

Coronavirus infection of the central nervous system: host–virus stand-off

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Abstract | Several viruses infect the mammalian central nervous system (CNS), some with devastating consequences, others resulting in chronic or persistent infections associated with little or no overt pathology. Coronavirus infection of the murine CNS illustrates the contributions of both the innate immune response and specific host effector mechanisms that control virus replication in distinct CNS cell types. Despite T-cell-mediated control of acute virus infection, host regulatory mechanisms, probably designed to protect CNS integrity, contribute to the failure to eliminate virus. Distinct from cytolytic effector mechanisms expressed during acute infection, non-lytic humoral immunity prevails in suppressing infectious virus during persistence.

Neurotrophins

A large family of proteins required for cell survival and differentiation within the vertebrate nervous system.

Blood–brain barrier

(BBB). Permeability barrier that comprises endothelial cells that line cerebral capillaries. Regulates the transport of cells and large-molecule transport into the CNS parenchyma.

Interactions between the immune system and the central nervous system (CNS) constitute the most complex and interactive regulatory network in mammals. The high degree of specialization of cell types that comprise the CNS, and their intricate communication, controls both cognitive and vital functions. Disruption of the communication network and poor CNS regenerative properties make this organ vulnerable to microbial as well as physical injury. Although it is known that host responses must be strictly regulated to preserve CNS function and to minimize the incidence of autoimmunity, the factors regulating CNS immune and repair responses are not well understood. In addition to the absence of a dedicated lymphatic drainage system, CNS cells express few, if any, molecules encoded by the major histocompatibility complex (MHC)^{1–3}. Therefore, in the quiescent CNS there is little endogenous antigen presentation or potential to activate T cells. Although the underlying basis for this limited immunological activity is not completely understood, interactions between both neurons and the glial population represented by microglia, astrocytes and oligodendrocytes (BOX 1), as well as constitutive secretion of neurotrophins and transforming growth factor- β , might contribute to this quiescent resting state^{1,4–7}. The limited expression of adhesion molecules by endothelial cells of the blood–brain barrier (BBB) and the presence of tight junctions between these cells also limit or prevent large molecules, such as antibodies, and T cells from entering the CNS^{2,8}. Despite this, a few activated/memory CD4⁺ and CD8⁺ T cells randomly patrol the CNS in the absence of ‘danger’ signals, and either exit or die *in situ* in the absence of antigen recognition^{2,8}.

Mammals have evolved many immune effector mechanisms to eliminate pathogens that infect the CNS^{7,9}. The vigorous inflammatory responses that are induced during many CNS infections contrast dramatically with its quiescent steady state. These inflammatory responses include rapidly induced, non-specific cellular and soluble effectors that provide an innate antimicrobial defence and facilitate development of antigen-specific effectors, which exert antimicrobial function and establish long-lived immunological memory. Some effectors mediate specific functions, whereas others mediate pleiotropic effects. Furthermore, several regulatory mechanisms limit immune responsiveness to avoid damage of uninfected host cells or the induction of autoimmunity^{10,11}.

The conflicting needs for pathogen elimination and protection from cellular damage make the mammalian CNS a partially protected environmental niche that is a prime target for persistent viral infections. Viruses that persist in the human CNS include DNA viruses, as exemplified by herpes simplex virus and JC polyomavirus; RNA viruses, such as measles virus; and retroviruses, such as HIV and HTLV-1 (REFS 9, 12–14). Several viruses that establish chronic infections in the rodent CNS provide useful models to examine both the roles and regulation of immune effectors in this vital organ. Collectively, these models have provided a wealth of information about the genetics of host resistance, acute and chronic viral infection as well as host defence mechanisms. Chronic, viral rodent CNS pathogens that are associated with myelin loss include two well characterized RNA virus models: Theiler’s murine encephalomyelitis virus (TMEV), a member of the non-enveloped Picornaviridae, and mouse hepatitis

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Box 1 | CNS cell types

The central nervous system (CNS) is composed of two main cell types: neurons and glial cells. The three main types of glial cells are astrocytes, oligodendrocytes and microglia.

Glial cells

Microglia. Microglia are the myelomonocytic lineage-derived resident ‘macrophage’ population of the CNS. They have many characteristics common to other tissue macrophages. However, microglia express only low levels of **CD45**, a marker of bone-marrow-derived cells, and unlike tissue macrophages, they also proliferate. Major histocompatibility complex (MHC) molecules are not expressed on microglia in the undisturbed CNS, but are rapidly expressed following exposure to IFN- γ . In addition to mouse hepatitis virus (MHV), murine microglia are the targets for Theiler’s murine encephalomyelitis virus (TMEV) infection, and human microglia are the targets of HIV and JC polyomavirus infection.

Astrocytes. Astrocytes are the most abundant CNS cell population. Astrocytes interact with CNS endothelial cells to form the blood–brain barrier. *In vitro* data suggests a potential role for astrocytes in antigen presentation; however, little *in vivo* data is currently available. Expression of MHC molecules *in situ* is also controversial. Astrocytes support the replication of the John Howard Mueller (JHMV) strain of MHV in the murine CNS and of HIV and adenoviruses in the human CNS.

Oligodendrocytes. Oligodendrocytes produce a lipid- and protein-rich laminated myelin membrane that surrounds axons and promotes axonal conduction. In addition to supporting JHMV replication in the murine CNS, oligodendrocytes support JC-polyomavirus and measles-virus infection in the human CNS.

Neurons

Neurons are the main cell type involved in motor and cognitive function. The JHMV variants differ in their ability to infect neurons. Neurons are primary targets in the murine CNS for many diverse viruses, including **Japanese encephalitis virus**, **Sindbis virus**, West Nile virus, vesicular stomatitis virus and lymphocytic choriomeningitis virus. Human neurons are targets of herpesvirus, **poliovirus** and **rabies virus** in the human CNS.

virus (MHV), a member of the enveloped *Coronaviridae*. Although CD8⁺ T cells are important in controlling the acute phase of both infections, these viruses can escape immune surveillance and establish chronic CNS infection with ongoing myelin loss^{14–18}.

Despite similar disease pathologies during chronic disease, infectious TMEV is present in the CNS during chronic disease¹⁹. By contrast, infectious MHV remains undetectable during persistence although MHV viral antigens and RNA are retained^{16–18}. Another distinguishing characteristic during chronic TMEV infection is that chronic inflammation involves activation of self-reactive T cells^{20,21}. By contrast, control of infectious MHV results in a slowly resolving, but chronic, CNS disease that is associated with minimal inflammation^{16–18}. These chronic pathological changes in the absence of overt infectious virus are similar to human CNS diseases with suspected or potential viral aetiologies, such as multiple sclerosis. Therefore, MHV infection of the CNS provides a unique model in which viral replication is controlled by a vigorous immune response but the host is unable to achieve a sterile immunity, resulting in a persistent infection that is associated with ongoing pathology in the apparent absence of infectious virus.

Here, we discuss the interplay between the neurotropic viral pathogen MHV, with emphasis on the neurotropic John Howard Mueller (JHMV) strain, and the immune-mediated mechanisms that control acute and persistent CNS infection.

