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New advances in immune components mediating viral control in the CNS

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Protective immune responses in the central nervous system (CNS) must act efficiently but need to be tightly controlled to avoid excessive damage to this vital organ. Under homeostatic conditions, the immune surveillance of the CNS is mediated by innate immune cells together with subsets of memory lymphocytes accumulating over lifetime. Accordingly, a wide range of immune responses can be triggered upon pathogen infection that can be associated with devastating clinical outcomes, and which most frequently are due to neurotropic viruses. Here, we discuss recent advances about our understanding of anti-viral immune responses with special emphasis on mechanisms operating in the various anatomical compartments of the CNS.

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CNS-infiltrating leukocytes, as recently summarized in more detail [1,2]. Depending on whether viruses spread mainly in the brain coverings or parenchyma, such infections either result in meningitis, encephalitis or both [3]. Animal models of CNS infections have fundamentally shaped our understanding of immune responses governed by the nature of the virus, the type of infected cells and the affected region in the CNS.

For many viruses, a robust innate immune response already at CNS barriers (comprising the meninges, perivascular space and ventricular system) prevents further spread into the subjacent parenchyma (Table 2). At these CNS barriers, specialized macrophage populations, including meningeal, perivascular and choroid plexus macrophages, are collectively referred to as CNS-associated macrophages (CAMs) [4]. Early pathogen detection by CAMs and CNS-resident microglia triggers a disease-associated signature and the release of pro-inflammatory cytokines and chemoattractants [5,6]. Thereby, CAMs initiate an inflammatory response by recruiting other immune cells, including T cells, neutrophils and monocytes. In line with this, CAMs depletion during CNS infection reduces cell recruitment, while it does not affect local viral load [7]. By contrast, the control and elimination of most viral CNS infections depend on the adaptive immune system which generates distinct memory lymphocyte subsets (Table 2). The latter surveils the CNS to rapidly detect invading or re-activating pathogens and provides immediate responses toward previously encountered antigens.

This review highlights recent findings on immune components mediating viral control during CNS infections taking into consideration the affected anatomical compartment.

Introduction

In contrast to most other organs, the adult mammalian central nervous system (CNS) has a highly limited regenerative capacity and any damage to its structure may result in permanent neurological impairments. It is therefore hardly a coincidence that nature has implemented functional and anatomical barriers to prevent CNS access for pathogens, including viruses. Yet, despite this containment, a wide range of neurotropic viruses are capable to infect the CNS, especially in immunocompromised hosts (Table 1). Thereby, viruses can exploit various entry routes, including transcellular or paracellular transport across CNS barriers or by Trojan horse infection of

Innate immune responses involved in CNS anti-viral responses

Meninges

The meninges refer to distinct membranes enveloping the CNS parenchyma, namely the dura mater, the arachnoid mater and the pia mater that altogether provide a tiered layer of defense. Within the dura mater, a lymphatic system is connected to the cerebrospinal fluid (CSF) via subarachnoid extensions and drains brain interstitial fluid and macromolecules into deep cervical lymph nodes [8,9]. Tight junctions of arachnoid mater cells physically restrict CNS access for viruses [10].

Table 1**Viruses infecting the central nervous system (CNS) with immune cells implicated in viral control and viral entry routes**

Virus family	Human virus	Mouse model virus	Immune cells implicated	CNS access	References
Arenavirus	Lymphocytic choriomeningitis virus (LCMV)	LCMV	T cells	Transcellular	[1]
Coronavirus	Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)	Mouse hepatitis virus (MHV)	Unclear	Retrograde axonal transport	[2]
Enterovirus	Coxsackie virus (CV)	CV	Unclear	Transcellular	[3]
	Poliovirus	Poliovirus	Unclear	Retrograde axonal transport Paracellular	[4,5]
Filovirus	Ebola virus (EBOV)	EBOV	Unclear	Spread from retinal epithelium Leukocyte infection (monocytes)	[6,7]
Flavivirus	Japanese encephalitis virus (JEV)	JEV	T cells	Transcellular Paracellular Leukocytes infection Spread from nasal cavity	[8–10]
	Tick-born encephalitis virus (TBEV)	TBEV	T cells, B cells, innate	Transcellular Paracellular	[11–13]
	West Nile Virus (WNV)	WNV	CD8+ T cells	Transcellular Paracellular Leukocyte infection (neutrophils) Retrograde axonal transport	[14–16]
	Zika virus (ZIKV)	ZIKV	T cells	Paracellular	[17,18]
Herpesvirus	Cytomegalovirus (CMV)	Mouse cytomegalovirus (MCMV)	T cells	Spread from meninges in IC hosts	[19,20]
	Epstein Barr Virus (EBV)	EBV	Unclear	Leukocyte infection (B cells)	[21]
	Herpes simplex virus (HSV-1/HSV-2)	HSV	T cell	Paracellular Retrograde axonal transport	[5,22]
Orthomyxovirus	Influenza A virus (IAV)	IAV	Unclear	Paracellular Retrograde axonal transport	[23,24]
Paramyxovirus	Measles virus	Measles virus	T cells	Transcellular	[5]
Parvovirus	Adeno-associated virus (AAV)	AAV	Unclear	Leukocyte infection (T cells)	[25]
Picornavirus	Theiler's encephalomyelitis virus (TEV)	Theiler's murine encephalomyelitis virus (TMEV)	Unclear	Retrograde axonal transport	[26]
Polyomavirus	John Cunningham Virus (JCV)	Murine Polyomavirus (MuPyV)	T cells	Leukocyte infection	[27]
Retrovirus	Human immunodeficiency virus (HIV)	HIV	Unclear	Paracellular Leukocytes infection (CD4 cells)	[28,29]
	Simian immunodeficiency virus (SIV)	SIV	Unclear	Leukocyte infection (PVM)	[30]
Rhabdovirus	Rabies virus	Rabies virus	Unclear	Paracellular Retrograde axonal transport	[31,32]
	Vesicular stomatitis virus (VSV)	VSV	Unclear	Retrograde axonal transport	[33]
Togavirus	Chikungunya virus	Chikungunya virus	Unclear	Transcellular	[34]
	Sindbis virus (SINV)	SINV	Unclear	Retrograde axonal transport	[34]

PVM: perivascular macrophages.

IC: immune-compromised.

1. Kim JV, Kang SS, Dustin ML, McGavern DB: Myelomonocytic cell recruitment causes fatal CNS vascular injury during acute viral meningitis. *Nature* 2009, 457:191–195.

