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INVITED REVIEW

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# B cells going viral in the CNS: Dynamics, complexities, and functions of B cells responding to viral encephalitis

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## Summary

A diverse number of DNA and RNA viruses have the potential to invade the central nervous system (CNS), causing inflammation and injury to cells that have a limited capacity for repair and regeneration. While rare, viral encephalitis in humans is often fatal and survivors commonly suffer from permanent neurological sequelae including seizures. Established treatment options are extremely limited, predominantly relying on vaccines, antivirals, or supportive care. Many viral CNS infections are characterized by the presence of antiviral antibodies in the cerebral spinal fluid (CSF), indicating local maintenance of protective antibody secreting cells. However, the mechanisms maintaining these humoral responses are poorly characterized. Furthermore, while both viral and autoimmune encephalitis are associated with the recruitment of diverse B cell subsets to the CNS, their protective and pathogenic roles aside from antibody production are just beginning to be understood. This review will focus on the relevance of B cell responses to viral CNS infections, with an emphasis on the importance of intrathecal immunity and the potential contribution to autoimmunity. Specifically, it will summarize the newest data characterizing B cell activation, differentiation, migration, and localization in clinical samples as well as experimental models of acute and persistent viral encephalitis.

**KEYWORDS**
antibodies, B cells, CNS, inflammation, neuroimmunology, virus

## 1 | INTRODUCTION

### 1.1 | Viral infections of the CNS as a human health burden

Viral infections are a common cause of encephalitis, a seriously debilitating disease with high mortality rates.[1-5] During viral encephalitis, injury to non-regenerative neuronal tissue caused directly by the infection or indirect by the inflammatory response can lead to long-term neurological disability. Therefore, while the neurological impairments experienced during the acute phase of infection may resolve in some survivors, many patients develop at least one permanent neurocognitive impairment.[6] CNS infections largely fall into several categories: (i) DNA viruses generally acquired during

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childhood or adolescence that are associated with latency, but can reactivate; (ii) RNA viruses, including enteroviruses or arthropod-borne viruses, that cause acute encephalitis or meningoencephalitis; (iii) Retroviruses including HIV-1 and HTLV1.

Notable DNA viruses include *Herpesviruses* and human polyomavirus 2, also known as John Cunnigham (JC) virus, to which 50%-90% of the world population is estimated to have antibody seropositivity.[7] Herpes simplex virus 1 and 2 (HSV-1 and 2) establish latency in the sensory ganglia neurons. Reactivation generally leads to lesions at peripheral mucosal sites innervated by affected neurons. However, HSV encephalitis, while rare, causes serious neurological disease.[8,9] Notable RNA viruses include members of the insect borne *Togaviridae*, *Bunyaviridae*, and *Flaviviridae* families, as well as the zoonotic *Rhabdoviridae* family.[5] 30%-50% of encephalitis survivor afflicted by the Japanese encephalitis virus (JEV) *(Flavivirus)* will suffer long-term neurological impairment.[10,11] Lastly, the retrovirus HTLV-1 can cause HTLV-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP), a progressive neurological disease associated with perivascular leukocyte infiltration in the CNS in a small number of carriers.[12]

### 1.2 | Brief overview of peripheral B Cells

The bone marrow (BM), including the skull cap BM, is a lifelong source of continuous B cell generation from pluripotent hematopoietic stem cells (HSC) residing in specialized niches.[13] B cell development and maturation have recently been extensively reviewed.[14-16] Briefly, HSC in the BM move through the pro-B to the pre-B cell stage to become immature B cells that express IgM B cell receptors (BCRs). After leaving the BM, circulating immature B cells populate secondary lymphoid organs, where they undergo further development and selection processes during transitional stages, and ultimately become mature marginal zone B cells or follicular B cells expressing surface IgM and IgD. Within lymph nodes (LNs), mature naive B cells express the CXCR5 receptor allowing them to follow a CXCL13 gradient to the B cell zone, where they survey subcapsular macrophages and specialized stromal cells for foreign antigen. In the absence of antigenic stimulation over several hours, naive B cells egress back into the circulation to migrate to other lymphoid tissue.[16] However, upon antigenic activation through the BCR while engaging various coreceptors, B cells upregulate the CCL19 and CCL21 chemokine receptor CCR7 allowing them to migrate to the interphase of the B cell follicle and T cell zone. Depending on the type of antigen and cognate CD4 T cell interaction, B cells either participate in a short-lived extrafollicular response or in germinal center (GC) responses.[15] Extrafollicular B cell responses typically give rise to low-affinity IgM secreting plasma blasts, also termed short-lived antibody secreting cells (ASCs) that mostly remain in the lymphoid tissue. During GC formation, CD4 T follicular helper cells and cognate B cells migrate deeper into the B cell follicle to propagate GC reactions, which involve isotype class switching and somatic hypermutation to increase antibody avidity. The GC response generates two major types of antigen-specific B cells: high-affinity, class-switched plasma blasts that secrete antibodies and have transient migratory capacity, or memory B cells (Bmem cells) that express high-affinity cell-surface antibodies. Bmem cells can be found in the circulation, strategically positioned within LN niches that allow rapid foreign antigen encounter, or resident in non-lymphoid tissue. A hallmark of Bmem cells is their rapid differentiation into ASCs upon antigen re-encounter, thus providing a major source of long-lived terminally differentiated plasma cells in the BM.[15]

### 1.3 | B Cells in the homeostatic CNS

The CNS has historically been considered an immune-privileged site with peripheral leukocytes and lymphocytes being excluded by the blood brain barrier (BBB). Breach of the BBB by infection, endothelial cell damage, and/or signals from various cytokines (e.g., TNF and IL-6) was considered necessary for leukocytes to infiltrate into the parenchyma. More recently, it has become appreciated that the meningeal compartment, particularly the meningeal dura mater, harbors immune cells that constitutively survey the CNS from within. During homeostasis, B cells make up to 30% of the total immune cells in the dura and single-cell RNAseq analysis revealed the presence of both mature and immature B cells in the dura.[13] More in-depth analysis showed that the distribution of B cell developmental stages in the meninges resembled that of the BM, thereby implicating the dural meninges as a possible site of B cell development.[13] Focused examination of the mature B cell compartment in young mice demonstrated that most were $IgD^+IgM^+$ naive B cells.[13,17,18] BM chimera and parabiosis experiments revealed that most of the dural B cells did not infiltrate from the circulation, but originated from the calvarial BM.[13] However, as the mice aged, more antigen experienced B cells accumulate in the dura mater.[13,17] Interestingly, gut-educated $IgA^+$ plasma cells also accumulate along the dural venous sinuses in early adulthood and constituted the predominant plasma cell isotype in the dura. However, IgM became the predominant plasma cell isotype type with aging.[13,17]