Mouse hepatitis virus

MHV is a member of the *Coronaviridae* family in the Order Nidovirales. The replication cycle is depicted in FIG. 1. Clinically important human coronaviruses include those that cause ~30% of cases of the common cold and that cause severe acute respiratory syndrome (SARS)²². Bovine, porcine and avian coronaviruses also produce economically important diseases. MHV is a natural pathogen of mice that primarily infects the gastrointestinal tract. It produces a self-limiting infection with residual systemic immunological defects including reduced rejection of histo-incompatible tissues^{17,18}. In common with many viruses, pathogenesis and immune responses depend on the viral strain, route of inoculation, age and genetic background of the host. Different MHV isolates induce various acute and chronic diseases in the murine host, including hepatitis, vasculitis, acute fatal encephalitis and encephalomyelitis associated with acute and chronic CNS demyelination^{17,18} (BOX 2).

Pathogenic strategy

MHV initiates intracellular infection by interaction of the viral-envelope spike protein (S) with its cellular receptor, the **CEACAM-1** molecule²³. Analysis of S genes from MHV strains that exhibit varied pathogenesis²⁴, selection of viruses with S-gene mutations²⁵ and recombinant viruses with modified S genes²⁶ all confirm that the S protein is the main determinant of cell tropism and pathogenicity. But analysis of recombinant MHV that shows a high degree of tropism for neurons indicates that, in the absence of the dominant CD8⁺ T-cell epitope, other viral genes in addition to S genes also influence pathogenesis^{27,28}. Adaptation to non-CEACAM-1-bearing cells can be achieved by co-culture with infected, susceptible CEACAM-1-expressing cells²⁹, and CEACAM-1-independent infection *in vitro* has also been described³⁰. These data indicate that alterations in tropism or host range might be achieved *in vivo*. Also, it has been suggested that receptor–S-protein affinity might contribute to the variable pathogenesis of some MHV strains³¹. However, not all cells that express CEACAM-1 (for instance, B cells) support MHV replication³², indicating that other (co-)receptors and intracellular factors influence productive virus replication. This is supported by the efficient replication of JHMV in the CNS, despite extremely low levels of receptor mRNA and protein expression relative to other tissues^{33,34}. Interestingly, receptor expression on microglia decreases during CNS inflammation³⁵, indicating that inflammatory mediators might manipulate the reservoir of susceptible cells by altering receptor expression.

Following direct intracranial injection, JHMV infection is rapidly established in the ependymal cells that line the brain ventricles³⁶ (FIG. 2). As replication increases, virus spreads from the ependyma into the brain parenchyma. The cell types that support replication include macrophages, microglia and astrocytes, with a small number of infected oligodendrocytes in the periventricular white matter. Virus subsequently spreads down the central canal of the spinal cord, and moves out into the white matter, where it predominantly infects

Tight junctions

Intercellular junctions where adjacent plasma membranes are joined and occlude the intercellular space, resulting in limited intercellular passage of molecules.

Ependymal cells

Cells that line the CNS ventricles as a single cell layer.

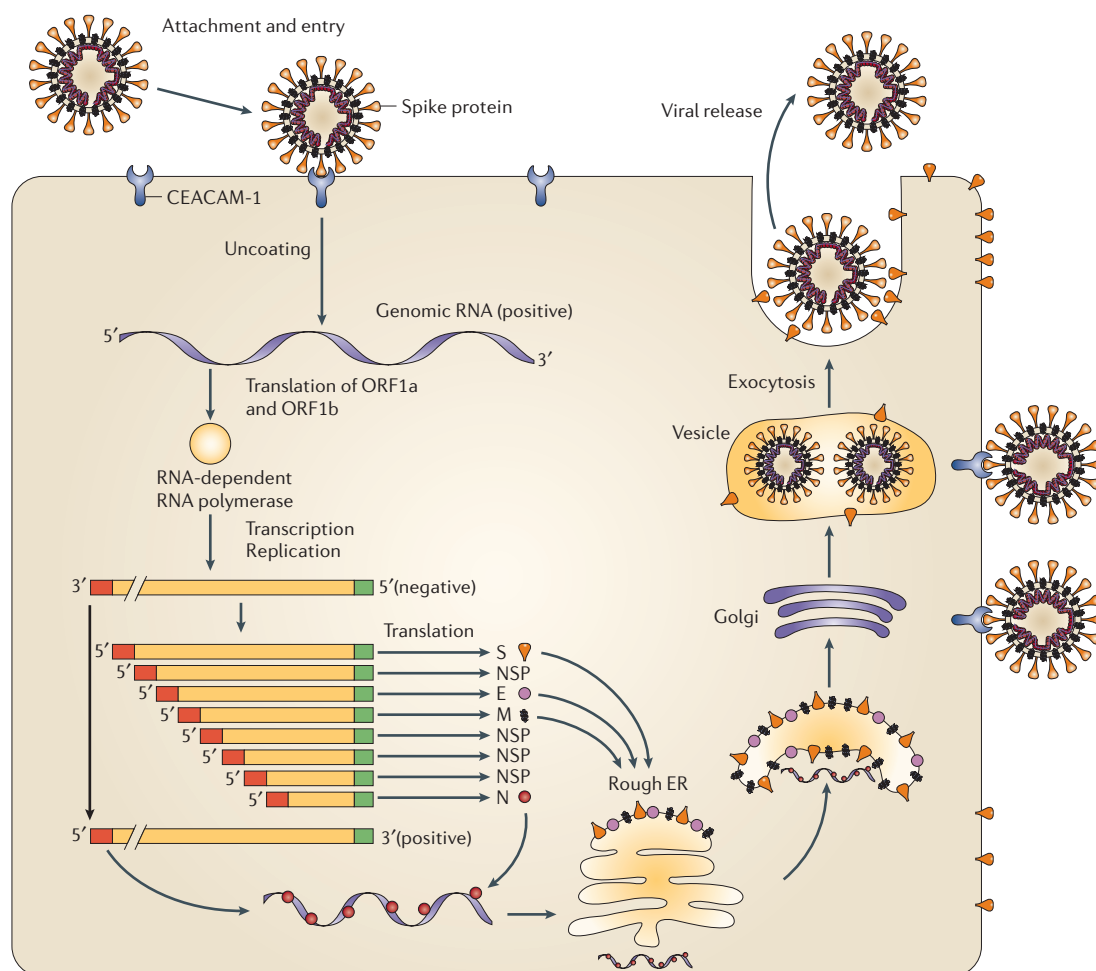


Figure 1 | Summary of mouse hepatitis virus (MHV) replication. MHV binds to the host-cell receptor CEACAM-1 through interaction of the spike (S) glycoprotein. Virus entry into the host cell can occur through fusion with the surface of the host cell, with the subsequent release of the genomic RNA into the cytoplasm. Alternatively, MHV can enter the host cell through the formation of endocytic vesicles, and genomic RNA is released into the cytoplasm following fusion with the vesicle membrane (not shown). Translation of the positive-strand genomic RNA gives rise to a large polyprotein that undergoes proteolytic processing to generate an RNA-dependent RNA polymerase. Through the action of the RNA polymerase, a full-length, antisense negative-strand template is generated. Subgenomic mRNAs are synthesized, presumably from subgenomic negative-strand templates. Translation of subgenomic mRNAs gives rise to structural viral proteins. S glycoprotein is expressed on the surface of the host cell and this might contribute to fusion with neighbouring uninfected cells by binding to CEACAM-1. Virus assembly occurs within vesicles, followed by virus release by fusion of virion-containing vesicles with the plasma membrane. Released virus can infect other cells and can replicate within the parent cell through binding to CEACAM-1. E, envelope protein; ER, endoplasmic reticulum; M, membrane protein; N, nucleocapsid protein; ORF, open reading frame. Modified with permission from REF. 22 © (2003) Macmillan Publishers Ltd.

