CORONAVIRUS IMMUNITY
From T cells to B cells

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1. INTRODUCTION

Coronavirus infections affect a variety of organs including the respiratory and enteric tracts as well as the central nervous system (CNS). Infections of the CNS provide a unique challenge to the host. Rapid responses are vital to control the pathogen yet also affect the communication network between highly specialized cells that control all host cognitive and vital functions.[1-3] CNS resident cells express few, if any, major histocompatibility complex (MHC) molecules in the quiescent state, thus minimizing the potential to activate T cells. Immunological activity in the CNS is also restrained by the absence of a dedicated lymphatic drainage system and constitutive secretion of neurotrophins and TGF-β.[3-5] Lastly, tight junctions between endothelial cells associated with the blood-brain barrier (BBB) and the limited expression of adhesion molecules limit large molecules, e.g., antibodies, as well as T cells from entering the CNS.[2, 5] Although a small number of activated/memory T cells randomly patrol the CNS in the absence of 'danger' signals, they disappear in the absence of antigen recognition.[2] This quiescent steady state contrasts dramatically with the vigorous inflammatory responses induced following many CNS infections.[3, 4] Infections of the murine CNS by neurotropic coronaviruses provide excellent model systems that illustrate the interactions of innate immune responses with adaptive host effector mechanisms and the control of virus replication at the cost of immune pathology.

Infection by the neurotropic mouse hepatitis virus (MHV) strain JHMV produces an acute demyelinating encephalomyelitis in mice.[6-8] Survivors have no detectable infectious virus by approx. 2 weeks postinfection (p.i.), yet viral antigen and more prominently RNA remain detectable exclusively within the CNS up to 2 years p.i. Despite exploitation of various T cell–mediated functions to control acute virus infection in distinct cell types, host regulatory mechanisms, presumably designed to protect CNS integrity, contribute to the failure to eliminate virus. An enigma has been the inability to isolate persisting virus

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from CNS explants or from immunosuppressed mice perfused to remove neutralizing antibody (Ab) prior to explant. Although survivors exhibit little or no clinical abnormalities, histological examination shows ongoing primary CNS demyelination, similar to the pathological changes associated with multiple sclerosis. Clues that virus may persist in a replication competent form were provided by mice genetically impaired in anti-viral Ab production.[9,10] Despite initial effective clearance of infectious virus, virus recrudesced in the absence of anti-viral Ab. The majority of data discussed in this review pertains to a monoclonal Ab neutralization JHMV escape mutant, designated 2.2v-1.[11] Microglia, astrocytes, and oligodendroglia are primary targets of infection. Neurons are only rarely infected, sparing mice from death due to neuronal dysfunction. Survival and demyelination associated with chronic infection is thus significantly enhanced. Furthermore, hepatitis, potentially interfering with CNS specific immune responses, is extremely rare after intracerebral (i.c.) infection.

2. LINK BETWEEN INNATE AND ADAPTIVE RESPONSES

JHMV CNS infection induces rapid, coordinated expression of chemokines, matrix metalloproteinases (MMPs), the tissue inhibitor of MMPs (TIMP-1), and pro-inflammatory cytokines.[12-18] MMP activation is associated with several physiological processes, including the influx of inflammatory cells into tissues, activation of cytokines, and CNS pathology.[19] Neutrophils, macrophages, and natural killer (NK) cells are the initial inflammatory cells recruited into the MHV-infected CNS.[20, 21] Secretion of pre-packaged MMP-9 by neutrophils contributes to a loss of BBB integrity and facilitates subsequent entry of inflammatory cells into the infected CNS.[20] JHMV infection itself only induces a subset of MMPs in CNS resident cells, namely MMP-3 and MMP-12.[18, 22] However, their roles in cell migration or pathology are unclear. A potential mechanism for counteracting MMP-mediated CNS pathology may reside in sustained elevation of mRNA encoding the MMP inhibitor, TIMP-1.[18, 19]

