Chapter

Neurotropic Virus-Induced Meningoencephalomyelitis

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Abstract

Meningoencephalomyelitis emanates under the umbrella relating inflammatory changes of the Central Nervous System (CNS). Meningitis denotes inflammation in the meningeal layers, encephalitis is an acute diffuse inflammation of the brain, and inflammation in the spinal cord is denoted as myelitis. These can be interrelated or independent of each other depending on the etiology. The entire mechanism of meningoencephalomyelitis is governed by an acute innate inflammatory branch followed by a chronic progressive, adaptive branch of immunity with clinical signs like hyperthermia, weight loss, hypoxia, leukocytosis. This book chapter will focus on viral-induced meningitis, encephalitis, and myelitis. Thirty years of experience working with a murine-β-coronavirus (m-CoV); Mouse hepatitis virus (MHV)-A59 induced experimental model system provided us a thorough understanding of neuroglial cell-mediated acute neuroinflammation, denoted by the accumulation of leukocyte-common-antigen (LCA) positive or $CD45^+$ leukocytes in perivascular infiltrates referred to as perivascular cuff formation and microglial nodules in the brain parenchyma, which mimics specific pathology of human neurological disease multiple sclerosis (MS). Additionally, in this chapter, we summarized the role of CNS resident microglial activation and its interaction with peripheral migratory T cells in mounting neuropathogenesis and host immunity in different families of neurotrophic encephalomyelitis viruses that cause CNS inflammation.

Keywords: meningioencephalomyelitis, demyelination, axonal loss, murine-β-coronavirus (m-CoV), neuroinflammation, multiple sclerosis (MS)

1. Introduction

Encephalitis is a pathological entity that refers to the inflammation of the *encephalon* or the brain parenchyma due to infection, autoimmunity, and brain injury. It is a rare medical condition with clinically serious consequences ranging from headaches, fever, seizures, permanent disability, and brain damage. Encephalitis majorly affects infants and the elderly above the age of 65, whereas, the incidence is transitional in the youth and is of significant public health importance because of the associated morbidity and mortality [1]. It results in varied clinical symptoms, such as mild fever and headaches to severe cognitive impairment accompanied by loss of physical vigor

and unconsciousness or even life-threatening symptoms that can result in permanent brain damage [2, 3]. It is believed that the severe inflammation associated with encephalitis causes swelling in the brain, which in turn gives rise to headaches, stiff neck, mental confusion, and even seizures [1, 4]. Though cases are recognized in all populations and ages, pediatric populations, young adults, and especially males have a higher propensity to encephalitis [5–7].

Infection by a virus is the most common and important cause of encephalitis [8]. Virus infections can also cause aseptic meningitis and myelitis [9, 10]. Research on viral encephalitis has gained much momentum with the recognition of encephalitis in human immunodeficiency virus (HIV) infection of the CNS and the emerging viruses such as West Nile virus (WNV), Nipah virus, and severe acute respiratory syndrome viruses (SARS-CoV and SARS-CoV-2) [11]. Members of several virus families like flaviviruses, paramyxoviruses, alphaviruses, bunyaviruses, orthomyxoviruses, arenaviruses, enteroviruses, rhabdoviruses, and astroviruses are also known to cause encephalitis [12].

Viruses may directly enter into the CNS or replicate away from the CNS at first and gain entry to the CNS through various routes [13, 14]. Local factors, like pH, mucosal immune responses, or the integrity of skin and mucosal barriers, govern the entry of the virus into the CNS and resultant encephalitis. Virus entry and replication activate the CNS resident immune cells, which, together with peripheral leukocytes, induce host immune response and promote encephalitis and neuroinflammation, resulting in multiple changes to the CNS physiology [15]. Histopathologically, characteristic microglial nodule formation, i.e., the accumulation of activated microglia in the brain parenchyma and the perivascular cuff formation, is observed in encephalitic brains [16–18]. Once the virus clears, the acute behavioral symptoms are resolved; however, long-term psychiatric, neurocognitive, and degenerative issues persist due to the ongoing immune responses in the CNS even after pathogen clearance [18, 19].

Interestingly, people infected with neurotropic viruses may not always develop encephalitis, indicating that host cell factors may play a critical role in regulating the outcome of the disease process. While mounting shreds of evidence are available to understand these host factors, limited information is available to understand the genomic control of the pathogenic properties and host factors that mediate a balance between neurovirulence and neuroprotection. Host cell response pathways like UPR and ER stress and oxidative stress may alter due to robust viral replication and intracellular assembly, causing imbalance between reactive oxygen species and antioxidants. These are governed by a battery of cellular mediators like DJ-1, Nrf-2, catalase, HMOX, MMPs, NADPH oxidase, cytokines, chemokines, and secondary messengers. These mediators are either CNS resident proteins or may be produced by the CNS resident neuro-glial cells like microglia, astrocytes, endothelial cells, and peripherally derived leukocytes that enter the CNS upon inflammation and breaching of the blood-brain barrier [13]. The nexus of these immune-inflammatory mediators with endogenous host proteins are well studied for ages in mounting host immunity. The question lies in whether host immunity plays a protective or pathogenic role.

Moreover, understanding the inflammatory mechanisms of meningioencephalomyelitis is a challenge in human patients due to the unavailability of the high throughput data from non-invasive techniques like MRI, fMRI, CT, and detailed invasive histopathological data from punched-biopsy/autopsy tissues. Thus, a thorough understanding of the cellular factors ranging from genomic control of pathogenic properties to viral host factors and immunomodulatory effects requires cause-effect

relationship experimental animal models/systems that can provide detailed insight into the disease process instrumental in the diagnosis and designing therapeutics. Though the use of mouse models to understand human disease has its limitations, critical pathological features of encephalitis can be efficiently reproduced in viral-induced experimental models. This book chapter will majorly summarize studies on viral encephalitis and its consecutive neuroinflammatory demyelination and axonal loss.

2. Routes of infection emanating to neurotropic virus infection

The term "neurotropic" refers to affinity towards the nervous system and displays the properties of neuroinvasion (entry into the CNS), and has direct neuroglial tropism. The viral entry to the neuroglial cells may be via receptor-mediated endocytosis and its fusion with the cell cytoplasm. It may also enter via direct endocytosis irrespective of engaging a receptor. Mounting shreds of evidence reported that neurotropic virus enters the brain parenchyma from the olfactory epithelium or retinal ganglionic cells via retrograded axonal transport. Upon entry to the brain parenchyma, infectious virus particles may also follow anterograde axonal transport via the optic nerve to reach retinal ganglionic cells and also can spread to different anatomic regions of the brain like the hippocampus, cortex, anterior commissure, basal forebrain, amygdala, brain stem as well as down the spinal cord. The neurotropic viruses can also travel through the lung-brain axis and cause inflammation in the brain stem region, the respiratory center. Neurotropic viruses can also access brain parenchyma via the gut-brain axis through the vagus nerve.

3. Neurotropic virus infection in mice is employed as an experimental model system to understand the underlying mechanisms of encephalitis, poliomyelitis, neuroinflammation, and demyelination concurrent with axonal loss

Neuroinflammation is responsible for initiating direct neuroglial dystrophy, which in turn can activate CNS resident microglia to release immune modulators. Microglia, the major resident immune cells in the central nervous system (CNS), are considered as the key cellular mediators of neuroinflammatory processes. Microglial research has become a central focus in cellular neuroimmunology and neuroinflammation in the past few years. Chronic/remitting neurological disease such as multiple sclerosis (MS) has long been considered an inflammatory autoimmune disease with the infiltration of peripheral myelin-specific T cells into the CNS. With the rapid advancement in the field of microglia and astrocytic neurobiology, the term neuroinflammation progressively started to denote chronic CNS cell-specific inflammation in MS. The direct glial responses in MS are different from conventional peripheral immune responses. This book chapter attempts to summarize current findings of neuroinflammatory responses within the CNS by direct infection of neural cells by mouse hepatitis virus (MHV) and the mechanisms by which glial cell responses ultimately contribute to the meningioencephalomyelitis and demyelination concurrent with axonal loss (**Figure 1**). Microglia can be persistently infected by MHV. Microglial activation and phagocytosis are recognized to be critically important in the pathogenesis of