## 2 | CLINICAL RELEVANCE OF B CELLS DURING VIRAL ENCEPHALITIS

### 2.1 | CNS humoral immunity in patients with viral encephalitis

The involvement of B cells in CNS infections has long been evident from the detection of antiviral oligoclonal immunoglobulin (Ig) bands in the CSF of patients afflicted by CNS disease during measles, mumps, and Rubella virus infections.[19-22] The oligoclonal nature inferred local Ig production, but the source, mechanisms of maintenance, and localization of the ASCs remain unknown. Many other CNS infections are also associated with the detection of virus-specific antibodies of several isotypes in the CSF, which can

be reliable diagnostic tools.[23] To what extent the CNS localized antibodies participate in suppressing persistent infections is difficult to assess due to sampling logistics. Nevertheless, the potency of B cells to minimize local virus reactivation or virus CNS invasion from the periphery is clearly evident by studies of patients receiving anti-CD20 monoclonal antibody (trade name Rituximab) B cell depletion therapy to treat rheumatoid arthritis, MS or some cancers.[24-31] While extremely rare, prolonged treatment with Rituximab is associated with progressive multifocal leukoencephalopathy (PML), an often-lethal opportunistic infection resulting from replication of JC virus in the CNS. While lifelong persistent JC virus infection in the kidney is generally asymptomatic, the virus may gain access to the CNS in immunocompromised individuals. Interestingly, PML-associated JC variants carry mutations promoting virus replication in the CNS.[32,33] PML survival has been associated with the presence of high neutralizing antibody titers in both the plasma and CSF and JC virus-neutralizing monoclonal antibodies isolated from patients who recovered from PML appear to block virus cell entry.[32-34] Other CNS infections reported to be linked to anti-CD20 antibody treatment are enteroviruses and, in isolated cases, HSV and deer tick virus encephalitis.[28-31] This highlights the need to understand how B cells contribute to suppressing latent and persistent viral infections from entering the CNS or already within the CNS.

During acute infections caused by some arthropod-borne *Flaviviridae*, non-class-switched IgM B cells are associated with limiting CNS infection.[35] For example, during infection with tick-borne encephalitis virus (TBEV), encephalitic symptoms are inversely correlated with IgM in the CSF.[36] Virus-specific IgM antibodies can also be found in the CSF during the very early phase of the majority of JEV encephalitis cases.[37,38] Interestingly, West Nile virus (WNV)-specific IgM antibodies have even been detected in the CSF of patients as far out as 100 days after acute encephalitis.[39] These data suggest WNV persistence may sustain local IgM production within the CNS, or that IgM ASCs are long lived in the CNS. *Flavivirus* humoral responses are not just limited to IgM production, however, as human CSF also harbors WNV-specific IgA antibodies, implicating a possible mucosal associated B cell response.

Intrathecal humoral immunity has also been studied in HTLV-1 induced HAM/TSP where HTLV-1-specific antibodies and oligoclonal IgG bands in the CSF also indicate intrathecal synthesis.[40-43] HTLV-1-specific antibodies in the CSF inversely correlate with higher HTLV-1 viral loads and a worse prognostic outcome.[44] Direct analysis of B cells in the CSF further revealed that, compared to asymptomatic HTLV-1 carriers, ASCs were elevated in CSF of HAM/TSP patients and correlated with the presence of intrathecal HTLV-1-specific antibodies.[44] However, HTLV-1 antibodies have also been demonstrated to cross-react with host-antigens, suggesting that some antibodies may be pathogenic.[45,46] Although it remains unclear to what extent virus-specific antibody production contributes to viral control or inflammatory-mediated injury, the data reveal that the B cell compartment contributed to HAM/TSP.

Aside from antibody-mediated functions, B cells can contribute to virus protection or inflammatory-mediated injury via production of lytic and soluble factors including granzyme B, GM-CSF, IL-6, TNF, LTβ, and IL-10 production.[47,48] B cells with antiviral granzyme B activity respond to HIV antigens and participate in virus control through a cytotoxic mechanism.[48] Granzyme B production in B cells has also been observed in patients with multiple sclerosis (MS), a CNS autoimmune disease discussed in greater detail below.[49] However, the BCR specificity and propensity of the granzyme B expressing B cells to migrate to the CNS are unknown. Regrettably, the participation of this B cell subset during human viral encephalitis remains unexplored and granzyme B producing B cells do not appear to develop in murine models making them particularly hard to study.[50] Taken together, the studies support an overall protective role of antibody responses in the CNS in controlling human encephalitic viruses.

## 2.2 | Autoimmune encephalitis linked to viruses

Acute peripheral viral infections can lead to neurological symptoms associated with autoimmune responses to CNS or peripheral nervous system (PNS) antigens. Two neurological autoimmune diseases associated with viruses in the CNS are MS and the anti-N-methyl-d-aspartate (NMDA) receptor encephalitis.

MS is a chronic, neurodegenerative inflammatory disease of the CNS characterized by demyelination, axonal loss, and progressive neurological disability. The etiology of MS is unknown, but has been associated with several CNS viral infections.[22] Infection by Epstein Barr virus (EBV) provides the strongest correlation to development of MS. EBV is a *Herpesvirus* that infects B cells and can cause asymptomatic infection, acute mononucleosis, or viral encephalitis. Serum EBV and antibody titers positively correlate with relapsing MS disease.[51] A large cohort study of Danish participants across multiple decades found that infectious mononucleosis was associated with increased risk of MS diagnosis.[52] A more recent study of samples from US military service members found that nearly all patients diagnosed with MS became EBV seropositive prior to their MS diagnosis.[53] The mechanism underlying the link between EBV and MS are under intense investigation. One potential mechanism was identified when T and B cells specific for the EBV protein EBNA1 were found to cross-react with the self-cell adhesion molecule GlialCAM.[54] Furthermore, autoreactive antibodies and T cells specific for other self-proteins, including myelin basic protein and alpha B-crystallin, were also found.[55,56] A second possible mechanism involves the migration of circulating latently infected Bmem cells into the CNS, thereby recruiting EBV-specific cytotoxic T cells primed during peripheral infection. In this scenario, latently infected B cells in the periphery and the CNS may act as antigen-presenting cells to trigger pathogenic T cell responses (recently reviewed in[57]). Transcriptional profiles of laser captured white matter perivascular and intrameningeal immune infiltrates revealed that latent EBV infection coinciding with immune activation was more prominent in meningeal samples

supporting potentially enhanced EBV propagation and lymphocyte activity in the meninges.[58] More recent data further suggest that epigenetic adaptation in EBV-infected B cells induces an altered trafficking phenotype promoting CNS infiltration.[59]

A murine model was developed to study how EBV infection may promote CNS autoimmune disease using the murine gamma herpesvirus 68 (γHV-68), the murine homolog to EBV.[60] Mice latently infected with γHV-68 developed more severe experimental autoimmune encephalitis (EAE) with loss of myelin in the brain and spinal cord as well as lesions composed of macrophages, CD8 T cells, and CD4 T cells. Virus infection further indicated skewing toward Th1 T cell responses, suggesting that the virus induced immune status may influence the subsequent CNS autoimmune inflammatory skewing.