The earliest chemokines induced following infection are CXCL10 and CCL3.[12, 23] CXCL10 overexpression in the CNS using a recombinant MHV revealed that excessive NK cell accumulation in immunodeficient RAG1$^{-/-}$ mice may contribute to virus clearance.[24] By contrast, despite early NK cell recruitment, there is little evidence of a direct anti-viral role to combat wildtype virus. Their potential to secrete IFN-γ may, however, facilitate antigen presentation via upregulation of MHC class I and class II molecules. Increased CCL3 expression appears to link the innate and adaptive immune responses by stimulating recruitment, activation, and maturation of dendritic cells and T cells.[23, 25] Accumulation of macrophages, comprising the largest fraction of innate infiltrates, is enhanced by CCL5.[12,26,27] Cytokines rapidly induced within the MHV infected CNS include IL-1α, IL-1β, IL-6, and IL-12.[13-16] These cytokines vary in expression levels, but not overall pattern between distinct MHV variants.[15] TNF-α, IL-12, and IL-1β are induced in resident CNS cells in response to viral infection.[13,16] The absence of TNF-α neither alters MHV replication *in vivo* nor CNS pathology.[28, 29]

The innate CNS inflammatory response induced by MHV infection is succeeded by a prominent adaptive response, which peaks at 7–10 days p.i.[20,21,30,31] Although JHMV replication is undetectable at peripheral sites, virus-specific T cells are detected in the cervical lymph nodes (CLN) and spleen, prior to the CNS.[31] Initial virus replication in

ependymal cells lining the ventricles[32] is likely to facilitate the peripheral activation of adaptive immune responses by antigen drainage into the CLN via the cerebral spinal fluid.[4, 5] The early detection of cells with a dendritic cell–like phenotype in the CNS parenchyma and CLN alternatively supports acquisition of viral antigens in the CNS by antigen presenting cells, followed by migration to CLN.[25] Activation of adaptive immune components alters both the composition of CNS infiltrating cells and chemokine expression. Although CXCL9, CCL2, CCL3, CCL4, CCL5, and CCL7 are expressed during acute infection, CXCL10 is the most prominent and sustained chemokine.[12] In addition to attracting NK cells,[24] CXCL9 and CXCL10 recruit activated T cells[33,34] and potentially plasmablasts,[35] via the CXCR3 receptor. Increasing T-cell accumulation coincides with a decline in neutrophils and NK cells; however, macrophages persist in the CNS.[21] The T cell–mediated reduction in CNS viral burden results in a decline in CXCL9, CCL2, CCL3, and CCL7[12] as well as IL-1α, IL-1β, IL-6, IL-12, and IFN-β mRNA.[13] By contrast, the T-cell chemoattractants CXCL10 and CCL5 remain elevated[12] correlating with increased T-cell recruitment and IFN-γ expression.[13,30] Peak IFN-γ mRNA coincided with peak T-cell infiltration and is functionally evident by maximal expression of both MHC class I and II on microglia.[13,21,36] In the absence of IFN-γ, MHC class I expression is reduced and MHC class II remains undetectable on microglia and most macrophages.[21,36] IFN-γ is thus crucial in facilitating interactions between immune effectors and CNS resident cells.

3. T CELL–MEDIATED IMMUNE CONTROL

T cells, most prominently the $CD8^+$ subset, provide the most critical anti-viral functions.[7] $CD4^+$ T cells provide crucial accessory function by enhancing virus-specific $CD8^+$ T-cell expansion and maintaining $CD8^+$ T-cell viability within the CNS.[37,38] The distinct localization of T-cell subsets early during inflammation constitutes an enigma. $CD4^+$ T cells cross the BBB and accumulate around blood vessels.[37] By contrast, $CD8^+$ T cells migrate into the parenchyma potentially guided by sites of virus replication. The differential ability of T-cell subsets to traffic through the infected tissue is linked to TIMP-1 expression by $CD4^+$ but not $CD8^+$ T cells.[22] These findings implicate the novel concept that migration into the CNS parenchyma is not only controlled by proteases that promote migration but also by protease inhibitors potentially stalling migration.