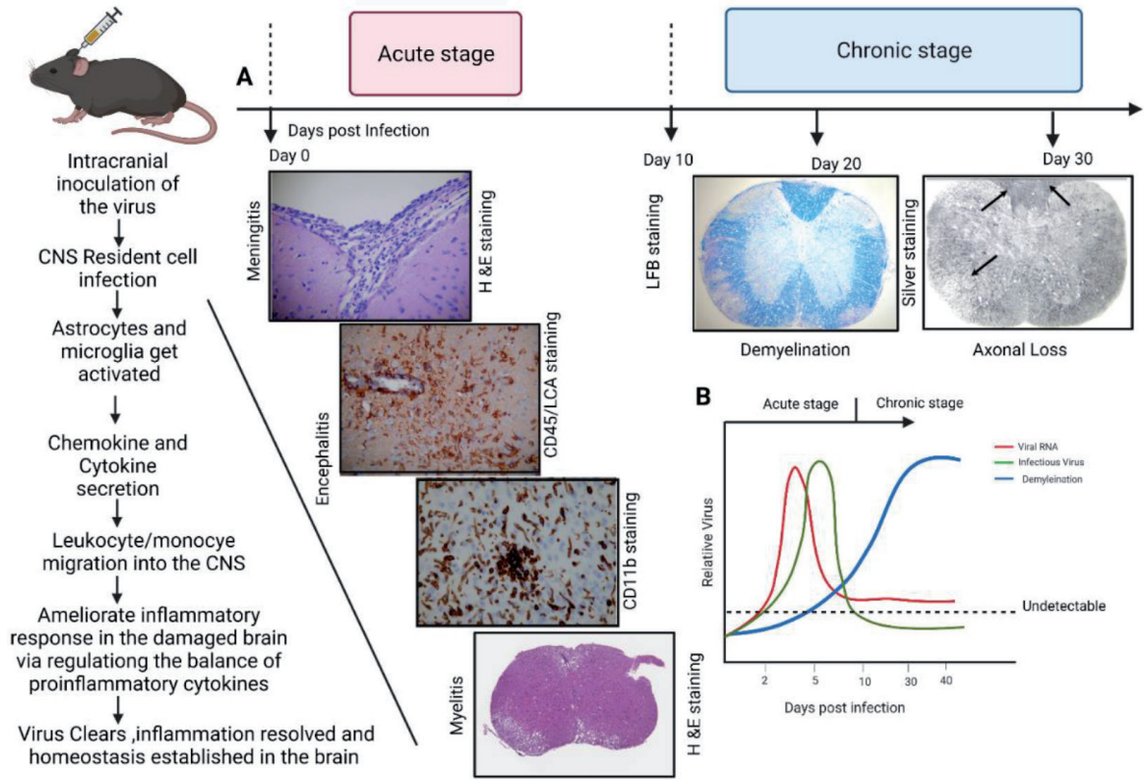

Figure 1.
Disease kinetics and pathological manifestations of murine β-CoV, MHV-A59 intracranial inoculation in C57BL/6 mice, a model to understand viral induced meningioencephalomyelitis and demyelination concurrent with axonal loss. (A) Shows the timeline of infection namely acute stage and its corresponding neuropathologies: meningitis denoting inflammation in the meningeal layers, encephalitis,an acute diffuse inflammation of the brain, and inflammation in the spinal cord denoted as myelitis and chronic progressive demyelination characterized by myelin loss concurrent with axonal loss. (B) Kinetics of MHV-A59 replication and viral clearance represented by viral RNA and infectious viral particles respectively and occurrence of demyelination [20].

demyelination. Emerging evidence for the pathogenic role of microglia and the activation of inflammatory pathways in these cells in MHV infection supports the concept that microglia-induced neuroinflammation is an amplifier of virus-induced neuropathology. Conventional understanding was that the peripheral immune cells are the major players for mounting CNS inflammatory responses. But the current studies revealed that if microglial activation and its immune modulators can check the infection, then the peripheral immune system need not be involved in mounting host immunity, and meningioencephalomyelitis may not shadow. In most cases, CNS resident microglial activation sets the stage for innate immune inflammation that results in the proinflammatory milieu of cytokines and chemokines, known as cytokine storm, which, while trying to combat pathogens, also causes damage to the CNS tissues. Amelioration of the proinflammatory condition requires anti-inflammatory cytokines where the peripheral immune system plays a major role. A series of recent studies on neurotropic murine β-coronavirus demonstrated acute-innate neuroinflammation mediated by CNS resident microglial interplay with peripheral leucocytes comprising monocytes and neutrophils NKT cells, $CD4^+$ and $CD8^+$ T cells to eradicate the pathogen and protect host tissue against aberrant tissue damage (**Figure 2**). In the below-mentioned sections of this book chapter, we are discussing in detail MHV infection as a prototype of β-coronavirus infection and its pathogenesis to understand the underpinning mechanism of meningioencephalomyelitis, demyelination and axonal loss.

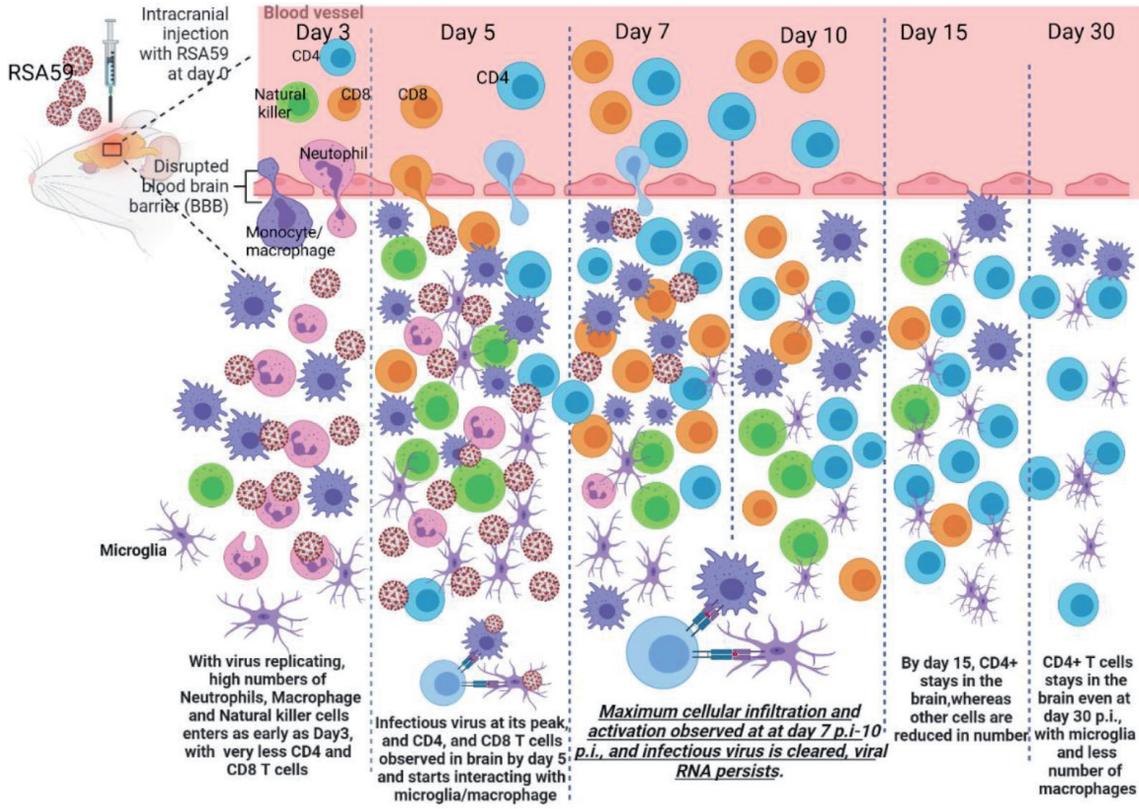

Figure 2.
Temporal kinetics of CNS resident glial cell activation associated with peripheral cell migration in response to RSA59 infection in the mouse CNS is key to cause meningioencephalomyeltits. Intracranial inoculation of RSA59 directly infects CNS resident neuroglial cells that in-turn activates CNS resident immune-glial cells like astrocytes and microglia. Activated CNS resident cells secrete a large number of inflammatory mediators like pro-inflammatory cytokines and chemokines. Microglial activation and its pro-inflammatory mileu in the inflamed CNS make a chemoattractant gradient to help the migration of peripheral leukocytes in the CNS. A differential infiltration of total myeloid (neutrophils, macrophages/monocytes, and microglia) and lymphoid (CD4, CD8, and NKT) cell populations observed at different time post infection is critical for orchestration of the clearance of the viral particle mounting host immunity by balancing the pro-inflammatory condition with the anti-inflammatory condition and restoring the CNS homeostasis.

