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Review

Beneficial and detrimental functions of microglia during viral encephalitis

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Microglia are resident immune cells of the central nervous system (CNS) with multiple functions in health and disease. Their response during encephalitis depends on whether inflammation is triggered in a sterile or infectious manner, and in the latter case on the type of the infecting pathogen. Even though recent technological innovations advanced the understanding of the broad spectrum of microglia responses during viral encephalitis (VE), it is not entirely clear which microglia gene expression profiles are associated with antiviral and detrimental activities. Here, we review novel approaches to study microglia and the latest concepts of their function in VE. Improved understanding of microglial functions will be essential for the development of new therapeutic interventions for VE.

Highlights

During homeostasis, microglia are the most abundant immune cell type within the central nervous system. Different microglia subsets in different brain areas maintain brain function and integrity.

During virus-induced encephalitis, microglia are essential for antiviral defence and protection of the brain.

Microglia activation and antiviral defence are regulated by crosstalk between neurons, astrocytes, and other cell types in the brain.

Microglia activation may have detrimental effects by causing direct or indirect loss of neurons and disturbance of tissue integrity, eventually causing long-term sequelae.

Innovative methodologies are being used to study microglial function in experimental and clinical settings to refine concepts about the roles of microglia in viral encephalitis.

Improved understanding of microglial function in the virus-infected brain is essential for the development of novel treatments for viral encephalitis.

Microglia and their multiple functions

Microglia are resident myeloid cells of the CNS. They are part of the innate immune system and have miscellaneous functions. Microglia are considered glial cells and mononuclear phagocytes. They are involved in pathogen recognition as well as in the initiation and maintenance of local immune responses. Microglia have been shown to be crucially involved in orchestrating responses to viral infections of the CNS, which are mostly associated with brain inflammation and accordingly are often referred to as 'viral encephalitis' (VE) [1,2]. Under homeostatic conditions, microglia contribute to the maintenance of brain plasticity and function by supporting synaptic wiring and spatial patterning of the developing and adult CNS [3–6].

In some situations, myeloid cells other than microglia, such as peripheral macrophages and dendritic cells, are recruited to the CNS. Although microglia share several characteristics with infiltrating myeloid cells in the CNS, microglia are unique in terms of their ontogeny. Fate-mapping studies and developmental analyses revealed that microglia are yolk sac derived. Specifically, yolk sac-derived myeloid precursors (i.e., mesodermal progenitors) differentiate to yolk sac-derived macrophages that, during early embryonic development, travel to the brain, where they differentiate to immature microglia [7–10].

Microglia also share functions and phenotypes with other macrophages located within the CNS, including perivascular, meningeal, and choroid plexus macrophages, which are also referred to as 'border-associated macrophages' and 'CNS-associated macrophages' (BAMs and CAMs, respectively) [11,12]. Therefore, particularly under inflammatory conditions, it is difficult to unambiguously classify different myeloid cell subsets within the CNS. Recent transcriptional analyses revealed genes that are specifically expressed by microglia and that allow classification of myeloid cells. Core signature genes of homeostatic BAMs/CAMs comprise *Mrc1*, *Pf4*, *Ms4a7*, *Stab1*, *Cbr2*, and *CD163*, whereas homeostatic microglia express *Hexb*, *Cx3cr1*, *Csf1r*, *P2ry12*, *Tmem119*, *Gpr34*, *Tgfbr1*, *Fcrls*, *Siglech*, *Slc2a5*, and *Sall1* (see following text) [11–13]. Since microglia play multiple roles during health and disease, there is tremendous interest in better understanding their diverse functions, particularly during VE. Here, we review concepts of microglial

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functions during VE, including insights from recent studies gained through novel methodologies in *in vivo* as well as *ex vivo* settings.

Methods of microglia investigation and profiling

Microglial biology and functions have been studied for many decades, which brought up essential understanding of this unique brain-resident immune cell type (reviewed in [14]). Notable methodologies in the study of microglia include immunohistochemical visualisation and flow cytometric analysis, as well as imaging of microglia both *in vitro* and *in vivo*. Recent progress in microglia classification using the aforementioned methods allowed an improved understanding of microglial morphology (e.g., by quantitative analysis of ramification and cell shape), activation status (e.g., by analysis of cell surface marker expression profiles and cell motility), and functions (e.g., by cell–cell interaction analysis by *in vivo* imaging). However, differentiating microglia from other myeloid cell subsets within the CNS continues to be challenging, underscoring the need for further methodology refinement.

