Journal of NeuroVirology (1997) 3, 428–434

http://www.jneurovirol.com

Role of virus receptor-bearing endothelial cells of the blood-brain barrier in preventing the spread of mouse hepatitis virus-A59 into the central nervous system

Catherine Godfraind[1], Nathalie Havaux[2], Kathryn V Holmes[3] and Jean-Paul Coutelier[2]

[1]Laboratory of Pathology, St-Luc Hospital, [2] Unit of Experimental Medicine, International Institute for Cellular and Molecular Pathology, Catholic University of Louvain, 1200 Bruxelles, Belgium, and [3]Department of Microbiology, University of Colorado Health Sciences Center, 80262 Denver, Colorado, USA

BALB/c mice develop a neurologic demyelinating disease after inoculation of mouse hepatitis virus (MHV), strain A59, by the intracranial, but not by the intraperitoneal route. To determine the mechanisms that prevent virus spreading through the blood-brain barrier, we analyzed expression of MHVR, a glycoprotein that serves as receptor for mouse hepatitis virus on endothelial cells of cerebral blood vessels. Our results indicated that MHVR was strongly expressed on the endoluminal pole of these cells. In addition, a direct virus binding assay showed that mouse hepatitis virus was able to bind endothelial cells via this receptor. Despite this expression of a functional viral receptor, in normal mice infected with mouse hepatitis virus by the contra-peritoneal route, no *in vivo* viral replication could be detected in endothelial cells from the brain, contrasting with the equivalent cells from the liver. However, shortly after i.v. administration of sodium dodecylsulfate detergent to the mice, virus infection of some cerebral endothelial cells was detected in a few mice. As a consequence of detergent treatment, virus infection was able to cross the blood-brain barrier. These results suggest that the protective role of the blood-brain barrier against spreading of mouse hepatitis virus A59 into the central nervous system is determined by a specific restriction of viral entry into the endothelial cells of cerebral origin.

Keywords: mouse hepatitis virus; virus receptor; endothelial cell; blood-brain barrier; central nervous system

Introduction

Viral pathogenicity depends on the ability of infectious agents to reach their cellular targets, then to infect and either destroy or functionally impair these cells. This double requirement is well illustrated in the central nervous system (CNS): to induce neurological lesions, a virus must be both neurotropic and neurovirulent. For viruses present in the general circulation, neurotropism often implies that they can cross the blood-brain-barrier (BBB). Accordingly, several studies have shown that some viruses that were not able to induce neuropathology by themselves when administered in normal mice by a general route, triggered lesions in the CNS when the BBB was concomitantly breached mechanically, or by treatment with lipopolysaccharide or detergent (Hase *et al*, 1990; Kobiler *et al*, 1989; Lustig *et al*, 1992). On the other hand, ability of a virus to disrupt the BBB, which can result from infection of the brain endothelial cells (Cosby and Brankin, 1995; Krakowka *et al*, 1987; Soilu-Hänninen *et al*, 1994; Zurbriggen and Fujinami, 1988), may lead to dissemination of the virus into the CNS. Therefore, a better understanding of the mechanisms by which the BBB prevents the passage of viruses into the CNS could improve our knowledge of viral neuropathogenicity.

Recent evidence points to human coronaviruses as potential etiologic agents for multiple sclerosis (Murray *et al*, 1992; Stewart *et al*, 1992; Talbot *et al*, 1996). The availability of an experimental model to study the relationship between demyelinating disease of the CNS and coronavirus infection, and especially the mechanisms responsible for neurotropism of these viruses, is therefore of prime interest. Mouse hepatitis viruses (MHV) are coronaviruses that trigger various murine diseases, including hepatitis and alterations of the immune