3.1 MHV

Mouse hepatitis virus (MHV) is a β-CoV of the family Coronaviridae. It poses no threat to humans but shows similarities with other human viruses of the same family, such as SARS-CoV, MERS-CoV, and SARS-CoV-2 though they are evolutionarily distinct. MHV can infect the CNS and cause white matter lesions, which makes it an excellent viral model of neuroinflammatory demyelinating disease. Depending on the inoculation route and the strain of MHV-CoV, different outcomes are expected [21].

For example, a highly neurovirulent strain of MHV, JHM, J2.2-V-1, upon intracranial inoculation, induces a monophasic disease course, characterized by inflammatory cell infiltrates in the CNS with subsequent demyelination and clinical symptoms of hind limb weakness, ataxia, and paralysis [22, 23]. No auto-reactive T cells have ever been found in the CNS of J2.2-V-1-infected mice, and the disease is the resultant of virus-specific T cells, which indicated that virus alone can cause myelin destruction. Earlier it was believed that demyelination in JHM infection may be solely due to the lytic oligodendrocyte infection [24], but with the application of immune-deficient animal models, it became clear that immune-mediated mechanisms may be more important [25].

MHV-A59, a hepatotropic and neurotropic MHV strain, caused demyelination in C57BL/6 mice even in the absence of B and T cells [20]. The disease upon MHV-A59 intracranial administration also follows a biphasic course, where encephalomyelitis is characteristic of an acute phase peaking during days 5/6 post infection (p.i.) and chronic stage where demyelination and axonal degeneration peak on day 30 p.i. [26–29]. Thus, it can be said that different, but related MHV strains may induce demyelination via distinct mechanisms. MHV-A59 induced neuroinflammation and neuroimmune modulation mediated neuroglial dystrophy is triggered by the activation of cellular censors like Toll-like receptor (TLRs)/Rig-I-like receptor (RLRs)/synthase for the second messenger cyclic GMP-AMP and the cyclic GMP-AMP receptor stimulator of interferon genes (cGAS-STING) which can further activate the interferon regulatory factors (IRFs), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and downstream type I interferon (IFN) genes. Acute-innate neuroinflammation is rather dependent on the CNS resident immune cell activation in association with peripheral derived myeloid cells, which in turn involve lymphoid cells in ameliorating proinflammatory condition and bring the anti-inflammatory condition in order to restore the homeostasis of the CNS compartment [30].

Studies are focused on understanding mechanisms from cellular sensing to the disease outcome comprising the MHV-A59 induced neuroinflammation encompassing encephalitis and microglial nodule formation and its progressive myelin pathology concurrent with axonal pathology. We have used and compared spike gene recombinant strains of MHV, a demyelinating strain (DM) RSA59, and non-demyelinating strain (NDM) RSMHV2 to understand the genomic control of encephalitic properties. A plethora of studies from the eminent scientists in this field, along with ours, have contributed to understanding the pathogenesis of MHV infection. Strains of MHV can cause direct CNS cell infection or access the CNS via retrograde axonal transport, but irrespective of the route, they cause encephalitis [31–33].

3.1.1 Comparative studies between spike gene recombinants murine coronaviruses RSA59 and RSMHV2 to understand the genomic control of meningioencephalomyelitic properties

Using targeted RNA recombination, two isogenic spike protein recombinant strains of MHV, RSA59, and RSMHV2 (background is from demyelinating strain MHV-A59) were generated. For RSA59, the spike was taken from the parental demyelinating strain MHV-A59, and RSMHV2 had the spike from the parental non-demyelinating MHV-2 strain. Enhanced green fluorescent protein (EGFP) was also inserted in the recombinants by replacing the nonessential gene 4a and part of 4b in the MHV-A59 genome by heterologous targeted recombination [34].

Comparative studies between the two recombinants revealed similar pathology to their parental strains for RSA59 and RSMHV2, respectively. Intracerebral (IC) inoculation of RSA59 in 4-week-old C57BL/6 mice caused acute hepatitis, neuroinflammation comprising of meningitis, encephalitis, myelitis, and chronic demyelination and axonal loss, characterized by lymphocytic infiltrates and microglial nodules with focal neuronophagia associated lepto-meningitis [29, 35]. IC inoculation of RSMHV2 caused acute stage hepatitis, meningitis, and encephalitis but no myelitis or chronic demyelination. The encephalitis was indeed more robust compared to RSA59. MHV-2 does not induce encephalitis; it cannot even enter the brain parenchyma and restricts to the meninges inducing meningitis alone [34].

Both RSA59 and RSMHV2 showed similar infection in the brain where they successfully infected and replicated in meninges, the site of inoculation (near the lateral geniculate nucleus), ventral striatum/basal forebrain, hippocampus, and brainstem, and infect the neurons, astrocytes, microglia, and oligodendrocytes in the brain. However, they show different tropism in the spinal cord. DM infects the grey matter neurons and takes an axonal route to be released at the nerve end, whereas in the white matter, it preferentially infects oligodendrocytes [36]. By day 7 p.i. most of the viruses have traversed to the spinal cord white matter. Though NDM can also infect the neurons in the grey matter, they fail to infect the white matter oligodendrocytes [35, 36] due to their inefficiency to translocate through neurites and fusion at the nerve end [37]. The difference in the disease outcome can be attributed to their differential spinal cord tropism and persistence. Thus, it can be inferred that the combined action of spike mediated axon transport of DM strain to evade the heightened immune response and ability to infect the white matter oligodendrocytes and persist in the white matter could be the key to inducing demyelination and axonal loss during the chronic phase of neuroinflammation. DM strain-induced axonal loss and myelin loss are associated with profuse accumulation of macrophages filled with myelin debris within the demyelinating plaques which is not observed in NDM strain infection. High resolution TEM microscopy revealed that microglia/macrophages are indeed responsible for direct myelin stripping, leading to demyelination [29, 35, 38].

It is important to note that neuroinflammation and encephalitis in MHV infection is accompanied with pronounced activation of CNS resident immune cells, microglia, and astrocytes. Upon activation, they take their characteristic activated phenotype and start expressing microglia/macrophage-specific protein Iba1 (ionized calcium-binding adaptor molecule 1), which promotes ruffling and phagocytosis [20, 38]. Detailed Affymetrix microarray analysis revealed that both RSA59 and RSMHV2 initiate innate immune responses during the acute phase with the expression of chemokines like CXCL10, CXCL9, CCL5, and CCL12 and other CD molecules that represent activation of microglia/macrophages [39]. Results also showed the induction of antiviral host response represented by the expression of perforins and IFN gamma signaling genes. Together, the acute stage innate-immune responses and encephalitis were comparable in both RSA59 and RSMHV2 infection [39].

The inflammatory responses gradually declined in RSMHV2 infection following virus clearance, but RSA59 chronic infection showed persistent microglia in the demyelinating plaques and the production of microglia-associated inflammatory mediators. Studies have shown that IFN responses can promote phagolysosomes maturation and autophagy in the persistently activated microglia/macrophages, which can promote myelin sheath engulfment leading to demyelination. This evidence demonstrated that RSA59 induced demyelination could occur through innate immune neuroinflammation denoted by meningioencephalomyelitis triggered during the acute infection stage. Although innate immune responses contribute partially towards controlling of initial virus spread, virus-specific T cell effector functions are essential to eliminate the infectious virus load during most acute infections. Control of m-CoV spread requires the functioning of both $CD4^+$ and $CD8^+$ T cells [40]. $CD8^+$ T cells are the primary effectors but require support from $CD4^+$ T cells. A recent study in $CD4^{-/-}$ mice showed impaired RSA59 clearance, despite the presence of functional $CD8^+$ T cells, demonstrating the importance of $CD4^+$ T cells for the efficient functioning of $CD8^+$ T responses [41].

The distinct cause-effect relationship-driven studies demonstrate that RSA59 infection can be instrumental towards understanding a direct CNS resident immune cell-mediated as well as antibody-mediated encephalomyelitis and demyelination pathologies.

Both DM and NDM strains show a reduction in the expression of genes responsible for innate immune response, and this reduction is more pronounced in the NDM strain-infected mice. In contrast, the genes involved in adaptive immune cell response are upregulated only in DM strain, specifically during the chronic stage of spinal cord infection. A significant upregulation of genes involved in T helper cell signaling pathways, B-cell development, and communication between innate and adaptive immune cells as well as of the expression of IgG genes are observed in the DM strain infection leading to chronic pathology but not in NDM strain [42].