Substantial efforts have been undertaken to generate transgenic mouse lines showing microglia-selective Cre expression in order to target genes of interest selectively in this cell type. The most robust and frequently used models are conventional *Cx$_3$cr1*-Cre and tamoxifen-inducible *Cx$_3$cr1*-Cre^{ERT2} mice, both expressing Cre under the promoter of the Cx$_3$ chemokine receptor 1 gene (*Cx$_3$cr1*) [15]. Even though microglia show more abundant CX$_3$CR1 expression than other myeloid cells, the expression of this receptor is not strictly limited to microglia, highlighting an important limitation of these models [16–19]. This limitation can be overcome by using *Cx$_3$cr1*-Cre^{ERT2} mice 8 weeks after tamoxifen treatment, when bone marrow-derived myeloid cells have been renewed by bone marrow-derived precursors, whereas microglia did not change, because they are not bone marrow derived and renew locally at extremely slow rates (for the most part) [20]. Another mouse line was generated based on inducible Cre expression under the control of the *Sall1* promoter, which was described as being microglia specific [21]. However, especially under varying conditions, the specificity and stability of *Sall1* expression in microglia has not yet been fully validated. More recently, two additional transgenic mouse lines based on microglia-specific genes have been generated. In one of these lines, *P2ry12*-Cre^{ER} mice, an inducible Cre was inserted into the gene of the purinergic receptor P2Y12 (*P2ry12*), which has been reported to be expressed during the entire lifespan of microglia [22]. The other transgenic line is based on the hexosaminidase subunit beta (*Hexb*) gene, which is specifically expressed by microglia. In brief, in these mice, the *Hexb* locus is modified in such a manner that (i) the open reading frame of *Hexb* is replaced by a cassette consisting of a T2A peptide that has self-cleaving activity and that is combined with the tdTomato reporter (T2A-^{tdTomato}) (*Hexb*^{tdTomato}) or (ii) the T2A-Cre^{ERT2} cassette is introduced into the *Hexb* locus directly before the *Hexb* stop codon (Hexb^{CreERT2}) [18]. In the first case, the Cre-expressing allele is associated with a *Hexb* knockout, whereas in the second case, translation of the resulting polycistronic RNA results in a fusion protein that, due to the self-cleaving activity of the T2A peptide, gives rise to HEXB and the Cre^{ERT} protein. Despite the potential of these two mouse lines to address microglia's roles in a wide range of scenarios, their utility in the context of infectious and noninfectious encephalitis models remains to be proven. Thus, depending on the scientific question at hand, each of the various aforementioned transgenic mouse lines can be useful to study certain aspects of microglia's phenotype and function during development, health, and disease.

In recent years, single-cell RNA sequencing (scRNA-seq) provided deeper insight into the transcriptomic profiles of microglia [11,12,18,23]. One of the main conclusions from these studies is that, under homeostatic conditions, microglia consist of at least two different subpopulations

[23–27]. In contrast, during sterile and infectious neuroinflammation as well as during aging, microglia show a high extent of plasticity and heterogeneity. Depending on the context in which microglia are activated, they may show major changes in their transcriptomic profiles, and even their core signature genes, which until recently were considered to be stably expressed under all conditions, can be downmodulated. Earlier studies proposed that M1 and M2 microglia polarisation is of key relevance for the control of VE (Box 1). However, the concept of M1/M2 polarisation has been challenged [28–30], and inspection of transcriptional profiles of microglia during viral infection generally does not support the concept of M1/M2 polarisation [13,31,32]. Clarifying how precisely microglia respond to viral CNS infection, including in terms of transcriptional profiles, will require further investigations.

Another strategy to perform microglia-specific gene profiling is the RiboTag approach. This strategy relies on Cre recombinase-induced expression of a haemagglutinin tag in conjunction with the major ribosomal subunit (*Rpl22* gene) [33]. Crossing this mouse line with a microglia-specific Cre line (e.g., *Cx$_3$cr1*-Cre^{ERT}) allows pull-down of microglia-derived ribosomes from whole-brain lysates. Following isolation of mRNA attached to tagged ribosomes, the microglia-associated translatome can be studied [34]. By using this approach, cell isolation techniques that might affect gene expression profiles of the cells of interest can be avoided. Considering the aforementioned methodologies that enhanced microglia understanding under various conditions, this review focuses on new developments in elucidating the roles of microglia under homeostatic conditions as well as in sterile inflammation and VE, with a special emphasis on new methodologies (e.g., transcriptomics). Notably, only few studies using such methodologies to investigate microglia upon viral CNS infection are currently available.

Microglia under homeostatic conditions

Microglia are the most abundant resident phagocytic cell type within the brain and represent approximately 10% of all cells in the CNS [4,5]. During homeostatic conditions in the healthy CNS, microglia constantly scavenge the tissue and support neuronal functions, thus maintaining neuronal networks and connectivity [35,36]. During normal brain function, the main roles of microglia are removal of cell debris, synaptic engulfment and pruning, myelin homeostasis, and tissue surveillance. Morphologically, homeostatic microglia are typically small, rod-shaped cells with numerous thin and highly ramified processes [37]. Despite their homeostatic status, these microglial cells are not resting. On the contrary, they are continuously scanning and monitoring the proximal tissue to get alerted in case of detection of any irregularity. Notably, microglia express several core genes, which are specifically but not exclusively expressed in this cell type, such as *Hexb*, *Cx3cr1*, *Csf1r*, *P2ry12*, *Tmem119*, *Gpr34*, *Tgfbr1*, *Fcrls*, *Siglech*, *Slc2a5*, and *Sall1* [11,18]. scRNA-seq studies revealed that homeostatic microglia are rather heterogeneous

Box 1. M1 and M2 polarisation of microglia during VE?