Correspondence: C Godfraind, Laboratory of Pathology, St Luc Hospital, UCL, Av. Hippocrate 10, B-1200 Bruxelles, Belgium.
Received 6 May 1997; revised 14 July 1997; accepted 1 October 1997

system, depending on the mouse and virus strains used (reviewed in Wege *et al*, 1982). In the CNS, infection with the JHM strain of MHV is followed by acute, and often lethal encephalomyelitis. After intracerebral inoculation, MHV strain A59 induces in susceptible mice, such as BALB/c, a milder transient demyelinating disease (Godfraind *et al*, 1989; Lavi *et al*, 1984). However, so far, no evidence has been reported for a passage of this virus through the BBB (Robb *et al*, 1979). The recent isolation of a glycoprotein in the carcinoembryonic antigen family, called MHVR or Bgp1[a] that serves as receptor for MHV-A59 (Dveksler *et al*, 1991, 1993a, b) and the availability of antibodies reacting with this molecule have led to a better understanding of the role of this receptor in MHV-A59-induced pathology. The purposes of this study were to further analyze the efficiency of the BBB in preventing the spread of MHV-A59 from the circulation into the CNS and to determine whether this was related to the density of viral receptors expressed on the cells constituting the BBB.

Results and Discussion

To determine the ability of the BBB to block passage of MHV-A59 into the CNS, BALB/c mice were infected either by the intracranial or the intraperitoneal route. Tissue sections of the CNS were prepared at different times after infection and analyzed either by immunohistochemistry or by electron microscopy for the presence of virally-induced lesions. As shown in Table 1, most of the mice that received MHV-A59 by the intracranial route developed a neurological disease that was characterized by demyelinating lesions. In contrast, after intraperitoneal administration of the virus, no CNS lesions could be detected in a total of 45 mice from nine independent experiments, neither by electron microscopy nor by immunohistochemistry. This observation, which confirms and extends previous results published by others (Robb *et al*, 1979) suggests that the BBB efficiently prevents MHV-A59 spreading from the general circulation into the CNS.

Since infection of endothelial cells leads to passage of other viruses than MHV-A59 through the BBB (Cosby and Brankin, 1995; Krakowka *et al*, 1987; Soilu-Hänninen *et al*, 1994; Zurbriggen and Fujinami, 1988), one could postulate that the inability of MHV-A59 to cross the BBB could be related to resistance of these cells to infection. We examined by immunohistochemistry whether infection of the endothelial cells of the brain followed administration of the virus by the intraperitoneal (i.p.) route. Tissues obtained at different times (3, 4, 5 and 6 days) after ip administration of large doses of the virus ($10^4 - 10^6$ $TCID_{50}$) to BALB/c mice were labeled with anti-S antibody. As shown in Figure 1b, no infected endothelial cells could be detected in the CNS, at any time after infection. In contrast, clear evidence of infection was obtained in endothelial cells from the liver (Figure 1a). This observation suggests that some characteristic of BALB/c CNS endothelial cells allows them to escape infection by MHV-A59 administered by the ip route and therefore to prevent passage of this virus into the brain.

The resistance of brain endothelial cells to infection by MHV-A59 could be due to the absence of suitable viral receptors expressed on these cells. Therefore, we examined this hypothesis by immunohistochemistry performed with anti-MHVR antibodies as indicated previously (Godfraind *et al*, 1995b). As shown in Figure 2, MHVR was strongly expressed in the brain only on endothelial cells. The localization of MHVR on the apical pole of these endothelial cells (Figure 2a), suggested that

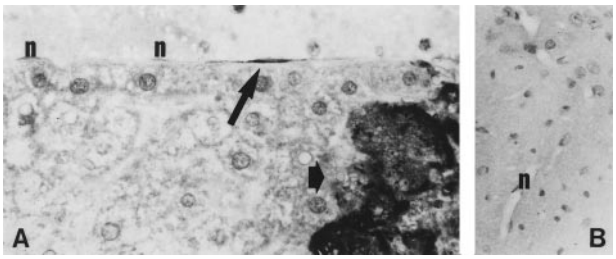

Figure 1 Infection of endothelial cells by MHV-A59. MHV-A59 was detected by immunohistochemistry with an anti-S antibody in sections of the liver (a) and CNS (b) obtained 3 days after infection of BALB/c mice. Infected liver endothelial cells (long arrow) were observed, especially near foci of infected hepatocytes (large arrow). In contrast, no infected endothelial cells could be detected in the CNS, even after biotinylated tyramine enhancement of the labeling (b). Nuclei of uninfected cells are shown (*n*). Magnification: a: 420×; b: 270×.