While MHV infection in mouse is a prototype to understand the cellular and molecular consequences of encephalitis and demyelination, Theiler's murine encephalomyelitis virus (TMEV) in SJL mice also serves as another excellent experimental model of MS because of its histopathological and immunological similarities with MS.

3.2 Theiler's murine encephalomyelitis virus (TMEV) induced encephalitis

TMEV is a non-enveloped, single-stranded positive-sense RNA virus belonging to the family Picornaviridae and also used as a model to understand the immune-mediated mechanism of demyelination [43, 44].

Upon intracranial (i.c.) infection in SJL mice induces characteristic polioencephalomyelitis in the CNS. In a biphasic CNS disease, first, during the acute phase immune cells including the $CD4^+$ T cells and $CD8^+$ T cells infiltrate the CNS in response to profuse virus replication, and inflammation [45]. They exert rather protective effects by helping clear the virus from the grey matter and result in immune-mediated encephalomyelitis [46]. During the chronic phase, TMEV persistently infects glial cells and macrophages in the white matter, further, there is an infiltration of leukocytes, including macrophages, TMEV-specific T cells and antibodies. The immune effectors ($CD4^+$ and $CD8^+$ T cells) that exerted protective functions during the acute phase exert detrimental effects in the chronic phase by participating in epitope spreading to myelin antigens resulting in severe immune pathology in the white matter of the spinal cords, which causes demyelination [47]. White matter lesions harbor monocytes/macrophages and a few B cells in addition to the T lymphocytes [48]. Initially, the $CD4^+$ T cells recognized the abundant myelin protein PLP, but later, the $CD4^+$ T cells subsets start to recognize subdominant myelin protein epitopes and led to autoreactivity [47]. Additionally, the $CD8^+$ T cells have also been suggested to function as autoreactive cytotoxic cells or regulatory cells in TMEV infection [49]. TMEV induced CNS pathology is immunologically mediated like in MS, wherein MHC plays an important role, and substantial similarities exist in neuropathology, including axonal damage and remyelination [43]. Also, like MS, T-cell apoptosis is less in TMEV induced disease [50].

Cytokines are known to play important functions in the induction and regulation of immune responses against the neurotropic virus. Similarly, the cytokine production by the CNS resident cells astrocytes and microglia, as well as the peripheral immune cells such as T cells and macrophages heavily, influence the encephalitic response induced by TMEV infection [51, 52]. Studies have shown that a critical balance between the inflammatory cytokines governing the propagation of antiviral response to clear the virus during early infection and controlling of immune

pathology and establish homeostasis during the late stage. That being said, no particular cytokine pattern is yet established and successfully associated with resistance or susceptibility to TMEV-induced encephalitis and demyelination by the different strains of the virus. A study compared the cytokine response between TMEV DA-infected susceptible (SJL) and resistant (B6) mice. Results showed a high expression of proinflammatory cytokines (IFN-γ, TNF-α, IL-1, IL-2, and IL-6) and low levels of anti-inflammatory cytokines (IL-4, IL-5, and IL-10) in the brains of both SJL and B6 mice during the early acute phase decreasing thereafter [51]. However, only in the SJL mice after a peak of the inflammatory response during day 8–12 p.i. in the brain with a minimum recorded during days 20–25 p.i., the second wave of inflammatory cytokine production was observed later in the spinal cord, which could explain the inflammatory demyelination only in the SJL mice. TGF-β, an important anti-inflammatory cytokine, has shown a significantly higher upregulation in SJL mice compared to B6 [51]. TGF-β can specifically inhibit the cytotoxic T lymphocyte (CTL) response [53] which is noted to be significantly impaired in the SJL mice resulting in reduced TMEV clearance and persistence, leading to virus-mediated encephalitis and demyelination.

4. Overview of microglial activation in encephalomyelitis: amplifier of virus-induced neuropathology

In the context of viral encephalitis that is characterized by an inflammatory response with meningeal, perivascular, and parenchymal infiltrates of peripheral leukocytes, studies have revealed that microglial activation acts as a double-edged sword [16]. On the one hand they promote multiple antiviral functions; microglia sense the ATP released by virus-infected neurons through the purinergic receptor P2Y12 and quickly migrate towards the infected neurons to exert their phagocytic activity [54]. They directly exert their antiviral effect by producing type 1 interferon (IFN-1), inducing IFN-stimulated gene (ISG) to activate corresponding signaling pathways [55]. Additionally, microglia induce autophagy and secrete cytokines to clear the virus from the tissue [56–58]. On the other hand, their persistent activation leads to tissue damage due to autophagy and apoptotic pathway activation, presynaptic membrane damage in the hippocampus mediated by the complement system activation, which further results in long-term memory impairment and cognitive dysfunction in patients with viral encephalitis [59–62].

The most common and important example of virus-induced chronic brain infection is the HIV [63]. HIV-induced encephalitis is typified by the accumulation of activated microglia in nodules-like phenotype throughout the parenchyma [64, 65]. HIV enters the CNS via the myelomonocytic cells such as monocytes, perivascular cells, and microglia [66]. HIV particularly targets and disables microglia in the CNS and T cells in the periphery, the key players in neuroinflammation [64, 67]. In fact, the persistence of HIV in microglia indicates that the virus uses the cells as the reservoir [68]. Even though reports suggest that microglia may perform protective functions early on during HIV infections, their functions are considerably compromised. Most studies suggest that active infection of microglia results in their secretion of a variety of neurotoxins, increasing neural apoptosis and neuronal autophagy [16]. Astrogliosis is another characteristic pathology following microglial activation that, together with microgliosis, ultimately leads to myelin paleness and neuronal loss. Patients with advanced AIDS are likely to develop severe encephalitis upon human cytomegalovirus

(HCMV) infection [69, 70]. HCMV infection is characterized by microglial nodular encephalitis consisting of microglia, astrocytes, and giant cells, and ventriculoencephalitis and is the main cause of dementia in AIDS patients [71].

Microglia are susceptible to congenital Zika virus (ZIKV) infection [61]. Histopathological analysis showed that ZIKV infects and activates microglia in the perivascular regions causing localized neuroinflammation [61]. Further, the virus is disseminated throughout the parenchyma, which is later associated with neuron damage, especially in the cortical regions [72]. Pronounced neuronal injury results in microcephaly noted in many cases of congenital ZIKV infection [72].

E3 ubiquitin ligase pellino (pelia) expressed by microglia promotes the replication of West nile virus (WNV) in microglia and neurons [73]. It also induces NF-κB and/or p38-MAPK signaling in the microglia that causes an upregulation of inflammatory cytokines and chemokines, leading to peripheral leukocytes infiltration [73, 74]. The robust neuroinflammation may lead to lethal WNV encephalitis.

It is interesting to note that many viruses from diverse families of viruses have been studied in microglia depletion models. Results from most studies showed increased viral replication upon microglial depletion [16, 75–77]. Additionally, microglial depletion was also associated with overt neurological symptoms and/or death along with high viral burden, which indicated their importance in survival in encephalitis [77]. Though confirmatory results on how these protective functions are exerted by microglia are lacking, some studies show a dependence of T cell responses on microglia activation.

Studies on several mouse models of viral encephalitis have shown that viral clearance depends on efficient T cell responses, including WNV, MHV, and TMEV [78–81]. TMEV model shows strain-specific differences in disease phenotype and viral clearance, which was associated with underlying differences in $CD8^+$ T cell responses subject to Treg suppression [82, 83]. Further investigation revealed that microglia depletion did not impact $CD8^+$ T cell recruitment but resulted in increased infiltration of Tregs, which caused clinical severity in C57BL/6 mice which is normally not susceptible to TMEV induced disease [84]. In the MHV-induced neuroinflammation model, both $CD8^+$ and $CD4^+$ T cells are implicated in viral clearance, but $CD4^+$ T cells have also been reported to contribute to pathogenesis [80, 85, 86]. A study showed that microglial depletion in mice infected with JHMV strain of MHV, rJ2.2 significantly reduced the infiltration of $CD4^+$ T cells and Tregs in the CNS along with a significant reduction in IFN-γ expression by $CD4^+$ T cells, but there was no impact on the $CD8^+$ T cell population [77]. Thus, showing the importance of microglia in especially orchestrating virus-specific $CD4^+$ T cell response.