Although the majority of early T-cell infiltrates are memory T cells specific for irrelevant antigens, these are replaced by virus-specific T cells.[39] During peak T-cell accumulation the majority of both $CD8^+$ and $CD4^+$ T cells within the CNS are virus-specific.[7,30] Virus-specific $CD8^+$ T cells accumulate to 10-fold higher frequencies in the CNS compared with the periphery.[30,31] This high frequency correlates with virus specific *ex vivo* cytolysis and efficient control of viral replication.[10,30,40,41] Susceptibility of astrocytes, microglia/macrophages, and oligodendroglia to distinct T-cell effector function is cell type specific.[42,43] Replication in astrocytes and microglia, but not oligodendrocytes is controlled via perforin mediated cytolysis.[42] By contrast, IFN-γ controls replication in oligodendrocytes, but is insufficient for virus elimination in astrocytes and microglia.[43] The absence of the Fas/FasL pathway does not alter virus clearance or pathology.[44] Distinct anti-viral efficiencies of T-cell effector mechanisms were confirmed in infected, immunodeficient recipients of $CD8^+$ T cells lacking IFN-γ or perforin.[21,36] Overall a more

prominent role of IFN-γ compared with perforin is evident by enhanced virus control and reduced mortality in the sole presence of either function alone.[21, 36, 42, 43] Importantly, infection of mice with an IFN-γ signaling defect selectively in oligodendroglia support a direct role for IFN-γ rather than a secondary effect in controlling oligodendroglial infection.[45] The basis for oligodendroglial resistance to perforin-mediated cytolysis is unclear, especially as MHC class I is up regulated on oligodendroglia during JHMV infection.[46] Irrespectively, perforin-mediated control of virus replication in astrocytes and macrophage/microglia implicates MHC class I–mediated cytolysis.

After infectious virus is eliminated, inflammatory cells, viral antigen, and viral mRNA persist. The inability to achieve sterile immunity suggests viral evasion from, or loss of, T-cell function. Indeed, responsiveness by virus-specific $CD8^+$ T cells is lost at the cytolytic level concomitant with virus clearance.[30, 47] Loss of function is independent of either demyelination or antigen load[10, 41] and unlikely due to anergy, as $CD8^+$ T cells are not impaired in IFN-γ secretion.[30, 47] The loss of $CD8^+$ T cell–mediated cytolysis during resolution of primary MHV infection and throughout persistence further contrasted with retention of cytolytic function in reactivated memory cells after neurotropic influenza virus challenge.[48] In an analogous study, MHV-specific memory $CD8^+$ T cells from the inflamed CNS of previously immunized mice exhibit increased IFN-γ and granzyme B production, as well as enhanced cytolysis at a single cell level compared with naïve mice after challenge.[47] Enhanced effector function resulted in more effective virus control. Importantly, reactivated memory $CD8^+$ T cells retained cytolytic function coincident with increased granzyme B levels compared with primary $CD8^+$ T cells.[47] Loss of virus-specific cytolytic function thus appears to reflect distinct differentiation states of primary compared to memory $CD8^+$ T cells rather than an intrinsic property of the inflamed CNS environment.

Despite their significant decline following clearance of infectious virus, persisting T cells are a hallmark of viral persistence and ongoing demyelination.[6, 7] The percentages of virus-specific T cells within the $CD8^+$ compartment remain remarkably stable throughout infection[10, 30, 47] suggesting indiscriminate homeostatic retention or turnover. A role for viral persistence and/or continuing pathology in maintaining T-cell retention is supported by the complete loss of T cells from the CNS after infection with a neurotropic MHV not associated with persistence or myelin loss.[40] A virus driven component was also suggested by selection of $CD8^+$ T-cell populations with limited T-cell receptor specificities during persistence compared with the acute infection.[49] Finally, virus-induced TNF-α secretion by $CD8^+$ T cells during both acute infection and persistence is low,[47] suggesting that T-cell retention within the CNS may be due to decreased secretion of apoptosis inducing factors. The contribution of local homeostatic proliferation or ongoing recruitment to T-cell maintenance in the CNS remains unclear. Preliminary evidence suggests that IL-15, which regulates antigen-independent homeostasis of memory cells in lymphoid organs,[50] is not required (Bergmann, unpublished). Memory cells traffic poorly into the CNS[51] and activated T cells recruited in response to acute infection are only retained within the CNS upon cognate antigen recognition.[2, 39] These recent observations support both very limited local turnover and peripheral recruitment.