Early studies proposed the polarisation of microglia into proinflammatory M1 microglia, which are CD14-, CD16-, CD32-, CD40-, CD86-, MHC-II-, translocator protein-, and inducible nitric oxide synthase-positive, and anti-inflammatory M2 microglia expressing CD163, CD206, Arg1, and other markers [108]. In the context of VE, it has been shown, for instance, that upon WNV infection of the CNS, microglia are activated and display upregulation of certain M1-like markers, such as Iba-1, as well as chemokines such as CCL2, CCL3, CCL5, and CCL7 [109]. In contrast, treatment with the anti-inflammatory drug minocycline reduced the expression of M1-like markers and increased the expression of M2-like markers. Such M2-like microglia were associated with neuroprotective effects in WNV infection of the CNS [110], suggesting that M1-like microglia are needed for the initial control of WNV infection, whereas M2-like microglia prevent excessive tissue damage during CNS inflammation. However, in recent years, the relevance of the M1/M2 microglial polarisation paradigm has been challenged [28], and in particular, transcriptomic analyses of microglia did not indicate a clear separation into M1- and M2-like microglia (see also main text).

and consist of several subsets. Initial studies proposed that homeostatic microglia comprise at least two subsets that are located in different brain areas and that presumably have overall similar functions [12,18,19,38]. Following activation, the morphology of microglia changes significantly (i.e., they show a swollen and larger cell body with thicker ramifications). Major effort has been invested in the characterisation of microglia by means of morphology, including analysis of process motility and ramifications under different conditions [29], which allows determining microglia status (i.e., homeostasis versus activation) [39]. Similar to the morphological changes, also expression profiles of core signature genes change upon microglial activation, highlighting again the challenges associated with targeting and tracing these cells under experimental conditions [18].

VE is a complex syndrome

Viral infections of the brain, which mostly are associated with the induction of inflammatory processes, are defined as VE and are the most common cause of encephalitis [40,41]. Many viruses that can enter the brain exhibit neurotropic characteristics, indicating that they predominantly infect neurons and other cells from the CNS [42]. Under certain conditions, also opportunistic viral infections can cause VE (e.g., in immunosuppressed patients). Mostly, VE is undiagnosed due to the unspecific, mild, flulike symptoms that patients develop (see also Box 2). Normally, VE is self-limiting [43–45], but still, severe and even fatal cases of encephalitis can occur after viral infection of the CNS. In some cases, patients affected by viral infection of the CNS can develop long-term neurological sequelae, such as epilepsy and cognitive dysfunctions, that often manifest years after the initial insult. In-depth understanding of the association between CNS infection and long-term sequelae is crucial for the development of new intervention strategies, and, accordingly, many ongoing preclinical studies are focussing on studying viral CNS infections and their effects on neural function.

A variety of neurotropic and non-neurotropic viruses can enter the CNS and cause encephalitis (Box 2) [40,42]. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a virus initially thought to cause primarily respiratory symptoms in infected patients, is now recognised as a pathogen affecting multiple organ systems, including the nervous system. The extent of SARS-CoV-2's ability to enter the CNS continues to be a matter of debate, but at least in some cases, the virus has been reported to cause acute encephalitis as well as long-term cognitive impairments [46,47]. So far, it remains unclear whether such long-term deficits, which more generally are also referred to as 'long COVID syndrome', are caused by direct infectious pathology, including in the CNS, or indirectly, via immune reactions in the periphery.

Viruses can enter the brain via different routes of infection, such as the cerebrospinal fluid, the bloodstream, or neuronal retrograde transport. Upon entering the patient's body, viruses are

Box 2. Clinical outcomes of VE

Among viruses that cause encephalitis, the most common known ones are herpes simplex virus (HSV) and other viruses of the *Herpesviridae* family, JEV, rabies virus, HIV, and measles virus [40,42]. More recently, other emerging viruses that predominantly are vector-borne diseases mostly transmitted by insects, such as tick-borne encephalitis virus, DENV, Chikungunya virus, and WNV, have been reported more frequently as the causative agent in patients with VE. Symptoms in patients range from headache and mild flulike symptoms to confusion, amnesia, personality changes, seizures, paralysis, coma, and even death [40]. CNS infection with HSV causes the most serious cases of VE with a mortality of approximately 70% in untreated patients [40,44]. VE is often self-limiting and even undiagnosed [41]. Histopathological hallmarks of VE in CNS tissue are leucocyte infiltration, astrogliosis, microglial activation, and nodule formation, as well as neuronal loss [59,119]. Notably, 20–30% of patients who survive VE experience long-term sequelae, such as neurological difficulties, chronic recurrent seizures (epilepsy), and movement disorders [120,121]. The precise mechanisms linking VE with neurological dysfunctions are not fully understood yet and require further investigations.

sensed by pattern recognition receptors that trigger downstream signalling cascades, which eventually induce antiviral responses [6,48–50]. Depending on which pathogen causes viral CNS infection, robust local immune responses can be triggered. Proinflammatory cytokines such as interferons (IFNs) are produced early on and orchestrate the immune response. At later time points, recruitment of peripheral leucocytes is initiated, which is essential for host protection [51]. Of note, a fine-tuned balance in the immune system between pro- and anti-inflammatory processes is crucial to successfully control viral infections not only in the periphery [52] but also in the CNS [53].

