Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Journal of Neuroimmunology, 20 (1988) 111–116
Elsevier

JNI 00700

Coronavirus-induced CNS disease: a model for virus-induced demyelination

Michael J. Buchmeier [1], Robert G. Dalziel [2] and Marck J.M. Koolen [3]

[1] *Scripps Clinic and Research Foundation, La Jolla, CA 92037, U.S.A.,* [2] *Royal (Dick) School of Veterinary Studies, Edinburgh, U.K., and* [3] *Netherlands Bloodtransfusion Service, Amsterdam, The Netherlands*

(Received 26 July 1988)
(Accepted 27 July 1988)

Key words: Coronavirus; Demyelination, virus-induced; Central nervous system disease

Introduction

Although viruses offer an attractive explanation for the etiology of some forms of chronic progressive central nervous system (CNS) diseases in man, etiology has only been proven in a handful of cases. Among them, subacute sclerosing panencephalitis (SSPE) is a rare late complication of childhood measles characterized by a long time span between the initial attack and the onset of SSPE. This latent or silent phase may be a decade or longer, and the interaction between virus and host during this interval is poorly understood (Ter Meulen and Hall, 1978). Progressive multifocal leukoencephalopathy (PML) is another virus-induced chronic progressive disease of the CNS (Weiner et al., 1972). In this case, the infectious agent is a DNA-containing papovavirus. Infection with the PML agent is common but progression to the fatal chronic gray matter disease is rare, occurring most frequently in association with immunosuppression or as a complication of acquired immunodeficiency syndrome. As in SSPE, the factors which separate the self-limiting benign infection, which occurs in most individuals, from the lethal disease are poorly understood.

There are circumstantial data to support the suggestion that viruses cause human demyelinating disease. Norrby and coworkers (Norrby, 1978) described oligoclonal IgG specific for measles and other common human viruses in the cerebrospinal fluid of MS patients. Measles virus and the closely related paramyxovirus canine distemper have been implicated by epidemiological studies showing an association of antibodies to these viruses with MS but insufficient evidence is available to establish a causal link. It is precisely these difficulties in establishing a causal relationship between viruses and MS that has led us and others to focus on well-established animal models of CNS disease (Lampert et al., 1973; Weiner, 1973; Sturman and Holmes, 1982; Stroop and Baringer, 1982; Koolen and Buchmeier, 1986). Using such models, factors such as the age, genetic background and immune status of the host and the strain, dose and route of inoculation of the infecting virus can be controlled and manipulated. A detailed understanding of the functions of specific macromolecular components and genes of viruses and their interactions with the host in disease will yield greater insight about the molecular basis of viral CNS pathogenesis.

Address for correspondence: Michael J. Buchmeier, Scripps Clinic and Research Foundation, 10666 N. Torrey Pines Rd., La Jolla, CA 92037, U.S.A.

Mouse hepatitis virus strain JHM is a neurotropic coronavirus, a group which also includes a number of human common cold agents (Sturman and Holmes, 1982). Infection of susceptible 4- to 6-week-old mice by the intracerebral or intranasal route with wild-type virus causes a fatal encephalomyelitis characterized by widespread destruction of CNS neurons, particularly in brainstem and hippocampus, accompanied by demyelination. Resistance to the lethal infection was shown by Knobler et al. (1981) to be controlled by a single autosomal dominant gene located on chromosome 7, and the effect is seen both at the level of macrophages and neurons. A small fraction of these mice survive encephalitis and go on to develop chronic demyelination. Several laboratories have demonstrated attenuation of the encephalitogenic properties of the virus using ts and spontaneously arising mutants of MHV-JHM. In these models infection with the attenuated virus results in a high incidence of demyelinating disease without encephalitis. The best characterized mutant is the ts 8 mutant of Haspel et al. (1978). In ts 8 infection, demyelination is a direct result of infection of the oligodendrocyte, the cell which forms and maintains the myelin sheath in the CNS, thus the disease is referred to as a primary demyelination. Similar attenuation with demyelination has been reported for an MHV-JHM variant ds (Stohlman et al., 1982) and for the ts 342 mutant of MHV-A59 (Koolen et al., 1983). In the latter case revertants of ts 342 regained wild-type pathogenicity, suggesting that the mutation responsible for ts phenotype was linked to the gene responsible for attenuation.