In addition to these studies using the JHMV strain, MHV-A59 or its isogenic recombinant strain RSA59 have also elucidated a critical communication between microglia and $CD4^+$ T cell response. Using a $CD4^{-/-}$ mice model very recent study demonstrated for the first time that the mice are highly susceptible to RSA59 induced chronic demyelination with axonal loss [80]. Though the overall inflammation was not affected during the early time-points (day 5–6) i.e., the acute neuroinflammation phase, the CD11b + microglial activation was significantly impaired. The entire inflammatory response was skewed towards an M2 type which was also reflected in the persistence of characteristic amoeboid shaped phagocytic microglia in the CNS of the mice during the chronic phase (day 30 p.i.). Encephalitis, which usually resolves after the acute phase in RSA59 infection, persisted for as long as day 30 p.i. The brain stems of $CD4^{-/-}$ mice were populated with CD11b + microglia surrounding bulbar vacuolated pathology, which signified axonal death and damage [80]. Additionally,

$CD4^+$ T cell deficiency resulted in severe grey matter inflammation in the form of poliomyelitis in the spinal cords as well as the dorsal root ganglion (**Figure 3**). Together these results showed a critical interdependence of microglia and $CD4^+$ T cells in RSA59 infection. Typically, M2 microglial activation fails to resolve during the chronic infection, rendering mice more susceptible to demyelination and axonal bulbar vacuolation [80].

A very recent study on neurotropic coronavirus MHV-RSA59 infection in $Ifit2^{-/-}$ mice revealed that Ifit2 protects mice from uncontrolled replication and spread throughout the brain parenchyma as well as the spinal cord. Ifit2 deficiency showed pronounced morbidity and mortality in RSA59 infected mice. Furthermore, microglial activation in the CNS was impaired in infected $Ifit2^{-/-}$ mice compared to WT infected mice, and as a consequence, peripheral lymphocyte specifically NK1.1 T

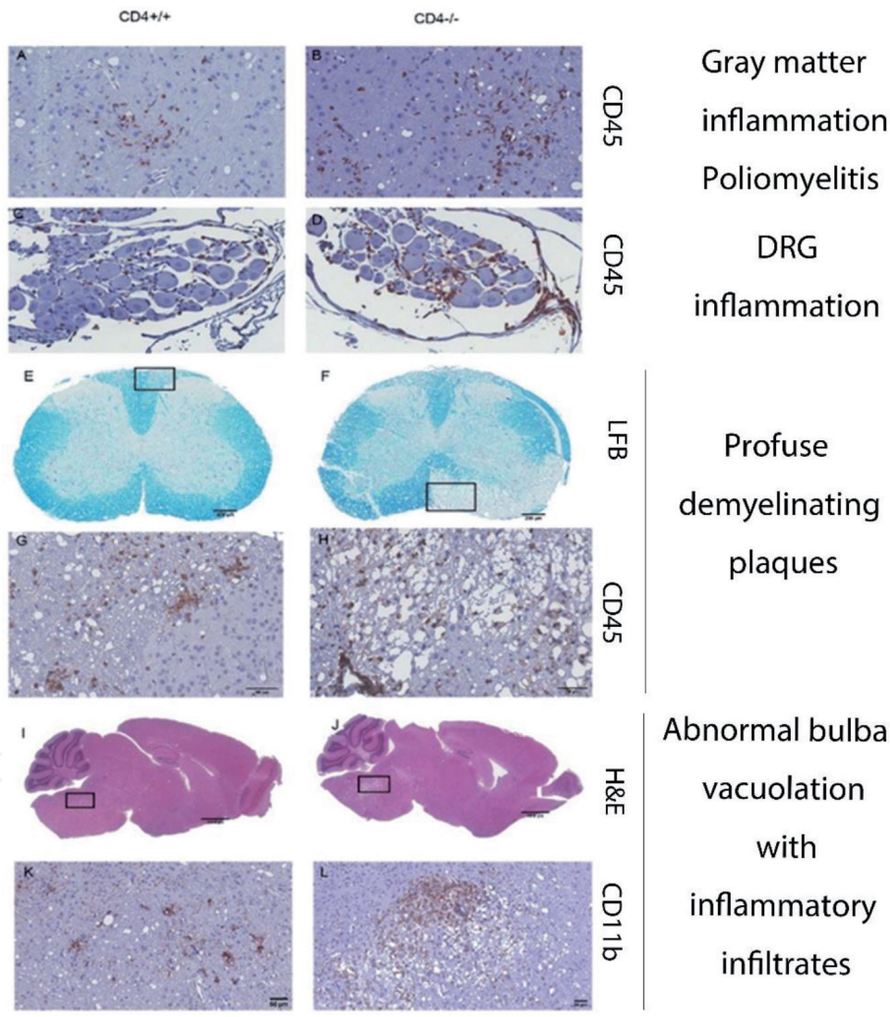

Figure 3.
CD4 deficiency causes poliomyelitis and the dorsal root ganglionic inflammation at acute phase and abnormal bulbar vacuolation at chronic phase of RSA59 infection. Serial sections of spinal cord, dorsal root ganglion and brain from $CD4^{+/+}$ and $CD4^{-/-}$ mouse were immunostained with anti-CD45, LFB and/or H&E. CD45 immunostining showed heightened poliomyelitis/inflammation of gray matter and inflammation of the dorsal root ganglia in the $CD4^{-/-}$ mice compared to $CD4^{+/+}$ mice at the acute phase of infection. Spinal cord sections form these mice when further analyzed at chronic stage for demyelination by LFB showed increased myelin loss in $CD4^{-/-}$ mice compared to $CD4^{+/+}$ mice, CD45 + inflammatory cells were observed in demyelinating lesions of both wildtype and CD4 deficient mice but were elevated in case of $CD4^{-/-}$ mice. Sagittal sections of the brain at chronic stage, stained with H&E showed large number of vacuoles in the brain stem region denoting abnormal bulbar vacuolation which was populated with and Cd11b + micgrogila/monocyte macrophages. Adapted from Chakravarty et al [41].

cells and $CD4^+$ T cells migration to the CNS was restricted in the $Ifit2^{-/-}$ mice possibly contributing to the lack of viral clearance. Impaired microglial activation and reduced migration of inflammatory cells in the CNS may be associated with less encephalitis and devoid of mounting host immunity. These deficiencies were associated with a lower level of microglial expression of CX3CR1, the cognate receptor of the CX3CL1 (fractalkine) chemokine, which plays a critical role in both microglial activation and leukocyte recruitment. These findings highlighted a pivotal role of interferon stimulating genes and its tetratricopeptide protein as host cell factors in the induction of encephalitis and uncovered a new potential role of an interferon-induced protein in immune protection (**Figure 4**) [30].

Taking the above-mentioned experimental evidence of the role of $CD4^+$ T cells and monocyte/macrophage activation in viral-induced neuroinflammation, further studies were geared to explore the interactome between the $CD4^+$ T cell expressed CD40 Ligand and CD40 expressed on microglia. CD40-CD40L dyad is an important immune dyad that controls both $CD4^+$ T cell and microglia functions [87, 88]. Our studies in $CD40L^{-/-}$ mice showed that the absence of CD40L renders mice highly susceptible to RSA59 infection due to reduced microglia/macrophage activation during the acute phase of infection required to eliminate the virus (**Figures 5**) [89]. Effector $CD4^+$ T recruitment to the CNS is significantly dampened, and due to the