oligodendrocytes³⁶. Although direct CNS injection initially disrupts BBB integrity, it is rapidly re-established and then progressively lost as inflammation increases³⁷. Virus replication peaks at ~5 days post infection (p.i.) but infectious virus cannot be recovered from immunocompetent hosts by ~2 weeks p.i.^{16–18,38–40} (FIG. 3a). As viral titres increase, physiological changes such as alterations in BBB integrity and glial-cell activation occur in the host, even in the absence of overt clinical signs of disease. As immunity controls infectious virus, clinical signs of disease increase^{25,38–40}. Infection of immunodeficient mice indicates that clinical signs are dependent on the inflammatory response, especially the CD4⁺ T-cell component^{41,42}. Prolonged detection of

viral antigen and mRNA in immunocompetent mice for >1 year p.i.^{1,16–18,40,43–45} implies that there is incomplete immunological control of CNS virus replication. A portion of the persisting viral RNAs are defective^{44,45}, which might contribute to the failure to recover infectious virus during persistence. Virus control by inflammatory cells is associated with primary demyelination, which is ameliorated but sustained during the persistent state. Ongoing demyelination might be associated with limited virus replication and concomitant immune control.

The innate immune response to acute infection

In common with other models of viral-induced encephalitis^{7,9}, intranasal or direct intracranial MHV infection

induces a vigorous CNS inflammatory response composed of both innate and adaptive immune components that peaks at 6–8 days p.i.^{37,46}. CNS infection is initially manifested by rapid, dynamic and coordinated expression of chemokines, matrix metalloproteinases (MMPs), a tissue inhibitor of MMPs (**TIMP-1**) and pro-inflammatory cytokines (FIG. 3b,c; 0–5 days p.i.). Upregulation of these factors has largely been characterized by mRNA analysis of whole organs to reveal overall signal strength and patterns. However, more detailed analysis in a limited number of studies clearly indicates that both virus-infected and uninfected glial cells, most prominently astrocytes, provide early inflammatory signals^{41,47,48}. Together, these molecules facilitate BBB disruption and attract innate immune effectors, which express inflammatory factors. MMP expression is associated with tissue influx of inflammatory cells, activation of cytokine secretion and CNS damage⁴⁹. JHMV infection induces expression of **MMP-3** mostly in astrocytes and **MMP-12** mostly in oligodendrocytes, independent of the inflammatory response^{47,50}. By contrast, a broad range of MMPs are induced in mouse models of CNS autoimmune disease⁴⁹, emphasizing the distinction between CNS infection and autoimmunity as well as the complexity of CNS responses. Neutrophils, macrophages and natural killer (NK) cells are the initial inflammatory cells recruited into the MHV-infected CNS^{37,40} (FIGS 2,4a). Secretion of pre-packaged **MMP-9** by neutrophils, upregulation of adhesion molecules on CNS endothelium and, possibly, the action of **IL-6** (REF. 51) contribute to a loss of BBB integrity that facilitates the subsequent entry of further inflammatory cells into the infected CNS³⁷. **MMP-3**, **MMP-9** and **MMP-12** mRNAs decrease either at the peak of JHMV-induced inflammation or rapidly thereafter^{47,50}, supporting an early role in shaping the CNS environment. However, with the exception of **MMP-9** (REF. 37), their role(s) in innate inflammatory-cell trafficking and CNS pathology is unclear.

The earliest chemokines induced in the CNS following MHV infection are **CXCL10** and **CCL3** (REFS 48,52). **CXCL10** is expressed by both infected and uninfected glial cells as early as day 1 p.i. (FIGS 2,3b) and recruits NK cells by signalling through **CXCR3** (REF. 53). Despite rapid but transient NK-cell recruitment into the CNS, there is little direct evidence for an antiviral role; however, their potential to secrete **IFN- γ** might facilitate antigen presentation through upregulation of MHC class I and class II molecules. **CCL3** might enhance the adaptive immune response by stimulating T-cell activation and recruitment⁵². Macrophages comprise the largest component of innate CNS infiltrates (FIG. 4a). Their accumulation is enhanced by **CCL5** (REFS 41,54), which is induced with slightly delayed kinetics relative to **CXCL10** and **CCL3** (REF. 48). Infection of the CNS with other neurotropic viruses, for example, **lymphocytic choriomeningitis virus**, TMEV and measles virus, induces chemokine-gene-expression profiles that are similar to MHV, which indicates that CNS-resident cells respond in a similar manner to viral infection, possibly through the expression of type I interferons (IFNs)^{55–58}.

Cytokines that are rapidly induced by MHV, predominantly in astrocytes and microglia, include **IL-1 α** , **IL-1 β** , **IL-6** and **IL-12** (REFS 59–62) (FIG. 3c). Similar innate cytokine patterns, albeit with modified relative levels, are also characteristic of other viral CNS infections, including TMEV, **vesicular stomatitis virus**, HIV and **West Nile virus**^{63–65}. This indicates that the secretion of these cytokines is a general, rather than pathogen-specific or cell-type-specific, antiviral response and is consistent with their role in the subsequent activation of adaptive immunity. **TNF- α** , **IL-12** and **IL-1 β** mRNA levels increase, even in the absence of inflammation^{59,60,62}, which indicates a resident CNS cell response to MHV infection. Induction of the pleiotropic cytokine **IL-6** might enhance inflammatory-cell passage across the BBB, similar to its role in the CNS autoimmune model, experimental allergic encephalomyelitis (EAE)⁵¹.

Chemokines

Small, mostly soluble proteins that induce directional migration of innate and adaptive immune cells to sites of infection or injury.

Matrix metalloproteinases (MMPs)

Proteases that contribute to tissue remodelling, wound healing and cell trafficking. MMPs enhance the activity of cytokines and growth factors but also degrade these factors.

Tissue inhibitors of MMPs (TIMPs)

A small family of specific matrix metalloproteinase (MMP) inhibitors that regulate MMP activity.

Cytokine

A member of a large family of secreted proteins that bind immune cells through specific receptors. Cytokine production results in the activation of an intracellular-signalling cascade that commonly regulates processes such as immune function and inflammation.

Type I interferons

Interferons **IFN- α** and **IFN- β** , produced by most nucleated cells to resist viral replication. By contrast, type II interferon (**IFN- γ**) is secreted by activated T cells and NK cells and activates many responding cell types, including macrophages and microglia.

Adaptive Immunity

Represented by B and T cells that express antigen-specific receptors. Memory lymphocytes persist, providing lifetime immunity against re-infection.

Box 2 | Varied central nervous system (CNS) pathogenesis of murine hepatitis viruses

There are many different biotypes of mouse hepatitis virus (MHV), creating both confusion to the casual observer and a wealth of information on the interactions of related viruses with their natural hosts^{17,18}. The many isolates, variants and subtypes are categorized by serological relatedness, cellular tropism and molecular genetics that correlate with distinct virulence and pathogenesis. Basic tropism and pathogenesis generally reflect the original serotype: MHV-1, MHV-2, MHV-3 and MHV-A59 are generally hepatotropic; whereas the John Howard Mueller (JHMV) and MHV-4 strains are generally neurotropic. Plasticity of MHV pathogenesis has long been apparent, based on plaque-size variants¹⁰⁷. The most detailed genetic and biological information is available for the relatively neurotropic JHMV and the dual hepato- and neurotropic A59 strains. Data have focused on the spike (S) protein, as JHMV variants with S-protein alterations or deletions show different tropism^{24–26} and spread³⁴. Recombinants that express distinct S proteins on heterologous viral backgrounds confirm that the **JHMV S protein** confers neurovirulence²⁶, although JHMV background genes override **A59 S protein** in determining hepatotropism¹⁰⁸. Deletion of a dominant CD8⁺ T-cell epitope from the S protein, characteristic of several less pathogenic strains and variants, further complicates pathogenesis. In general, more virulent strains infect neurons, rapidly leading to fatal encephalitis. Less pathogenic strains are predominantly glial-tropic, leading to persistent CNS infection.