4. HUMORAL IMMUNITY CONTROLS PERSISTENCE

A protective role of T cells during persistence is disputed by viral recrudescence in mice devoid of B cells. Mice lacking humoral immunity mount a normal inflammatory response during acute infection and control CNS infectious virus with kinetics similar to immunocompetent mice.[9, 10] However, unlike recovery of wild-type mice, mice unable to secrete Ab exhibit increased mortality associated with the reemergence of infectious virus within the CNS. By contrast, the MHV A59 strain, which infects both the liver and CNS, fails to reactivate in the liver in the absence of humoral immunity.[52] 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 unclear. Preexisting virus neutralizing Ab provides protection against MHV-induced CNS,[6] presumably by limiting virus replication after transport into the CNS parenchyma while BBB integrity is compromised.[18] Similarly, virus recrudescence in the CNS of Ab deficient mice can be prevented by transfer of polyclonal Ab at the time infectious virus is initially cleared.[9] Dissection of the specificities and mechanisms of Ab-mediated protection revealed that only spike (S) protein specific neutralizing IgG prevented recrudescence.[53] Non-neutralizing Ab specific for S, matrix, or nucleocapsid proteins had no anti-viral effect,[53] distinct from the apparent protective role for non-neutralizing Ab prior to infection.[6] Despite the initial promise of protection, antiviral control waned as transferred Ab decayed in B cell–deficient recipients.[53] These data suggested that virus is maintained in a replication competent form within the CNS and that sustained intrathecal Ab is crucial to maintain virus at undetectable levels during persistence.

Accumulation of virus-specific Ab secreting cells (ASC) within the CNS confirmed intrathecal Ab synthesis.[54] Unlike T cells, virus-specific IgG ASC accumulate prominently in the CNS approximately 1 week after clearance of infectious virus. Although virus specific ASC are barely detectable in the CNS during the virus clearance phase numerous heterologous ASC are already present, implicating nonspecific recruitment.[54] The delayed peak in virus-specific IgG ASC in the CNS compared with CLN, suggests maturation in secondary lymphoid organ germinal centers precedes migration into the CNS. Consistent with the meager presence of virus-specific ASC in either the CNS or lymphoid ogans during acute infection,[54] virus-specific serum Ab, including neutralizing Ab, is undetectable prior to the complete elimination of infectious virus. However, sustained serum Ab levels after clearance of infectious virus supports a role in controlling persistence. Virus-specific ASC are retained in the CNS at high frequencies for at least 3 months p.i. implicating ASC-specific survival factors in the CNS during viral persistence. Despite their progressive decline, virus-specific ASC are maintained at higher levels than virus-specific T cells. The CNS as a survival niche for ASC has previously been observed following other virus-induced CNS infections.[4]

5. BYSTANDER RECRUITMENT AND PATHOLOGY

Numerous approaches have been applied to assess the roles of individual effector molecules in the demyelinating process; however, a unifying mechanism has not emerged.[8] Despite their protective role in controlling acute virus replication, T cells are prominent mediators of demyelination.[6, 8, 34, 55] Immunocompromised SCID or RAG1$^{-/-}$ mice develop little if any demyelination, despite uncontrolled virus replication. Mice

deficient for IFN-γ, perforin, inducible nitric oxide synthase, $CD4^+$, $CD8^+$, or B cells all develop demyelinating disease.[6, 42, 43, 56] Similarly, immunodeficient recipients of $CD4^+$ T cells, $CD8^+$ T cells, or γδ T cells are all susceptible to immunopathology.[21,29,36,55,57,58] Interpretation is further confounded by distinct abilities of transferred populations to control virus. The common theme emerging is that recruitment of most immune cell populations as a single entity or overexpression of a specific chemokines can lead to demyelination.[59] A separate issue still unresolved is the role of bystander cells. Bystander cells of irrelevant specificity are commonly recruited by chemokines. Their activation within the CNS environment after microbial infection may influence subsequent CNS inflammation and/or enhance pathogenesis. Enhanced CNS pathology via activation of bystander $CD8^+$ T cells has recently been demonstrated after JHMV infection of LCMV-specific TCR/Rag $2^{-/-}$ mice.[60] By contrast, investigation of bystander effects in mice with a wild-type TCR repertoire demonstrated that although JHMV-induced encephalitis recruits heterologous memory T cells, they are not activated in the process.[39] The vast majority of CNS infiltrating $CD8^+$ T cells express the $CD44^{hi}$, $CD62L^{-/lo}$, $CD11a^{hi}$, and CD49d (VLA-4) activation/memory phenotypic markers.[30,39] However, $CD43^{hi}$, $CD127^{-/lo}$ expression discriminates virus-specific $CD8^+$ T cells within the CNS from cells specific for irrelevant antigens, which retain a $CD43^{int}$, $CD127^+$ phenotype. Bystander cells further did not acquire expression of *ex vivo* cytolytic activity, suggesting that bystander $CD8^+$ T-cell recruitment alone is unlikely to directly result in immune pathology in the absence of cognate or cross-reactive antigen.[39] These results are consistent with the requirement for an antigen-driven component in contributing to $CD8^+$ T cell–mediated CNS damage observed in other models.[61,62]