MHV-JHM contains three major structural proteins including a 56–60 kDa nucleoprotein and two glycoproteins, E1 and E2 (Sturman et al., 1980; Niemann and Klenk, 1981; Sturman and Holmes, 1982). Glycoprotein E1 is a 25 kDa transmembrane glycoprotein which bears O-linked oligosaccharides. E2 is a 180 kDa glycoprotein which forms the peplomer or spike of the virion and is composed of two non-identical 90 kDa polypeptide chains in a dimer (Sturman et al., 1985). E2 is responsible for a number of important biological activities of the virus including neutralization by antibody, attachment to cellular receptors, and cell–cell fusion (Collins et al., 1982). We have raised a library of monoclonal antibodies against the MHV-4 structural proteins and used these to study the formation of the viral polypeptides as well as the influences of specific immune responses against them on development and course of acute and chronic disease (Buchmeier et al., 1984). Using this approach we have shown that antibody against specific determinants on glycoprotein E2 were sufficient to alter the course of MHV-4 infection from fatal encephalitis to chronic demyelinating disease.

Results and discussion

Monoclonal antibodies (MAb) mapping to three topographically linked sites on MHV-4 E2 have

TABLE 1

PROPERTIES OF ANTI-E2 MONOCLONAL ANTIBODIES

BALB/c mice were given 150 μl of ascites fluid containing the indicated antibody, then challenged one day later with 100 LD_{50} of MHV-4. Protected mice (+) survived indefinitely while unprotected mice (−) died within 7 days after challenge.

	Monoclonal [a]							
	5B19.2	5B170.3	5A13.5	4B11.6	5B21.5	5B93.9	5B207.7	5B216.8
Epitope	E2A	E2A	E2B	E2C	E2D	E2D	E2E	E2E
Western blot	+	+	−	−	+	+	+	−
In vitro neutralization	+	+	+	+	−	−	−	−
In vivo protection	+	+	+	−	−	−	−	−

[a] PRD_{50} titer + = ≥ 1/100; − = ≤ 1/20.

TABLE 2

PRD_{50} NEUTRALIZATION TITERS OF MONOCLONAL ANTIBODIES MEASURED AGAINST VARIANT VIRUSES

MAb	Virus strain						
	wt MHV-4	V5A13.1	V5A13.2	V5A13.3	V4B11.1	V4B11.2	V4B11.3
5B19.2	7900	12600	17800	11200	6300	7900	7900
5A13.5	31600	250	200	400	125	160	140
4B11.6	15800	36	90	80	36	40	20

been identified (Talbot et al., 1984b). Two of these, 5B19.2 (epitope E2A) and 5A13.5 (E2B) were previously shown in passive transfer experiments to protect mice from lethal encephalitis (Buchmeier et al., 1984). The third MAb, 4B11.6 (E2C) neutralized virus in vitro but did not protect mice against challenge in vivo. These properties are summarized in Table 1.

We selected variants from 3× cloned virus stocks by incubating in excess antibody for 30 min at 37°C, then plaqued the surviving virus in the presence of antibody. Plaques which escaped neutralization were selected and subjected to a second round of neutralization. Stocks were then grown and rechecked for resistance to antibody. We observed a frequency of true variants in the initial population of $10^{-4.3}$ to $10^{-4.6}$, a rate consistent with other RNA virus systems (Laver et al., 1979; Dietzschold et al., 1983; Minor et al., 1983). Three variants each selected using MAb 5A13.5 and 4B11.3 were chosen from a panel of over 30 for further study. We were surprised to observe that variants selected with either antibody were resistant to neutralization by both (Dalziel et al., 1986) (Table 2) and further studies with a panel of monoclonals raised against MHV-JHM E2 independently by Dr. John Fleming further substantiated this observation (Fig. 1). It appeared then that selection of neutralization-resistant variants at either the E2B or E2C sites induced conformational changes reflected in both sites. We know from previous studies (Talbot et al., 1984a) that both of these sites are predominantly conformational in nature.