Efforts to correlate enhanced neurovirulence with immunity revealed no correlation with **TNF- α** , **CCL5** or **CCL2** mRNA expression^{61,109}. However, lethal JHMV infection induces higher **IL-6**, **IL-1 α** , **IL-1 β** , **IFN- β** , **CCL3**, **CCL4** and **MIP-2** levels compared with MHV-A59 infection, which is characterized by prominent **IFN- γ** mRNA induction^{61,109}. Surprisingly, **IL-6** induction correlates with JHMV background genes, rather than with the S protein¹⁰⁹. In contrast to the unique chemokine and cytokine induction associated with infection of the central nervous system by JHMV and MHV-A59, non-lethal JHMV variants that differ only in S protein induce no significant differences, except for the magnitude of the immune response⁶⁰. JHMV/A59 recombinants using a highly virulent JHMV parent are providing insights into the role of the S protein and the other viral proteins in pathogenesis^{26,28,109}.

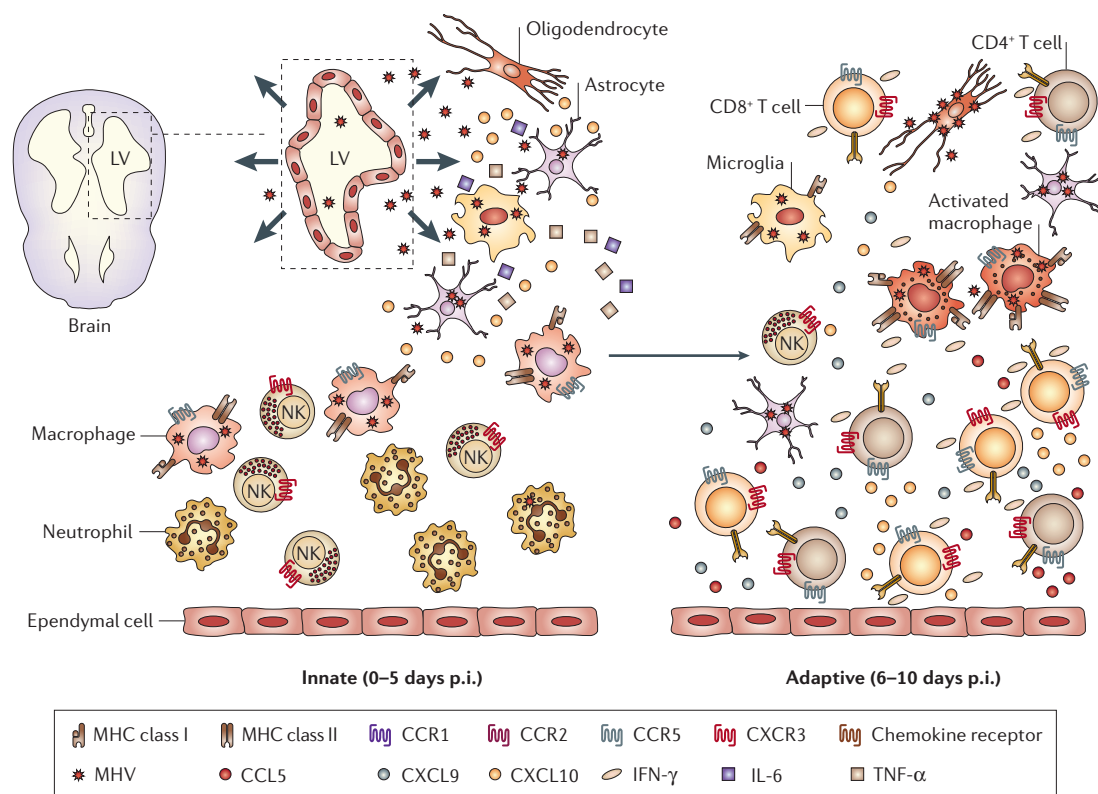


Figure 2 | Kinetics of viral spread and central nervous system (CNS) tropism. Overview of viral spread following intracranial inoculation of mouse hepatitis virus (MHV) into susceptible mice. Ependymal cells lining the lateral ventricles (LV) are the initial targets of replication, followed by spread of virus into the parenchyma and viral infection of resident glial cells of the CNS — astrocytes, oligodendrocytes and microglia. Early during acute infection, the inflammatory infiltrate consists primarily of innate components, that is, neutrophils, macrophages and natural killer (NK) cells, which presumably respond to proinflammatory signals such as TNF- α , IL-6 and CXCL10 released by glia. These proinflammatory signals enhance trafficking and accumulation of cells within the CNS. The adaptive stage of acute infection is characterized by rapid spread of virus throughout the parenchyma and increased infiltration of virus-specific CD4⁺ and CD8⁺ T cells that secrete IFN- γ , and subsequently increase expression of additional proinflammatory chemokines such as CXCL9, CXCL10 and CCL5 from astrocytes as well as inflammatory cells. Accumulation of virus-specific T cells, especially CD8⁺ T cells, ultimately results in a decrease in virus replication in glia. As virus replication is controlled, the number of inflammatory cells decreases, but viral persistence is associated with the retention of immune effectors in the CNS.

Two rapidly induced antiviral molecules, TNF- α and nitric oxide synthase-2 (iNOS, the inducible NOS isoform), which influence immunity to other CNS viral infections^{62–65}, seem to have no role in the anti-MHV host response. Although iNOS mRNA levels increase in the CNS of MHV-infected immunocompromised mice and although iNOS suppresses virus replication *in vitro*⁶⁶, no role for iNOS in controlling CNS virus replication was detected *in vivo*^{67,68}. The reduced mortality of MHV-infected iNOS-deficient mice might be due to its contribution to neuronal apoptosis⁶⁹. Despite increased TNF- α transcription during MHV infection *in vitro* and *in vivo*, translation is inhibited in MHV-infected cells⁷⁰. However, TNF- α is produced by uninfected microglia within the inflamed CNS, indicating that translation might only be impaired in the minor fraction of MHV-infected microglia *in vivo*⁷⁰. In contrast to its role as an antiviral effector and mediator of myelin loss during EAE⁷¹, neither MHV replication *in vivo* nor CNS pathology are altered in the absence of TNF- α ^{70,72}.

The adaptive immune response

Virus replication and spread increases despite the innate response, although innate immunity facilitates the induction, recruitment and effector function of adaptive immune components. Accumulation of virus-specific T cells, especially the CD8⁺ T-cell component, correlates with a marked decrease in virus replication in astrocytes, microglia, macrophages and oligodendrocytes. Distinct antiviral mechanisms control virus replication in a CNS-cell-type-specific manner. As virus replication is controlled, the number of inflammatory cells decreases; however, viral persistence is associated with the CNS retention of immune effectors.