2. Meinhardt J, Radke J, Dittmayer C, Franz J, Thomas C, Mothes R, Laue M, Schneider J, Brunink S, Greuel S, et al.: **Olfactory transmucosal SARS-CoV-2 invasion as a port of central nervous system entry in individuals with COVID-19.** *Nat Neurosci* 2020.
3. Bozym RA, Morosky SA, Kim KS, Cherry S, Coyne CB: Release of intracellular calcium stores facilitates coxsackievirus entry into polarized endothelial cells. *PLoS Pathog* 2010, **6**:e1001135.
4. Ohka S, Sakai M, Bohnert S, Igarashi H, Deinhardt K, Schiavo G, Nomoto A: **Receptor-dependent and -independent axonal retrograde transport of poliovirus in motor neurons.** *J Virol* 2009, **83**:4995–5004.
5. Dahm T, Rudolph H, Schwerk C, Schrotten H, Tenenbaum T: **Neuroinvasion and Inflammation in Viral Central Nervous System Infections.** *Mediators Inflamm* 2016, **2016**:8562805.
6. Smith JR, Todd S, Ashander LM, Charitou T, Ma Y, Yeh S, Crozier I, Michael MZ, Appukuttan B, Williams KA, et al.: **Retinal Pigment Epithelial Cells are a Potential Reservoir for Ebola Virus in the Human Eye.** *Transl Vis Sci Technol* 2017, **6**:12.
7. Menicucci AR, Versteeg K, Woolsey C, Mire CE, Geisbert JB, Cross RW, Agans KN, Jankeel A, Geisbert TW, Messaoudi I: Transcriptome Analysis of Circulating Immune Cell Subsets Highlight the Role of Monocytes in Zaire Ebola Virus Makona Pathogenesis. *Front Immunol* 2017, **8**:1372.
8. Hsieh JT, St John AL: Japanese encephalitis virus and its mechanisms of neuroinvasion. *PLoS Pathog* 2020, **16**:e1008260.
9. Yamada M, Nakamura K, Yoshii M, Kaku Y, Narita M: Brain lesions induced by experimental intranasal infection of Japanese encephalitis virus in piglets. *J Comp Pathol* 2009, **141**:156–162.
10. Aleyas AG, Han YW, George JA, Kim B, Kim K, Lee CK, Eo SK: Multifront assault on antigen presentation by Japanese encephalitis virus subverts CD8+ T cell responses. *J Immunol* 2010, **185**:1429–1441.
11. Palus M, Vancova M, Sirmarova J, Elsterova J, Perner J, Ruzek D: Tick-borne encephalitis virus infects human brain microvascular endothelial cells without compromising blood-brain barrier integrity. *Virology* 2017, **507**:110–122.
12. Ruzek D, Salat J, Singh SK, Kopecky J: Breakdown of the blood-brain barrier during tick-borne encephalitis in mice is not dependent on CD8+ T-cells. *PLoS One* 2011, **6**:e20472.
13. Bogovic P, Lusa L, Korva M, Pavletic M, Rus KR, Lotric-Furlan S, Avsic-Zupanc T, Strle K, Strle F: **Inflammatory Immune Responses in the Pathogenesis of Tick-Borne Encephalitis.** *J Clin Med* 2019, **8**.
14. Suen WW, Prow NA, Hall RA, Bielefeldt-Ohmann H: **Mechanism of West Nile virus neuroinvasion: a critical appraisal.** *Viruses* 2014, **6**:2796–2825.
15. Paul AM, Acharya D, Duty L, Thompson EA, Le L, Stokic DS, Leis AA, Bai F: **Osteopontin facilitates West Nile virus neuroinvasion via neutrophil “Trojan horse” transport.** *Sci Rep* 2017, **7**:4722.
16. Samuel MA, Wang H, Siddharthan V, Morrey JD, Diamond MS: Axonal transport mediates West Nile virus entry into the central nervous system and induces acute flaccid paralysis. *Proc Natl Acad Sci U S A* 2007, **104**:17140–17145.
17. Papa MP, Meuren LM, Coelho SVA, Lucas CGO, Mustafa YM, Lemos Matassoli F, Silveira PP, Frost PS, Pezzuto P, Ribeiro MR, et al.: **Zika Virus Infects, Activates, and Crosses Brain Microvascular Endothelial Cells, without Barrier Disruption.** *Front Microbiol* 2017, **8**:2557.
18. Hassert M, Wolf KJ, Schwetke KE, DiPaolo RJ, Brien JD, Pinto AK: **CD4+ T cells mediate protection against Zika associated severe disease in a mouse model of infection.** *PLoS Pathog* 2018, **14**:e1007237.
19. Reuter JD, Gomez DL, Wilson JH, Van Den Pol AN: Systemic immune deficiency necessary for cytomegalovirus invasion of the mature brain. *J Virol* 2004, **78**:1473–1487.
20. Slavuljica I, Kvestak D, Huszthy PC, Kosmac K, Britt WJ, Jonjic S: Immunobiology of congenital cytomegalovirus infection of the central nervous system—the murine cytomegalovirus model. *Cell Mol Immunol* 2015, **12**:180–191.
21. Serafini B, Severa M, Columba-Cabezas S, Rosicarelli B, Veroni C, Chiappetta G, Magliozzi R, Reynolds R, Coccia EM, Aloisi F: **Epstein-Barr virus latent infection and BAFF expression in B cells in the multiple sclerosis brain: implications for viral persistence and intrathecal B-cell activation.** *J Neuropathol Exp Neurol* 2010, **69**:677–693.
22. Miranda-Saksena M, Denes CE, Diefenbach RJ, Cunningham AL: Infection and Transport of Herpes Simplex Virus Type 1 in Neurons: Role of the Cytoskeleton. *Viruses* 2018, **10**.
23. Chaves AJ, Vergara-Alert J, Busquets N, Valle R, Rivas R, Ramis A, Darji A, Majo N: Neuroinvasion of the highly pathogenic influenza virus H7N1 is caused by disruption of the blood brain barrier in an avian model. *PLoS One* 2014, **9**:e115138.
24. van Riel D, Leijten LM, Verdijk RM, GeurtsvanKessel C, van der Vries E, van Rossum AM, Osterhaus AD, Kuiken T: **Evidence for influenza virus CNS invasion along the olfactory route in an immunocompromised infant.** *J Infect Dis* 2014, **210**:419–423.
25. Huser D, Khalid D, Lutter T, Hammer EM, Weger S, Hessler M, Kalus U, Tauchmann Y, Hensel-Wiegel K, Lassner D, et al.: High Prevalence of Infectious Adeno-associated Virus (AAV) in Human Peripheral Blood Mononuclear Cells Indicative of T Lymphocytes as Sites of AAV Persistence. *J Virol* 2017, **91**.
26. Roussarie JP, Ruffie C, Brahic M: The role of myelin in Theiler’s virus persistence in the central nervous system. *PLoS Pathog* 2007, **3**:e23.
27. Major EO, Amemiya K, Tornatore CS, Houff SA, Berger JR: Pathogenesis and molecular biology of progressive multifocal leukoencephalopathy, the JC virus-induced demyelinating disease of the human brain. *Clin Microbiol Rev* 1992, **5**:49–73.
28. Xu R, Feng X, Xie X, Zhang J, Wu D, Xu L: HIV-1 Tat protein increases the permeability of brain endothelial cells by both inhibiting occludin expression and cleaving occludin via matrix metalloproteinase-9. *Brain Res* 2012, **1436**:13–19.
29. Burdo TH, Lackner A, Williams KC: **Monocyte/macrophages and their role in HIV neuropathogenesis.** *Immunol Rev* 2013, **254**:102–113.
30. Williams KC, Corey S, Westmoreland SV, Pauley D, Knight H, deBakker C, Alvarez X, Lackner AA: Perivascular macrophages are the primary cell type productively infected by simian immunodeficiency virus in the brains of macaques: implications for the neuropathogenesis of AIDS. *J Exp Med* 2001, **193**:905–915.
31. Chai Q, She R, Huang Y, Fu ZF: Expression of neuronal CXCL10 induced by rabies virus infection initiates infiltration of inflammatory cells, production of chemokines and cytokines, and enhancement of blood-brain barrier permeability. *J Virol* 2015, **89**:870–876.
32. Klingen Y, Conzelmann KK, Finke S: Double-labeled rabies virus: live tracking of enveloped virus transport. *J Virol* 2008, **82**:237–245.
33. Beier KT, Borghuis BG, El-Danaf RN, Huberman AD, Demb JB, Cepko CL: **Transsynaptic tracing with vesicular stomatitis virus reveals novel retinal circuitry.** *J Neurosci* 2013, **33**:35–51.
34. Passoni G, Langevin C, Palha N, Mounce BC, Briolat V, Affaticati P, De Job E, Joly JS, Vignuzzi M, Saleh MC, et al.: **Imaging of viral neuroinvasion in the zebrafish reveals that Sindbis and chikungunya viruses favor different entry routes.** *Dis Model Mech* 2017, **10**:847–857.

Table 2**CNS location of innate and adaptive immune cell types**

CNS compartment	Innate immune cell types	Adaptive immune cell types
Meninges	Dural M, pial M, cDC1, cDC2, pDC, migDC?, mast cells?	circT cells, ASC
Choroid plexus	CPM, epi-CPM, cDC1, cDC2, migDC?, PVM	circT cells
Perivascular space		ASC
Parenchyma	Microglia, blood-derived macrophages	TRM, TEM?, ASC

M: macrophages.

cDC: conventional dendritic cells.

pDC: plasmacytoid dendritic cells.

migDC: migratory dendritic cells.

CPM: choroid plexus macrophages.

Epi-CPM: macrophages located in the apical surface of the choroid plexus epithelium.

PVM: perivascular macrophages.

circT: circulating T cells.

ASC: antibody-secreting cells.

TRM: tissue-resident memory T cells.

TEM: effector memory T cells.

The meninges harbor dense populations of CD206+ dural and pial macrophages [11] (meningeal macrophages, MMs) that survey their environment and support robust inflammatory responses. MMs are tissue-resident cells that are not replenished by blood monocytes in the healthy CNS [12]. Those long-lived MMs can be infected by viral pathogens such as lymphocytic choriomeningitis virus (LCMV) [13^{••}] and are also potential reservoirs for persistent infections such as human immunodeficiency viruses (HIV) [14]. Furthermore, following infection with attenuated LCMV in mice, MMs are depleted and replaced by inflammatory monocytes that locally differentiate into CD206+ macrophages [13^{••}]. Of note, following engraftment, macrophages retained an altered signature that affected their microbial sensing, highlighting the dynamism of the meningeal niche in the context of infections and supporting the concept of innate memory in this compartment. Multiple subsets of dendritic cells (DCs) are identified in murine meninges, including conventional DCs (cDC1 and cDC2) and plasmacytoid DCs (pDCs) [6^{••}]. Interestingly, a subset with a gene signature resembling migratory DCs was detected in the dural meninges as well. Upon infection, these DCs may access dural lymphatics and reach draining lymph nodes to initiate adaptive immune responses. These recent findings reshaped our understanding how meningeal immune responses are connected to the peripheral immune system and about the immune privilege attribute of the CNS.