6. CONCLUSIONS

Several novel concepts have emerged from MHV-induced CNS infection. T-cell subsets appear to have differential abilities to migrate within the CNS. Cross-talk is indicated by enhanced $CD8^+$ T-cell function and survival in the presence of $CD4^+$ T cells. Distinct cellular targets of infection have differential susceptibility to T-cell effector function. The accumulation and maintenance of virus-specific ASC 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 absolutely critical for the control of MHV CNS persistence. Lastly, retention of both T cells and ASC in the CNS during persistence suggests myelin loss is associated with an ongoing immune response, sustained by low-level oligodendroglial infection.

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8. REFERENCES

1. Z. Fabry, C. S. Raine, and M. N. Hart, Nervous tissue as an immune compartment: the dialect of the immune response in the CNS, *Immunol. Today* **15**, 218-224 (1994).
2. W. F. Hickey, Basic principles of immunological surveillance of the normal central nervous system, *Glia* **36**, 118-124 (2001).
3. R. Dorries, The role of T-cell-mediated mechanisms in virus infections of the nervous system, *Curr. Top. Microbiol. Immunol.* **253**, 219-245 (2001).
4. D. E. Griffin, Immune responses to RNA-virus infections of the CNS, *Nat. Rev. Immunol*. **3**, 493-502 (2003).
5. R. M. Ransohoff, P. Kivisakk, and G. Kidd, Three or more routes for leukocyte migration into the central nervous system, *Nat. Rev. Immunol.* **3**, 569-581 (2003).
6. S. Stohlman, C. Bergmann, and S. Perlman, in: *Persistent Viral Infection*, edited by R. Ahmed and I. Chen (John Wiley, New York, 1999), pp. 537-558.
7. N. W. Marten, S. A. Stohlman, and C. C. Bergmann, MHV infection of the CNS: mechanisms of immune-mediated control, *Viral Immunol.* **14**, 1-18 (2001).
8. M. J. Buchmeier and T. E. Lane, Viral-induced neurodegenerative disease, *Curr. Opin. Microbiol.* **2**, 398-402 (1999).
9. M. T. Lin, D. R. Hinton, N. W. Marten, C. C. Bergmann, and S. A. Stohlman, Antibody prevents virus reactivation within the central nervous system, *J. Immunol.* **162**, 7358-7368 (1999).
10. C. Ramakrishna, S. A. Stohlman, R. Atkinson, M. Schlomchik, and C. C. Bergmann, Mechanisms of central nervous system viral persistence: critical role of antibody and B cells, *J. Immunol.* **168**, 1204-1211 (2002).
11. J. O. Fleming, M. Trousdale, F. El-Zaatari, S. A. Stohlman, and L. P. Weiner, Pathogenicity of antigenic variants of murine coronavirus JHM selected with monoclonal antibodies, *J. Virol.* **58**, 869-875 (1986).
12. T. E. Lane, V. C. Asensio, N. Yu,. A. D., Paoletti, I. L.Campbell, and M. J. Buchmeier, Dynamic regulation of alpha- and beta-chemokine expression in the central nervous system during mouse hepatitis virus-induced demyelinating disease, *J. Immunol.* **160**, 970-978 (1998).
13. B. Parra, D. R. Hinton, M. T. Lin, D. J. Cua, and S. A. Stohlman, Kinetics of cytokine mRNA expression in the CNS following lethal and sublethal coronavirus-induced encephalomyelitis, *Virology* **233**, 260-270 (1997).
14. B. D. Pearce, M. V. Hobbs, T. S. McGraw, and M. J. Buchmeier, Cytokine induction during T-cell-mediated clearance of mouse hepatitis virus from neurons in vivo, *J. Virol.* **68**, 5483-5495 (1994).
15. J. D. Rempel, S. J. Murray, J. Meisner, and M. J. Buchmeier, Differential regulation of innate and adaptive immune responses in viral encephalitis, *Virology* **318**, 381-392 (2004).
16. J. D. Rempel, L. A. Quina, P. K. Blakelu-Gonzales, M. J. Buchmeier, and D. L. Gruol, Viral induction of central nervous system innate immune responses, *J. Virol.* **79**, 4369-4381 (2005).
17. T. E. Lane and M. J. Buchmeier, in: *Universes in Delicate Balance: Chemokines and the Nervous System*, edited by R. Ransohoff, et al. (Elsevier Press, New York, 2002), pp. 191-202.
18. J. Zhou, S. Stohlman, R. Atkinson, D. Hinton, and N. Marten, Matrix metalloproteinase expression correlates with virulence following neurotropic mouse hepatitis virus infection, *J. Virol.* **76**, 7373-7384 (2002).
19. V. W. Yong, C. Power, P. Forsyth, and D. R. Edwards, Metalloproteinases in biology and pathology of the nervous system, *Nature* **2**, 502-511 (2001).
20. J. Zhou, S. Stohlman, D. R. Hinton, and N. Marten, Neutrophils modulate inflammation during viral induced encephalitis, *J. Immunol.* **170**, 3331- 3336 (2003).
21. C. C. Bergmann, B. Parra, D. Hinton, C. Ramakrishna, M. Morrison, and S. A. Stohlman, Perforin mediated effector function within the CNS requires IFN-γ mediated MHC upregulation, *J. Immunol.* **170**, 3204-3213 (2003).
22. J. Zhou, N. W. Marten, C. C. Bergmann, W. B. Macklin, D. R. Hinton, and S. A. Stohlman, Expression of matrix metalloproteinases and their tissue inhibitor during viral encephalitis, *J. Virol*. **79**, 4764-4773 (2005).
23. M. J. Trifilo, C. C. Bergmann, W. A. Kuziel, and T. E. Lane, CC chemokine ligand 3 (CCL3) regulates $CD8^+$-T-cell effector function and migration following viral infection, *J. Virol.* **77**, 4004-4014 (2003).
24. M. J. Trifilo, C. Montalto-Morrison, L. N. Stiles, K. R. Hurst, J. L. Hardison, J. E. Manning, P. S. Masters, and T. E. Lane, CXC chemokine ligand 10 controls viral infection in the central nervous system: Evidence for a role in innate immune response through recruitment and activation of natural killer cells, *J. Virol.* **78**, 585-594 (2004).