NMDA receptor encephalitis is caused by an autoantibody response to the NMDA receptor in the CNS resulting in a range of symptoms including headache, lethargy, seizures, and psychosis that result in severe disability and death in 25% of patients.[61] Some of these cases have been linked to acute or reactive EBV infection as indicated by the presence of EBV antigen in the serum and anti-EBV antibodies in the CSF.[62] The link between EBV and NMDA receptor autoantibodies may reside in production of anti-EBV antibodies that cross-react with self-NMDA receptor, or the release of self-antigens during neuronal cell damage. In this context, it is interesting to note that JEV,[63] HSV1,[64] and SARS-CoV2,[65] as well as other viral and nonviral CNS infections, have also been associated with anti-NMDA receptor encephalitis.[66] These clinical data implicate that acute infections can trigger activation of self-reactive B cells that cause debilitating neurological disease. Whether humoral autoimmunity to self-antigen arises from tissue damage or cross-reactivity requires further analysis in animal models.

## 3 | B CELLS DURING EXPERIMENTAL VIRAL ENCEPHALITIS

Our understanding of clinical CNS infections is mainly based on genetic and brain imaging studies, as well as the examination of CSF and autopsy samples. Such data reveal excellent associations between clinical disease and inflammation. However, the elucidation of pathological cellular and molecular mechanisms relies on animal models of viral encephalitis. While no animal model truly reflects all hallmarks of the human disease, they are invaluable to complement the data gathered from patient samples and provide new cues for treatment strategies.

### 3.1 | Neurotropic mouse hepatitis virus

Mouse hepatitis virus (MHV) is an enveloped, single-stranded positive sense RNA virus with large genome of approximately 31 kb belonging to the *Coronaviridae* family. While most MHV strains are enterotropic, two strains of MHV have been commonly used to study viral encephalomyelitis[67-69]: An attenuated variant of the highly neurovirulent John Howard Muller (JHM) strain that demonstrates little evidence of hepatitis (JHMv2.2-1) and the A59 variant, which is both hepato- and neurotropic. Upon intracranial infection, replication within the CNS resolves into a low-level persistent infection characterized by presence of viral RNA in the absence of detectable infectious virus. Concurrent with the initiation of viral persistence, there is demyelination in the spinal cord white matter that is primarily immune-mediated. Acute virus replication is controlled by type I IFNs as well as CD4 and CD8 T cell effector functions.[67,70,71] Reemergence of infectious virus during the persistent phase is controlled by virus-specific ASCs.[67,72]

The majority of early studies characterizing humoral CNS responses relating to ASCs have used the JHMv2.2-1 variant and have been previously reviewed.[72] The importance of ASC recruitment to and maintenance within the CNS became evident by the reemergence of infectious virus in B cell deficient mice after T cell mediated control of infectious virus. Transferred hyperimmune serum or anti-spike neutralizing IgG monoclonal antibody only transiently prevented viral re-emergence in mice deficient in B cells or with B cells unable to mount virus-specific responses. A requirement for sustained local antibody production was supported by the accumulation of virus-specific IgM and IgG ASCs in the CNS during the establishment of persistent infection. Lastly, consistent with ASC expression of the chemokine receptor CXCR3, mice deficient in CXCR3 had reduced ASC recruitment to the CNS, which coincided with a significant reduction of antiviral antibodies in the CNS, but not serum.[72,73] Together, these results imply that ASCs are recruited from the periphery to locally produce MHV-specific antibodies in order to prevent the reemergence of infectious virus.

Efforts to characterize a potential contribution of other B cell subsets to viral pathogenesis revealed that $IgD^+IgM^+$ naive/early activated B cells emerge in the CNS as early as Day 7 post infection (the earliest timepoint monitored), and are gradually replaced by $IgD^-IgM^+$ B cells, followed by isotype-switched $IgG^+$ Bmem cells and ASCs. This progression was accelerated in spinal cords relative to the brain and coincident with elevated mRNA expression of chemokines known to support B cell recruitment, differentiation, and survival, but not with altered BBB integrity.[74] Focused mRNA expression analysis of FACS purified B cell subsets supported that all CNS B cells expressed the CXCL12 receptor CXCR4, with the highest expression levels in the $IgD^+$ population. ASCs expressed the highest levels of CXCR3 mRNA, with lower expression in Bmem cells.[74] Complementary analysis of the ligands for CXCR3, CXCL10, and CXCL9 characterizing both their expression and chemokine-deficient mice indicated that local production of CXCL10 by astrocytes is critical not only for the recruitment of ASCs to the spinal cords, but also for their extravasation into the parenchyma.[75] CXCL9 was redundant to ASC migration despite its expression in the microvasculature of infected spinal cords. Transcripts for CCR7, the chemokine receptor engaged by the lymphoid chemokines CCL19 and CCL21, was also highest in $IgD^+$ B cells, with lower expression in Bmem cells. Lastly, relative to their draining LN counterparts, $IgD^-$ B

cells, as well as Bmem cells, in the CNS expressed modest levels of CXCR5 mRNA, which was barely detectable in ASCs.[74]

The CXCR5 ligand CXCL13 is associated with lymphoid tissue organization and ectopic follicle formation in various chronic inflammatory conditions, including MS.[76,77] As CXCL13 is also upregulated in the CNS following MHV infection, its contribution to humoral immunity was assessed in CXCL13-deficient mice, which lack most LNs with the notable exception of cervical (cLNs).[78] The migration capacity of $IgD^+$ naive/early activated B cells to the infected CNS appeared independent of CXCR5/CXCL13 signaling, implicating CXCR4 and/or CCR7 in driving recruitment.[79] In this context, it is interesting to note that significantly elevated CCL21 mRNA levels in the spinal cords of naive CXCL13-deficient mice were maintained throughout infection, potentially favoring CCR7-mediated recruitment of early B cells. Although GC formation in the cLNs was impaired in CXCL13-deficient mice, serum titers of virus-specific IgG antibodies were not significantly altered. However, reduced numbers of virus-specific IgG ASCs in the CNS underscored a role for CXCL13 in accumulation of $IgG^+$ ASCs during persistence.[79]