Activation of naive T cells. Initial virus replication in the ependymal cells that line the cerebral ventricles³⁶ (FIG. 2) probably facilitates the activation of adaptive immune responses by drainage of antigen into the cervical lymph nodes (CLN) through the cerebrospinal fluid, which connects the CNS to the lymphatic system^{7,8}. This pathway is consistent with a model in

Innate immunity

The first line of defence after infection. Macrophages, neutrophils and natural killer cells as well as acute-phase proteins and cytokines participate partly by signalling through non-antigen-specific receptors, including Toll-like receptors.

Cerebrospinal fluid

Fluid produced by the secretory epithelium of the choroid plexus, which lines the ventricles of the brain.

CNS parenchyma

Tissue space occupied by resident cells of the CNS and separated from peripheral space by the blood–brain-barrier.

which initial virus-specific T-cell activation occurs in the CLN, followed by chemokine-directed T-cell trafficking into the CNS. By contrast, stereotactic instillation of antigens, viruses or viral vectors directly into the CNS under conditions that maintain BBB integrity elicits poor adaptive immune responses, presumably owing to the relative isolation of the CNS and immune systems^{73,74}. No detectable JHMV replication occurs at peripheral sites; however, virus-specific T cells are detected in the CLN prior to detection in the CNS or spleen⁷⁵. Although adaptive immunity seems to be initiated in the CLN, whether infectious virus or only viral antigens are present in CLN and the identity and origin

of MHV-specific antigen-presenting cells are unclear. Bone-marrow-derived circulating monocytes that are recruited into the CNS as an innate immune component might differentiate into macrophages or dendritic cells and present antigen in the CNS^{21,76}. Alternatively, antigen-presenting cells might acquire viral antigens within the CNS and subsequently enter the CLN. The latter possibility is supported by detection of cells with a dendritic-cell-like phenotype in the CNS parenchyma and CLN as early as two days p.i.⁷⁷. Therefore, it is plausible that, following phagocytosis of viral antigens and exit from the CNS, dendritic cells or macrophages in CLN provide an initial source of antigen presentation that is required for activation and expansion of virus-specific T cells.

Alterations in chemokine and cytokine patterns. Chemokine expression by infected and uninfected CNS cells and changes in receptor expression by peripherally activated adaptive immune components alter the dynamics of CNS-infiltrating cell populations. Chemokines that are expressed during the adaptive immune response to acute MHV infection include CXCL9, CXCL10, CCL2, CCL3 and CCL5, and there is corresponding expression of the chemokine receptors CCR1, CCR5 and CXCR3 (FIG. 3b) (REF. 48). This chemokine pattern in the infected CNS is not specific for MHV infection; microglia and astrocytes synthesize chemokines following infection with both RNA and DNA viruses in the absence of inflammatory cells^{63,64}. Similar to the innate immune response, CXCL10 is the prominent chemokine expressed during the adaptive phase of acute infection, consistent with an important role in promoting neuroinflammation. CXCL9 and CXCL10 attract activated NK and T cells that express CXCR3 (REFS 53,78,79). Supporting their central role in effector recruitment, inhibition of CXCL9 and CXCL10 increases MHV-induced mortality^{78,79}.

Increasing accumulation of T cells as BBB integrity becomes compromised at 6–8 days p.i. coincides with a decline in neutrophils and NK cells (FIG. 4), although it is not clear if these cells exit the CNS or die *in situ*. By contrast, macrophages persist in the CNS; however, their phenotype alters owing to increased MHC class II expression that is driven by increasing concentrations of T-cell-derived IFN- γ . Although most early T-cell infiltrates are memory T cells specific for irrelevant antigens, these are replaced by virus-specific T cells, which expand in secondary lymphoid organs and migrate into the CNS parenchyma⁸⁰. As antiviral T cells accumulate in the CNS, there is a concomitant decline in infectious virus (FIG. 3a). The reduction in CNS viral burden is reflected in the modulation of immunological markers associated with maximal viral replication. For example, chemokine transcripts that encode CXCL9, CCL2, CCL3 and CCL7 are notably reduced⁴⁸. Similarly, proinflammatory cytokines (IL-1 α , IL-1 β , IL-6, IL-12 and IFN- β) decline⁶⁰. By contrast, the T-cell chemoattractant chemokines CXCL10 and CCL5 remain elevated⁴⁸, correlating with increased T-cell recruitment and IFN- γ expression^{40,60} (FIG. 3b,c). Unexpectedly,

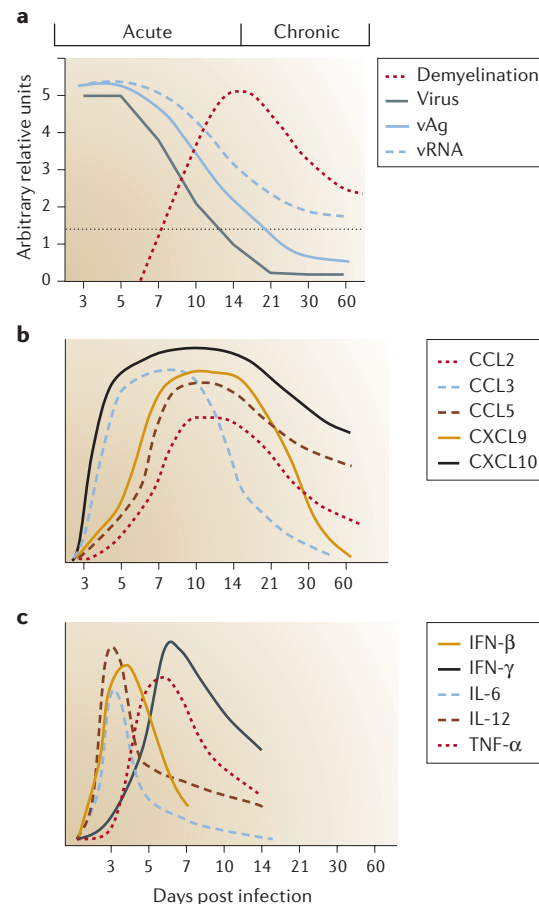


Figure 3 | Host responses in the central nervous system (CNS) associated with neurotropic coronavirus replication. **a** | John Howard Mueller (JHMV) strain replication is controlled by two weeks post infection (p.i.), but viral antigen (vAg) and viral RNA (vRNA) persist. Clearance of infectious virus is accompanied by primary demyelination, which is most severe between days 14 and 21 p.i. **b** | Overview of the relative levels of chemokine mRNA expressed within the CNS following JHMV infection of mice. A systematic analysis of the functional contributions reveals both redundant and non-redundant roles for these molecules in participating in host defence by linking innate and adaptive immune responses (CCL3), promoting T-cell infiltration (CXCL9, CXCL10 and CCL5) and macrophage accumulation (CCL2 and CCL5). **c** | Schematic of cytokine mRNA kinetics during acute JHMV infection.