25. M. J. Trifilo and T. E. Lane, The CC chemokine ligand 3 regulates CD11c+CD11b+CD8alpha-dendritic cell maturation and activation following viral infection of the central nervous system: implications or a role in T cell activation, *Virology* **327**, 8-15 (2004).
26. T. E. Lane, M. T. Liu, B. P. Chen, V. C. Asensio, R. M. Samawi, A. D. Paoletti, I. L. Campbell, S. L. Kunkel, H. S. Fox, and M. J. Buchmeier, A central role for CD4(+) T cells and RANTES in virus-induced central nervous system inflammation and demyelination, *J. Virol.* **74**, 1415-1424 (2000).
27. W. G. Glass, M. J. Hickey, J. L. Hardison, M. T. Liu, J. E. Manning, and T. E. Lane. Antibody targeting of the CC chemokine ligand 5 (CCL5) results in diminished leukocyte infiltration into the central nervous system and reduced neurologic disease in a viral model of multiple sclerosis, *J. Immunol.* **172**, 4018-4025 (2004).
28. S. A. Stohlman, D. R. Hinton, D. Cua, E. Dimacali, J. Sensintaffar, S. Tahara, F. Hofman, and Q. Yao, Tumor necrosis factor expression during mouse hepatitis virus induced demyelination, *J. Virol.* **69**, 5898-5903 (1995).
29. L. Pewe and S. Perlman, Cutting edge: CD8 T cell-mediated demyelination is IFN-gamma dependent in mice infected with a neurotropic coronavirus, *J. Immunol.* **168**, 1547-1551 (2002).
30. C. C. Bergmann, J. Altman, D. Hinton, and S. A. Stohlman, Inverted immunodominance and Impaired cytolytic function of CD8+ T cells during viral persistence in the CNS, *J. Immunol.* **163**, 3379-3387 (1999).
31. N. Marten, S. A. Stohlman, Z. Zhou, and C. C. Bergmann, Kinetics of virus specific CD8+ T cell expansion and trafficking following central nervous system infection, *J. Virol.* **77**, 2775-2778 (2003).
32. F. I. Wang, D. R. Hinton, W. Gilmore, M. D. Trousdale, and J. O. Fleming, Sequential infection of glial cells by the murine hepatitis virus JHM strain (MHV-4) leads to a characteristic distribution of demyelination, *Lab. Invest.* **66**, 744-754 (1992).
33. M. T. Liu, B. P. Chen, P. Oertel , M. J. Buchmeier, D. Armstrong , T. A. Hamilton, and T. E. Lane, The T cell chemoattractant IFN-inducible protein 10 (IP-10) is essential in host defense against viral-induced neurologic disease, *J. Immunol.* **165**, 2327-2330 (2000).
34. M. T. Liu, H. S. Keirstead, and T. E. Lane, Naturalization of the chemokine CXCL10 reduces inflammatory cell invasion and demyelination and improves neurological function in a viral model of multiple sclerosis, *J. Immunol.* **167**, 4091-4097 (2001).