A requirement for GC formation in draining cLNs for ASC migration to the CNS during MHV infection is supported by the relative kinetics for the recruitment of class-switched ASCs in the CNS, studies in CXCL13 and CD19-deficient mice, as well as the absence of detectable tertiary lymphoid structures.[80,81] Based on GL7 expression, a marker of GC B cells, GC formation in the cLN is initiated as early as Day 7 post infection, but is not robust until Day 21 post infection.[80] Virus-specific ASCs emerged in the CNS approximately 14 days post infection and increased out to Day 28 post infection.[80-82] The expression of CD19 lowers the threshold of antigen-driven activation and promotes GC reactions.[16] MHV-JHMv2.2-1infected CD19-deficient mice exhibit severely reduced numbers of virus-specific $IgM^+$ and $IgG^+$ ASCs whose accumulation in the CNS was significantly impaired after Day 14 post infection.[81] This was attributed to a decrease in the short-lived phenotype rather than limited intrinsic migration capacity or cues from the CNS. Recruitment of early activated $IgD^+$ B cells during acute infection was not affected by CD19 deficiency, suggesting B cell receptor independent cues promote their migration to the CNS.[81] This was further supported by the inability to differentiate these cells into virus-specific ASCs.[83]

The contribution of ongoing peripheral GC reactions to MHV-specific ASC and Bmem cell recruitment to the CNS was assessed using mice expressing tamoxifen-inducible Cre recombinase (Cre-ERT2) under the *Aicda* promoter crossed with Rosa26-loxP-tdTomato reporter mice ($AID^{Cre}$-$Rosa26^{tdTomato}$). These mice provide tools to mark B cells that have undergone antigen induced activation-induced cytidine deaminase (AID)-mediated somatic hypermutation following tamoxifen administration. AID detection via tdTomato expression allowed tracking of virus-specific ASCs and Bmem cells in cLNs and the CNS throughout infection.[84] Continuous tamoxifen treatment throughout infection revealed that $tdTomato^+$ B cells only emerged in the CNS following peripheral GC formation and continue to accumulate well into the persistent phase of infection. Notably, early GC-independent $tdTomato^+$ ASCs in the cLN did not appear to migrate to the CNS. Furthermore, the spinal cord harbored a larger proportion of $tdTomato^+$ B cells earlier and at higher frequencies compared to brains. Bmem cells dominated the $tdTomato^+$ population in cLNs throughout GC activity, but not at later stages of viral persistence in the CNS. Distinct labeling periods further showed that ASCs and Bmem cells are being continuously recruited to the CNS after Day 21. Nearly 50% of ASCs and 25% of Bmem cells originated from late GC reactions into the persistence stage. Overall, the tdTomato labeling studies suggested that the vast majority of ASCs recruited to both the brain and spinal cord were virus-specific, with limited accumulation of ASCs of heterologous specificity.[84] Whether Bmem cells can directly convert to ASCs within the CNS upon chemokine or viral antigen exposure remains to be tested. Irrespectively, the data reveal ongoing active communication between the cLNs and the persistently infected CNS.

While ASCs are protective by controlling persistent MHV, the functions of the $IgD^+$ $IgM^+$ B cells and isotype-switched Bmem cells within intrathecal compartments remain to be resolved. Early accumulating B cells were confined to perivascular and pial sites in the brain, and only rarely formed clusters.[80] Isotype-switched B cells, on the contrary, localized progressively to parenchymal areas. This migration pattern may be guided by chemokine gradients and differential receptor expression on distinct B cell subsets. It is also consistent with upregulation of CCL19 and CCL21 in meningeal stromal cells,[85] and CXCL13 expression in microglia.[74,79] Moreover, B cells at perivascular sites appeared to engage multiple CD4 T cells, suggesting additional imprinting/activation. Importantly, analysis of sorted B cell populations from the CNS could not detect expression of mRNA for AID.[74] It is thus unlikely that early precursors undergo affinity maturation in the CNS. Whether they act as antigen-presenting cells (APC) or execute immunomodulatory roles through production of cytokines remains to be resolved.

### 3.2 | Theiler's murine encephalomyelitis virus

Theiler's murine encephalomyelitis virus (TMEV) is a *Picornavirus* that causes acute encephalitis that is resolved in C57BL/6 mice, but results in a persistent CNS infection associated with inflammatory demyelinating disease in the SJL mouse strain.[86-88] WT mice treated with anti-CD8 depleting antibody, as well as B cell deficient μMT mice, all on the C57BL/6 background, are relatively resistant to TMEV-induced demyelination.[89] CD8 depletion in C57BL/6 μMT mice leads to prolonged viral persistence and severe disease of the spinal cord gray matter. These data indicated that the humoral response is essential to preventing severe disease in C57BL/6 mice in the absence of CD8 T cells. While this observation may be attributed to enhanced viral titers in the CD8-depleted μMT mice, the still resistant isotype control treated μMT mice also displayed higher viral titers, albeit to a lower extent.[89] Infection of the susceptible SJL mouse background suggested a pathogenic role for B cells based on the finding that serum from chronically infected mice, but not from uninfected controls, contained autoantibodies that recognized white

and gray matter antigens in the spinal cord of uninfected mice.[90] This autoreactive antibody component is presumably linked to the autoreactive CD4 T cell response, which is mounted to myelin-specific epitopes and has been well characterized in the TMEV-infected SJL model.[91,92] Recent studies focusing on B cell localization have shown that the majority of B cells infiltrating the spinal cord were class-switched ASCs or Bmem cells during the demyelinating disease stage.[93] At this time, intrathecal IgG antibodies were also readily detectable. Imaging studies found both $B220^+$ and $IgG^+$ cells in the meninges, parenchyma, and perivascular spaces with evidence of perivascular cuffing, but not ectopic lymphoid follicle-like structures during demyelination. Taken together, these data indicate that myelin damage during TMEV infection leads to autoreactive CD4 T cells and autoimmune humoral responses.