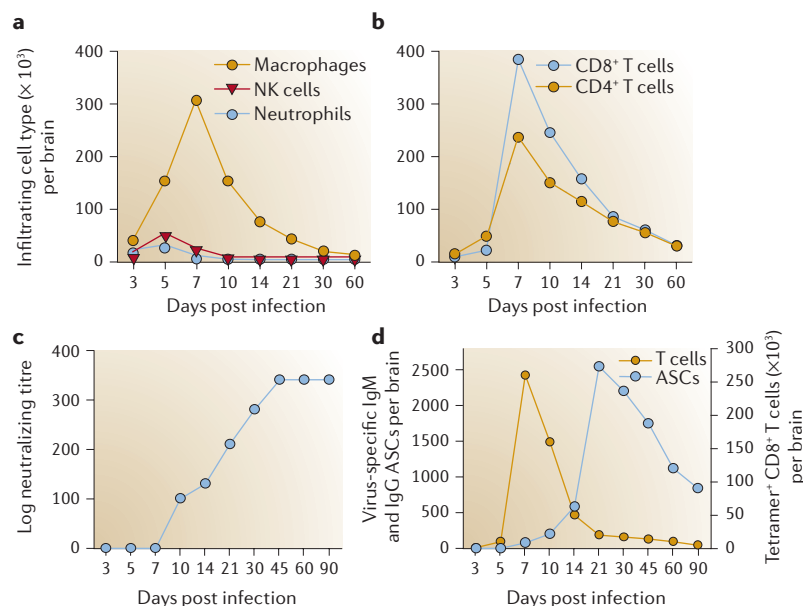


Figure 4 | Kinetics of the cellular and humoral inflammatory response to neurotropic coronavirus infection. Infiltrating cells following infection of the central nervous system (CNS) with the John Howard Mueller (JHMV) strain are identified by flow cytometric analysis. Bone-marrow-derived infiltrates are distinguished from resident cells by their CD45^{hi} phenotype and other surface markers that characterize distinct myeloid and lymphoid populations. Symbols depict representative numbers of individual cell populations within total brain cells. **a–b** | Macrophages make up the vast majority of early infiltrates up to day 5 following infection, whereas T cells are most abundant during peak inflammation and thereafter. **c** | Humoral responses emerge after infectious virus is cleared. Neutralizing antibodies in serum emerge following clearance of infectious virus and stay elevated. **d** | Virus-specific antibody-secreting cells (ASCs) do not emerge in the CNS until after infectious virus is cleared, and ASCs peak ~2 weeks after maximal T-cell inflammation. Virus-specific CD8⁺ T cells, measured by major histocompatibility complex (MHC) class I tetramer staining, decline rapidly as virus is cleared. Compared to virus-specific CD8⁺ T cells, virus-specific ASCs persist at high frequencies and decline slowly, supporting a role in preventing virus recrudescence.

TNF- α mRNA levels decrease before those of IFN- γ ⁶⁰, although virus-specific T cells can secrete TNF- α . Among its many biological activities, IFN- γ has direct antiviral activity and induces MHC expression on CNS-resident cells, facilitating interactions between immune effectors and CNS-resident cells. In the absence of IFN- γ , MHC class I expression is reduced and MHC class II remains undetectable on microglia^{46,81} and most macrophages⁸¹ during JHMV infection. Indeed, peak IFN- γ mRNA levels coincide with peak T-cell infiltration, and IFN- γ protein is functionally evident in the inflamed CNS by maximal expression of both MHC class I and II on microglia^{46,60,81}.

T-cell infiltration and antiviral effector functions. Novel concepts emerging from MHV-induced CNS infection are the differential abilities of T-cell subsets to migrate within the CNS and the crosstalk between T-cell subsets. CD4⁺ T cells cross the BBB, but instead of trafficking to parenchymal sites of virus replication, they accumulate around blood vessels⁸². By contrast, CD8⁺ T cells enter the parenchyma after migrating through the BBB. The differential ability of CD4⁺ T cells versus CD8⁺ T cells

to traffic through the infected tissue is associated with expression of TIMP-1 by CD4⁺ T cells but not CD8⁺ T cells⁴⁷. These data indicate that, rather than expression of a protease to promote migration, expression of a protease inhibitor prevents migration of CD4⁺ T cells into the CNS parenchyma. In the absence of CD4⁺ T cells, parenchymal CD8⁺ T-cell infiltration is dramatically decreased and is associated with increased apoptosis⁸², indicating that CD4⁺ T cells, either directly or indirectly, provide factors that are required for both the migration and the survival of CD8⁺ T cells within the CNS. Although IL-2 has been excluded, other survival factors remain unidentified⁸³.

During peak T-cell accumulation, most CD8⁺ and CD4⁺ T cells within the CNS are virus specific^{16,40}. Virus-specific CD8⁺ T cells accumulate to 10-fold higher frequencies in the CNS compared with the periphery and they express the CD44^{hi}, CD62L^{-/lo}, CD11a^{hi} and CD49d (VLA-4) activation/memory phenotypic markers⁴⁰, which is consistent with their crucial role in controlling acute MHV replication^{40,75}. CD43^{hi} and CD127^{-/lo} expression discriminates virus-specific CD8⁺ T cells within the CNS from those T cells specific for irrelevant antigens, which retain a CD43^{int}, CD127⁺ phenotype⁸⁰. Although the early activation marker CD69 is only transiently upregulated early during priming and expansion of T cells in secondary lymphoid organs, CD8⁺ T cells recruited into the CNS during JHMV infection retain CD69 expression⁴⁰, consistent with other CNS-inflammation models⁸⁴.

Virus-specific CD8⁺ T cells isolated from the acutely inflamed CNS secrete IFN- γ , express granzyme B and are efficient cytolytic effectors^{40,85}. These T cells accumulate within the CNS coincident with inhibition of infectious virus, and transferred memory CD8⁺ T cells control virus replication in immunodeficient hosts^{40,46}, confirming their role as primary effectors of virus clearance. Compared with highly activated CD8⁺ T cells obtained during acute infection, virus-specific memory T cells are superior at controlling virus replication in immunodeficient hosts^{42,72}. This enigma might reflect an increased sensitivity of highly activated CD8⁺ T cells to activation-induced apoptosis, or their preferential accumulation in peripheral compartments⁸⁶.

T-cell antiviral effector mechanisms are cell-type specific. In mice deficient in perforin-mediated cytotoxicity, viral replication is uncontrolled in macrophages, microglia and astrocytes³⁸. However, infection of oligodendrocytes is controlled in the absence of cytotoxicity³⁸. These results indicate that an effector mechanism distinct from MHC class I recognition by CD8⁺ T cells controls virus replication in oligodendrocytes. By contrast, the absence of the Fas/FasL cytolytic pathway does not alter pathogenesis, virus clearance or pathology⁸⁷. In IFN- γ -deficient mice that are competent for perforin-mediated cytotoxicity, virus replication is controlled in astrocytes and microglia, but not oligodendrocytes³⁹. The distinct use of effector mechanisms in the control of viral replication by CD8⁺ T cells was confirmed by adoptive transfer of CD8⁺ T cells deficient in either

Perforin-mediated cytotoxicity

The release of perforin and granzymes from stored granules within cytotoxic T cells and NK cells on contact with target cells, resulting in death, primarily by apoptosis.

Fas/FasL cytolytic pathway

Signalling pathway evoked by binding of Fas ligand (FasL) on surface of effector cell to Fas (a member of the TNF receptor family) expressed on surface of target cells, inducing apoptosis of the Fas-bearing cell.

cytolytic activity or IFN- γ secretion into infected immunodeficient hosts^{40,46}. Furthermore, infection of mice with a selective defect in IFN- γ signalling in oligodendrocytes confirms that direct IFN- γ signalling is required to control oligodendrocyte infection⁸⁸. These data support the concept that the mechanisms of CD8⁺ T-cell-dependent control of virus replication are cell-type dependent.

Pathway to persistent infection

After infectious MHV is eliminated at ~2 weeks p.i., inflammatory cells, viral antigen and viral mRNA persist in the CNS (FIG. 3a). Virus-specific CD8⁺ T-cell cytolytic activity is rapidly lost by day 14 p.i., as viral-antigen concentrations decrease^{40,85}. Whether the loss of cytolytic function is due to decreased antigen⁸⁹ or reflects an attempt to limit the potential adverse effects of cytolysis on CNS cells is not clear.