35. A. E. Hauser, G. F. Debes, S. Arce, G. Cassese, A. Hamann, A. Radbruch, and R. A. Manz, Chemotactic responsiveness toward ligands for CXCR3 and CXCR4 is regulated on plasma blasts during the time course of a memory immune response, *J. Immunol.* **169**, 1277-1282 (2002).
36. C. C. Bergmann, B. Parra, D. R. Hinton, C. Ramakrishna, K. C. Dowdell, and S. Stohlman, Perforin and interferon gamma mediated control of coronavirus central nervous system infection by CD8 T cells in the absence of CD4 T cells, *J. Virol.* **78**, 1739-1750 (2004).
37. S. A. Stohlman, C. C. Bergmann, M. T. Lin, D. J. Cua, and D. R. Hinton, CTL effector function within the CNS requires CD4+ T cells, *J. Immunol.* **160**, 2896-2904 (1998).
38. J. Zhou, D. R. Hinton, S. A. Stohlman, and N. Marten, Maintenance of CD8+ T cells during acute viral infection of the central nervous system requires CD4+ T cells but not interleukin-2, *Virol. Immunol.* **18**, 162-169 (2005).
39. A. M. Chen, N. Khanna, S. A. Stohlman, and C. C. Bergmann, Virus-specific and bystander CD8 T cells recruited during virus-induced encephalomyelitis, *J. Virol.* **79**, 4700-4708 (2005).
40. N. Marten, S. Stohlman, and C. C. Bergmann, Role of Viral Persistence in Retaining CD8+ T cells within the central nervous system, *J. Virol.* **74**, 7903-7910 (2000).
41. N. W. Marten, S. A. Stohlman, R. Atkinson, D. A. Hinton, and C. C. Bergmann, Contributions of $CD8^+$ T cells and viral spread to demyelinating disease, *J. Immunol.* **164**, 4080-4088 (2000).
42. M. Lin, S. Stohlman, and D. Hinton, Mouse hepatitis virus is cleared from the central nervous system of mice lacking perforin-mediated cytolysis, *J. Virol.* **71**, 383-391 (1997).
43. B. Parra, D. R. Hinton, N. Marten, C. C. Bergmann, M. Lin, C. Yang, and S. A. Stohlman, Gamma interferon is required for viral clearance from central nervous system oligodendroglia, *J. Immunol.* **162**, 1641-1647 (1999).
44. B. Parra, M. Lin, S. Stohlman, C. Bergmann, R. Atkinson, and D. Hinton, Contributions of Fas-Fas ligand interactions to the pathogenesis of mouse hepatitis virus in the central nervous system, *J. Virol.* **74**, 2447-2450 (2000).
45. J. M. Gonzalez, C. C. Bergmann, B. Fuss, D. R. Hinton, W. B. Macklin, and S. A. Stohlman, Expression of a dominant negative IFN-γ receptor on mouse oligodendrocytes, *Glia* **51**, 22-34 (2005).
46. J. M. Redwine, M. J. Buchmeier, and C. F. Evans, In vivo expression of major histocompatibility complex molecules on oligodendrocytes and neurons during viral infection, *Am. J. Pathol.* **159**, 1219-1224 (2001).
47. C. Ramakrishna, S. Stohlman, R. Atkinson, D. H. Hinton, and C. C. Bergmann, Differential regulation of primary and secondary CD8+ T cells in the CNS, *J. Immunol.* **173**, 6265-6273 (2004).

