include luminal expression of CXCL10 and abluminal expression of CXCL12. CXCL10 expression at the BBB is associated with upregulation of barrier destabilizing cytokines, such as TNF, which increases BBB permeability [26–28], while CXCL12 ensures T cells localize to perivascular spaces where they may obtain pro- or anti-inflammatory cues from resident or infiltrating leukocytes [29]. The tight regulation of T-cell entry and cellular encounter supports the efficiency of the antiviral response, which likely prevents excessive bystander T-cell entry and injury, and may exhibit virus-specific effects (Table 1).

CD8 T-CELL SUBSETS: ROLES BEYOND VIRAL CLEARANCE?

A subset of CD8 T cells, brain-resident memory T cells (bTrm), can still persist at the sites of original infection even after viral clearance and remain within the parenchyma unlike other circulating lymphocytes, which is observed in both murine and human [30]. bTrm have been often reported to express CD103 and CD69, which is usually involved in the downregulation of sphingosine-1-phosphate receptor (S1PR1) to prevent tissue egress and to promote retention in lymphoid organs [24**,31,32*]. The necessity of CD103 expression on bTrm is not well determined while studies have shown divergent capacity of CD103+ and CD103− bTrm [33,34**]. In congenital murine cytomegalovirus (MCMV)-infected newborn mice, CD103+ sub-subset showed higher proliferation potential indicated by increased Ki-67 expression than its CD103− counterpart after reinfection while the protective ability was competent in both populations [33]. More IFN-γ production was also detected in CD103+ T cells compared to CD103− T cells in Murine polyomavirus (MuPyV)-induced encephalitis, a model for the DNA virus JC virus, which causes multifocal leukoencephalopathy (PML) in patients with severe T cell deficiencies [34**].

The inquiry of how bTrms are developed is still an active area of investigation. Local infection and direct antigen encounter (or in the deep cervical lymph nodes) in the CNS have been demonstrated to be crucial for formation, but not maintenance of CD8 bTrms since neuroinflammation in the absence of cognate antigen for T cells only induced transient CD8 T-cell infiltration [32*]. However, effects inflicted upon individuals by the residual T cells postinfection appear to be a double-edged sword. Survivors of previous neuropathic infections will manifest a stronger and more rapid immune
response against reinfection because of bTrms [35]. In the meantime, IFN-γ derived from persisting T cells in the CNS could drive microglia to promote cognitive impairment during recovery from neuro-pathogenic flaviviruses such as WNV and ZIKV [21,71]. The increase of cytokine production in CNS-residing lymphocytes is also associated with postoperative cognitive dysfunction [36], which indirectly emphasized the role of the cytokine milieu maintained by immune cells in the brain.

**CD4 T CELLS: PROTECTIVE AND REGULATORY FUNCTIONS**

Recent studies have been focusing on the role of CD4 T cells in the context of neurotropic infections, especially some flavivirus infections [37–40]. Mariah et al. [14**] showed that CD4 T cells are able to confer protection against a lethal ZIKV challenge. Antibody depletion of CD4 T cells in Ifnar−/− mice showed a significant weight loss, higher viral titers in the brains and spinal cords, more severe clinical phenotypes and more deaths compared to control animals [14**]. Adoptive transfer of ZIKV-experienced CD4 T cells ensured survival of most mice under lethal i.v. ZIKV infection while all the mice that received the naive CD4 cells succumbed [14**]. Although these immunodeficient mice may not faithfully reproduce host immune responses observed in humans, as described above, these experiments support multiple prior studies demonstrating a critical role for CD4 T cells in antiviral immunity in the CNS. Transforming growth factor beta (TGF-β) produced by Treg inducing CD103 expression on CD8 T cells has been well examined [41]. CD103 (i.e., integrin αEβ7) is the ligand for an adhesion molecule E-cadherin, which could be related to T cell retention within the brain. In Treg-depleted mice, CD103+ CD8 bTrms are significantly reduced following MCMV infection from 7 days post infection (dpi) to 30 dpi [42], which greatly supports the notion that Tregs are engaged in the development, perhaps even the maintenance of bTrm.