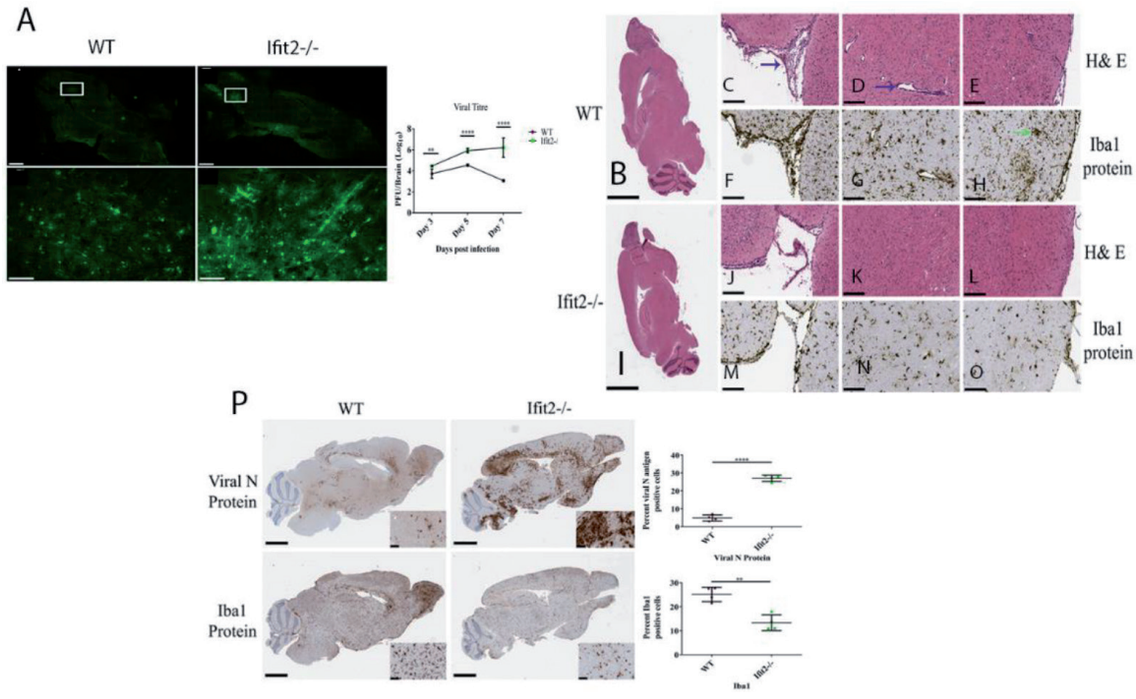

Figure 4.
$Ifit2^{-/-}$ mice upon a murine β-CoV RSA59 infection show increased viral spread and decreased microglia/macrophage activation. About 4–5-week-old $Ifit2^{-/-}$ mice upon RSA59 infection showed a robust viral replication and antigen spread throughout the brain parenchyma compared to the wildtype mice infection. Infectious viral load was significantly higher in the $Ifit2^{-/-}$ mice when assessed by plaque assay (A). The cryosections form $Ifit2^{-/-}$ and wildtype mice brain sections showed similar overall distribution of RSA59 but the total EGFP expression (viral antigen) was more in $Ifit2^{-/-}$ mice (A). At day 5 p.i. H&E staining for the sagittal sections of the whole brain form WT (B) and $Ifit2^{-/-}$ mice (I) showed much milder meningitis (J) and encephalitis characterized by perivascular cuffing (K) and microglial nodule (L) formation in the $Ifit2^{-/-}$ compared to WT (C–E) mice. Similarly activated microglia/macrophage were much less in the $Ifit2^{-/-}$ mice (M–O) compared to the WT (F–H) mice as seen in Iba1 immunostaining. (P) Brain section of $Ifit2^{-/-}$ mice showed heightened viral infection as evident by the profuse viral N protein immunostaining. $Ifit2^{-/-}$ mice however showed a comparatively decreased Iba1 immunostaining indicative of impaired activation of microglia/macrophages compared to the wildtype mice. Adapted form Das Sarma et al [30].

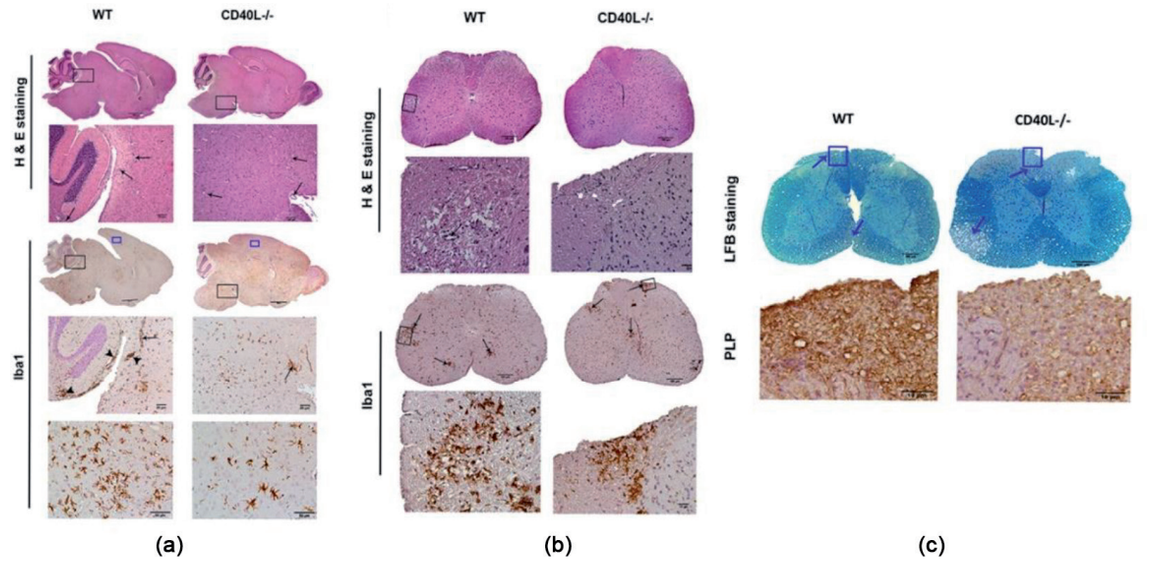

Figure 5.
RSA59 infection in CD40L deficient mice showed impaired microglia/macrophage activation during acute phase of neuroinflammation but causes profuse chronic demyelination concurrent with diminished PLP staining. (a and b) CD40L$^{-/-}$ and WT mice at day 5 upon RSA59 infection showed acute encephalitis and myelitis.Iba1 immunostaining in these brain and spinal cord sections showed that the CD40L$^{-/-}$ mice showed reduced Iba1+ cells compared to WT mice. (c) At day 30 p.i., spinal cord of CD40L$^{-/-}$ mice showed more intense demyelination and reduced PLP staining compared to wildtype mice. Adapted from Saadi et al [89].

impaired CD40-CD40L signaling in CD40L$^{-/-}$ mice, their priming is reduced substantially in the draining lymph nodes [89]. Effector CD4^{+} T cell population was reduced as well as the antiviral response was diminished, and phagocytic microglia persisted in the CNS at a substantial amount in the CD40L$^{-/-}$ mice. As a result, CD40L$^{-/-}$ mice exhibited greater demyelination, axonal loss, and persistent poliomyelitis at the chronic phase of infection [89]. Together, these studies highlight that migration of peripheral T cells and their interaction with microglia via CD40-CD40L is essential to eliminate the virus and provide long-term neuroprotection.

Independent of the effect on T cells, IFN produced by microglia acts on other cells that exert indirect antiviral effects [90]. For example, microglia induce antiviral functions in neurons via STING signaling and stimulate IFN-1 production in astrocytes by the TLR3 pathway [91]. Studies have shown that the Type 1 IFNAR signaling in astrocytes helps to protect the blood-brain barrier against virus infection and immunopathology [92]. Depletion of IFNAR signaling in astrocytes resulted in increased inflammatory cytokines and chemokines production, which caused blood-brain barrier inflammation during neurotropic viral infection [92].

Additionally, microglia also mediate viral clearance by autophagy [59]. Viral infections induce NFkB-dependent inflammatory effectors that produce antiviral molecules, including those promoting autophagy. ZIKV infection in Drosophila induces a stimulator of interferon genes (dSTING) in the brain, which promotes autophagy and helps protect the brain [93]. In mammals, autophagy has been shown to restrict HSV-1 infection [94]. Autophagy by microglia helps to clear the virus without causing cell death which protects mature neurons. Microglial phagocytosis is another mechanism that helps protect the neurons from severe damage. Microglia and neurons express C3aR that recognizes C3 cleavage products. In response to C3 and its cleavage products, microglia surround the neurons and phagocytose the presynaptic ends of the neurons [95]. This prevents the trans-synaptic spread of the virus and keeps neurons from firing abnormal signals that may result in cognitive impairment and physical disabilities.