## 4 | ARTHROPOD AND ZOONOTIC NEUROTROPIC RNA VIRUSES

### 4.1 | Sindbis virus

Sindbis virus (SINV) is an *Alphavirus* of the *Togaviridae* family that is transmitted by mosquito bites. SINV primarily targets neurons in the brain and spinal cord, which causes significant encephalitis in mice.[94] Clearance of infectious virus in mice occurs between Days 3-7 post infection, followed by declining viral RNA from Days 8-60, and continuous maintenance of low levels of persistent viral RNA thereafter.[95] The humoral response is absolutely essential to SINV control, as well as the prevention of reemergence of infectious virus during the persistent phase, as demonstrated by the inability of μMT mice to clear infectious virus from the brain.[96] Furthermore, passive transfer of hyperimmune serum or treatment with a monoclonal antibody directed to the SINV E2 envelope glycoprotein was sufficient for viral control in SCID (adaptive immune deficient) mice.[97-99] Repeated treatment of infected SCID mice with hyperimmune serum could also prevent reemergence of infectious virus, but this protection waned as levels of passively transferred antibodies declined.[97,99,100] The necessity for ongoing maintenance of antibodies in the CNS to prevent virus recrudescence is remarkably similar to MHV and demonstrates that viral RNA genomes can persist in a replication competent form.

Similar to MHV infection, T and B cell activation and expansion were observed in the cLNs, but not in the spleen, prior to CNS infiltration.[95] CD8 T cells were the first to infiltrate the brain, peaking at Day 5 post infection and steadily declining thereafter. Subsequently, CD4 T cells appeared in the brain, peaking at Day 10 post infection and slowly tapering off while remaining more numerous than CD8 T cells. A first wave of B cells was generated in the cLNs as extrafollicular low-affinity SINV-specific $IgM^+$ ASCs. The total number of the $IgM^+$ ASCs peaked in the cLN by Day 7 post infection, which coincided with their arrival in the CNS and clearance of infectious virus.[95] $GL7^+$ B cells could be detected by Day 5 post infection in the cLNs and peaked at Day 10 post infection. The appearance of SINV-specific IgG ASCs in the cLNS paralleled that of GC B cells and were detectable in the brain as early as Day 10 post infection. At Day 10, $CD19^+$ B cells in the brain were shown to express the chemokine receptors CXCR3, CCR5, and CCR7. At the same time, mRNA expression of these receptors' respective ligands increased in both the brain and spinal cord, providing possible mechanisms of B cell recruitment.[101] SINV-specific IgG ASCs accumulated, proliferated, and were maintained in the brain for at least 6 months after the infection, with IgG2a and IgG2b being the more predominant subclasses,[95,100,101] similar to MHV infection.[82] IgG Bmem and ASCs had similar kinetics of infiltration into the brain after infection.[95] Further characterization revealed the majority of the ASCs in the brain were plasmablasts, with very few terminally differentiated plasma cells. The frequency of splenic SINV-specific ASCs remained low out to Day 180 post infection, while IgM, IgG, and IgA ASCs were readily detected in the BM beginning around 30 days post infection.[95] During SINV infection, the interferon responsive factor 2 (IRF2) was shown to contribute to the B cell response as IRF2 knockout mice succumbed to peripheral SINV infection, but not when WT B cells were adoptively transferred prior to infection. Surprisingly, SINV-specific IgG antibodies at Day 7 post infection were diminished in the brain, but not the serum of IRF2 KO mice while SINV-specific IgM antibodies remained unchanged.[102]

Interestingly, while SINV-specific IgA ASCs were observed in the brain by Day 30 post infection, they were not found in the cLNs or spleen. While this observation may be explained by local differentiation within the CNS itself, there was no evidence of local ectopic follicle formation or GC formation. Furthermore, avidity index analysis revealed that there was no further affinity maturation via somatic hypermutation once ASCs entered the CNS.[95]

### 4.2 | Rift valley fever virus

Rift valley fever virus (RVFV) belongs to the *Bunyaviridae* family and is transmitted through mosquitos. RVFV infections are generally benign, particularly in immunocompetent patients. However, encephalitis has been observed in up to 20% of the severe cases that require hospitalization. This most predominantly occurs in patients who have some form of adaptive immune suppression.[103] Experimental murine RVFV infections were able to model these clinical findings, as depletion of CD4 T cells in C57BL/6 mice lead to a 30% mortality rate that was caused by late-onset encephalitis.[104] Further analysis to define the protective CD4 T cell subset revealed that the GC $CD40^+$ CD4 T cells required for high-affinity antibody responses, but not effector CD4 T cells, prevented RVFV encephalitis. In fact, blockade of CD40-CD40L interactions alone was sufficient to decrease RVFV survival from 100% to 80 or 60% depending on the blocking antibodies used. The increased fatality specifically correlated with the presence of viral genome copies in the brain and diminished levels of RVFV-specific IgG antibodies in the serum.[103] Importantly, whether these IgG antibodies prevent viral dissemination to the brain or control the infection once it has already been established in the brain

remains undetermined. Further investigation into the requirement for local IgG ASCs versus Bmem cells, as well as their origin from peripheral lymphoid organs, may provide further insight to prevent encephalitic disease.

### 4.3 | West Nile virus and other related *Flaviviruses*

West Nile virus (WNV) is a F*lavivirus* primarily transmitted by mosquitoes that causes severe CNS infection in immunocompromised patients. In mouse models of systemic WNV infection, a polyclonal B cell response is rapidly activated in a type I IFN-dependent manner in the LNs, but not the spleen.[105] Infection of μMT mice is 100% lethal, whereas 80% of WT controls survive. In the absence of B cells, a rapid increase in serum viral titers is followed by elevated titers in the brain by Day 6 post infection. This rapid increase in viral titers occurs when only low-affinity IgM antibodies are detectable and prior to GC responses in WT controls.[106] Solely abrogating IgM secretion, while preserving surface IgM and all other antibody isotypes, was sufficient to enhance viral dissemination to the CNS and cause 100% lethality. These data demonstrated that the early IgM ASCs are essential to control early viral dissemination to the brain and prevent mortality.[107] The protective role of IgM in mice is consistent with the clinical data on *Flaviviruses* discussed in Section 2.1.