**CROSS-REACTIVE T CELLS AND VACCINE DEVELOPMENT FOR FLAVIRUSES**

Both ZIKV and four serotypes of dengue viruses (DENV1–4) are members of the *Flaviviridae* family. These viruses share over half of the homology in amino acid sequences [43*], which lays the foundation of their cross-reactive immune response. T cell depletion and adoptive transfer studies have shown that ZIKV protection was mainly conferred by DENV-experienced CD8 T cells [44]. ZIKV-exposed T cells isolated from human doners’ peripheral blood mononuclear cells (PBMCs) also exhibited reactivity against both ZIKV and DENV [45,46]. Supporting the

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**Table 1. Studies on roles of T cells in viral infections of the CNS**

<table>
<thead>
<tr>
<th>Virus</th>
<th>T cell studies/characteristics</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rift valley fever virus (RVFV)</td>
<td>Depletion of CD4 T cells results in worse survival rate than CD8 depletion</td>
<td>[69]</td>
</tr>
<tr>
<td>Congenital murine cytomegalovirus (MCMV)</td>
<td>Virus-specific T cells persist in the brain</td>
<td>[31,33]</td>
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<tr>
<td></td>
<td>&gt;90% virus-specific CD8 T cells upregulate CD69 expression at 3 wks p.i.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Trms exhibit slower proliferation rate than of spleen counterpart</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Persistent T cells control viral reactivation and activation state of microglia</td>
<td></td>
</tr>
<tr>
<td>Theiler’s encephalomyelitis virus (TMEV)</td>
<td>Ratio of CD8:CD4 T cells in the brain is ~13:9 at 7 dpi</td>
<td>[70]</td>
</tr>
<tr>
<td></td>
<td>T cells not required for acute seizure development</td>
<td></td>
</tr>
<tr>
<td>West Nile virus (WNV)</td>
<td>CD8 T cells</td>
<td>[21**]</td>
</tr>
<tr>
<td></td>
<td>Increased CD69 expression on Trms</td>
<td></td>
</tr>
<tr>
<td>Human immunodeficiency virus (HIV-1)</td>
<td>Decreased number of CD4 T cells p.i.</td>
<td>[72**]</td>
</tr>
<tr>
<td></td>
<td>Increased number of CD8 T cells p.i.</td>
<td></td>
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<tr>
<td></td>
<td>T cells act as viral reservoirs in the CNS</td>
<td></td>
</tr>
<tr>
<td>Zika virus (ZIKV)</td>
<td>High level of CCR2 expression in CD8 T cells at 25 days p.i.</td>
<td>[14**,21**]</td>
</tr>
<tr>
<td></td>
<td>Effector CD8 T cells induce ZIKV-associated paralysis</td>
<td></td>
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<tr>
<td></td>
<td>CD4 T cell</td>
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<tr>
<td></td>
<td>Control viral titers in the CNS</td>
<td></td>
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<tr>
<td></td>
<td>Confer protection against lethal challenge post-ZIKV-immunization</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: wk: week; p.i.: postinfection.
cross-reactive immunity between ZIKV and DENV, another investigation has been conducted using a Zika DNA vaccine candidate (pV-ZME) expressing ZIKV premembrane and envelope proteins will elicit robust both humoral and cellular immune response in BALB/c mice against DENV1-4 where immunized mice had limited body loss, better survival rates and increased IFN-γ-producing CD8 T cells compared to the control mice [47*].

**RECOVERY FROM FLAVIVIRUS VIRAL ENCEPHALITIS**

In addition to the acute neuroinvasive syndromes and persistent motor deficits, patients that recover from WN neuroinvasive disease (WNND) experience significant long-term cognitive sequelae, including high rates of memory impairment and abnormalities in executive function [48–58]. Thus, although approximately 90% of patients survive WNND, 50–70% of survivors develop memory disorders that worsen over time [59]. New memory disorders have also been reported in adolescents and adults that recovered from ZIKV meningoencephalitis [60,61], and animal models also demonstrate synapse loss and cognitive dysfunction [62]. Few studies have examined mechanisms of postinfectious cognitive dysfunction after viral encephalitis, which might be generalizable to other neuroinflammatory diseases of cognition.

**PD1 PATHWAYS AND RECOVERY FROM VIRAL ENCEPHALITIS**

There is increasing evidence that PD1 and programmed death ligand 1 (PDL1) interaction could be related to T-cell functionality within the CNS. PD1, an inhibitory receptor expressed by all activated T cells, regulates T-cell effector functions during various physiological responses, including acute and chronic infections. Viral-peptide-specific CD8 T cells in the brain expressed PD1 during the acute phase of mouse MuPyV infection and showed sustained expression under persistent infection whereas their splenic counterparts only exhibited transient and low expression of PD1 during the acute phase [63**]. These data suggest that PD1 may exhibit a specialized function within the CNS environment. In MyPyV model of encephalitis, PDL1 was expressed on infiltrating myeloid cells, tissue-resident microglia and astrocytes. PDL1−/− mice upon acute MyPyV infection showed increased frequency of CD103+CD8 T cells and CD25 +Foxp3 +CD4 T cells in the brain. CD103 is an integrin protein that binds integrin beta 7 (β7–ITGB7), promoting retention of CD8 T cells in tissues. The association of CD103 expression on CD8 T cells and PD1 signaling has also been demonstrated in murine cytomegalovirus (MCMV) brain infection [64]. PD-1-deficient CD8 T cells also exhibit significant decrease in CD103 expression with mixed glia in vitro [64]. These data suggest that PD1:PDL1 interactions may contribute to effective T-cell defense in circumstances of acute virus infection, but promote chronic activation of microglia in the postinfectious state. The functional interpretation of the expression of PD1 on CNS-infiltrating CD8 T cells regardless of acute or chronic infection or postinfection remains to be unraveled. However, one can assume that PD1 signaling in brain-residing T cells depend on dichotomous function that will establish a balance between the control of viral infection and the potential immunopathology from over-reactive T cells. Of interest, PD1 levels on T cells are elevated in aged individuals [65], which may impact virologic control and recovery from viral encephalitis.