Additionally, detrimental effects of microglia are also reported in many studies on viral encephalitis. Microglia are reported to remain persistently activated in several viral infections [80, 89]. Their activation is further associated with the production of TNF-α [96, 97], which can activate the astrocytic TNFR-1 pathway [98]. This signaling accentuates their crosstalk with neurons leading to modification of the excitatory synapses, which emerges in cognitive disabilities. TNF-α secreted by microglia can directly affect synaptic transmission and plasticity [62]. ZIKV infection has been associated with neurological damage among infants. Studies have found that ZIKV majorly infects the fetal microglia and activates them [61]. This induces an intense pro-inflammatory response by the secretion of mediators like IL-6, TNF-α, IL-1β, and MCP-1 [61]. Also, in HIV encephalitis, many microglia genes undergo significant changes, including immune activation and function, kinases, phosphatases, and pro-/anti-apoptotic and neurotrophic factors, which indicates that microglia functions are compromised and skewed towards pro-inflammation [99].

Thus, it can be ascertained that, microglia are not only important to maintain homeostasis in the CNS but are also critical for responding to injury, infection, and neurodegeneration. Often microglia act quickly in response to injury but with varied stimuli received, their activation profile can differ and may result in harmful or beneficial effects. It is true that viral encephalitis has caused high morbidity, which is a grave concern, but it is also imperative that research on microglia and viral encephalitis can provide new and efficient targets for treatment. Considering the unique response of microglia with different viral infections and at different stages of encephalitis, it is needless to say that the current research on microglia and viral encephalitis remains is at a nascent state. Depending on the type of encephalitis, careful fine-tuning of microglial activation has the potential to improve the therapeutic effect of encephalitis, its prognosis and also reduce the sequelae of encephalitis.

5. Encephalitis caused by several other neurotropic virus families

Many viruses from numerous virus families in different geographical areas can induce immediate or delayed neuropathological manifestations in humans and animals [18, 100]. Infection by neurotropic viruses and their resultant immune response has the potential to irreversibly disrupt the complex structural and functional dynamics of the central nervous system, frequently leaving the patient with a poor or fatal prognosis. The incidences of virus-induced CNS disorder are significantly higher than the damage caused by other pathogens.

Members of several virus families are known to be neurotropic, e.g., herpes family viruses, flaviviruses, paramyxoviruses, alphaviruses, bunyaviruses, orthomyxoviruses, arenaviruses, enteroviruses, rhabdoviruses, coronaviruses, and picornaviruses. Specifically, some of the viruses from these families are viruses like SARS-CoV, MERS-CoV, herpes simplex virus, poliovirus, West Nile virus (WNV), Chikungunya virus (CHIKV), Zika virus (ZIKV), Japanese encephalitis virus (JEV), La Crosse encephalitis (LACV), Epstein-Barr virus (EBV), measles, and mumps viruses, among many others [100, 101]. These viruses have been frequently associated with significant encephalitis, as well as meningitis and myelitis in the CNS. The clinical disease outcome of the CNS virus infection depends on several factors, like the host immune status, viral genomic constitution, and other environmental factors [100]. There is also evidence suggesting coronaviruses such as α-CoVs (NL63 and 229E) and the β-CoVs (OC43, HKU1) being positive-sense single-stranded, enveloped RNA viruses,

can induce numerous neurological manifestations along with systemic inflammations in humans. A very recent outbreak of human β coronavirus SARS-CoV-2 is primarily known for its ARDS and paramount evidence suggest that it may enter the brain via olfactory route or can enter through the lung brain axis or gut-brain axis and is also known to cause meningitis, encephalitis, and demyelination [102–104].

The etiology of CNS infections induced by viruses can also depend on and vary across variable geographical locations. For example, reports have shown that herpes simplex viruses are the most common pathogens observed among both children and adults in the United States (US), Australia, and Italy. In contrast, in Southeast Asian countries like southern Vietnam, the Japanese encephalitis virus has been shown to be one of the most frequent inducers of viral encephalitis, especially among children. Enteroviruses have been commonly isolated to be involved in causing encephalitis in several parts of India. At the same time, HSVs have been observed to be more prevalent in the eastern parts of India, both among adults as well as children. Furthermore, the virulence of the viruses also varies geographically [105]. Therefore, understanding the etio-biology and epidemiology of neurotropic viruses is paramount in designing the targeted intervention.

Therefore, based on epidemiological prevalence and episodic occurrence evidence as well as the employment of these viruses as experimental model systems for human disorders, this book chapter will also briefly discuss some of the other viruses.

Flaviviruses are the enveloped, single-stranded, positive-sense RNA viruses that consist of the world's most clinically critical viruses like the following species: Japanese encephalitis virus (JEV), tick-borne encephalitis virus (TBEV), and Powassan encephalitis virus (POWV), as well as other mosquito-borne viruses, like Dengue virus (DENV), yellow fever virus (YFV), West Nile virus (WNV), St. Louis encephalitis virus (SLEV), and Zika virus (ZIKV) [106, 107]. JEV is highly prevalent in the Southeast Asian countries as well as the Indian subcontinent, affecting infants and children, and can also be transmitted to the fetus during pregnancy [108, 109]. The incidences of Tick-borne encephalitis (transmitted to humans by the bites of ticks) are progressively expanding in European and Asian countries with severe neurological complications [110]. WNV infection is endemic in temperate and tropical regions throughout the world, triggering yearly outbreaks of encephalitis [111]. St. Louis encephalitis virus (SLEV) is found predominantly in North, Central, and South America and accounts for nearly 35–60% of meningitis in all symptomatic cases in children [112, 113]. Zika virus is also an emerging pathogen with substantial clinical impact significantly on the CNS, as reported, in the form of severe congenital malformations (microcephaly) and neurological complications, mainly Guillain-Barré syndrome (GBS) [114]. It has shown explosive outbreaks in African, South, and Central American countries [112, 115].

Alphaviruses like Eastern equine encephalitis virus (EEEV), Western equine encephalitis virus (WEEV), and Venezuelan equine encephalitis virus (VEEV), as well as the Mayaro virus (MAYV), Una virus (UNAV), and Chikungunya virus (CHIKV) are the small, enveloped viruses with a single-stranded, positive-sense RNA [100, 113]. The most critical neurotropic alphavirus is VEEV, which induced many outbreaks in South, Central, and North America [116]. CHIKV has also caused severe neurological complications in humans at all age groups, especially in infants, in Europe, Asia, and Africa [117, 118]. EEEV can also induce encephalitis in humans in about 50–75% of the cases [8, 119].

Herpes family viruses which are double-stranded DNA viruses, have been commonly associated with severe encephalitis and meningitis in the CNS and have been

distributed globally. The members of herpes family viruses that are shown to be neurotropic include HSV types 1 and 2, varicella-zoster, Epstein-Barr virus, and cytomegalovirus [120, 121]. Both children and immunocompromised individuals are most vulnerable to herpes simplex meningoencephalitis [120]. Another critical property to herpes family viruses, especially varicella-zoster virus, is reactivation [122]. Primary infection with VZV during childhood induces chickenpox, but the virus becomes latent in the spinal and cranial ganglia. However, deteriorating cellular immunity with senescence or immunocompromised conditions may lead to virus reactivation that promotes zoster (shingles) [123, 124].

Paramyxoviruses that induce neurological diseases are from genera Rubulavirus (consisting of the mumps virus), which is neurotropic [125]; Morbillivirus genera (consisting of measles virus) [126] and Henipavirus with the emerging Nipah virus (NiV) being one of the neurotropic variants [127]. These are single-stranded, nonsegmented RNA viruses [128]. Measles virus-induced encephalitis is one of the leading causes of morbidity and mortality in the developing world [129]. Nipah virus is one of the emerging viruses that present with numerous cases of acute encephalitis in humans [127, 130].

Lymphocytic choriomeningitis virus (LCMV) belonging to the family Arenaviridae is an enveloped, single-stranded RNA virus. Although its primary host is mice, it is also present in other rodents and has the ability to infect humans, especially laboratory workers, pet owners, and individuals living in impoverished conditions. It is predominant in Europe, Asia, American continents, and Africa [131–133].

Picornaviruses are single-stranded, non-enveloped RNA viruses encompassing enteroviruses (echoviruses, coxsackieviruses) and parechoviruses (PeVs) pathogenic against humans. Infants and children are highly susceptible to human pathogenic picornaviruses that induce aseptic meningitis and meningoencephalitis [134, 135]. It is highly predominant in the UK, Ireland, the US and some Southeast Asian countries [105, 136, 137].