Perivascular space

Cerebral vessels possess ramifications penetrating the CNS that form the neurovascular unit (NVU) [2]. This

highly specialized microvasculature is an integral component of what is commonly referred to as the blood brain barrier (BBB), a potent physical barrier that restricts CNS access. Infections with neurotropic viruses are often associated with a disruption of the BBB, either by infection of cell types forming the NVU or by the release of cytokines and/or chemokines from infiltrating activated leukocytes [15]. For instance, in the rabies-virus-infected mouse brain, the pro-inflammatory cytokine interferon-gamma (IFN- γ) has the potential to interfere with the BBB permeability *in vivo* and to disrupt tight junctions *in vitro* [16].

Perivascular macrophages (PVMs), similarly to MMs, are long-lived CD206+ tissue-resident cells that act as first line defense against invading pathogens and regulate cell infiltration within the CNS [17]. During neuroinflammation in mice, PVMs were recently shown to acquire a disease-associated signature, characterized by upregulation of CD74 and CCL5 [5[•]], likely contributing to leukocyte recruitment. Although the main role of PVMs is to prevent viruses from accessing the CNS, they can also serve as a reservoir for latent viral infections such as HIV and can become the immunological target of CD8+ T cells [18]. Additionally, during simian immunodeficiency virus (SIV) infection in macaques, the proliferation of infected CNS PVMs results in macrophages' accumulation in the perivascular space and development of encephalitic lesions [19].

Ventricular system

The CSF is secreted by the choroid plexus (CP) situated in ventricles of the brain. The fenestrated CP capillaries represent an important entry site for pathogens into the CP stroma while epithelial cells lining the CP stroma are interconnected by tight junctions and represent the blood-CSF-barrier (B-CSF-B) [20]. Since pathogens can access the CP stroma, their detection at this site must be rapid in order to avoid as much as possible viral spread in the CSF and in the subventricular zone (SVZ). Stromal CP macrophages (CPMs) have a common origin with other CAMs, while macrophages located in the apical surface of the CP epithelium (epi-CPMs) were recently proposed to be ontogenically related to microglia [6^{••}]. In the same study, epi-CPMs have a very high phagocytic activity *in vitro*, suggesting an efficient sampling of their environment. In the healthy mouse brain, subsets of cDC1 and cDC2 are also described in the CP [6^{••},21]. Recently, single-cell RNA-sequencing (scRNA-seq) analysis of the mouse brain identified DCs with a gene signature evocative of migratory DCs in the CP [6^{••}], although their functional ability to migrate and reach draining lymph nodes has not been evaluated. Altogether, the recent identification of such CP-associated phagocytes revealed the complexity underlying CNS border immunity, which needs to be elaborated in future studies.

Ependymal cells that line the ventricles of the brain represent an additional physical barrier to restrict the spread of viruses within the SVZ. The SVZ, located just below the layer of ependymal cells, contains neural stem cells and progenitor cells that ensure neurogenesis in the adult brain [22–24]. In humans, the CSF is in close vicinity to those neural stem cells and can directly impact their proliferation [25]. Thus, damages to this neurogenic niche and in particular the destruction of ependymal cells during viral infections might expose SVZ cells to CSF and have disastrous consequences for brain homeostasis and repair.

Furthermore, the murine CP is the preferential location of group 1 innate lymphoid cells (ILC1) during steady-state, although also detectable in the meninges and to a lower extend in the brain parenchyma [26]. Their early release of IFN- γ upon neuroinflammation likely stimulates mononuclear phagocytes and initiates a tissue-wide anti-viral state in the infected tissue. Since ILC1 and natural killer (NK) cells express similar levels of NKp46 and NK1.1 [27], they were often confounded and evaluated as a common population. Nowadays, ILC1 and NK cells are discriminated by expression of surface markers CD49a and CD49b, respectively [26]. However, effector mechanisms of ILC1 during CNS viral infection has not been specifically addressed. During autoimmune CNS inflammatory disease in mice, ILC1 are recruited from the CP and the meninges to the brain parenchyma, a phenomenon associated with local upregulation of CXCL9, CXCL10 and CXCL16 in the inflamed brain [26]. Thus, one may speculate that ILC1 serve as migratory cells rapidly producing IFN- γ at infection site.

Olfactory bulb neuroepithelium

The nasal cavity is in close proximity to the olfactory bulb (OB) and represents an entry portal to reach the CNS through retrograde axonal transport via olfactory sensory neurons (OSNs). Respiratory viruses, including influenza A virus, reach the CNS mainly in immunocompromised hosts [28], suggesting that appropriate immune responses are key in preventing virus entry at this border site. A recent study of intra-nasal vesicular stomatitis virus (VSV) infection in mice revealed an important role of microglia in orchestrating neuroprotection from the nasal cavity [29^{••}]. In this setting, microglia acquired viral antigens from infected neurons and presented them to infiltrating virus-specific CD8⁺ T cells, eliciting potent virus elimination.

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is causing the worldwide pandemic, has been detected in the brain of some of the COVID 19 patients [30]. SARS-CoV-2 uses angiotensin-converting enzyme 2 (ACE2) as a cellular receptor for host cell entry [31]. This receptor is expressed at varying levels in the olfactory epithelium [32], suggesting a

possible CNS portal of entry. CNS access of SARS-CoV-2 via the nasal cavity is furthermore supported by the recent identification of SARS-CoV-2 RNA and S protein in the OSNs of the olfactory epithelium of COVID-19 patients that succumbed to infection [33]. Additionally, the expression of ACE2 seems to be expressed in the human brain microvasculature [34], which could explain why a fraction of COVID-19 patients suffer from cerebrovascular complications [35]. Beyond ACE2, neuropilin-1 (NRP-1) is an additional host factor involved in SARS-CoV-2 cell entry, since blocking its interaction with SARS-CoV-2 results in reduced viral infection *in vitro* [36]. Its *in vivo* role as receptor for SARS-CoV-2 remains however to be elucidated.

The emergence of novel viruses targeting the brain via the OB neuroepithelium highlights the necessity to better understand immune reactions occurring at this interface.

CNS parenchyma

NK cells represent 1% of total leukocytes in the healthy mouse brain [37] and are one of the first effector cells at sites of inflammation. In human, NK cells deficiencies are associated with high susceptibility to herpes virus encephalitis [38,39] and are described for several CNS infections [40–45]. NK cells produce IFN- γ in the brain during viral encephalitis [46], stimulating macrophages and microglia and thereby shaping anti-viral responses. In addition, NK cell activation is sufficient to protect mice from fatal neurotropic Sindbis virus infection [47], highlighting NK cells crucial role in such infections.

Microglia are a well-studied parenchymal resident population of innate immune cells ontogenetically distinct from other tissue macrophages [48] and are among the first responders to pathogen invasion [49]. In herpes simplex virus (HSV)-infected mice, microglia are essential for protective antiviral immunity as their depletion is accompanied by higher viral load and an increase of disease severity [50]. During CNS viral infection and the resulting inflammation, the pool of microglia is often enriched by engrafted blood-born monocytes which participate to the host defense in the CNS [51]. Thereby, microglia adopt an activated phenotype partially via interferon alpha/beta receptor (IFNAR) signaling characterized by proliferation, acquisition of an amoeboid morphology and the downregulation of homeostatic markers [52,53]. However, microglia also contribute to immunopathology during viral encephalitis. Of note, complement proteins mediate synaptic pruning by microglia during early stages of CNS development [54,55]. During peripheral arbovirus and flavivirus infections in mice, complement activation is essential to minimize viral spread to the CNS [56,57]. Unfortunately, although complement proteins are increased in the CNS in mouse models of encephalitis [58,59], their local implications in viral

control remain elusive. By contrast, microglia participate to complement-dependent synapse elimination post West Nile virus (WNV) encephalitis in mice [59]. During recovery from WNV and ZIKV infections in mice, T-cell-derived IFN- γ signaling in microglia results in hippocampal synaptic stripping that is associated with cognitive sequelae [60^{*}]. Synaptic loss mediated by microglia and blood-derived macrophage is also observed following neuronal infection by LCMV in mice, where it occurs via neuronal IFN- γ receptor (IFNGR) signaling and downstream STAT1 activation [61,62^{*}]. One may speculate that such a defense mechanism serves to limit retrograde trans-synaptic viral spread at the expense of disconnecting neurons from their functional network. However, neurotropic viruses have developed strategies to overcome interferon signaling by inhibiting or degrading key molecules (e.g. TYK2, STAT2, IFIT, etc.) involved in the downstream signaling cascade [63,64]. In an autopsy of human fetal brain infected with ZIKV, microglia released high levels of pro-inflammatory mediators including IL-6, TNF, IL-1 β , and CCL2 and participated to the loss of neuronal progenitors, thus contributing to microcephaly [65]. Thus, it appears clear that while beneficial in creating an anti-viral state during viral infection, IFN- γ signaling can also have detrimental effect in the CNS by interfering with existing synaptic network.