The contribution of CD8⁺ T-cell escape variants to persistent infection depends on mouse strain, age and immune status. Little evidence for escape mutants has been detected during virus persistence in naive mice infected as adults⁴⁵ or in mice undergoing reactivation owing to the absence of humoral immunity³⁸. Nevertheless, progressive accumulation of viral quasi-species with deletions in the S-protein hypervariable domain, which contains the immunodominant H-2^b CD8⁺ T-cell epitope, was found in persistently infected H-2^b mice. Secondary-structure analysis indicated that the deleted regions reside in an RNA stem-loop structure that forms a 'hot spot' for RNA recombination⁹⁰, questioning the extent to which the S mutants emerged from immune pressure. Mutations in this S-protein epitope were clearly associated with increased infectious virus in the CNS following infection of neonatal mice protected by maternal antibody⁹¹. A potential for immune escape was also shown when pre-immune mice that harboured CD8⁺ T cells specific for a novel epitope were challenged with the recombinant MHV-A59 strain that expressed the same epitope²⁷. Taken together, these data indicate that T-cell escape variants do not have a prominent role in the persistence of virus after infection of naive adult mice, but might readily emerge in genome regions that do not affect viral fitness, especially under conditions of pre-existing antibody or T-cell memory.

CD8⁺ T cells that are found in persistent CNS MHV infection are not impaired in IFN- γ secretion, which indicates that loss of cytolytic function is not due to the induction of an anergic state. However, impaired virus-induced TNF- α secretion by CD8⁺ T cells during both acute infection and persistence⁸⁵ indicates that T-cell retention within the CNS might be due to decreased secretion of apoptosis-inducing factors. The loss of CD8⁺ T-cell-mediated cytolysis during resolution of primary MHV infection and throughout persistence contrasts with the retention of cytolytic effector function in reactivated T-memory cells following neurotropic influenza-virus challenge⁸⁴. However, increased granzyme B levels in reactivated MHV-specific memory CD8⁺ T cells, compared with primary CD8⁺ T cells isolated from the CNS following challenge, supported the retention of intrinsic

cytolytic function⁸⁵. These data show that the loss of virus-specific cytolytic function is not an intrinsic property of the inflamed CNS environment, but reflects distinct differentiation states of primary CD8⁺ T cells compared with vaccine-induced memory CD8⁺ T cells.

Virus-specific T cells decline markedly between 10 and 21 days p.i., but are retained for at least 3 months following clearance of infectious virus^{43,85}. The initial T-cell decline in the CNS is similar to, but not as prominent as, the decline of T-cell effector populations in peripheral lymphoid organs following antigen elimination and withdrawal of cytokine survival factors^{92,93}. Nevertheless, CNS retention of small numbers of both CD4⁺ and CD8⁺ T cells^{40,43} indicates that the myelin-loss characteristic of the persistent phase of MHV infection is associated with a continuing immune response, sustained by low-level oligodendrocyte infection. Sustained CD69 expression also distinguishes CD8⁺ T cells that are retained within the CNS from resting peripheral memory cells in lymphoid organs, and suggests chronic activation⁴⁰ or an effector memory phenotype characteristic of memory T cells residing in non-lymphoid tissues⁹². Antigen-driven T-cell persistence was indicated by the limited T-cell-receptor specificities found in CD8⁺ T-cell populations isolated during MHV persistence compared with T cells isolated during acute infection⁹⁴. Complete disappearance of both CD8⁺ and CD4⁺ T cells from the CNS following infection with a neurotropic MHV⁴³ not associated with viral persistence or myelin loss²⁵ supports a role for viral persistence or continuing pathology in maintaining T-cell retention.

The contribution of local proliferation or ongoing recruitment to the T-cell population that persists in the CNS remains unclear. Indeed, IL-15, which regulates antigen-independent homeostasis of memory cells in lymphoid organs⁹³, is not required for CD8⁺ T-cell retention in the CNS (C.C.B., unpublished data). Adoptive transfer of CD8⁺ T cells into persistently infected mice further indicates that there is limited recruitment to the CNS compared with the acute phase (C.C.B., unpublished data). These data are consistent with the recent observations that memory T cells traffic poorly into the CNS⁹² and that activated T cells recruited in response to acute infection are only retained within the CNS on cognate antigen recognition^{2,7,8}. Overall, analysis of persistent MHV infection indicates that CD8⁺ T-cell turnover within the CNS is limited and does not comprise significant ongoing peripheral recruitment.

Humoral effectors and control of CNS persistence

Serum antibody that is present prior to MHV infection, either due to systemic administration or immunization, provides protection, although not necessarily by inhibition of virus replication. Virus-neutralizing antibody and antibodies with no apparent neutralizing activity modify MHV-induced CNS disease if passively transferred prior to infection^{17,18}. Transport of neutralizing antibody into the CNS parenchyma owing to the loss of BBB integrity⁵⁰ might limit the replication of challenge virus by inhibiting receptor binding. A complement-dependent role in

Energy

State of non-responsiveness of T cells and B cells characterized by their inability to respond to specific antigen under optimal stimulation conditions.

protection for antibodies lacking neutralizing activity is less clear⁹⁵, although at least one nucleocapsid-protein-derived epitope is expressed on the MHV-infected cell surface⁹⁶, providing a potential recognition structure.

Antibody responses in infected naive animals are delayed relative to the vigorous cell-mediated immune response (FIG. 4c,d). Serum antibody, including neutralizing antibody, is virtually undetectable and predominantly limited to IgM prior to the complete elimination of infectious virus (FIG. 4c,d). Furthermore, mice that lack humoral immunity control CNS-infectious virus with kinetics similar to immunocompetent mice, accompanied by a normal inflammatory response during acute infection^{97,98}. These data are consistent with the concept that control of acute infection is independent of humoral immunity^{17,18}. However, in contrast to wild-type mice that recover, mice that are unable to secrete antibody show increased mortality after resolution of acute disease, associated with the re-emergence of infectious virus within the CNS^{97,98}. Interestingly, the A59 strain of MHV, which infects both the liver and CNS, fails to reactivate in the liver in the absence of humoral immunity⁹⁹. Whether this is due to the absence of viral persistence in liver or reflects a fundamental difference in immune control in these two organs is not clear. Passive transfer of neutralizing, but not non-neutralizing, viral-specific antibody into B-cell-deficient mice following initial virus clearance prevents virus reactivation, confirming the crucial role of antibody in regulating CNS viral persistence¹⁰⁰. The inability of transferred non-neutralizing antibody to prevent virus recrudescence is inconsistent with the apparent protective role for non-neutralizing antibody prior to infection. Interestingly, infectious virus reactivates as passive antibody levels decline, supporting a requirement for CNS retention of antibody-secreting cells (ASCs) in providing long-term control of persistence.

MHV-specific ASCs accumulate rapidly after control of infectious virus during persistence¹⁰¹ (FIGS 4c,5). Although ASCs that are not specific for MHV are present in the CNS during the virus-clearance phase, only a few virus-specific ASCs are detectable in either the CNS or peripheral lymphoid system during acute infection¹⁰¹, consistent with the inability to detect serum antibody. Both populations are retained after virus is cleared¹⁰¹. The preceding peak of virus-specific IgG ASCs in CLN ~1 week prior to peak CNS accumulation indicates initial ASC activation and differentiation in CLN and spleen prior to CNS migration. Virus-specific ASCs are retained in the CNS at high frequencies for at least 3 months p.i., indicating that ASC-specific survival factors are present in the CNS during viral persistence. Despite their progressive decline, virus-specific ASCs are maintained at higher levels than virus-specific T cells^{40,101}. The CNS has been shown to be a survival niche for ASCs following Sindbis-virus- and Semliki-Forest-virus-induced encephalitis^{102,103}. The accumulation and maintenance of virus-specific ASCs in the CNS, coupled with reactivation of infectious virus in the absence of antibody, indicates that antibody secretion within the CNS, and not T-cell immunity, is crucial for the control of MHV CNS persistence.