Microglia in VE

As part of the innate immune response, microglia build the first line of defence against viral invasion within the CNS [6,48]. Upon CNS viral invasion, microglia are heavily activated, and they clonally expand and migrate to the site of infection. Their morphological state changes from a homeostatic rod-shaped phenotype to an enlarged cell body with retracted cell processes and finally to a more amoeboid shape [29]. Their transcriptomic profiles drastically change by upregulation of several cytokine- and chemokine-associated genes and pathways that are involved in antiviral defence and immune response induction (Figure 1). To follow up on currently ongoing debates on microglial functions during homeostasis and VE, in this review, we aim at reconciling the available information and to propose a unified model of the many functions that microglia have during VE.

Microglia confer protection against viral infection of the CNS

Microglia have been shown to critically contribute to survival during the acute phase of VE. In several mouse models of CNS viral infection, including Theiler's murine encephalomyelitis virus, vesicular stomatitis virus (VSV), West Nile virus (WNV), Japanese encephalitis virus (JEV), Dengue virus (DENV), pseudorabies virus, and mouse hepatitis virus, the crucial role of microglia has been demonstrated by pharmaceutical depletion of microglia [54–60]. Upon microglial depletion, the mortality rate upon viral infection increased in all the aforementioned CNS infection models, reaching between 50% and 100% lethality, depending on the studied model and the time point of microglial depletion. Furthermore, in these studies, viral loads within the CNS were significantly enhanced under conditions of microglial depletion, highlighting a protective effect of microglia during acute VE. In contrast, depletion of microglia was shown to potentially reduce neuronal loss and neuroinflammation in noninfectious models of CNS diseases and especially in Alzheimer's disease [61–64]. In a mouse model of temporal brain injury, where inflammation of the brain tissue occurs after the insult, microglial depletion did not alter spatial learning or memory abilities. Nonetheless, in a mouse model of ischaemic stroke, where sterile tissue inflammation is observed, microglial depletion exacerbated brain injury after cerebral ischaemia and facilitated excitotoxicity [65]. Thus, direct comparison of models of infectious and aseptic CNS inflammation highlights major differences in the roles of microglia during these conditions (Figure 1). In one of the infection studies, a mouse model of VE-induced acute seizures and epilepsy was used, and the impact of microglial depletion on seizure development was investigated in depth [57]. Under conditions of microglial depletion, the virus-infected brain showed accelerated development of seizures as well as enhanced degeneration of hippocampal neurons, which in patients is a hallmark of temporal lobe epilepsy [66]. Another study that deployed a mouse model of VSV infection via the olfactory route showed that microglia react to locally produced antiviral type I IFNs (IFN-I) and are able to form a physiological immune barrier upon infection, which prevents the virus from disseminating to other brain regions, again highlighting the protective role of microglia under such conditions [58,67,68]. The IFN-I signalling pathway has been described as the most upregulated one in microglia upon viral infection, indicating the relevance of IFN-α and IFN-β for antiviral immunity in the brain [55,58]. Interestingly, upon microglia-selective deletion