Adaptive immune cells involved in CNS anti-viral responses

T cells

Following CNS infections, both CD4⁺ T helper type 1 (T_H1) cells and CD8⁺ T cells infiltrate the infected area and contribute to the anti-viral response. It was recently described in an *in vitro* study that human T_H1 cells from patients with a neuroinflammatory disease have a better ability to cross the BBB than the B-CSF-B [66]. T_H1 cells mainly produce inflammatory cytokines to activate other immune cells, while CD8⁺ T cells exert in addition cytotoxic functions to directly eliminate infected cells. However, recent studies suggest that this classical view may not reflect the full spectrum of CD4⁺ T cell functions and propose instead that these cells could also exert cytotoxic function in the CNS [67^{*},68,69], but the formal demonstration thereof is still awaiting further investigations.

T cells use different mechanisms to fight viral infections in the CNS. Among them, T cells release IFN- γ , a critical cytokine for innate and adaptive responses. Functions of IFN- γ include the induction of a tissue-wide anti-viral state [70,71] and the local activation of immune cells, such as microglia and macrophages [72]. Following mouse hepatitis virus (MHV) infection in mice, T cells in the brain not only promote inflammatory reactions but also impede them by the production of anti-inflammatory cytokine such as IL-10 to avoid excessive tissue injury

[73]. In this regard, antibody-mediated blockade of the IL-10R signaling in mice exacerbates brain tissue damage and enhances immune cell infiltration in the brain following Theiler's murine encephalomyelitis virus (TMEV) infection [74].

The cytotoxic capability of CD8⁺ T cells mainly relies on the release of perforin-containing and granzyme-containing granules, inducing apoptosis-mediated cell death via several distinct mechanisms [75–77]. Degranulation-mediated cytotoxicity involves the recognition of the cognate antigen on MHC-I-expressing cells [78] and a single CD8⁺ T cells can kill numerous infected cells [79]. Depending on the virus and cell-type infected in the CNS, the prevailing immune mechanism responsible for viral clearance can vary. For instance, while CD8⁺ T cells eliminate LCMV and MHV from microglia and astrocytes through cytotoxic mechanisms, non-cytolytic viral clearance has been observed in infected ependymal cells and oligodendrocytes after LCMV and MHV, respectively [80,81]. Furthermore, granzyme-B-expressing CD8⁺ T cells were found to cluster around infected neurons without inducing cell death during reactivation of HSV-1 [82,83], whereas, in other virus infection contexts destruction of infected neurons were more extensive [84,85]. Of note, neurons can adopt an antiviral metabolic state to restrict ZIKV replication in mice [86^{**}], reflecting CNS inherent strategies to resist virus infection. A role for T cell-derived IFN- γ has been proposed to mediate non-cytolytic viral clearance [70]. Altogether, these observations suggest that immune responses are tailored to minimize irreversible cell loss in the fight against CNS infection. Yet, factors favorizing either cytotoxic or non-cytolytic virus clearance in the CNS remain incompletely understood. In this regard, neuronal and glial cells participate in the maintenance of tissue integrity by expressing programmed death-ligand 1 (PD-L1) [70] and/or Fas-L [87–89], which interfere with T cell activation and T cell survival, respectively. Hence, T cells and CNS resident cells both contribute to fine-tuning anti-viral immune responses that either resolve acute infections or control persistent infections, with the subsequent generation of T cell memory subsets.

Subsets of circulating and non-circulating memory T cells surveil distinct anatomical niches in the CNS during steady state (Table 2). In contrast, naive T cells hardly patrol the CNS under homeostatic condition. Circulating memory T cells predominantly patrol the CP and the meninges [90–93], while non-circulating tissue-resident memory T (T_{RM}) cells inspect the brain parenchyma [94]. As in mice, T cells populating the human brain with a T_{RM} phenotype have been identified [95^{**}]. Although brain T_{RM} (bT_{RM}) were originally thought to reflect traces of past neurotropic viral infections, they are also described during persistent CNS infection in mice [96] and it was recently shown that peripheral infections can

also generate bT_{RM} [97^{••}]. Of note, in mice, during persistent polyomavirus infection of the CNS, bT_{RM} differentiation requires local virus-specific CD4-derived IL-21 [98], while this prerequisite for CD4-help is not needed following acute LCMV infection [99]. Interestingly, bT_{RM} cells are not disseminated through the brain parenchyma but are preferentially localized in vicinity to surface-associated structures, such as perivascular and periventricular regions [99]. This localization at pathogen entry sites seems preferred by bT_{RM} to immediately detect viral reinfection [94,99] or reactivation [96,100]. Of note, bT_{RM} are sufficient to protect mice from lethal LCMV re-infection, using a combination of perforin and IFN- γ mechanisms [99]. Similarly, bT_{RM} can control murine cytomegalovirus (MCMV) reactivation in neonatally infected mice [100]. Since bT_{RM} express high-affinity T cell receptors (TCRs) [96], they might be able to be reactivated by low levels of viral antigens. In contrast to T_{RM} from other organs, bT_{RM} express high levels of the inhibitory receptor programmed cell death protein 1 (PD-1) [101]. Lung T_{RM} are rapidly reactivated by non-professional antigen-presenting cells during infection [102]. Thus, maintenance of PD-1 expression on bT_{RM} could constitute a safety mechanism by which reactivation of these highly cytotoxic and potential harmful cells may be prevented in the CNS. Additionally, CD8⁺ T cells with a T_{RM} phenotype were shown to mediate persistent neurological deficit following WNV and ZIKV infections by continuously producing IFN- γ and thereby promoting microglia-mediated synaptic stripping [60[•]]. Thus, while playing an important protective role against recurrent CNS infections, bT_{RM} appear to represent a double-edged sword which might be involved in chronic neuroinflammatory diseases, including CNS autoimmunity [103[•]]. However, bT_{RM} can also be found in the CNS of healthy donors [95^{••}], and thus their implications for such chronic neuroinflammatory conditions require further investigations.

B cells (Abs)

Viral infections of the CNS trigger virus-specific antibodies as can be detected in the blood serum but also in the CSF of infected hosts. With regard to the latter, B cell accumulation has been described in the CNS during the acute phase and chronic stages of CNS infections in which these cells are retained as antibody-secreting cells (ASCs) and memory B cells (Bmem) [104–106] (Table 2). Those ASCs exert local protective role after numerous neurotropic infections, including Sindbis virus, Semliki forest virus, WNV, rabies virus, coronavirus and MCMV infections [104,107,108], although precise mechanisms conferring protection remain unclear. Of note, those studies were performed on entire brain tissue and the exact localization of ASCs was not assessed. Recently, ASCs subsets were described in the meninges, the perivascular space and in the brain parenchyma [109] and they seemed to be maintained by homeostatic

proliferation, independently of follicle formation [109]. Since

T-cell dependent priming of virus-specific B cells is crucial for their differentiation [110], B cell priming likely occurs in secondary lymphoid organs and only after ASCs subsets enter the CNS. Since pDCs are well known to be involved in the generation of plasma cells [111], one may speculate that pDCs localized in the meninges could also be involved in local plasma cells differentiation, although this requires further investigation.

Production of neutralizing antibodies has been suggested to be involved in controlling persistent CNS viral infections at latent [110,112] and chronic stages [113] but little is known concerning their precise contribution. For instance, in MCMV chronic infection, B cell numbers increase throughout the latent phase of infection and control the spread of reactivated virus [114]. In MHV-A59 strain infection, a persistent gliatropic virus causing a chronic demyelinating disease, persistence of IgM-producing B cells in the brain is crucial for long-term viral protection [110]. Thus, the role of antibodies in the CNS following infections is probably to reduce viral spread rather than to contribute to local elimination of infected cells.

A more precise understanding of B cell behavior during CNS infections is therefore necessary to better understand how these cells could be involved in anti-viral immune responses of the CNS.