48. S. Hawke, P. G. Stevenson, S. Freeman, and C. R. M. Bangham, Long term persistence of activated cytotoxic T lymphocytes after viral infection of the central nervous system, *J. Exp. Med.* **187**, 1575-1582 (1998).
49. N. Marten, S. Stohlman, W. Smith-Begolka, S. Miller, M. Dimicali, Q. Yao, S. Stohl, J. Goverman, and C. Bergmann, Selection of CD8+ T cells with highly focused specificity during viral persistence in the central nervous system, *J. Immunol.* **162**, 3905-3914 (1999).
50. D. Masopust and R. Ahmed, Reflections on CD8 T-cell activation and memory, *Immunol. Res.* **29**, 151-160 (2004).
51. L. Lefrancois and D. Masopust, T cell immunity in lymphoid and non-lymphoid tissues, *Curr. Opin. Immunol.* **14**, 503-508 (2002).
52. A. E. Matthews, S. R. Weiss, M. J. Shlomchik, L. G. Hannum, J. L. Gombold, and J. Y. Paterson, Antibody is required for clearance of infectious murine hepatitis virus A59 from the central nervous system, but not the liver, *J. Immunol.* **167**, 5254-5263 (2001).
53. C. Ramakrishna, C. Bergmann, R. Atkinson, and S. Stohlman, Control of central nervous system viral persistence by neutralizing antibody, *J. Virol.* **77**, 4670-4678 (2003).
54. S. I. Tschen, C. Bergmann, C. Ramakrishna, R. Atkinson, and S. Stohlman, Recruitment kinetics of antibody secreting cells within the CNS following viral encephalomyelitis, *J. Immunol.* **168**, 2922-2929 (2002).
55. G. F. Wu, A. A. Dandekar, L. Pewe, and S. Perlman, CD4 and CD8 T cells have redundant but not identical roles in virus-induced demyelination, *J. Immunol.* **165**, 2278-2286 (2000).
56. G. F. Wu, L. Pewe, and S. Perlman, Coronavirus-induced demyelination occurs in the absence of inducible nitric oxide synthase, *J. Virol.* **74**, 7683-7686 (2000).
57. L. Pewe, J. Haring, and S. Perlman, CD4 T-cell-mediated demyelination is increased in the absence of gamma interferon in mice infected with mouse hepatitis virus, *J. Virol.* **76**, 7329-7333 (2002).
58. A. A. Dandekar, K. O'Malley, and S. Perlman, Important roles for gamma interferon and NKG2D in gammadelta T-cell-induced demyelination in T-cell receptor beta-deficient mice infected with a coronavirus, *J. Virol.* **79**, 9388-9396 (2005).
59. T. S. Kim and S. Perlman, Viral expression of CCL2 is sufficient to induce demyelination in RAG1-/- mice infected with a neurotropic coronavirus, *J. Virol.* **79**, 7113-7120 (2005).
60. J. S. Haring, L. L. Pewe, and S. Perlman, Bystander $CD8^+$ T cell-mediated demyelination after viral infection of the central nervous system, *J. Immunol.* **169**, 1550-1555 (2002).
61. J. Cabarrocas, J. Bauer, E. Piaggio, R. Liblau, and H. Lassman, Effective and selective immune surveillance of the brain by MHC class I-restricted cytotoxic T lymphocytes, *Eur. J. Immunol.* **33**, 1174-1182 (2003).
62. D. B. McGavern and P. Truong, Rebuilding an immune-mediated central nervous system disease: weighing the pathogenicity of antigen-specific versus bystander T cells, *J. Immunol*. **173**, 4779-4790 (2004).