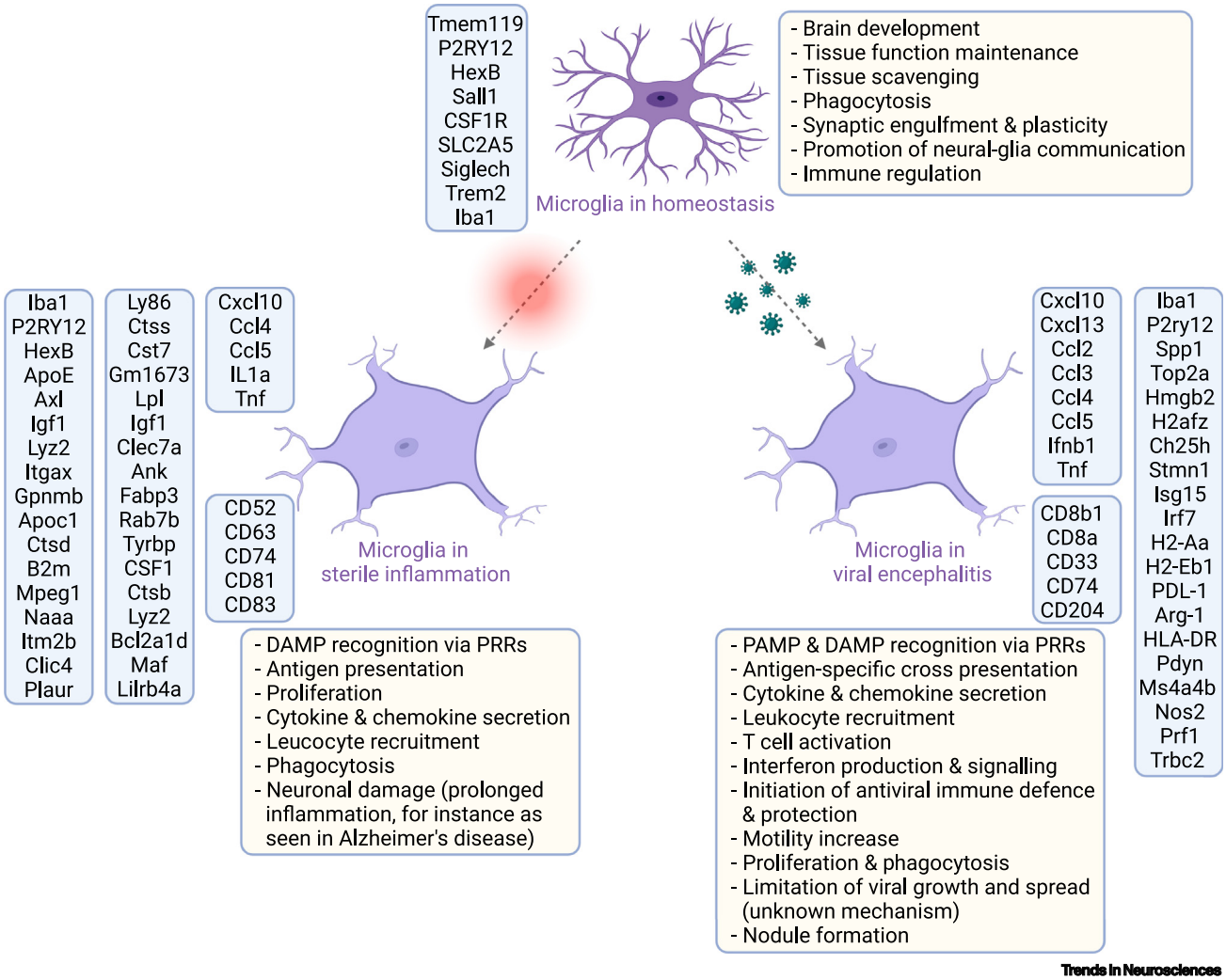

Figure 1. Microglial characteristics under homeostatic conditions and during sterile inflammation and viral encephalitis. Microglia are extremely plastic and dynamic. They rapidly adapt to changes in their local environment. Under homeostatic conditions, they fulfil miscellaneous tasks to ensure CNS tissue integrity and function. Under conditions of sterile inflammation, they get highly activated and initiate proinflammatory responses as a first line of defence against endogenous noxious agents. Microglia get activated also upon viral CNS infection. However, their transcriptomic profiles and antiviral defence mechanisms differ from those of microglia during sterile inflammation. The blue boxes depict a variety of microglial signature genes under homeostatic conditions and sterile and infectious inflammation. Understanding these differences will help to develop a more nuanced concept about the specific roles of microglia during viral encephalitis. Figure created with BioRender.com. Abbreviations: CNS, central nervous system; DAMP, damage-associated molecular pattern; PAMP, pathogen-associated molecular pattern; PRR, pattern recognition receptor.

of the type I IFN receptor (IFNAR), normal microglial activation and protection was detected, indicating that microglial function is independent of IFNAR signalling [58]. Notably, microglia proceed with phagocytosis during acute VE [3,5]. They engulf and eliminate dysfunctional and dying neurons at the site of infection or injury [5,16,35,69]. These processes are important for preventing neuronal hyperexcitability and tissue damage [3]. Moreover, in parallel with the functions summarised previously, microglia secrete cytokines, which leads to further activation of the immune system and recruitment of other immune cells, especially T cells, which are essential for host protection in VE (see following text). This, however, can also lead to exacerbated and unbalanced immunopathology and further brain damage, especially in the long-term perspective [70], as discussed in the following section.

Microglia induce detrimental effects during VE

While the previous section highlighted many antiviral functions of microglia during VE, there are also data implying potentially detrimental roles of microglia under such conditions. This double-edged sword of microglial function has also been described in various models of brain injury and neurodegenerative diseases [71–73], although in the context of infectious insults to the brain, the literature is more limited. During the acute phase of VE, the early immune reaction is characterised by the production and secretion of pro- and anti-inflammatory cytokines [48]. Within the brain, microglia are the main producers of the proinflammatory cytokines tumour necrosis factor α (TNF-α), interleukin 6 (IL-6), and IL-1, as well as complement factor 3 (C3) [7,74,75]. In JEV infection, TNF-α signalling in microglia can lead to glutamate-release induced neuronal death [76]. In addition to TNF-α, IL-6, and IL-1, microglia can also produce IFN-β, type II IFN (IFN-γ), C-C motif ligand 2 (CCL2), and CCL5 that can induce direct neurotoxic effects [77–80]. Interestingly, some of these factors, including TNF-α, IL-1, IL-6, and C3, have also been discussed as key triggers of acute seizure induction during VE, and it has been noted that an excessive production of these factors can lead to neuronal dysfunction and hyperexcitability [81,82]. Another study investigating the role of microglia in virus-induced development of acute seizures found reduced neurodegeneration during CNS infection in *Cx$_3$cr1*-knockout mice, in which microglial activation is prevented [83]. Notably, in these studies, there was no effect of *Cx$_3$cr1* deletion on seizure development, once again highlighting the complexity of microglial functions during neurodegenerative diseases and VE. In a model of WNV infection, microglia have been shown to facilitate viral entry into the CNS. Deletion of the expression of matrix metalloproteinases (MMPs), more specifically MMP9, that are produced by microglia led to decreased viral loads within the CNS and increased survival of mice [84,85]. Previously, microglia have also been described to have a potential neurotoxic effect during viral infections, often but not exclusively in the context of crosstalk with other cell types in the brain (see following text). Notably, these observations suggest an impact on the outcome of infections and the long-term consequences, rather than on the acute phase of VE. Infection with WNV or Zika virus (ZIKV) can be associated with neurological sequelae and cognitive decline long after recovery from VE [86]. It has been reported that after WNV and ZIKV infections, IFN-γ-activated microglia conferred reduction of post-synaptic termini in parallel with the appearance of cognitive defects and impaired spatial learning. This phenotype was reversed by microglia-selective deletion of the IFN-γ receptor, which illustrated the potentially detrimental effects of microglial activation during VE. The authors concluded that sustained activation of microglia by neurotropic viral infection could lead to long-lasting cognitive deficits despite effective viral clearance. Concordantly, another study showed that in mice that recovered from WNV infection, microglia seem to exhibit a chronically phagocytic phenotype. These animals showed decreased spatial learning and carried microglia with an upregulated complement C1q subcomponent subunit A [70]. Furthermore, mice with a deficiency in complement C3 or the C3 receptor showed reduced WNV-induced synaptic loss, supporting the concept that microglia can promote elimination of synapses during the disease.