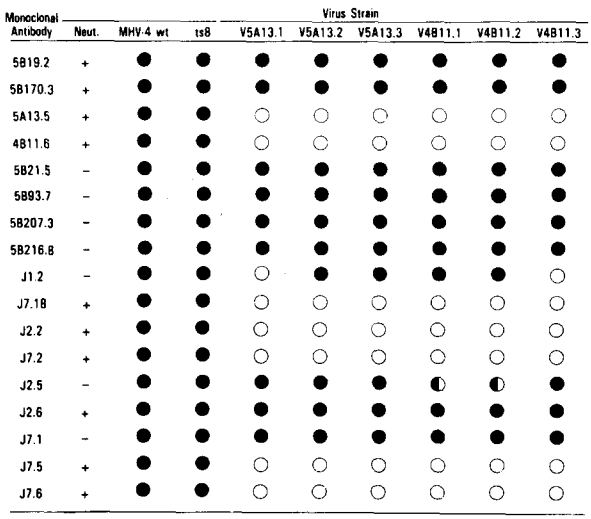

Fig. 1. Reaction of variant and wild-type MHV-4 strains with monoclonal anti-E2 antibodies. ● indicates strong positive reaction; ◐ indicates weak positive reaction; ○ indicates no reaction.

With the knowledge that we had selected variants, we were interested in determining whether the antigenic changes resulted in altered virulence. We determined LD_{50} values for representative variants (Table 3) and observed 200- to > 4000-fold decreases in neurovirulence following i.c. inoculation relative to wild-type MHV-4.

At the histopathologic level, mice infected with either class of variant had moderate inflammatory lesions in the white matter of their spinal cords and brains early in the infection at days 4–15, and subsequently developed extensive demyelinating

TABLE 3

LD_{50} VALUES OF WILD-TYPE AND ATTENUATED MHV-4 STRAINS

Virus	pfu/LD_{50} [a]	Attenuation factor relative to weight
wt	< 0.45	1
V5A13.1	> 1800	> 4000
V4B11.3	> 95	211

[a] Measured by Reed and Muench assay on BALB/c ByJ mice.

TABLE 4

VIRUS RECOVERY FROM TISSUES FOLLOWING WILD-TYPE AND VARIANT INFECTIONS

Virus	Tissue	Day of isolation [a]			
		2	4	7	15
wt	Brain	5×10^7	1×10^6	4×10^5	NA [b]
	Liver	5×10^2	1×10^4	3×10^4	
V4B11.3	Brain	2.5×10^3	1.9×10^3	$< 4 \times 10^{1,c}$	cocultivation
	Liver	2×10^2	$< 4 \times 10^1$	$< 4 \times 10^1$	
V5A13.1	Brain	3.1×10^4	1.4×10^4	$< 4 \times 10^1$	-
	Liver	2×10^2	-	$< 4 \times 10^1$	

[a] Titer in pfu/g of tissue.
[b] NA, not available; wild-type-infected mice died prior to this time point.
[c] Limit of detection.

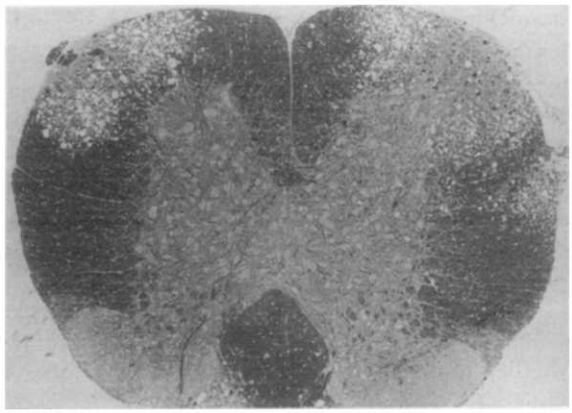

Fig. 2. Plaques of demyelination in the spinal cord of a mouse 32 days after infection. Reproduced with permission from J. Virol. (1986) 59, 463–471.

lesions (Fig. 2). We observed remyelination beginning around 3 weeks (Fig. 3) but demyelination apparently continued since lesions observed 50 and 65 days after infection were severe and showed evidence of both recent and old foci (Fig. 4). We found no significant evidence of neuronal involvement, suggesting that the E2 variants had lost neurovirulence in a manner similar to ts 8 (Haspel et al., 1978).