Conclusion

The last years have provided interesting new insights into regulation and processes of immune responses during viral CNS infections. It became apparent that the virus type and infected cells in the CNS strongly influence the resulting immune reactions with regard to their quality and quantity. However, our understanding about the individual cellular and molecular players contributing to protective immunity remains still incomplete. Thus, recent descriptions of ontogenically different innate immune cells but also resident T cells in different compartments of the CNS open up new possibilities to better dissect how the immune system copes with different viral CNS infections. In addition, high antiviral antibody titers and the recent description of ASCs persisting in the CNS during viral infections indicate local functions of B-cell subsets and antibodies for antiviral immune responses in the CNS. In this regard, we believe it is important to further investigate how such cellular and humoral immune responses may be involved in neurological short-term and long-term sequelae often observed after viral CNS infections, and how such potentially compartmentalized inflammatory responses, some of which are clinically silent, might represent a fertile ground for other neurological chronic diseases such as multiple sclerosis or schizophrenia [103[•],115,116].

Altogether, a better understanding of how anti-viral immune responses are tailored to rapidly detect viral invasion and strive for viral control while minimizing collateral tissue damage of the CNS may guide future protective and therapeutic interventions.

Conflict of interest statement

Nothing declared.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Cain MD, Salimi H, Diamond MS, Klein RS: **Mechanisms of pathogen invasion into the central nervous system.** *Neuron* 2019, **103**:771-783.
2. Forrester JV, McMenamin PG, Dando SJ: **CNS infection and immune privilege.** *Nat Rev Neurosci* 2018, **19**:655-671.
3. Dahm T, Rudolph H, Schwerk C, Schrotten H, Tenenbaum T: **Neuroinvasion and inflammation in viral central nervous system infections.** *Mediators Inflamm* 2016, **2016**:8562805.
4. Kierdorf K, Masuda T, Jordao MJC, Prinz M: **Macrophages at CNS interfaces: ontogeny and function in health and disease.** *Nat Rev Neurosci* 2019, **20**:547-562.
5. Jordao MJC, Sankowski R, Brendecke SM, Sagar, Locatelli G, • Tai YH, Tay TL, Schramm E, Armbruster S, Hagemeyer N *et al.*: **Single-cell profiling identifies myeloid cell subsets with distinct fates during neuroinflammation.** *Science* 2019, **363**
- This paper uses scRNAseq to describe CAM heterogeneity from different CNS compartments during homeostasis and neuroinflammation, their transcriptomic profiles and their dynamics.
6. Van Hove H, Martens L, Scheyltjens I, De Vlaminc K, Pombo • Antunes AR, De Prijck S, Vandamme N, De Schepper S, Van Isterdael G, Scott CL *et al.*: **A single-cell atlas of mouse brain macrophages reveals unique transcriptional identities shaped by ontogeny and tissue environment.** *Nat Neurosci* 2019, **22**:1021-1035
- This paper identifies for the first time a microglial subset residing on the apical surface of the CP epithelium by scRNAseq and high-dimension fluorescence cytometry.
7. Steel CD, Kim WK, Sanford LD, Wellman LL, Burnett S, Van Rooijen N, Ciavarrá RP: **Distinct macrophage subpopulations regulate viral encephalitis but not viral clearance in the CNS.** *J Neuroimmunol* 2010, **226**:81-92.
8. Louveau A, Herz J, Alme MN, Salvador AF, Dong MQ, Viar KE, • Herod SG, Knopp J, Setliff JC, Lupi AL *et al.*: **CNS lymphatic drainage and neuroinflammation are regulated by meningeal lymphatic vasculature.** *Nat Neurosci* 2018, **21**:1380-1391
- This paper identifies subarachnoid extensions of meningeal lymphatics that drain the CSF, unravelling a connection between CSF content and the periphery. Using *in vivo* cell tracing, authors identify drainage of naïve T cells from the CSF primarily in deep cervical lymph nodes via meningeal lymphatics.
9. Louveau A, Smirnov I, Keyes TJ, Eccles JD, Rouhani SJ, Peske JD, Derecki NC, Castle D, Mandell JW, Lee KS *et al.*: **Structural and functional features of central nervous system lymphatic vessels.** *Nature* 2015, **523**:337-341.
10. Rua R, McGavern DB: **Advances in meningeal immunity.** *Trends Mol Med* 2018, **24**:542-559.
11. Nayak D, Zinselmeyer BH, Corps KN, McGavern DB: **In vivo dynamics of innate immune sentinels in the CNS.** *Intravital* 2012, **1**:95-106.
12. Goldmann T, Wieghofer P, Jordao MJ, Prutek F, Hagemeyer N, Frenzel K, Amann L, Staszewski O, Kierdorf K, Krueger M *et al.*: **Origin, fate and dynamics of macrophages at central nervous system interfaces.** *Nat Immunol* 2016, **17**:797-805.
13. Rua R, Lee JY, Silva AB, Swafford IS, Maric D, Johnson KR, •• McGavern DB: **Infection drives meningeal engraftment by inflammatory monocytes that impairs CNS immunity.** *Nat Immunol* 2019, **20**:407-419
- This paper reveals the destruction of resident meningeal macrophages by virus-specific cytotoxic CD8+ T cells following acute LCMV infection and the unexpected subsequent long-term meningeal engraftment of inflammatory monocytes.
14. Lamers SL, Gray RR, Salemi M, Huysentruyt LC, McGrath MS: **HIV-1 phylogenetic analysis shows HIV-1 transits through the meninges to brain and peripheral tissues.** *Infect Genet Evol* 2011, **11**:31-37.
15. H S, RS K: **Disruption of the blood-brain barrier during neuroinflammatory and neuroinfectious diseases.** *Neuroimmune Diseases* 2019:195-234.
16. Chai Q, He WQ, Zhou M, Lu H, Fu ZF: **Enhancement of blood-brain barrier permeability and reduction of tight junction protein expression are modulated by chemokines/cytokines induced by rabies virus infection.** *J Virol* 2014, **88**:4698-4710.
17. Yang T, Guo R, Zhang F: **Brain perivascular macrophages: recent advances and implications in health and diseases.** *CNS Neurosci Ther* 2019, **25**:1318-1328.
18. Williams KC, Corey S, Westmoreland SV, Pauley D, Knight H, deBakker C, Alvarez X, Lackner AA: **Perivascular macrophages are the primary cell type productively infected by simian immunodeficiency virus in the brains of macaques: implications for the neuropathogenesis of AIDS.** *J Exp Med* 2001, **193**:905-915.
19. Filipowicz AR, McGary CM, Holder GE, Lindgren AA, Johnson EM, Sugimoto C, Kuroda MJ, Kim WK: **Proliferation of perivascular macrophages contributes to the development of encephalitic lesions in HIV-infected humans and in SIV-infected macaques.** *Sci Rep* 2016, **6**:32900.
20. Daneman R, Engelhardt B: **Brain barriers in health and disease.** *Neurobiol Dis* 2017, **107**:1-3.
21. Quintana E, Fernandez A, Velasco P, de Andres B, Liste I, Sancho D, Gaspar ML, Cano E: **DNGR-1(+) dendritic cells are located in meningeal membrane and choroid plexus of the noninjured brain.** *Glia* 2015, **63**:2231-2248.
22. Doetsch F, Caille I, Lim DA, Garcia-Verdugo JM, Alvarez-Buylla A: **Subventricular zone astrocytes are neural stem cells in the adult mammalian brain.** *Cell* 1999, **97**:703-716.
23. Obernier K, Alvarez-Buylla A: **Neural stem cells: origin, heterogeneity and regulation in the adult mammalian brain.** *Development* 2019, **146**.
24. Sanai N, Tramontin AD, Quinones-Hinojosa A, Barbaro NM, Gupta N, Kunwar S, Lawton MT, McDermott MW, Parsa AT, Manuel-Garcia Verdugo J *et al.*: **Unique astrocyte ribbon in adult human brain contains neural stem cells but lacks chain migration.** *Nature* 2004, **427**:740-744.
25. de Sonnaville S, van Strien ME, Middeldorp J, Sluijs JA, van den Berge SA, Moeton M, Donega V, van Berkel A, Deering T, De Filippis L *et al.*: **The adult human subventricular zone: partial ependymal coverage and proliferative capacity of cerebrospinal fluid.** *Brain Commun* 2020, **2**:fcaa150.
26. Romero-Suarez S, Del Rio Serrato A, Bueno RJ, Brunotte-Strecker D, Stehle C, Figueiredo CA, Hertwig L, Dunay IR, Romagnani C, Infante-Duarte C: **The central nervous system contains ILC1s that differ from NK cells in the response to inflammation.** *Front Immunol* 2019, **10**:2337.
27. Voshenrich CA, Di Santo JP: **Developmental programming of natural killer and innate lymphoid cells.** *Curr Opin Immunol* 2013, **25**:130-138.