The protection afforded by passive transfer of hyperimmune serum against many *Flaviviruses* has sparked a keen interest in the development of vaccines to these viruses. Humoral immunity-based vaccine strategies were bolstered by findings that antibody-mediated protection after exposure to one member of the *Flavivirus* family is also protective against other related members of this family. This was confirmed when it was demonstrated that vaccinating mice against JEV also established resistance to WNV infection. Furthermore, simply transferring B cells from mice vaccinated against JEV also provided protection against WNV.[108] Previous studies of long-term humoral responses generated after WNV infection revealed that the long-lived plasma cells only provided protection to a single neutralizing epitope and therefore could not protect against all WNV variants. By contrast, Bmem cells were polyclonal and could provide protection to WNV mutants.[109] Therefore, Bmem cells, and not the long-lived plasma cells, provide superior protection across related *Flavivirus* family members. Analysis of the Bmem cell response after vaccination with either WNV or Zika virus (a third related encephalitic *Flavivirus*) followed by vaccination against JEV revealed that antibody production after the JEV challenge was not dependent on GCs and did not require somatic hypermutation. Similar observations were made when mice were sequentially infected, rather than vaccinated, with the Dengue and Zika *Flaviviruses*.[110] Therefore, the heterologous protection afforded by the Bmem cells generated during the first challenge did not undergo further affinity maturation after exposure to a second related *Flavivirus*. Interestingly, this required the Bmem cells to maintain lower antigenic affinity than the long-lived plasma cells that were generated.[110] Given the importance of Bmem cells in preventing CNS infections upon secondary exposure, it is possible that a subset of the Bmem cells become CNS tissue resident where they can operate locally as a blockade to CNS virus invasion.

### 4.4 | Powassan virus

Powassan virus (POWV) is an increasingly common *Flavivirus* that cause encephalitis and is a member of the tick-borne encephalitis serocomplex.[111] Currently, there has been no observable protection to PWOV from other known *Flavivirus* family members.[112] Following POWV infection, virus-specific IgM can be detected as early as Day 3 post infection and virus-specific IgG antibodies as early as Day 7 post infection in the serum of mice. Passive transfer of hyperimmune serum to WT mice prior to infection increased POWV survival rates by 60% and prevented neurological sequelae in all surviving mice.[113] Adaptive immune deficient RAG1 KO mice had delayed morbidity compared to WT controls, but the infection remained 100% lethal. In these mice, transfer of hyperimmune serum only further extended the survival time, but was insufficient to prevent mortality at the dose tested.[113] It is therefore likely that the T cell response to POWV is both essential to virus control and a source of injury. While much still has to be understood about this emerging virus, it is clear that antibodies represent an important target for protection, particularly CNS protection, but are only optimally effective in the presence of T cells.

### 4.5 | Rabies virus

Rabies virus (RABV) is a single-stranded RNA virus of the *Rhabdoviridae* family that causes fatal encephalitis with an estimated more than 55 000 deaths globally a year. RABV enters nerve terminals at the site of infection and uses retrograde axonal transport to reach the spinal cord and brain.[114] As vaccination is the only line of defense against RABV, copious studies focus on how to best induce a strong protective humoral response. Toll-like receptor 7 (TLR7) is a pattern-recognition receptor that detects single-stranded RNA.[115,116] Upon intramuscular immunization against RABV, TLR7-deficient mice failed to generate strong GCs in LNs. As a result, TLR7-deficient mice generated fewer Bmem cells and ASCs in LNs, as well as circulating RABV-specific IgG antibodies compared to controls.[117] It has since been determined that plasmacytoid DCs utilize TLR7 to sense RABV and induce type I IFN responses in the brain after intracranial infection. TLR7 sensing of RABV also directly, and likely indirectly, regulated BBB permeability after infection. Therefore, the preservation of the BBB in TLR7 KO mice likely contributed to the decreased inflammatory cell (including B cell) infiltration into the infected brain parenchyma prior to any local differences in viral genome copies.[116] Plasmacytoid DC activation and their subsequent induction of type I IFN responses are also dependent on TLR7 during infection with vesicular stomatitis virus (VSV) (also of the *Rhabdoviridae* family).[118,119]

How TLR7 activation impacts the B cell and humoral response during VSV infection remains undetermined.

## 5 | DNA NEUROTROPIC VIRUSES

### 5.1 | HSV-1

It is estimated that greater than 60% of the world population is seropositive for the human neurotropic virus herpes simplex virus 1 (HSV-1).[120,121] While primary HSV-1 infections are often mild in immunocompetent patients, the primary lytic infection is followed by the establishment of a latent infection in sensory ganglia neurons for the entire life of the host.[121,122] Viral reemergence most commonly manifests as lesions in the oral mucosal membrane and genitals. However, HSV-1 is also the most common cause of sporadic encephalitis, which is often fatal. Furthermore, HSV-1 is known to cause corneal blindness and has been linked to a variety of peripheral nervous system disorders.[123] While the cellular and molecular mechanisms behind HSV encephalitis are unknown, genome wide studies in children have linked the manifestation of HSV-1 induced encephalitis to genetic defects in the TLR3 and IFN signaling pathways.[121,124,125] Infection of B cell deficient mice with HSV-1 lead to increasingly fatal viral encephalitis that could be prevented by the transfer of hyperimmune serum.[126] To establish a model of latent HSV-1 reactivation that is consistent with clinical infections where reactivation is enhanced in immunosuppressed hosts, RAG1-deficient mice were treated with intravenous Ig from human plasma 24 hours after infection. RAG1-deficient mice on the C57BL/6, but not the S129 background, were protected from lethality during primary HSV-1 infection by the intravenous Ig treatment. In this model, reactivation induced by transient hyperthermia was associated with the development of fatal HSV-1 encephalitis. How intravenous Ig protected RAG1-deficient mice is not completely understood; however, the mechanism was T cell dependent.[127]

### 5.2 | Cytomegalovirus

Cytomegalovirus (CMV) is another member of the *Herpesviridae* family. Like HSV-1, CMV is highly prevalent (it is estimated that 83% of world population is seropositive) and remains latent in its host for life.[128] While CMV infections go unnoticed in the majority of people, those who are immune-compromised can develop severe complications including encephalitis. CMV can also be transmitted intrauterine causing congenital CMV, the leading infectious cause of mental retardation and development delays in children.[129,130]

From the murine model of CMV, MCMV, we know that CD8 T cells are essential to controlling the primary infection and preventing fatal multiorgan failure.[131] Infection of uMT mice demonstrated that the humoral response was not necessary during primary MCMV infection, suggesting that by the time a strong antibody response could be generated the virus had already managed to enter latency in susceptible cells.[130,132] Furthermore, virus genome load, and not neutralizing antibodies, was the best predictor of virus reactivation and recurrence.[130,133] Despite an apparent redundant role of humoral responses in MCMV pathogenesis, their potential to regulate MCMV infection prophylactically is essential to CMV vaccine development. Indeed, transfer of serum or Bmem cells is protective in RAG1 KO mice, reducing morbidity and mortality in recipient mice.[134]