Table 1 Prevention by the BBB of MHV-A59 spreading into the CNS

Route of virus inoculation	*Total number of mice*[a]	*Number of mice with CNS lesions detected*[b] *by*	
		Electron microscopy	*Immunohistochemistry*
Intracranial	4	3	ND[c]
Intraperitoneal	18	0	ND
	27	ND	0

[a]Cumulative number from nine experiments. [b]Lesions specific of MHV-A59-induced CNS disease were detected 14 days after infection by electron microscopy, or 3–5 days after infection by immunohistochemistry. [c]ND: not done.

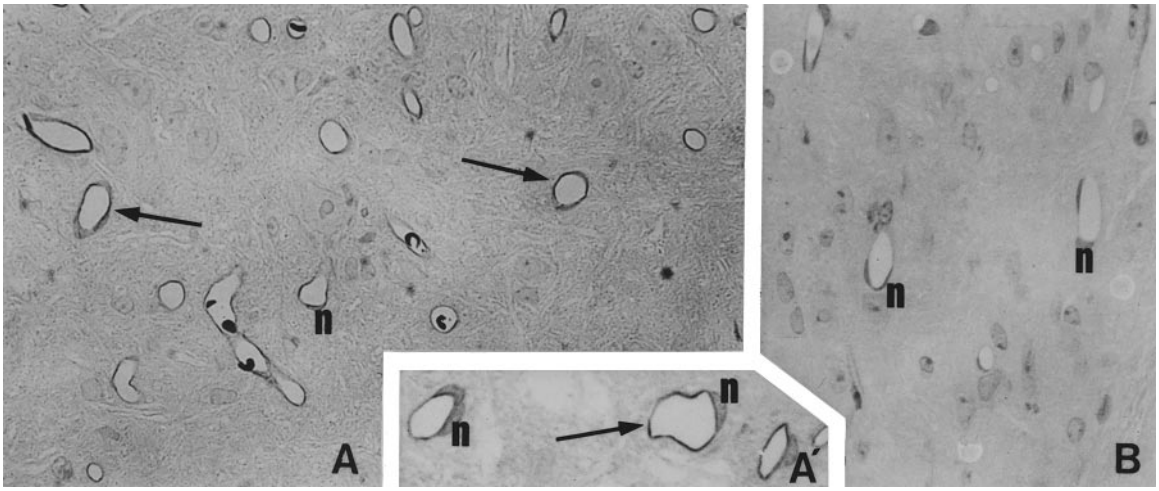

Figure 2 MHVR expression on CNS endothelial cells. Expression of MHVR was analyzed by immunohistochemistry with polyclonal Ab-655 anti-MHVR antibody. CNS sections from normal BALB/c mice were labeled either with anti-MHVR antibody (a) or with buffer followed by the secondary antibody (b). Endothelial cells expressing MHVR are shown (arrows). *n*=nuclei. Magnification: a, b: 410×; a′: 820×.

this molecule could serve as a receptor for circulating MHV-A59 particles. This was tested by a virus binding assay which indicated that endothelial cells of neurologic origin were functionally as able to bind MHV-A59 particles as were their counterparts from the liver (Figure 3). In addition, complete inhibition of MHV-A59 binding by treating tissues with anti-MHVR Mab-CC1 showed that MVHR was the only receptor for the virus on these endothelial cells (Figure 3b). However, this does not indicate what molecule on glial cells or neurons can serve as a MHV receptor, since MHVR was not detected in these cells. Thus, once MHV has entered into the CNS, its spread to other cell types than brain endothelial cells might require an additional MHV receptor such as bCEA (Chen *et al*, 1995).