28. van Riel D, Leijten LM, Verdijk RM, GeurtsvanKessel C, van der Vries E, van Rossum AM, Osterhaus AD, Kuiken T: **Evidence for influenza virus CNS invasion along the olfactory route in an immunocompromised infant.** *J Infect Dis* 2014, **210**:419-423.
 29. Moseman EA, Blanchard AC, Nayak D, McGavern DB: **T cell engagement of cross-presenting microglia protects the brain from a nasal virus infection.** *Sci Immunol* 2020, **5**.
- This paper identifies a key role for microglia in preventing viral spread from the neuroepithelium to the olfactory bulb during VSV infection via cross-presenting viral antigens uptaken from infected neurons to infiltrating CD8+ T cells, resulting in non-cytolytic viral clearance from infected neurons.
30. Matschke J, Lutgehetmann M, Hagel C, Sperhake JP, Schroder AS, Edler C, Mushumba H, Fitzek A, Allweiss L, Dandri M *et al.*: **Neuropathology of patients with COVID-19 in Germany: a post-mortem case series.** *Lancet Neurol* 2020, **19**:919-929.
 31. Hoffmann M, Kleine-Weber H, Schroeder S, Kruger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu NH, Nitsche A *et al.*: **SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor.** *Cell* 2020, **181**:271-280 e278.
 32. Bunyavanich S, Do A, Vicencio A: **Nasal gene expression of angiotensin-converting enzyme 2 in children and adults.** *JAMA* 2020.
 33. Meinhardt J, Radke J, Dittmayer C, Franz J, Thomas C, Mothes R, Laue M, Schneider J, Brunink S, Greuel S *et al.*: **Olfactory transmucosal SARS-CoV-2 invasion as a port of central nervous system entry in individuals with COVID-19.** *Nat Neurosci* 2020.
 34. Ghobrial M, Charish J, Takada S, Valiante T, Monnier PP, Radovanovic I, Bader GD, Wälchli T: **The human brain vasculature shows a distinct expression pattern of SARS-CoV-2 entry factors.** *bioRxiv* 2020. 2020.2010.2010.334664.
 35. Keller E, Brandi G, Winkhofer S, Imbach LL, Kirschenbaum D, Frontzek K, Steiger P, Dietler S, Haeblerlin M, Willms J *et al.*: **Large and small cerebral vessel involvement in severe COVID-19: Detailed clinical workup of a case series.** *Stroke* 2020, **51**:3719-3722.
 36. Cantuti-Castelvetri L, Ojha R, Pedro LD, Djannatian M, Franz J, Kuivanen S, van der Meer F, Kallio K, Kaya T, Anastasina M *et al.*: **Neuropilin-1 facilitates SARS-CoV-2 cell entry and infectivity.** *Science* 2020, **370**:856-860.
 37. Mrdjen B, Pavlovic A, Hartmann FJ, Schreiner B, Utz SG, Leung BP, Lelios I, Heppner FL, Kipnis J, Merkler D *et al.*: **High-dimensional single-cell mapping of central nervous system immune cells reveals distinct myeloid subsets in health, aging, and disease.** *Immunity* 2018, **48**:599.
 38. Almerigogna F, Fassio F, Giudizi MG, Biagiotti R, Manuelli C, Chiappini E, Galli L, Romagnani S, De Martino M: **Natural killer cell deficiencies in a consecutive series of children with herpetic encephalitis.** *Int J Immunopathol Pharmacol* 2011, **24**:231-238.
 39. Biron CA, Byron KS, Sullivan JL: **Severe herpesvirus infections in an adolescent without natural killer cells.** *N Engl J Med* 1989, **320**:1731-1735.
 40. Azeredo EL, De Oliveira-Pinto LM, Zagne SM, Cerqueira DI, Nogueira RM, Kubelka CF: **NK cells, displaying early activation, cytotoxicity and adhesion molecules, are associated with mild dengue disease.** *Clin Exp Immunol* 2006, **143**:345-356.
 41. Blom K, Braun M, Pakalniene J, Lunemann S, Enqvist M, Dailidyte L, Schaffer M, Lindquist L, Mickiene A, Michaelsson J *et al.*: **NK cell responses to human tick-borne encephalitis virus infection.** *J Immunol* 2016, **197**:2762-2771.
 42. Larena M, Regner M, Lobigs M: **Cytolytic effector pathways and IFN-gamma help protect against Japanese encephalitis.** *Eur J Immunol* 2013, **43**:1789-1798.
 43. Marquardt N, Ivarsson MA, Blom K, Gonzalez VD, Braun M, Falconer K, Gustafsson R, Fogdell-Hahn A, Sandberg JK, Michaelsson J: **The human NK cell response to yellow fever virus 17D is primarily governed by NK cell differentiation independently of NK cell education.** *J Immunol* 2015, **195**:3262-3272.
 44. Petitdemange C, Wauquier N, Devilliers H, Yssel H, Mombo I, Caron M, Nkoghe D, Debre P, Leroy E, Vieillard V: **Longitudinal analysis of natural killer cells in dengue virus-infected patients in comparison to chikungunya and chikungunya/dengue virus-infected patients.** *PLoS Negl Trop Dis* 2016, **10**:e0004499.
 45. Strauss-Albee DM, Fukuyama J, Liang EC, Yao Y, Jarrell JA, Drake AL, Kinuthia J, Montgomery RR, John-Stewart G, Holmes S *et al.*: **Human NK cell repertoire diversity reflects immune experience and correlates with viral susceptibility.** *Sci Transl Med* 2015, **7**:297ra115.
 46. Baxter VK, Griffin DE: **Interferon-gamma modulation of the local T cell response to alphavirus encephalomyelitis.** *Viruses* 2020, **12**.
 47. Manangeeswaran M, Lewkowicz AP, Israely T, Ireland DDC, Verthelyi D: **CpG oligonucleotides protect mice from alphavirus encephalitis: role of NK cells, interferons, and TNF.** *Front Immunol* 2020, **11**:237.
 48. Colonna M, Butovsky O: **Microglia function in the central nervous system during health and neurodegeneration.** *Annu Rev Immunol* 2017, **35**:441-468.
 49. Tufail Y, Cook D, Fourgeaud L, Powers CJ, Merten K, Clark CL, Hoffman E, Ngo A, Sekiguchi KJ, O'Shea CC *et al.*: **Phosphatidylserine exposure controls viral innate immune responses by microglia.** *Neuron* 2017, **93**:574-586 e578.
 50. Garcia JM, Stillings SA, Leclerc JL, Phillips H, Edwards NJ, Robicsek SA, Hoh BL, Blackburn S, Dore S: **Role of interleukin-10 in acute brain injuries.** *Front Neurosci* 2017, **8**:244.
 51. Prinz M, Erny D, Hagemeyer N: **Ontogeny and homeostasis of CNS myeloid cells.** *Nat Immunol* 2017, **18**:385-392.
 52. Nayak D, Johnson KR, Heydari S, Roth TL, Zinselmeyer BH, McGavern DB: **Type I interferon programs innate myeloid dynamics and gene expression in the virally infected nervous system.** *PLoS Pathog* 2013, **9**:e1003395.
 53. Schwartz M, Butovsky O, Bruck W, Hanisch UK: **Microglial phenotype: is the commitment reversible?** *Trends Neurosci* 2006, **29**:68-74.
 54. Bialas AR, Stevens B: **TGF-beta signaling regulates neuronal C1q expression and developmental synaptic refinement.** *Nat Neurosci* 2013, **16**:1773-1782.
 55. Schafer DP, Lehrman EK, Kautzman AG, Koyama R, Mardinly AR, Yamasaki R, Ransohoff RM, Greenberg ME, Barres BA, Stevens B: **Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner.** *Neuron* 2012, **74**:691-705.
 56. Colpitts TM, Conway MJ, Montgomery RR, Fikrig E: **West Nile Virus: biology, transmission, and human infection.** *Clin Microbiol Rev* 2012, **25**:635-648.
 57. Mehlhop E, Whitby K, Oliphant T, Marri A, Engle M, Diamond MS: **Complement activation is required for induction of a protective antibody response against West Nile virus infection.** *J Virol* 2005, **79**:7466-7477.
 58. De Chiara G, Piacentini R, Fabiani M, Mastrodonato A, Marocchi ME, Limongi D, Napoletani G, Protto V, Coluccio P, Celestino I *et al.*: **Recurrent herpes simplex virus-1 infection induces hallmarks of neurodegeneration and cognitive deficits in mice.** *PLoS Pathog* 2019, **15**:e1007617.
 59. Vasek MJ, Garber C, Dorsey D, Durrant DM, Bollman B, Soung A, Yu J, Perez-Torres C, Frouin A, Wilton DK *et al.*: **A complement-microglial axis drives synapse loss during virus-induced memory impairment.** *Nature* 2016, **534**:538-543.
 60. Garber C, Soung A, Vollmer LL, Kanmogne M, Last A, Brown J, Klein RS: **T cells promote microglia-mediated synaptic elimination and cognitive dysfunction during recovery from neuropathogenic flaviviruses.** *Nat Neurosci* 2019, **22**:1276-1288.
- This paper identifies that memory CD8+ T cells-derived IFN- γ acts on hippocampal microglia to trigger synapse elimination following WNV or ZIKV recovery in mice, resulting in post-infectious cognitive sequelae.