Apparently, replication of the variant viruses is not fulminant in the CNS. We were only able to isolate virus by cocultivation by 15 days after infection (Table 4), but the virus which was recovered retained the antibody-resistant variant phenotype. Thus, paradoxically, the virus-induced

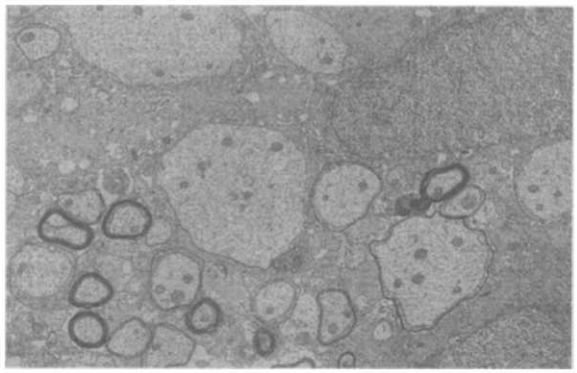

Fig. 3. Demyelination and early remyelination in the spinal cord of a mouse 22 days after infection. Paraphenylenediamine stain (×8000).

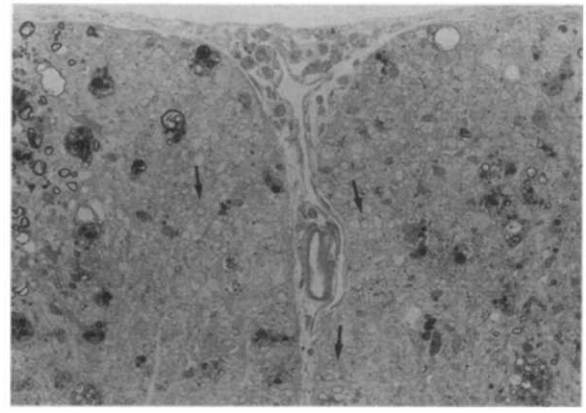

Fig. 4. Extensive demyelination in the spinal cord of a mouse 50 days after infection with remyelinating axons (arrows) (×500).

pathology persisted in the absence of demonstrable infectious virus. The possibility exists that stimulation of specific cell-mediated immune responses against viral antigens and/or host components such as myelin basic protein (MBP) are triggered in the infection and contribute to the chronic demyelinating disease. Alternatively virus may persist in limited cell populations such as the basal ganglia (Fishman et al., 1985) and continually re-initiate the demyelinating disease at the primary level. We have addressed the first of these two alternatives. In recent studies with the ts 342 strain of MHV-A59 we have demonstrated significant levels of proliferation measured as [^{3}H]thymidine incorporation when lymphocytes from virus-infected mice were cultured in the presence of either viral antigen or MBP. Responder cells were shown to be T-lymphocytes and were reduced to baseline levels by in vivo depletion of the L3T4 subset. We are currently studying this response at the cellular level, cloning the responder cells, and probing to determine the fate and expression of our attenuated variants of MHV-4 in the CNS during acute and chronic demyelinating disease.

These studies illustrate that mutational changes in a specific viral glycoprotein, E2, are reflected in pathogenicity differences in vivo. We hope that defining such changes at the molecular level and their effect on cellular tropism and replication in the CNS will lead to a better understanding of the pathogenesis of virus-induced CNS disease in man.

Acknowledgements

We thank Kaleo Wooddell, Gretchen Bloom, Leslie Igarashi, and Hanna Lewicki for expert technical assistance and Jim Johnston for manuscript preparation. This work was supported by USPHS grant No. NS-12428. M.J.M.K. was supported by an EMBO fellowship.

References

Buchmeier, M., Lewicki, H., Talbot, P. and Knobler R., (1984) Murine hepatitis virus-4 (strain JHM) induced neurologic disease is modulated in vivo by monoclonal antibody. Virology 132, 261.

Collins, A., Knobler, R., Powell, H. and Buchmeier, M. (1982) Monoclonal antibodies to murine hepatitis virus-4 (strain JHM) define the viral glycoprotein responsible for attachment and cell–cell fusion. Virology 119, 358.

Dalziel, R.G., Lampert, P.W., Talbot, P.J. and Buchmeier, M.J. (1986) Site specific alteration of murine hepatitis virus type-4 (MHV-4) peplomer glycoprotein E2 results in reduced neurovirulence. J. Virol. (in press).

Dietzschold, B., Wimmer, W., Wiktor, T., Lopes, A., Lafon, M., Smith, C. and Koprowski, H. (1983) Characterization of an antigenic determinant of the glycoprotein that correlates with pathogenicity of rabies virus. Proc. Natl. Acad. Sci. U.S.A. 80, 70.