In human HIV-1 infection, the role of microglia is diverse. As a target of the virus, microglia form inflammatory nodules within the brain [75]. These release neurotoxins, viral proteins, and cytokines that can eventually lead to neuronal death and cognitive impairment via inhibition of neuronal autophagy, a process that is crucial for maintenance of CNS functions [24,75,87,88]. In the context of SARS-CoV-2 infection, indications for potentially detrimental effects of microglia in hospitalised patients with coronavirus disease 2019 (COVID-19) have recently been reported using postmortem tissue analyses as well as cerebral magnetic resonance imaging and fluorodeoxyglucose positron emission tomography (FDG-PET) in the subacute phase of the infection [89]. FDG-PET scans revealed decreased glucose metabolism and suggested microglial activation, whereas postmortem analyses of brain tissue indicated microglial proliferation [89]. These data

suggested a role of microglia in the CNS of patients with COVID-19, although their exact functions remain elusive. Generally, the question whether the detrimental roles of microglia are solely a consequence of neuroinfection or if microglia can also by themselves exacerbate neuroinflammation during VE needs to be more thoroughly studied.

Microglia crosstalk with astrocytes, neurons, and infiltrating T cells

The aforementioned protective effects involving microglia during the acute phase of VE do not seem to exclusively rely on microglia, but additionally critically involve crosstalk between microglia and other cell types within the CNS (Figure 2).

Astrocytes

Astrocytes contribute to the maintenance of proper neuronal function and neural circuitry, including regulation of neurotransmitter release and synapse activities [90]. Interestingly, somewhat similar to