Our results suggest therefore that the BBB prevents spreading of MHV-A59 into the CNS because the virus cannot infect brain endothelial cells that form this structure even though they express the MHV receptor. That this resistance is characteristic of the BBB endothelial cells is indicated by the observation that the corresponding cells in the liver, obtained from similar mice, can be infected by MHV-A59. One could postulate that this difference could be due to a higher viral concentration in the liver that results from the release of virus from infected hepatocytes. However, the observation by Joseph *et al* (1995) that brain endothelial cells are relatively resistant to *in vitro* infection by MHV-3, whereas this virus easily replicates in liver endothelial cells, suggests that an intrinsic resistance to infection in cells forming the BBB prevents *in vivo* virus entry and replication. Resistance of cells expressing a virus receptor to infection by the corresponding virus has also been reported for several cell types in mice transgenic for the poliovirus receptor (Ren and Racaniello, 1992). This observation with MHV indicates that brain endothelial cells have potent and specific mechanisms that allow them to fulfil their function as barriers for passage of infectious agents between the circulation and the CNS.

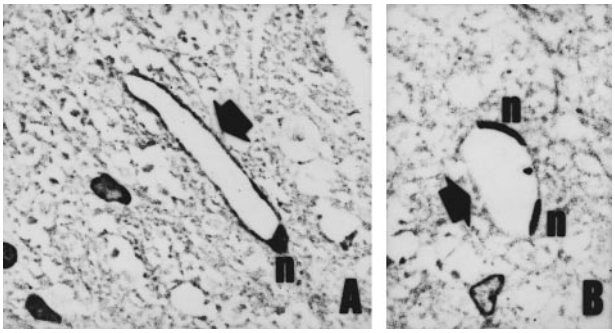

Figure 3 Binding of MHV-A59 to CNS endothelial cells. Binding of MHV-A59 was analyzed as described in Materials and methods on CNS sections from normal BALB/c mice (a). Bound virions are shown on endothelial cells (arrow in a). MHV-A59 binding was completely inhibited by competition with anti-MHVR MAb-CC1 monoclonal antibody (b, arrow). Unlabeled nuclei are shown (*n*). Magnification: 450×.

To determine whether this resistance of cerebral endothelial cells to MHV-A59 infection resulted from impairment of viral entry rather than from inhibition of intracellular viral replication, mice were treated with 3–7 intravenous injections of 50 ng sodium dodecylsulfate (SDS) detergent 3 days after infection with large doses of virus (10^5 to 10^7 $TCID_{50}$). This treatment has been shown previously to result in disruption of the BBB which allows a flavivirus to spread through this structure (Kobiler *et al*, 1989). In animals injected i.p. with MHV-A59,

but not treated with SDS, no MHV-A59 replication was observed in the brain (data not shown). In contrast, in about 10% of the MHV-infected mice treated with SDS, from three independent experiments, passage of MHV through the BBB into the CNS was detected (Figure 4). Moreover, in all of these animals MHV-A59 replication could be detected in brain endothelial cells (Figure 4). Depending on the CNS fields that were examined, infection was observed in isolated endothelial cells (Figure 4a), in small groups of cells including some endothelial cells and one or a few glial cells (Figure 4b) or in larger foci involving both many endothelial and glial cells (Figure 4c). These patterns indicated a progressive spread of virus from the endothelial cells into the brain tissue and strongly suggested that infection of endothelial cells is a necessary step for the successful passage of MHV-A59 through the BBB. In addition, these results suggested that the absence of endothelial cell infection in normal BALB/c mice was not a consequence of inhibition of virus replication, but was rather due to resistance to virus entry that could be overcome by increased cell membrane permeability after SDS treatment. In this context, it is quite possible that effective virus entry into other cells, such as liver endothelial cells requires the expression of a co-receptor in addition to Bgp1, like that reported for other viruses such as HIV (Choe *et al*, 1996; Doranz *et al*, 1996; Feng *et al*, 1996). Interestingly, resistance of SJL/J cells to MHV-A59 infection has been shown to depend on prevention of virus entry since these mice express a mutant MHVR, rather than to result from inability of these cells to carry on virus replication (Dveksler *et al*, 1993a; Yokomori and Lai, 1992).