61. Kreutzfeldt M, Bergthaler A, Fernandez M, Bruck W, Steinbach K, Vorm M, Coras R, Blumcke I, Bonilla WV, Fleige A *et al.*: **Neuroprotective intervention by interferon-gamma blockade prevents CD8+ T cell-mediated dendrite and synapse loss.** *J Exp Med* 2013, **210**:2087-2103.
 62. Di Liberto G, Pantelyushin S, Kreutzfeldt M, Page N, Musardo S, Coras R, Steinbach K, Vincenti I, Klimek B, Lingner T *et al.*: **Neurons under T cell attack coordinate phagocyte-mediated synaptic stripping.** *Cell* 2018, **175**:458-471 e419.
- This paper reveals that CD8-derived IFN- γ acts on persistently infected neurons to induce the production of CCL2, driving the recruitment of microglia and microglia-mediated synaptic loss.
63. Devasthanam AS: **Mechanisms underlying the inhibition of interferon signaling by viruses.** *Virulence* 2014, **5**:270-277.
 64. Samuel MA, Diamond MS: **Alpha/beta interferon protects against lethal West Nile virus infection by restricting cellular tropism and enhancing neuronal survival.** *J Virol* 2005, **79**:13350-13361.
 65. Mlakar J, Korva M, Tul N, Popovic M, Poljsak-Prijatelj M, Mraz J, Kolenc M, Resman Rus K, Vesnaver Vipotnik T, Fabjan Vodusek V *et al.*: **Zika virus associated with microcephaly.** *N Engl J Med* 2016, **374**:951-958.
 66. Nishihara H, Soldati S, Mossu A, Rosito M, Rudolph H, Muller WA, Latorre D, Sallusto F, Sospedra M, Martin R *et al.*: **Human CD4(+) T cell subsets differ in their abilities to cross endothelial and epithelial brain barriers in vitro.** *Fluids Barriers CNS* 2020, **17**:3.
 67. Patil VS, Madrigal A, Schmiedel BJ, Clarke J, O'Rourke P, de Silva AD, Harris E, Peters B, Seumois G, Weiskopf D *et al.*: **Precursors of human CD4(+) cytotoxic T lymphocytes identified by single-cell transcriptome analysis.** *Sci Immunol* 2018, **3**.
- This paper unravels the heterogeneity of cytotoxic CD4+ T cells in the blood of DENV-infected human donors by scRNA-seq and identifies the gene signature of cytotoxic CD4 cells and their precursors.
68. Sledzinska A, Vila de Mucha M, Bergerhoff K, Hotblack A, Demane DF, Ghorani E, Akarca AU, Marzolini MAV, Solomon I, Vargas FA *et al.*: **Regulatory T cells restrain interleukin-2- and blimp-1-dependent acquisition of cytotoxic function by CD4(+) T cells.** *Immunity* 2020, **52**:151-166 e156.
 69. Tian Y, Sette A, Weiskopf D: **Cytotoxic CD4 T cells: differentiation, function, and application to dengue virus infection.** *Front Immunol* 2016, **7**:531.
 70. Kang S, Brown HM, Hwang S: **Direct antiviral mechanisms of interferon-gamma.** *Immune Netw* 2018, **18**:e33.
 71. Zhou F: **Molecular mechanisms of IFN-gamma to up-regulate MHC class I antigen processing and presentation.** *Int Rev Immunol* 2009, **28**:239-260.
 72. Schachtele SJ, Hu S, Sheng WS, Mutnal MB, Lokensgard JR: **Glial cells suppress postencephalitic CD8+ T lymphocytes through PD-L1.** *Glia* 2014, **62**:1582-1594.
 73. Trandem K, Zhao J, Fleming E, Perlman S: **Highly activated cytotoxic CD8 T cells express protective IL-10 at the peak of coronavirus-induced encephalitis.** *J Immunol* 2011, **186**:3642-3652.
 74. Uhde AK, Ciurkiewicz M, Herder V, Khan MA, Hensel N, Claus P, Beckstette M, Teich R, Floess S, Baumgartner W *et al.*: **Intact interleukin-10 receptor signaling protects from hippocampal damage elicited by experimental neurotropic virus infection of SJL mice.** *Sci Rep* 2018, **8**:6106.
 75. Boivin WA, Cooper DM, Hiebert PR, Granville DJ: **Intracellular versus extracellular granzyme B in immunity and disease: challenging the dogma.** *Lab Invest* 2009, **89**:1195-1220.
 76. Kagi D, Seiler P, Pavlovic J, Ledermann B, Burki K, Zinkernagel RM, Hengartner H: **The roles of perforin- and Fas-dependent cytotoxicity in protection against cytopathic and noncytopathic viruses.** *Eur J Immunol* 1995, **25**:3256-3262.
 77. Voskoboinik I, Smyth MJ, Trapani JA: **Perforin-mediated target-cell death and immune homeostasis.** *Nat Rev Immunol* 2006, **6**:940-952.
 78. McGavern DB, Christen U, Oldstone MB: **Molecular anatomy of antigen-specific CD8(+) T cell engagement and synapse formation in vivo.** *Nat Immunol* 2002, **3**:918-925.
 79. Halle S, Keyser KA, Stahl FR, Busche A, Marquardt A, Zheng X, Galla M, Heissmeyer V, Heller K, Boelter J *et al.*: **In vivo killing capacity of cytotoxic T cells is limited and involves dynamic interactions and T cell cooperativity.** *Immunity* 2016, **44**:233-245.
 80. Bergmann CC, Parra B, Hinton DR, Ramakrishna C, Dowdell KC, Stohman SA: **Perforin and gamma interferon-mediated control of coronavirus central nervous system infection by CD8 T cells in the absence of CD4 T cells.** *J Virol* 2004, **78**:1739-1750.
 81. Pinschewer DD, Schedensack M, Bergthaler A, Horvath E, Bruck W, Lohning M, Merkler D: **T cells can mediate viral clearance from ependyma but not from brain parenchyma in a major histocompatibility class I- and perforin-independent manner.** *Brain* 2010, **133**:1054-1066.
 82. Knickelbein JE, Khanna KM, Yee MB, Baty CJ, Kinchington PR, Hendricks RL: **Noncytotoxic lytic granule-mediated CD8+ T cell inhibition of HSV-1 reactivation from neuronal latency.** *Science* 2008, **322**:268-271.
 83. Verjans GM, Hintzen RQ, van Dun JM, Poot A, Miliikan JC, Laman JD, Langerak AW, Kinchington PR, Osterhaus AD: **Selective retention of herpes simplex virus-specific T cells in latently infected human trigeminal ganglia.** *Proc Natl Acad Sci U S A* 2007, **104**:3496-3501.
 84. Doll JR, Hoebe K, Thompson RL, Sawtell NM: **Resolution of herpes simplex virus reactivation in vivo results in neuronal destruction.** *PLoS Pathog* 2020, **16**:e1008296.
 85. Shrestha B, Samuel MA, Diamond MS: **CD8+ T cells require perforin to clear West Nile virus from infected neurons.** *J Virol* 2006, **80**:119-129.
 86. Daniels BP, Kofman SB, Smith JR, Norris GT, Snyder AG, Kolb JP, Gao X, Locasale JW, Martinez J, Gale M Jr *et al.*: **The nucleotide sensor ZBP1 and kinase RIPK3 induce the enzyme IRG1 to promote an antiviral metabolic state in neurons.** *Immunity* 2019, **50**:64-76 e64.
- The authors describe here for the first time the generation of a particular metabolic state in ZIKV-infected neurons that restricts viral replication.
87. Flugel A, Schwaiger FW, Neumann H, Medana I, Willem M, Wekerle H, Kreutzberg GW, Graeber MB: **Neuronal FasL induces cell death of encephalitogenic T lymphocytes.** *Brain Pathol* 2000, **10**:353-364.
 88. Lee SJ, Zhou T, Choi C, Wang Z, Benveniste EN: **Differential regulation and function of Fas expression on glial cells.** *J Immunol* 2000, **164**:1277-1285.
 89. Medana I, Li Z, Flugel A, Tschopp J, Wekerle H, Neumann H: **Fas ligand (CD95L) protects neurons against perforin-mediated T lymphocyte cytotoxicity.** *J Immunol* 2001, **167**:674-681.
 90. de Graaf MT, Smitt PA, Luitwieler RL, van Velzen C, van den Broek PD, Kraan J, Gratama JW: **Central memory CD4+ T cells dominate the normal cerebrospinal fluid.** *Cytometry B Clin Cytom* 2011, **80**:43-50.
 91. Kivisakk P, Mahad DJ, Callahan MK, Trebst C, Tucky B, Wei T, Wu L, Baekkevold ES, Lassmann H, Staugaitis SM *et al.*: **Human cerebrospinal fluid central memory CD4+ T cells: evidence for trafficking through choroid plexus and meninges via P-selectin.** *Proc Natl Acad Sci U S A* 2003, **100**:8389-8394.
 92. Mundt S, Greter M, Flugel A, Becher B: **The CNS immune landscape from the viewpoint of a T cell.** *Trends Neurosci* 2019, **42**:667-679.
 93. Russo MV, McGavern DB: **Immune surveillance of the CNS following infection and injury.** *Trends Immunol* 2015, **36**:637-650.
 94. Wakim LM, Woodward-Davis A, Bevan MJ: **Memory T cells persisting within the brain after local infection show functional adaptations to their tissue of residence.** *Proc Natl Acad Sci U S A* 2010, **107**:17872-17879.