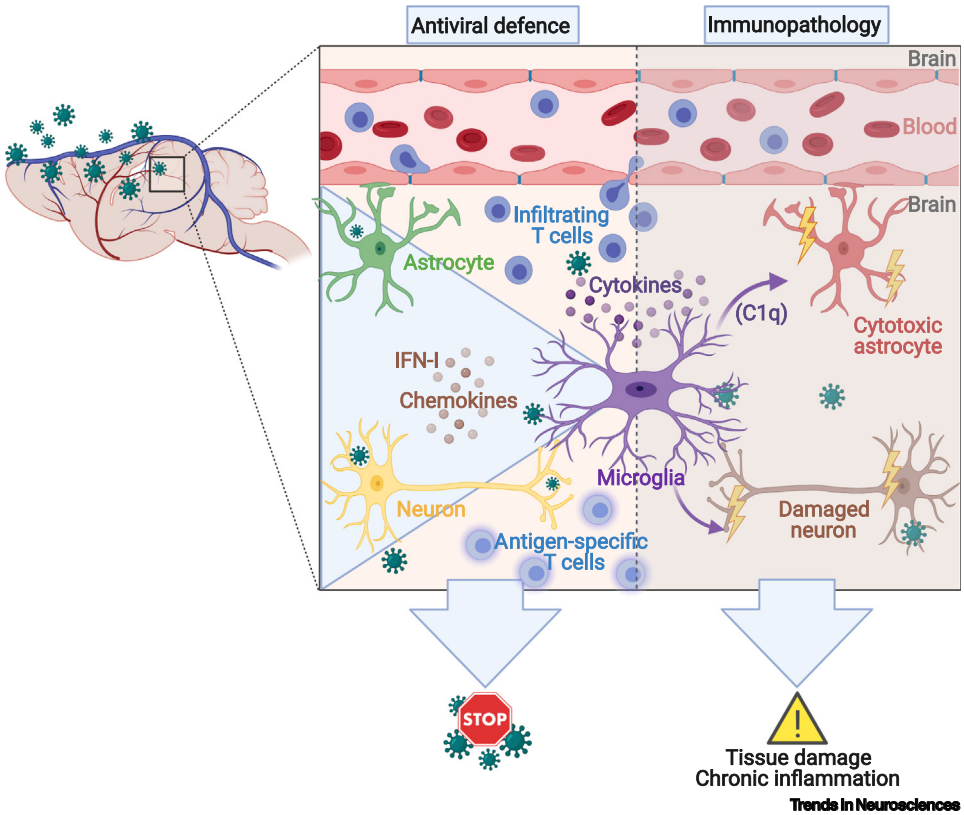

Figure 2. Microglial activation and cellular crosstalk during viral encephalitis (VE). During viral infection of the brain, microglia fulfil miscellaneous tasks. This schematic overview summarises the diverse cell–cell interactions and functions of microglia during VE. Activated microglia phagocytose cell debris and release cytokines, which activate other immune cells (purple dots). Furthermore, they can confer direct antiviral effects by barrier formation and virus trapping. Microglia also closely interact with other brain-resident cell types, as well as with infiltrating cells. Type I interferon (IFN-I) signalling by neurons and astrocytes (brown dots), as well as chemokine production by neurons, leads to microglial activation and antiviral defence. T cells are recruited to the central nervous system (CNS) and interact with microglia, presumably via MHC, which is a key mechanism to maintain T cell function within the CNS. However, microglial activation and cellular crosstalk during VE can also exhibit adverse effects. Upon complement factor secretion by microglia, astrocytes can develop a neurotoxic phenotype and may confer detrimental effects. Microglia can also perform uncontrolled elimination of synapses, thus harming neurons due to overshooting inflammatory reactions. Nevertheless, microglia and the crosstalk with the depicted cells in the CNS play a central role during antiviral defence that is essential to protect the brain from viral infection. Figure created with BioRender.com.

microglia, astrocytes can be beneficial or detrimental, depending on the local context. When activated by trauma, pathogen invasion, or other insults, astrocytes are important for scar formation and restriction of spread of inflammatory responses within the CNS parenchyma [91]. However, astrocytes can also exhibit neurotoxic functions by producing reactive oxygen species or proinflammatory cytokines and molecules [92]. Microglia–astrocyte crosstalk is initiated by secretion of mediators such as neurotransmitters, growth factors, and cytokines [90]. It has been proposed that during inflammation, microglia can sense astrocyte-derived signals such as ATP via the purinergic signalling pathway (e.g., P2RY12), which activates microglia and initiates phagocytosis [93]. A recent study showed that during VE, IFNAR signalling in astrocytes (and also neurons) is needed for full microglial activation and successfully controlling VSV infection [58]. Furthermore, that study revealed that in the presence of proper IFNAR signalling, astrocytes seem to produce so far unidentified factors that contribute to microglial activation. Conversely, there are also studies showing that microglial activation can lead to neurotoxicity of astrocytes via production of complement factor Cq1, IL-6, and TNF-α [94]. Also, during VE in mice, it has been shown that reactive astrocytes can decrease neurogenesis in adults, which can result in cognitive dysfunction [95]. Thus, microglia–astrocyte crosstalk is of key relevance during homeostasis and VE for ensuring tissue integrity and proper neuronal functions as well as for orchestrating antiviral responses.

Neurons

Neurons constitute the functional basis of cognition and behaviour. Neurons' deterioration due to infection or other insults can affect CNS integrity and functionality, including cognitive performance and neurological function, and eventually can also affect host survival. To maintain their functions, neurons heavily communicate with surrounding cells, especially glial cells. Furthermore, neurons often are the main target of neurotropic viruses, and therefore, they need to be efficiently protected from pathogen invasion [74]. In the early defence of viral invasion, neurons critically contribute to chemokine responses. In mouse studies investigating infections with various neurotropic RNA viruses, the most abundantly expressed chemokines are CCL5, CXCL10, and CCL2 [74,96]. These molecules are known to recruit peripheral immune cells, especially T lymphocytes, which hence are in close communication with microglia upon infiltration. Other studies reported that neurons are able to produce IFN-I, which is essential for antiviral defence [97–99]. A study addressing VSV infection showed that neuronal and astrocytic IFNAR signalling regulates microglial activation and barrier formation upon infection, a crucial mechanism for survival after intranasal VSV infection of mice [58]. Upon IFNAR signalling initiation, neurons and astrocytes release factors in an IFNAR-dependent manner that promoteinduction of protective microglial mechanisms against VSV, whereas IFNAR signalling by microglia is not needed for full microglial activation. In a recent study using VSV as a viral infection model [99], it was demonstrated that absence of MyD88 signalling by infected neurons does not have an impact on microglial activation and antiviral mechanisms, whereas this signalling pathway is essential in neurons for recruitment of peripheral immune cells. In conclusion, the full spectrum of neuronal production of chemokines and antiviral molecules is not fully understood yet and still needs to be further investigated. However, crosstalk between cell types, especially microglia and neurons, seems crucial for mounting an antiviral response in the CNS, highlighting in part the contribution of neurons to pathogen defence.

Infiltrating T cells

The aforementioned study of VSV infection [99] showed that the recruitment of T cells into the virus-infected CNS is critically dependent on neuronal chemokine production, including CXCL10 and CCL5. These data support the hypothesis that neuron-derived chemokines regulate T cell recruitment, although also microglia can produce chemokines such as CCL5. Notably,