MHV-A59 infection provides a useful model to understand the diverse mechanisms triggered by the interaction of a virus with cells expressing its receptor and leading to various types of pathologies. Indeed, in mice infected with MHV-A59, cellular expression of MHVR may lead to different outcomes depending on the cell type involved. Hepatocytes that express the receptor are directly killed by the virus, leading to hepatitis (Godfraind *et al*, 1995b). Indirect pathology may also develop after infection of some cells expressing MHVR. This seems to be the case in the thymus, where involution resulting from apoptosis of immature CD4+ CD8+ T cells, which do not bear the receptor for MHV-A59, was related to infection of thymic epithelial cells that express this molecule (Godfraind *et al*, 1995a). Alterations of cellular functions may also follow interaction of cells expressing MHVR with MHV-A59. In some mouse strains, infection triggers a polyclonal B lymphocyte activation leading to increase in concomitant IgG2a antibody responses that are not directed against viral antigens and that can include autoantibodies (Coutelier *et al*, 1991, 1988; Lardans *et al*, 1996). This immunostimulation involves a cascade of events such as proliferation of B lymphocytes and secretion of interleukin-12 by macrophages (Coutelier *et al*, 1995). Expression of MHVR has been found on those two types of cells (Coutelier *et al*, 1994). On the other hand, as shown here, some cells that express MHVR such as brain endothelial cells are resistant to infection with MHV-A59, whereas other cells, although very similar, like the hepatic endothelial cells are not. This contrasts with the

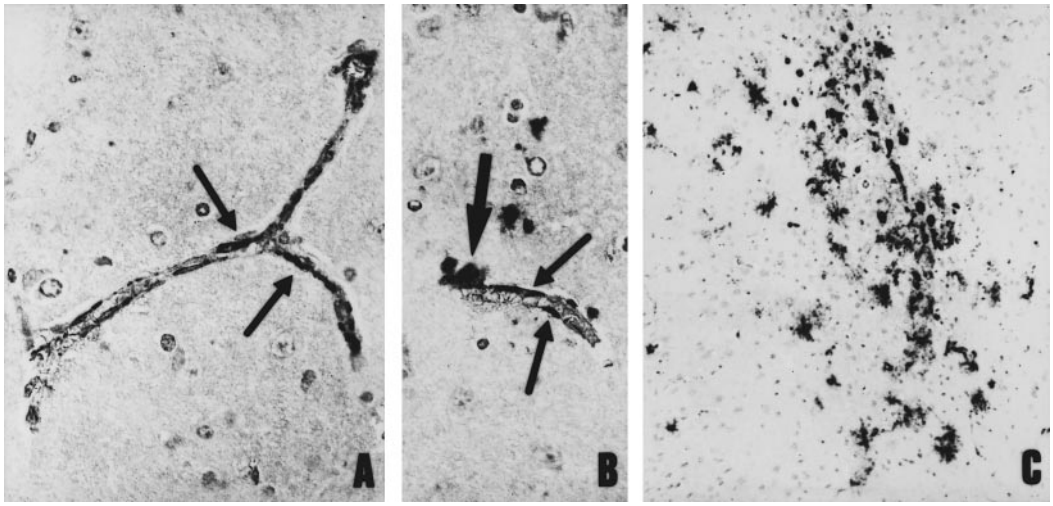

Figure 4 Passage of MHV-A59 through the BBB after SDS treatment. MHV-A59 infected cells were analyzed by immunohistochemistry with Ab-655 anti-S polyclonal antibody, enhanced by biotinylated thyramine treatment. CNS sections were obtained 6 days after infection and 3 days after repeated SDS injections. MHV-A59 replication (arrows) was observed in scattered endothelial cells (a), in endothelial cells adjacent to isolated infected glial cells (b) or in foci of infected endothelial and glial cells (c). No MHV-A59 replication was detected in endothelial cells of mice that were not treated with SDS. Magnification: a, b: 270×; c: 65×.

outcome of *in vitro* infection with a neurotropic MHV strain, namely MHV-4, which easily replicates in brain, but not in liver endothelial cells (Joseph *et al*, 1995). Thus, in addition to virus receptor expression, other factors may determine the regulation of viral tropism.