**CHRONIC MEMORY AND BEHAVIORAL DEFICITS**

Recent studies using attenuated strains of flaviviruses in mice indicate that antiviral T cells that promote virologic clearance during CNS infection may underlie neurocognitive sequelae in survivors. IFN-γ released by CNS-infiltrating, virus-specific CD8 T cells induces microglial activation, as evidenced by upregulation of MHC class II expression on these cells, which is normally expressed at lower levels [21**]. Microglial activation has been shown to be associated with a variety of neurotoxic effects including synapse elimination, neurodegeneration, and decreased adult neurogenesis [1]. CD3+ T cells that persist in the hippocampus were found to be the predominant source of IFN-γ after recovery in murine models of WNV and ZIKV encephalitis, which were both associated with elimination of synapses and cognitive dysfunction [18] (Fig. 1).

These T cells also expressed markers of Trm cells, including CD103 [21**]. Specific deletion of IFN-γ signaling in microglia protects mice from microglial activation, synapse elimination and promoted synaptic repair resulting in reinstatement of spatial learning post recovery, suggesting that the effect of Trm-derived IFN-γ on microglia may be the most proximal trigger in the development of memory disorders that emerge after flavivirus encephalitis. IFN-γ alone, or in combination with tumor necrosis factor-α, has also been shown to upregulate CXCL9 and CCL2 in subventricular zone-derived adult neural precursor cells [66]. This increase of CXCR3 ligand secretion may be responsible for further recruitment or maintenance of CXCR3+ or...
CCR2+ cells, which includes microglia and T cells, respectively [67,68]. This could potentially provide a feed-forward mechanism to maintain T cells in the parenchyma with continued activation of microglia.

CONCLUSION
Mosquito-borne and tick-borne neurotropic arboviruses cause annual epidemics of virus-induced encephalitis throughout the world and are considered some of the most rapidly spreading vector-borne diseases. Emerging Flaviviruses, New World Alphaviruses, and Bunyaviruses, cause neurologic illness at a rate of 50–100 000 cases/year with lasting neurocognitive sequelae in up to 70% of survivors [2]. Viral infections within the brain pose a unique challenge for the immune system: the host must trigger an effective immune response to control and clear the infection while minimizing neuronal damage. The studies reviewed here illustrate the complex interactions between antiviral T cells and infected and/or inflamed neural cells that orchestrate their effector functions and ultimate fate within the CNS. These effects, which depend on age and immune status of affected individuals, not only determine survival but whether recovery is associated with progressive memory disorders. They also identify potential therapeutic targets with *in vivo* demonstration that cytokine receptor inactivation may prevent loss of neural correlates of memory formation and spatial learning defects. Use of anticytokine receptor therapeutics may ultimately prove beneficial for these patients and those with other dementing illnesses.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING
Papers of particular interest, published within the annual period of review, have been highlighted as:
- of special interest
- of outstanding interest

This study shows that tissue-resident memory T cells in human brain express distinct patterns of gene expression compared to peripheral memory T cells, which may reflect differences in their function and localization within the brain. This finding is important in the context of viral infections of the brain, including those caused by enteroviruses, as it highlights the need for a more detailed understanding of the immune response in the CNS during neuroinvasive disease.

In conclusion, the study by Grant A, Ponia SS, Tripathi S, Campanella GSV, Tager AM, El Khoury JK, et al. highlights the importance of tissue-resident memory T cells in the regulation of host response and disease outcome during neuroinvasive infections. Further research is needed to elucidate the mechanisms by which these cells contribute to the control of viral infections of the brain, and to identify potential therapeutic targets for the development of new antiviral strategies.
This study demonstrates that EcoHIV infection in mice induces HIV-specific cognitive impairment that may be prevented by antiretroviral therapy.
This study illustrates that T cells infiltrate the brain of humanized mice infected with HIV and maintain infections as viral carriers.