95. Smolders J, Heutink KM, Fransen NL, Remmerswaal EBM, ●● Hombink P, Ten Berge IJM, van Lier RAW, Huitinga I, Hamann J: **Tissue-resident memory T cells populate the human brain.** *Nat Commun* 2018, **9**:4593

This paper analyzes the T cell repertoire populating the human white matter by flow cytometry and identifies subsets of CD8 and CD4 bT_{RM} residing in healthy and diseased brain.

96. Frost EL, Kersh AE, Evavold BD, Lukacher AE: **Cutting edge: resident memory CD8 T cells express high-affinity TCRs.** *J Immunol* 2015, **195**:3520-3524.
97. Urban SL, Jensen IJ, Shan Q, Pewe LL, Xue HH, Badovinac VP, ●● Harty JT: **Peripherally induced brain tissue-resident memory CD8(+) T cells mediate protection against CNS infection.** *Nat Immunol* 2020

This paper unravels the generation of CD8+ T_{RM} within the brain following peripheral infections with VSV, IAV, LCMV and LM and shows for the first time that local CNS infection is not required to generate CD8 bT_{RM} cells that protect from CNS reinfections.

98. Ren HM, Kolawole EM, Ren M, Jin G, Netherby-Winslow CS, Wade Q, Shwetank, Rahman ZSM, Evavold BD, Lukacher AE: **IL-21 from high-affinity CD4 T cells drives differentiation of brain-resident CD8 T cells during persistent viral infection.** *Sci Immunol* 2020, **5**.
99. Steinbach K, Vincenti I, Kreutzfeldt M, Page N, Muschaweckh A, Wagner I, Drexler I, Pinschewer D, Korn T, Merkler D: **Brain-resident memory T cells represent an autonomous cytotoxic barrier to viral infection.** *J Exp Med* 2016, **213**:1571-1587.
100. Brizic I, Susak B, Arapovic M, Huszthy PC, Hirsl L, Kvestak D, Juranic Lisnic V, Golemac M, Pernjak Pugel E, Tomac J *et al.*: **Brain-resident memory CD8(+) T cells induced by congenital CMV infection prevent brain pathology and virus reactivation.** *Eur J Immunol* 2018, **48**:950-964.
101. Wakim LM, Woodward-Davis A, Liu R, Hu Y, Villadangos J, Smyth G, Bevan MJ: **The molecular signature of tissue resident memory CD8 T cells isolated from the brain.** *J Immunol* 2012, **189**:3462-3471.
102. Low JS, Farsakoglu Y, Amezcua Vesely MC, Sefik E, Kelly JB, Harman CCD, Jackson R, Shyer JA, Jiang X, Cauley LS *et al.*: **Tissue-resident memory T cell reactivation by diverse antigen-presenting cells imparts distinct functional responses.** *J Exp Med* 2020, **217**.
103. Steinbach K, Vincenti I, Egervari K, Kreutzfeldt M, van der Meer F, ●● Page N, Klimek B, Rossitto-Borlat I, Di Liberto G, Muschaweckh A *et al.*: **Brain-resident memory T cells generated early in life predispose to autoimmune disease in mice.** *Sci Transl Med* 2019, **11**

This paper links CNS viral infections early in life with a predisposition to develop an autoimmune disease later in life. Authors unravel that early-life CNS infection in mice results in an imprinted chronic inflammatory signature in brains characterized by CD8 bT_{RM} expressing CCL5, which predispose to brain lesions later in life in a mouse model of autoimmune disease.

104. Atkinson JR, Hwang M, Reyes-Rodriguez A, Bergmann CC: **Dynamics of virus-specific memory B cells and plasmablasts following viral infection of the central nervous system.** *J Virol* 2019, **93**.
105. Knopf PM, Harling-Berg CJ, Cserr HF, Basu D, Sirulnick EJ, Nolan SC, Park JT, Keir G, Thompson EJ, Hickey WF: **Antigen-dependent intrathecal antibody synthesis in the normal rat brain: tissue entry and local retention of antigen-specific B cells.** *J Immunol* 1998, **161**:692-701.
106. Metcalf TU, Griffin DE: **Alphavirus-induced encephalomyelitis: antibody-secreting cells and viral clearance from the nervous system.** *J Virol* 2011, **85**:11490-11501.
107. Fragkoudis R, Ballany CM, Boyd A, Fazakerley JK: **In Semliki Forest virus encephalitis, antibody rapidly clears infectious virus and is required to eliminate viral material from the brain, but is not required to generate lesions of demyelination.** *J Gen Virol* 2008, **89**:2565-2568.
108. Stewart BS, Demarest VL, Wong SJ, Green S, Bernard KA: **Persistence of virus-specific immune responses in the central nervous system of mice after West Nile virus infection.** *BMC Immunol* 2011, **12**:6.
109. DiSano KD, Stohlman SA, Bergmann CC: **Activated GL7(+) B cells are maintained within the inflamed CNS in the absence of follicle formation during viral encephalomyelitis.** *Brain Behav Immun* 2017, **60**:71-83.
110. Gil-Cruz C, Perez-Shibayama C, Firner S, Waisman A, Bechmann I, Thiel V, Cervantes-Barragan L, Ludewig B: **T helper cell- and CD40-dependent germline IgM prevents chronic virus-induced demyelinating disease.** *Proc Natl Acad Sci U S A* 2012, **109**:1233-1238.
111. Jęgo G, Palucka AK, Blanck JP, Chalouni C, Pascual V, Banchereau J: **Plasmacytoid dendritic cells induce plasma cell differentiation through type I interferon and interleukin 6.** *Immunity* 2003, **19**:225-234.
112. Ramakrishna C, Bergmann CC, Atkinson R, Stohlman SA: **Control of central nervous system viral persistence by neutralizing antibody.** *J Virol* 2003, **77**:4670-4678.
113. Planz O, Ehl S, Furrer E, Horvath E, Brundler MA, Hengartner H, Zinkernagel RM: **A critical role for neutralizing-antibody-producing B cells, CD4(+) T cells, and interferons in persistent and acute infections of mice with lymphocytic choriomeningitis virus: implications for adoptive immunotherapy of virus carriers.** *Proc Natl Acad Sci U S A* 1997, **94**:6874-6879.
114. Mutnal MB, Hu S, Lokensgard JR: **Persistent humoral immune responses in the CNS limit recovery of reactivated murine cytomegalovirus.** *PLoS ONE* 2012, **7**:e33143.
115. Handel AE, Williamson AJ, Disanto G, Handunnetthi L, Giovannoni G, Ramagopalan SV: **An updated meta-analysis of risk of multiple sclerosis following infectious mononucleosis.** *PLoS One* 2010, **5**.
116. Arias I, Sorlozano A, Villegas E, de Dios Luna J, McKenney K, Cervilla J, Gutierrez B, Gutierrez J: **Infectious agents associated with schizophrenia: a meta-analysis.** *Schizophr Res* 2012, **136**:128-136.