similar to depletion of microglia, also depletion of $CD4^+$ and/or $CD8^+$ T cells resulted in decreased survival of VSV-infected mice [51,99–101]. Microglial depletion studies showed that the absence of microglia has massive effects on infiltrating cells, especially T cells [55,57,60,100,102]. Under such conditions, T cells are no longer fully functional, and the local responses of $CD4^+$ and $CD8^+$ T cells are massively impaired during acute VE [55,57,102]. This is in part due to the lack of MHC class II (MHC-II)-dependent antigen presentation by microglia, since these cells are the main MHC-II expressing cells within the brain. Concomitant with the changes in the tissue's cytokine milieu, MHC-II is known to be required for restimulation of $CD4^+$ T cells after recruitment to the brain [102–104], whereas fully functional $CD4^+$ T cell responses are needed to promote many $CD8^+$ T cell responses. The decreased functionality of antiviral T cells in the CNS is associated with impaired control of local viral infection, which eventually leads to increased viral loads within the infected CNS that ultimately may cause locally enhanced inflammatory reactions and tissue destruction. Concordantly, a recent study using the VSV model showed that microglia also present cognate MHC-I/peptide complexes to virus-specific $CD8^+$ T cells, supporting cytotoxic T cell-mediated killing of infected cells such as neurons via cross-presentation of internalised and processed extracellular antigens [100]. These findings highlight another dimension of crosstalk between microglia and T cells. It can be concluded that microglia are nonredundant antigen-presenting cells in the virus-infected brain and that this antigen presentation is essential to provide T cell-mediated host protection (Box 3) [2,100,105]. These mechanisms are critical for host protection during VE [100,106], where microglia and T cells are equally relevant to maintain fully functional local antiviral defence. Nonetheless, excessive T cell-mediated killing of neurons can have negative implications for tissue function in the long term. The crosstalk of microglia and T cells, as discussed earlier, emphasises the relevance of microglia not only as effector cells of innate immunity but also for their role in maintaining and adjusting the functions of infiltrating T cells.

Concluding remarks

Many of the processes operating within the CNS upon viral infection are only partially understood. In recent years, significant progress has been made in clarifying immune mechanisms within the CNS. This progress stems in part from methodological advances such as scRNA-seq as well as improved animal models for selective cell-type manipulations. One key conclusion relates to the centrality of interaction between different cell types within the CNS. Although the separate analysis of isolated specific cell types and subsets of the CNS provides important information, these

Box 3. Local T cell restimulation by microglia?

Upon viral infection, viral antigens are transported to draining lymph nodes, where MHC-I- and MHC-II-positive dendritic cells take up the antigens, process them, and present protein fragments in the context of MHC-I on the cell surface to $CD8^+$ T cells and MHC-II to $CD4^+$ T cells [104,111]. Upon priming of naive virus-specific T cells, they proliferate for several days in the lymph nodes and then expand into the bloodstream. From there, virus-specific T cells are recruited into the infected CNS. So far, there is no evidence (to our knowledge) that priming of naive T cells can take place within the CNS [112]. We and others showed earlier that virus-specific but not naive T cells are recruited to the infected CNS by local chemokine production and the expression of the corresponding receptors (e.g., CCR1, CCR2, CCR4, CCR5, CCR6, CXCR3) on antigen-specific T cells [113,114]. Indeed, our group has found that virus-infected neurons mount chemokine responses that are critically needed to recruit leucocytes to the infected CNS [99]. Recently, it was reported that upon microglial depletion, recruited T cells show reduced functionality [55,57,115]. Thus, it is likely that MHC I- and MHC II-positive microglia take up viral antigens and present antigen fragments to infiltrating $CD4^+$ and $CD8^+$ T cells. This way, infiltrating T cells can be restimulated in order to maintain their full functionality. Other studies showed that microglia-specific deletion of $H\text{-}2K^b$ MHC-I molecule reduced $CD8^+$ T cell responses and decreased the activation status of brain-infiltrating $CD8^+$ T cells upon viral challenge [116–118]. Therefore, microglia-dependent restimulation of antigen-specific T cells appears to be a safeguard mechanism to avoid immunopathology mediated by irrelevant antigen-specific T cells that entered the CNS, whereas local restimulation assures maintenance of T cell function only when it is needed (i.e., if virus antigen is present).

Outstanding questions

How are microglia activated and how is their function adjusted under conditions of viral encephalitis?

Is the protective function of microglia during CNS infection a cause or consequence of brain pathology?

Is the detrimental function of microglia during CNS infection a cause or consequence of brain pathology?

How exactly do microglia confer protection during viral CNS infection?

How is microglial function affected by the surrounding tissue?

What exactly are the signals from CNS-resident cells and cells that infiltrate the brain parenchyma that support the protective effect of microglia against viral infection of the CNS?

What are other protective factors and pathways in addition to type I interferon signalling that are needed for the induction of antiviral microglial function?

How exactly is the function of infiltrating T cells adjusted and maintained by microglia during CNS infection?

Can microglia be exploited as a pharmacological target for the treatment of viral encephalitis?

systems convey limited insight about communication between cells within the CNS. In particular in the case of microglia, the cells' activation and function as well as their overall phenotype is significantly affected by other cell types in their local environment, such as neurons, astrocytes, and infiltrating peripheral immune cells [107], as well as by the local cytokine milieu.

Currently, the complex roles of microglia in VE remain incompletely understood (see Outstanding questions). We hypothesise that in the acute phase of VE, microglia are essential for protection and survival, even though they can display direct neurotoxic effects in some scenarios. By contrast, prolonged and chronic microglial activation, as frequently observed during VE, can eventually lead to increased tissue damage and neurological long-term sequelae, as observed also in some aseptic neurodegenerative diseases. Future studies are needed to deepen current understanding of microglia's roles in VE, as well as their interaction with other cell types within the CNS. Ultimately, this understanding could support the development of new intervention strategies to improve the therapy of VE.

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Declaration of interests

The authors declare no competing interests in relation to this work.

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