In a model of congenital CMV infection, neonatal mice infected with MCMV had reduced CNS pathology and demonstrated improvements in developmental delays after being treated with hyperimmune serum or anti-MCMV monoclonal antibodies.[135] However, the ability of antibodies to provide protection in the clinical setting remains controversial. The presence of anti-CMV antibodies does not prevent maternal transmission to the fetus or the development of neurological sequelae in congenital CMV.[130,136,137] Moreover, studies of intrauterine transmissions showed little to no correlation with maternal anti-CMV antibody levels, specificity, or functional activity.[130,138] However, while maternal antibodies could not prevent fetal transmission, maternal antibodies did prevent the development of severe and fatal infections in new born infants in cases of acquired CMV from blood transfusions.[130,139]

## 6 | OVERALL CONCLUSIONS, CHALLENGES, AND OPPORTUNITIES TO UNDERSTAND B CELL IMMUNITY DURING CNS INFECTIONS

Human neurological diseases associated with CNS infections have revealed virus-specific oligoclonal Ig bands in the CSF almost five decades ago. More recent measurements of serum and CSF antibodies during a variety of viral encephalitic diseases further demonstrated elevated levels of virus-specific IgM, IgG, and IgA suggesting that recruitment is not isotype specific. Their protective role is largely implied by correlative studies, and places emphasis on animal models to better understand correlates of immunity and the communication between the CNS and the periphery.

Models of acute encephalitis developing from mosquito- or tick-borne virus infections, as well as human studies, both show that encephalitis is largely prevented by efficient peripheral immunity, especially IgM and IgG responses. With respect to vaccinations, establishment of a broadly specific Bmem cell compartment, enhanced by appropriate adjuvant, appears to provide the best strategy to optimize heterologous immunity, particularly for multiple *Flaviviruses*.

Analysis of mice infected with prototypic members of the *Togaviridae*, *Coronaviridae*, and *Picornaviridae* families reveals several surprising similarities. In all three models of viral encephalitis, B cells are essential to controlling persistent infection and mitigating disease. Specifically, the continued presence of local Ig in the CNS is essential to prevent persisting low-level MHV and SINV RNA from reemerging into infectious virus. The inability to provide sterile immunity supports that these viruses persistent in a replication competent form that is only partially susceptible to classic virus

neutralization. This finding has implications for underlying neurological symptoms in neurological post-acute sequelae of SARS CoV-2. While possible infection of the CNS by SARS CoV-2 remains inconclusive, sampling may fail to detect low levels of a potentially persistent form of SARS CoV2 in the CNS.

Another similarity in all three infection models is the recruitment of distinct B cell subtypes starting with naive/early activated phenotype $IgD^+$ $IgM^+$ transitioning to $IgD^-$ $IgM^+$ followed by isotype-switched $IgG^+$ ASCs and Bmem cells over time (see Figure 1). The emergence of the virus-specific ASCs and Bmem