6. Chronic viral encephalitis and neurodegeneration

The research on viral encephalitis is a rather dynamic and large field. However, its relationship with neurodegeneration is less explored. As research on viral encephalitis and neurodegenerative disease is progressing on diverse fronts many similarities are being identified between the classical neurodegenerative pathways and viral-induced neurodegeneration [138, 139]. It is well established that many neurotropic viruses result in neuronal dysfunction which can have devastating life threatening consequences for the host [138, 140, 141]. Virus can either directly infect the neurons and kill the cells directly by replication and lysis or by apoptosis as observed in poliomyelitis [139, 142]. Additionally viral-induced encephalitis can damage the neurons in an immune mechanism as all neurotropic virus infections irrespective of the route of entry can trigger both innate and adaptive immune responses [139, 143, 144]. Supporting this concept, a number of viruses have been associated with neurodegeneration outcomes [141]. For example, In addition to causing encephalitis, CMV infection can result in transverse myelitis [145], HIV is associated with severe dementia [146] and Echo virus can cause neuro-muscular disorder [147]. Additionally, yearlong infection with JEV in humans can cause postencephalitic parkinsonism (PEP) which shows symptoms similar to sporadic Parkinson's disease (PD) [148]. Evidence also suggests that H5N1 influenza virus induces many PD-like symptoms [149, 150].

Specifically, the virus first infects the peripheral nervous system (PNS) and later gains entry into the CNS where it causes degeneration of susceptible dopamine (DA) neurons in midbrain regions similar to PD patients [149, 151]. Another influenza virus strain H3N2 causes many neurodegenerative symptoms like amyotrophy, MS flares and relapsing delirium [152, 153]. However, the direct role of viruses in neurodegeneration is less understood. Most likely, several viruses have developed means to evade the immune response and are present in subclinical levels [154–156]. The local inflammatory response in the CNS in response to persistent virus infections results in chronic encephalomyelitis even without overt cell death which may lead to neuronal damage resulting in degeneration.

HSV-1 is one of the most common virus infections that can remain dormant in the neurons for life-long [157]. It is highly neurotropic and periodic reactivation is observed to establish productive infection of the neurons [140]. It is one of the largely associated viruses with Alzheimer's disease (AD) [158]. The virus presence is detected in the AD brains, in fact the presence of HSV-1 DNA on APOE gene carriers is a risk factor for AD [159]. Studies also showed HSV-1 DNA and amyloid β to be present in close proximity in AD plaques [160, 161]. Mechanistically, HSV-1 infection can promote neurotoxic Aβ accumulation, tau phosphorylation and cleavage as observed *in vitro* [161–163]. In addition to the direct interaction which the virus exploits to travel to the cell surface it also interferes with post-transcriptional regulation by up regulating microRNA-146a, which is another marker for AD [164].

Parkinson's disease (PD) the second most common neurodegenerative disorder has been linked to Influenza viruses [140, 165]. Highly neurotropic and pathogenic H5N1 virus can enter the CNS, induce encephalitis associated with microglial activation, loss of dopaminergic neurons and accumulation of α-synuclein aggregates in infected regions resembling PD symptoms and pathology [149, 166]. It is well documented that with the 1918 epidemic of H1N1 has greatly increased the incidence of PD [166]. Both H1N1 and H5N1 are found in the substantia nigra region which is also majorly affected in PD patients [167]. In fact, post-mortem brain sections from PD patients show the presence of influenza A virus [168]. No direct mechanism is yet established but is majorly thought to be a contribution of the neuroinflammation process activated by the virus in the CNS [151]. Moreover, HIV infection of the CNS is associated with amyotrophic lateral sclerosis (ALS), which is a fatal neurodegenerative disease with characteristic degeneration of the spinal cord and cortical neurons [169, 170]. Several other neurological symptoms are associated with HIV infections; the most common is HIV-associated dementia which shows certain complications presented with MS [171, 172]. HIV-associated dementia (HAD) also has many similarities with AD and PD including the target anatomical region hippocampus and substantia nigra [173]. The similarities of HAD with neurodegenerative disorders is reported at genomic, proteomic as well as transcription levels [140].

Modern times have seen a drastic increase in the average life expectancy which has come with its own limitation i.e., the incidence of ageing disorders. As discussed, virus infection can significantly act as co factors of neurodegeneration if not the causative agents. Also, the mechanism of viral and non-viral neurodegeneration is not very different [138, 140]. Both show an involvement of immune system by means of neuroinflammation and direct or indirect damage of neurons. Viruses can significantly modulate the structure and function of cytoskeletal proteins that are instrumental in neuronal dysfunction associated with neurodegeneration [174]. Thus, neurotropic virus infection for encephalitis and neuroinflammation can serve as excellent models for understanding neurodegeneration. Moreover, a better

understanding of targeting the immune system, which has profound implications, especially in viral-induced neurodegeneration and deciphering the critical overlaps and distinctions between classical neurodegeneration and viral-induced neurodegeneration, can lead to developing new and efficient therapeutic strategies.

7. Conclusion

Encephalitis was considered a rare syndrome, but the incidence of encephalitis is likely to be elevated than previously estimated. This underrated approach towards understanding encephalitis prevails because it is challenging to diagnose, manage and study. Encephalitis is a condition with multiple etiologies and pathogeneses, ranging from direct infectious to immune-mediated; however, each of these specific mechanisms is diverse and often incompletely understood. Accurate diagnosis of encephalitis cases is made complicated because of the difficulties involved in distinguishing between encephalitic and non-encephalitis mimics, autoimmune vs. infectious encephalitis, and the limitation of standard clinical case/laboratory definitions. Ancillary testing, and clinical correlations, along with a clinical follow-up, are important to establish more specific diagnoses. Determining the etiology is the key first step to improving patient outcomes, and it needs advanced neuropathologic and clinical algorithms. Furthermore, the most recent antibody-associated forms of encephalitis also pose a challenge due to their significantly varying clinical manifestations. Understanding the antigenic specificity of intrathecal IgGs found in the CSF may help to identify clues to the cause of infection or inflammation in several cases of encephalitis.

Also, it is critical to study host-immune responses and other host factors to design novel therapeutic interventions, given the paradoxical role of the immune system in encephalitis. The increased urbanization, travel, and climate change are some of the factors, which contribute to the evolution and spread of new pathogens. Infectious diseases are emerging profoundly. Neuroinfectious diseases might occur as occurrences in small, localized regions or may rapidly spread over large geographical areas as pandemics, just like SARS-CoV2. Understanding emerging viruses need better experimental animal models, which will help to comprehend the cause-effect relationship between the virus and its associated neuropathogenesis. Strong consideration should be given to trials of combination therapy that include treatment strategies with both anti-inflammatory and anti-pathogen drugs.

Acknowledgements

Neurotopic m-CoV research in an experimental animal model has provided a timely and appropriate platform to take a step toward writing this comprehensive book chapter on encephalitis. This work has been a collaborative effort for two decades between the Indian Institute of Science Education and Research Kolkata (IISER-K), India; Indian Institute of Sciences (IISc), India; University of Pennsylvania, USA; Thomas Jefferson University, USA and University of Colorado Denver, USA. The joint endeavor has been supported and nurtured by the Indo-U.S. Science and Technology Forum (IUSSTF) on several occasions. m-CoV research was generously funded by the Department of Biotechnology (DBT), India, Council for Scientific and Industrial Research (CSIR), India; National Multiple Sclerosis

Society, USA; M.E. Groff Surgical Medical Research and Education Charitable Trust; and Lindback Foundation Career Enhancement Award, USA. The authors would also like to express their gratefulness to Dr. Dhriti Chatterjee, Dr. Kaushiki Biswas, Dr. Abhinoy Kishore, Dr. Subhajit Das Sarma, Dr. Afaq Hussain, Mr. Soumya Kundu, Mr. Abhishek Bose, Mr. Sourodip Sengupta, Ms. Mithila Kamble, Ms. Vaishali Mulchandani, Mr. Saurav, Mr. A. Safiriyu Abass, Dr. Mahua Maulik, and Ms. Soma Nag who have contributed immensely to the generation of a large volume of work on m-CoV research over the years. Authors would like to thank Dr. Kenneth S. Shindler, Dr. Lawrence C. Kenyon, and Dr. Randall J. Cohrs for their long-standing collaboration on m-CoV research. The success of this work has been primarily dependent on the collaborative efforts of the national and international m-COVID team and many researchers whose names are listed in the references.

Ethical statement

Most of the m-CoV work discussed in this chapter either adopted from our published work or from other studies adhered to the experimental procedures and animal care and use in accordance with good animal ethics approved by the Institutional Animal Care and use Committee.

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