Fishman, P., Gass, J., Swoveland, P., Lavi, E., Highkin, M. and Weiss, S. (1985) Infection of the basal ganglia by a murine coronavirus. Science 229, 877.

Haspel, M., Lampert, P. and Oldstone, M. (1978) Temperature sensitive mutants of mouse hepatitis virus produce a high incidence of demyelination. Proc. Natl. Acad. Sci. U.S.A. 75, 4033.

Knobler, R., Haspel, M. and Oldstone, M. (1981) Mouse hepatitis virus type-4 (JHM strain)-induced fatal central nervous system disease. I. Genetic control and the murine neuron as the susceptible site of disease. J. Exp. Med. 153, 832.

Koolen, M. and Buchmeier, M. (1986) Experimental models of virus attenuation and demyelinating disease. Microbiol. Sci. 3, 68.

Koolen, M., Osterhaus, A., van Steenis, G., Horzinek, M. and van der Zeijst, B. (1983) Temperature sensitive mutants of mouse hepatitis virus strain A59: isolation, characterization and neuropathogenic properties. Virology 125, 393.

Lampert, P., Simms, J. and Kniazeff, A. (1973) Mechanism of demyelination in JHM virus encephalomyelitis. Electron microscopic studies. Acta Neuropathol. 24, 76.

Laver, W., Air, G., Webster, R., Gerhard, W., Ward, C. and Dopheide, T. (1979) Antigenic drift in type A influenza virus: sequence differences in the hemagglutinin of Hong Kong (H3N2) variants selected with monoclonal hybridoma antibodies. Virology 98, 226.

Minor, P., Schild, G., Bootman, J., Evans, D., Ferguson, M., Reeve, P., Spitz, M., Stanway, G., Cann, A., Hauptmann, R., Clarke, L., Mountford, R. and Almond, J. (1983) Location and primary structure of a major antigenic site for poliovirus neutralization. Nature 301, 674.

Niemann, H. and Klenk, H. (1981) Coronavirus glycoprotein E1, a new type of viral glycoprotein. J. Mol. Biol. 153, 993.

Norrby, E. (1978) Viral antibodies in multiple sclerosis. Prog. Med. Virol. 24, 1.

Stohlman, S., Brayton, P., Fleming, J., Weiner, L. and Lai, M. (1982) Murine coronaviruses: isolation and characterization of two plaque morphology variants of JHM neurotropic strain. J. Gen. Virol. 63, 265.

Stroop, W. and Baringer, J. (1982) Persistent, slow and latent viral infections. Prog. Med. Virol. 28, 1.

Sturman, L. and Holmes, K. (1982) The molecular biology of coronaviruses. Adv. Virus Res. 28, 33.

Sturman, L., Holmes, K. and Behnke, J. (1980) Isolation of

coronavirus envelope glycoproteins and interaction with the viral nucleocapsid. J. Virol. 33, 449.

Sturman, L., Ricard, C. and Holmes, K. (1985) Proteolytic cleavage of the E2 glycoprotein of murine coronavirus: activation of cell fusing activity of virions by trypsin and separation of two different 90k cleavage fragments. J. Virol. 56, 904.

Talbot, P., Knobler, R. and Buchmeier, M. (1984a) Western and dot immunoblotting analysis of viral antigens and antibodies: application to murine hepatitis virus. J. Immunol. Methods 73, 177.

Talbot, P., Salmi, A., Knobler, R. and Buchmeier, M. (1984b) Topographical mapping of epitopes on the glycoproteins of murine hepatitis virus-4 (strain JHM): correlation with biological activities. Virology 132, 250.

Ter Meulen, V. and Hall, W. (1978) Slow virus infections of the nervous system: virological, immunological and pathogenic considerations. J. Gen. Virol. 41, 1.

Weiner, L. (1973) Pathogenesis of demyelination induced by a mouse hepatitis virus (JHM virus). Arch. Neurol. 28, 298.

Weiner, L.P., Herndon, R., Narayan, O., Johnson, R., Shah, K., Rubinstein, L., Prezoisi, T. and Conley, F. (1972) Isolation of a virus related to SV-40 from patients with progressive multifocal leukoencephalopathy. New Engl. J. Med. 286, 385.