Materials and methods

Mice and virus

Specific-pathogen free female BALB/c mice were bred at the Ludwig Institute for Cancer Research by Dr G Warnier, and used when 6–8 week-old. Mice were infected with approximately 10^4-10^5 tissue culture infectious doses ($TCID_{50}$) of the MHV-A59 grown in NCTC 1469 cells, either by the intracranial or the intraperitoneal route.

Immunohistochemistry

For virus labelling, tissues were fixed in 4% paraformaldehyde and processed for paraffin embedding. 5 mμ sections were mounted on Superfrost slides. Immunolabelling was performed as described previously (Godfraind *et al*, 1995b) using a polyclonal goat antibody directed against the spike glycoprotein of MHV-A59, isolated from detergent-disrupted virions (anti-S) (Sturman *et al*, 1980). When indicated, enhancement of labelling was obtained by the biotinylated tyramine method described by others (Merz *et al*, 1995), using the TSA™-Indirect kit, following the recommendations of the manufacturer (DuPont NEN).

MHVR expression was examined by immunohistochemistry performed as indicated previously (Godfraind *et al*, 1995b). Briefly, tissues were fixed with paraformaldehyde, then incubated in increasing concentrations of sucrose, following the method of Tokuyasu (1973). Ultrathin frozen sections (1 μm) were cut with a Reichert-Jung Ultracut E microtome and transferred onto polylysine-coated slides in drops of 2.3 M sucrose in PBS. Slides post-fixed with 4% paraformaldehyde were incubated with rabbit polyclonal Ab-655 or mouse monoclonal MAb-CC1 anti-MHVR antibodies, followed by biotinylated secondary antibodies, and streptavidin-peroxidase. Sections were lightly counterstained with Mayer's Haematoxylin. Polyclonal rabbit anti-MHVR antibody (Ab-655) had been obtained after immunization of New Zealand White rabbits with immunoaffinity purified receptor from Swiss Webster mouse liver (Dveksler *et al*, 1991). Anti-receptor monoclonal antibody (MAb-CC1) had been derived from SJL/J mice immunized with protein extracted from BALB/c intestinal brush border membranes (Dveksler *et al*, 1993b). This antibody reacts with the N-terminal domain of MHVR and blocks binding of MHV-A59 to MHVR glycoproteins (Compton *et al*, 1992; Dveksler *et al*, 1993b).

Virus binding assay

Binding of virus to tissue sections was tested by a direct virus binding assay described previously (Godfraind *et al*, 1995b). Briefly, one micron cryosections were incubated with supernatant medium from cells infected with MHV-A59, or with control TRIS solution containing 1% BSA. After appropriate washing and post-fixation in 4% paraformaldehyde, sections were incubated with goat anti-S antibody, followed sequentially by peroxidase-conjugated rabbit anti-goat antibody, biotinylated anti-rabbit immunoglobulin goat Fab fragment (Boehringer Mannheim), streptavidin-peroxidase (Boehringer Mannheim), and diaminobenzidine (Boehringer Mannheim). Sections were lightly counterstained with Mayer Hemalun, dehydrated and mounted in Eukitt medium.

Acknowledgements

The authors are indebted to Drs RH vahns'Yqule and PL Masson for critical reading of this manuscript, to T Briet, Y De Craeye, M-D Gonzales, S Lagasse and J Van Broeck for expert technical assistance. This work was mainly supported by the association 'Recherche & Partage', Paris, France, and by the Fonds National de la Recherche Scientifique (FNRS), Fonds de la Recherche Scientifique Médicale (FRSM), Loterie Nationale and the State-Prime Minister's Office–SSTC (interuniversity attraction poles, grant no 44) and the French Community (concerted actions, grant no 88/93-122), Belgium. This work was supported in part by NIH grant # R01 AI 25231. J-PC is a senior research associate with the FNRS.

References

Chen DS, Asanaka M, Yokomori K, Wang FI, Hwang SB, Li HP, Lai MMC (1995). A pregnancy-specific glycoprotein is expressed in the brain and serves as a receptor for mouse hepatitis virus. *Proc Soc Natl Acad Sci USA* **92:** 12095–12099.