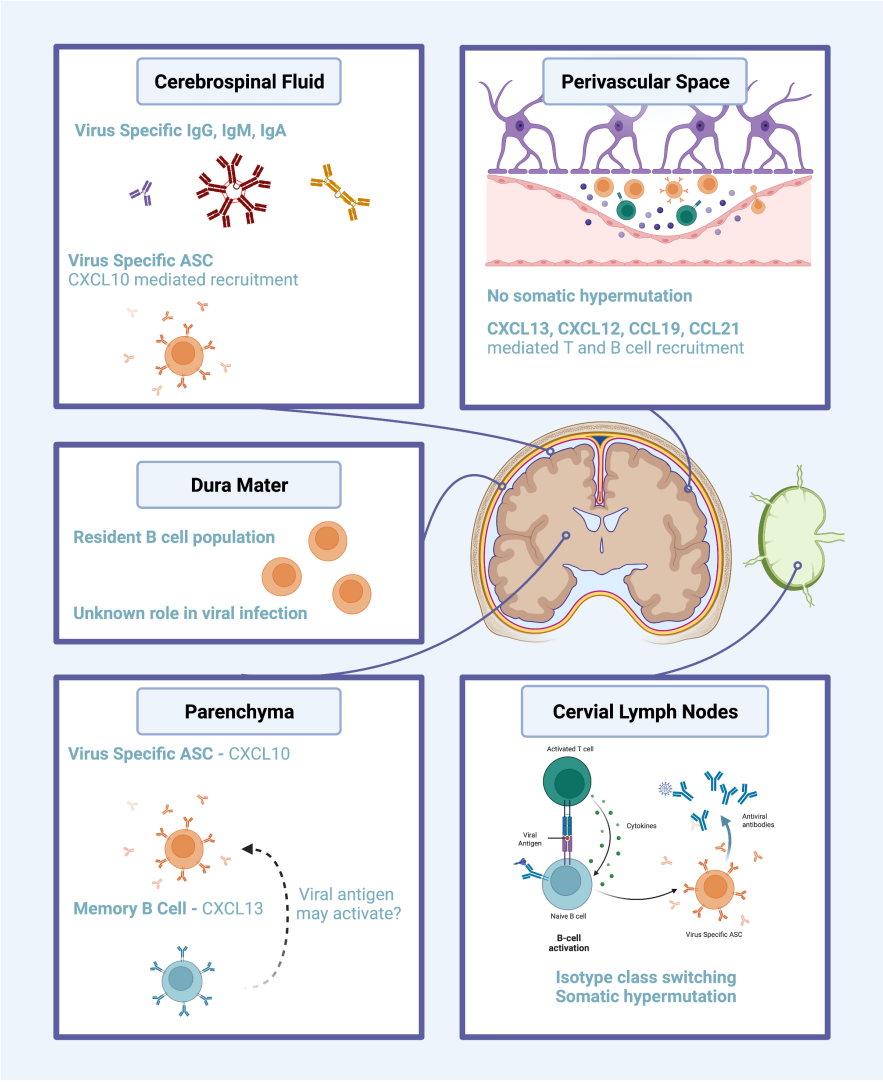


FIGURE 1 Overview of B cell subsets, localization, and functions during viral encephalitis. Following viral infections humoral responses are initiated in draining lymph nodes giving rise to both short-lived extrafollicular and germinal center (GC) derived antibody secreting cells (ASCs) and memory B cells (Bmem cells). Experimental CNS infections (TMEV; MHV) elicit sequential CNS recruitment of naive IgD + IgM+ B cells, followed by IgM+ B cells, and finally isotype-switched IgG+ ASCs and Bmem cells. Some infections are also associated with IgA+ ASC recruitment. The timing of the recruitment of the diverse B cell subsets varies depending on the virus. IgM+ B cells can be recruited prior to robust GC reactions, while IgG+ B cell accumulation generally correlates with formation of peripheral GCs. B cells initially start accumulating in meningeal or perivascular sites, where they can form clusters and potentially interact with CD4 T cells. Their location may be guided by activated meningeal stromal cells producing lymphoid chemokines akin to lymphoid tissue. However, neither formation of ectopic follicle-like structures or somatic hypermutation has been detected in the CNS during acute or persisting CNS infections with TMEV, MHV, or SINV. As isotype-switched B cells migrate to the CNS, they are equipped to migrate into the parenchyma, potentially guided by local production of chemokines and B cell survival factors. ASC migration to MHV-induced demyelinating lesions harboring persisting viral RNA require the chemokine receptor CXCR3 and its ligand CXCL10. The signals regulating Bmem cell localization to the parenchyma have not been characterized but may involve CXCR5/CXCL13. Antibodies found in the CSF are thought to be produced intrathecally by ASCs residing in the CNS tissue or the CSF. The prolonged persistence of antibodies in the CSF and the oligoclonal nature indicates prolonged survival of polyclonal ASCs and/or ongoing stimulation in the CNS. Whether Bmem cells contribute to the ASC pool by converting into ASCs upon exposure to persisting viral antigens is unclear. Furthermore, the source and function of early recruited IgD+ B cells remain to be determined. Lastly, a potential involvement of B cells and IgA+ ASCs residing in the dural meningeal immune niches remains to be explored.

cells in the CNS appears to coincide with formation of GCs in draining cLNs, although their recruitment during SINV infection is accelerated. Migration to the CNS is linked to B cell egress from cLNs into the circulation, concurrent with their active recruitment to the meninges and perivascular space. Within these niches, B cell clustering may promote interactions with CD4 T cells and antigen-presenting cells thereby providing survival signals or altered migratory capacity. While ASCs are recruited via signals through the CXCR3 receptor, the chemokines promoting $IgD^+$ B cell and Bmem cell recruitment remain to be defined, but may involve CXCR4 and CCR7 for $IgD^+$ B cells and CXCR4 and CXCR5 for Bmem cells. Interestingly, the loss of CXCL13 did not mitigate recruitment of early B cells during either MHV or SINV infection, as well as EAE.[79,140] All these data suggest that recruitment from the periphery, rather than local follicle formation, drives local CNS B cell responses.

The inability to detect ectopic follicle-like structures is a noticeable difference between viral and autoimmune encephalitis. Ectopic follicles are thought to exacerbate MS disease by fueling self-sustaining inflammatory reactions and releasing toxic mediators. These follicles require the support of activated fibroblastic stromal cells at meningeal or perivascular sites.[141] Although a reticular network providing support for lymphocyte recruitment is activated during MHV infection, there is no evidence for follicle-like structures during persistent infection despite ongoing recruitment of T and B cells. One difference may be different signals activating or maintaining these stromal cell niches. In EAE, CD4 Th17 cells produce both lymphotoxin beta and IL-22 to promote ectopic follicle-like structures. However, the viral models are not associated with a Th17 component and the initial activators of the reticular fibroblastic cells have not been characterized. The role of various stromal cells known to express lymphoid chemokines thus requires further investigation to better understand how manipulation of the stromal cell compartment may provide strategies to enhance and promote local humoral immunity, thereby accelerating viral control and counteracting the establishment of persistence. Further, while T cell interactions with the components of the neurovascular unit have been extensively studied,[142] the signals guiding B cell recruitment and infiltration into the CNS are less well characterized.

The diversity of B cell subsets and humoral response throughout TMEV, MHV, and SINV infection also merits further studies. For example, an enigma arises from the SINV μMT studies that demonstrate a need for antibody production to clear infectious virus from the brain, but not the spinal cord. The functions of early $IgD^+$ B cells and Bmem cells are also unresolved. Bmem cells may act similar to tissue-resident T cells, as a broadly reactive reservoir contributing to ASC renewal upon viral antigen restimulation. As is the case with *Flaviviruses*, Bmem cells may also provide a source of lower affinity, but more broadly reactive antibodies that have a greater potential to neutralize viral variants. The most enigmatic issue is the role of $IgD^+$ cells, as they may act as antigen-presenting cells or as modulators of the immune environment through production of cytokines, such as lymphotoxin beta, which promotes the activation of stromal cells in lymphoid organs and inflamed non-lymphoid tissue. There are also the broader questions as to whether B cells contribute to viral encephalitis disease pathology independent of antibody production. This remains technically difficult to elucidate given the absence of viral control without antibody production. Furthermore, outside of the *Flaviviradae* family, the contribution, if any, of the early low-affinity IgM response to virus control and pathology remains to be investigated. Lastly, how the CNS environment regulates entry and retention of distinct B cell subsets in the parenchyma remain to be elucidated.

Other new exciting dimensions for future studies are new insights into meningeal lymphatic drainage[143,144] as well as niches hosting a plethora of immune cells, including B cells, along the outer meningeal border.[13,17] A contribution of the dural meningeal immune compartment to viral encephalitis has not been elucidated to our knowledge. The more recent finding of immature and mature naive B cells emerging form the skullcap BM to seed the dural meninges suggests naive B cells may be recruited from these sites to perivascular locations. Furthermore, gut-derived IgA plasma blasts located in the dural meninges may participate in ameliorating complications imposed by circulating commensal bacterial or fungal products. Another question raised is whether virus-specific IgA cells preferentially localize to the dura, or reside in an anatomical distinct location from that of the IgG ASCs. It also questions the benefit of IgA ASCs in the presence of high-affinity IgG ASCs.

The route of viral antigen drainage into the cLNs, as well as the mechanisms activating and recruiting B cells to the infected CNS, also requires more characterization. The more recently appreciated meningeal lymphatics[143,144] likely play a prominent role in these processes; however, this has yet to be formally demonstrated and characterized. This is an intense area of investigation as viral encephalitis cases are expected to increase with the increased spread of mosquito- and tick-borne neurotropic viruses.[145] In this context, it is critical to note that VSV, JEV, and Zika virus are all capable of infecting human lymphatic endothelial cells providing a possible mechanism for these viruses to reach the cLNs.[146]

In summary, it is evident that virus-specific antibodies are essential for regulating CNS dissemination, limiting CNS injury, preventing neurological sequalae, and decreasing mortality in many animal models. B cells are thus clearly a potent therapeutic target for vaccines to combat primary, opportunistic, and recrudescing CNS infections. Given the increasingly commonality of clinical viral encephalitis, particularly with the increase in transmission of arboviruses, it is absolutely essential to deeply characterize how B cells can limit disease morbidity and mortality, both as a vaccine target and during active infections.

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## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

## DATA AVAILABILITY STATEMENT

Data sharing is not applicable—no new data generated.

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