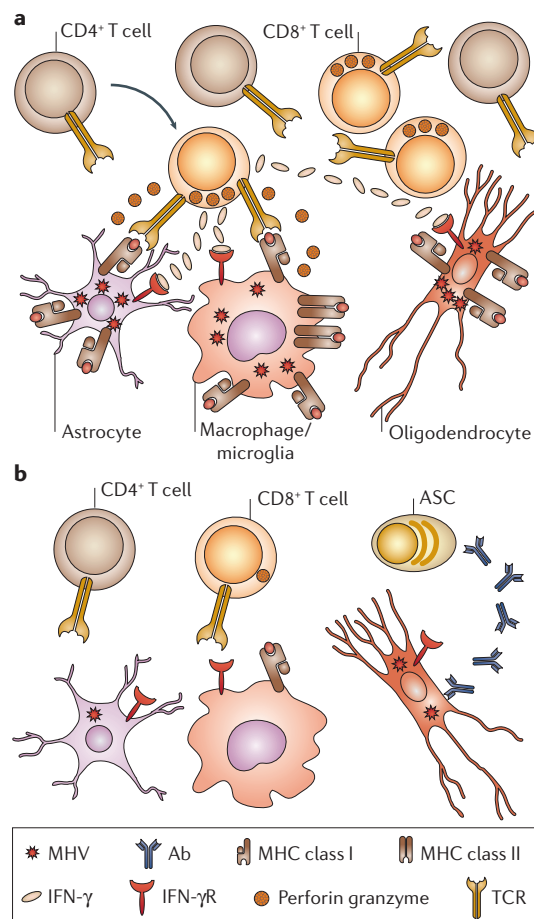


Figure 5 | Distinct immune effector mechanisms control acute and persistent infection. **a** | CD8⁺ T cells are crucial for elimination of replicating virus during acute infection. The direct antiviral role of CD4⁺ T cells is unclear; however, they enhance CD8⁺ T-cell survival and function by an unknown mechanism. Whereas perforin-mediated mechanisms, in the absence of IFN-γ, control virus in astrocytes and microglia, IFN-γ is crucial for reducing infection in oligodendrocytes. Major histocompatibility complex (MHC) class I expression on astrocytes is postulated, as perforin-mediated cytotoxicity requires class I expression; however, this has not been demonstrated *in vivo*. Increased IFN-γ release by T cells during interaction with virus-infected targets enhances class I expression on all glial cells and induces class II expression on microglia, therefore further enhancing target–T-cell interactions. **b** | As viral antigen is cleared, CD8⁺ T cells lose cytolytic function and virus persists predominantly in oligodendrocytes. IFN-γ secretion decreases, MHC is downregulated and T cells decline but persist at low frequencies. Virus-specific antibody-secreting cells are crucial to prevent virus recrudescence. Ab, antibody; IFN-γR, IFN-γ receptor; MHV, mouse hepatitis virus; TCR, T-cell receptor.

Conclusions and future perspectives

Analysis of the MHV model highlights the diversity of immune responses that is required to prevent subsequent pathology associated with a persistent infection confined to a single target organ. This model supports a paradigm in which cell-mediated immunity affects

clearance of infectious virus through mechanisms that are dictated by the specific cell type within the infected tissue (FIG. 5). Although effective in controlling acute virus replication, T cells are ultimately unable to achieve sterile immunity or suppress virus reactivation, most likely owing to downregulation or inhibition of destructive effector functions *in vivo*. However, cessation of T-cell function is complemented by a wave of virus-specific ASCs that are recruited into the CNS following resolution of acute infection. In contrast to T cells, ASCs are maintained within the CNS at high frequencies during virus persistence. These data indicate that local secretion of neutralizing antibody within the CNS maintains virus at low levels, thereby providing a protective *in situ* effector system preventing virus recrudescence (FIG. 5).

Many issues related to neurotropic MHV infections remain unresolved. Contributions of alternative receptors, co-receptors or receptor-independent spread to tropism and pathogenesis are still elusive. Viral components involved in cell signalling through viral receptors, Toll-like receptors or type I IFN pathways are also largely unexplored. Distinct MHV isolates, combined with powerful new genetic tools^{26–28}, promise to shed light on these pathways. Differential cell susceptibility to antiviral mechanisms also requires further investigation^{38,39,46}. Specifically, the ability of mature glial cells to present viral antigens¹⁰⁴, regulation of ligands affecting lymphocyte function, and factors involved in apoptosis are of interest. Similarly, the responsiveness of resident CNS cells to IFNs *in vivo* is largely unknown⁸⁸. The role

of dendritic cells during virus-induced CNS inflammation, as well as CD4⁺ T-cell contributions to CD8⁺ T-cell function within the CNS⁸², also requires evaluation. Last, an intriguing question is how, and in what form, virus persists, although a replication-competent form is implicated by virus recrudescence in the absence of humoral immunity^{97,98}. Resolving mechanisms of viral persistence might also elucidate events associated with ongoing immune activation and T-cell and ASC retention, all potentially contributing to demyelinating disease.

Acute, potentially lethal viral infections of the human CNS, for example, West Nile virus and **Saint Louis encephalitis virus**, primarily target neurons¹⁰⁵. Other human viruses, for example, herpes viruses, target and remain latent in neurons. HIV and JC polyomavirus primarily target other CNS cell types and are prone to producing latent or persistent CNS infections^{9,12,13}. Although it is unclear how SARS-virus CNS replication contributes to pathogenesis, recent data also confirm CNS virus infection¹⁰⁶.

Coronavirus infection of the CNS has provided unique insights into the immune regulation of acute and persistent infection at the cellular level of a natural rodent pathogen, and provides a model for studying chronic demyelinating diseases, such as multiple sclerosis. Delineation of the dynamic interactions that regulate acute and persistent infections of the CNS has implications for vaccine design as well as for the development of novel immunotherapeutics to limit viral replication and attenuate the potential damaging effects of the immune response within the CNS.

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Competing interests statement

The authors declare no competing financial interests.

DATABASES

The following terms in this article are linked online to:

Entrez Genome: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=genome>

HTLV-1 | Japanese encephalitis virus | JC polyomavirus | JHMV | lymphocytic choriomeningitis virus | measles virus | MHV-A59 | poliovirus | rabies virus | Saint Louis encephalitis virus | Sindbis virus | TMEV | vesicular stomatitis virus | West Nile virus

UniProtKB: <http://us.expasy.org/uniprot>

A59 S protein | CEACAM-1 | CCL2 | CCL3 | CCL4 | CCL5 | CCL7 | CCR1 | CCR5 | CD45 | CD69 | CXCL9 | CXCL10 | CXCR3 | granzyme B | IFN- β | IFN- γ | IL-1 α | IL-1 β | IL-2 | IL-6 | IL-15 | iNOS | JHMV S protein | MIP-2 | MMP-3 | MMP-9 | MMP-12 | spike protein | TIMP-1 | TNF- α

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