Choe H, Farzan M, Sun Y, Sullivan N, Rollins B, Ponath PD, Wu L, Mackay CR, LaRosa G, Newman W, Gerard N, Gerard C, Sodroski J (1996). The β-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. *Cell* **85:** 1135–1148.

Compton SR, Stephensen CB, Snyder SW, Weismiller DG, Holmes KV (1992). Coronarivus species specificity: murine coronavirus binds to a mouse-specific epitope on its carcinoembryonic antigen-related receptor glycoprotein. *J Virol* **66:** 7420–7428.

Cosby SL, Brankin B (1995). Measles virus infection of cerebral endothelial cells and effect on their adhesive properties. *Vet Microbiol* **44:** 135–139.

Coutelier JP, Godfraind C, Dveksler GS, Wysocka M, Cardellichio CB, Noël H, Holmes KV (1994). B lymphocyte and macrophage expression of carcinoembryonic antigen-related adhesion molecules that serve as receptors for murine coronavirus. *Eur J Immunol* **24:** 1383–1390.

Coutelier JP, Van Broeck J, Wolf SF (1995). Interleukin-12 gene expression after viral infection in the mouse. *J Virol* **69:** 1955–1958.

Coutelier JP, van der Logt JTM, Heessen FWA (1991). IgG subclass distribution of primary and secondary immune responses concomitant with viral infection. *J Immunol* **147:** 1383–1386.

Coutelier JP, van der Logt JTM, Heessen FWA, Vink A, Van Snick J (1988). Virally induced modulation of murine IgG antibody subclasses. *J Exp Med* **168:** 2373–2378.

Doranz BJ, Rucker J, Yi Y, Smyth RJ, Samson M, Peiper SC, Parmentier M, Collman RG, Doms RW (1996). A dual-tropic primary HIV-1 isolate that uses fusin and the β-chemokine receptors CKR-5, CKR-3, and CKR-2b as fusion cofactors. *Cell* **85:** 1149–1158.

Dveksler GS, Dieffenbach CW, Cardellichio CB, McCuaig K, Pensiero MN, Jiang GS, Beauchemin N, Holmes KV (1993a). Several members of the mouse CEA-related glycoprotein family are functional receptors for murine coronavirus MHV-A59. *J Virol* **67:** 1–8.

Dveksler GS, Pensiero MN, Cardellichio CB, Williams RK, Jiang GS, Holmes KV, Dieffenbach CW (1991). Cloning of the mouse hepatitis virus (MHV) receptor: expression in human and hamster cell lines confers susceptibility to MHV. *J Virol* **65:** 6881–6891.

Dveksler GS, Pensiero MN, Dieffenbach CW, Cardellichio CB, Basile AA, Elia PE, Holmes KV (1993b). Mouse hepatitis virus strain-A59 and blocking antireceptor monoclonal antibody bind to the N-terminal domain of cellular receptor. *Proc Natl Acad Sci USA* **90:** 1716–1720.

Feng Y, Broder CC, Kennedy PE, Berger EA (1996). HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. *Science* **272:** 872–877.

Godfraind C, Friedrich VL, Holmes KV, Dubois-Dalcq M (1989). *In vivo* analysis of glial cell phenotypes during a viral demyelinating disease in mice. *J Cell Biol* **109:** 2405–2416.

Godfraind C, Holmes KV, Coutelier JP (1995a). Thymus involution induced by mouse hepatitis virus A59 in BALB/c mice. *J Virol* **69:** 6541–6547.

Godfraind C, Langreth SG, Cardellichio CB, Knobler R, Coutelier JP, Dubois-Dalcq M, Holmes KV (1995b). Tissue and cellular distribution of an adhesion molecule in the carcinoembryonic antigen family that serves as a receptor for mouse hepatitis virus. *Lab Invest* **73:** 615–627.

Hase T, Dubois DR, Summers PL (1990). Comparative study of mouse brains infected with Japanese encephalitis virus by intracerebral or intraperitoneal inoculation. *Int J Exp Pathol* **71:** 857–869.

Joseph J, Kim R, Siebert K, Lublin FD, Offenbach C, Knobler RL (1995). Organ specific endothelial cell heterogeneity influences differential replication and cytopathogenicity of MHV-3 and MHV-4. *Adv Exp Med Biol* **380:** 43–50.

Kobiler D, Lustig S, Gozes Y, Ben-Nathan D, Akov Y (1989). Sodium dodecylsulphate induces a breach in the blood-brain-barrier and enables a West Nile virus variant to penetrate into mouse brain. *Brain Res* **496:** 314–316.

Krakowka S, Cork LC, Winkelstein JA, Axthelm MK (1987). Establishment of central nervous system infection by canine distemper virus: breach of the blood-brain barrier and facilitation by anti viral antibody. *Vet Immunol Immunopathol* **17:** 471–482.

Lardans V, Godfraind C, van der Logt JTM, Heessen FWA, Gonzalez MD, Coutelier JP (1996). Polyclonal B lymphocyte activation induced by mouse hepatitis virus A59 infection. *J Gen Virol* **77:** 1005–1009.

Lavi E, Gilden DH, Wroblewska Z, Rorke LB, Weiss SR (1984). Experimental demyelination produced by the A59 strain of mouse hepatitis virus. *Neurology* **34:** 597–603.

Lustig S, Danenberg HD, Kafri Y, Kobiler D, Ben-Nathan D (1992). Viral neuroinvasion and encephalitis induced by lipopolysaccharide and its mediators. *J Exp Med* **176:** 707–712.

Merz H, Malisius R, Mannweiler S, Zhou R, Hartmann W, Orscheschek K, Moubayed P, Feller AC (1995). ImmunoMax. A maximized immunohistochemical method for the retrieval and enhancement of hidden antigens. *Lab Invest* **73:** 149–156.

Murray RS, Brown B, Brian D, Cabirac GF (1992). Detection of coronavirus RNA and antigen in multiple sclerosis brain. *Ann Neurol* **31:** 525–533.

Ren R, Racaniello VR (1992). Human poliovirus receptor gene expression and poliovirus tissue tropism in transgenic mice. *J Virol* **66:** 296–304.

Robb JA, Bond CW, Leibowitz JL (1979). Pathogenic murine coronaviruses. III. Biological and biochemical characterization of temperature-sensitive mutants of JHMV. *Virology* **94:** 385–399.

Soilu-Hänninen M, Erälinna JP, Hukkanen V, Röyttä M, Salmi AA, Salonen R (1994). Semliki Forest Virus infects mouse brain endothelial cells and causes blood-brain-barrier damage. *J Virol* **68:** 6291–6298.

Stewart JN, Mounir S, Talbot PJ (1992). Human coronavirus gene expression in the brains of multiple sclerosis patients. *Virology* **191:** 502–505.

Sturman LS, Holmes KV, Behnke JN (1980). Isolation of coronavirus envelope glycoproteins and interaction with the viral nucleocapsid. *J Virol* **33:** 449–462.

Talbot PJ, Paquette JS, Ciurli C, Antel JP, Ouellet F (1996). Myelin basic protein and human coronavirus 229E cross-reactive T cells in multiple sclerosis. *Ann Neurol* **39:** 233–240.

Tokuyasu KT (1973). A technique for ultramicrotomy of cell suspension and tissues. *J Cell Biol* **57:** 551–565.

Wege H, Siddell S, ter Meulen V (1982). The biology and pathogenesis of coronaviruses. *Curr Top Microbiol Immunol* **99:** 165–200.

Yokomori K, Lai MMC (1992). The receptor for mouse hepatitis virus in the resistant mouse strain SJL is functional: implications for the requirement of a second factor for viral infection. *J Virol* **66:** 6931–6938.

Zurbriggen A, Fujinami RS (1988). Theiler's virus infection in nude mice: viral RNA in vascular endothelial cells. *J Virol* **62:** 3589–3596.