CHARACTERIZATION OF JHMV VARIANTS ISOLATED FROM RAT BRAIN AND CULTURED NEURAL CELLS AFTER WILD TYPE JHMV INFECTION

F. Taguchi,* S. Siddell, H. Wege, P. Massa, and V. ter Meulen

Institute of Virology, University of Wurzburg, Versbacher str.7, 8700 Wurzburg, West Germany and
* present address: National Institute of Neuroscience, NCNP, 4-1-1, Ogawa-higashi-machi, Kodaira, Tokyo 187, Japan

INTRODUCTION

Mouse hepatitis virus (MHV) is a member of the coronavirus family , which are enveloped RNA viruses. Among MHV strains JHMV is of particular interest since this virus can produce a chronic demyelinating disease in rats (1, 2, 3) and mice (4, 5, 6). The intracerebral infection of 4 to 5-week-old Lewis rats with JHMV produces two distinct disease patterns. A subacute demyelinating encephalitis which is seen in a minority of animals develops 12 to 14 days postinfection (p.i.)(2, 7). The transfer of lymphocytes from rats with subacute demyelinating encephalitis to uninfected recipient animals has recently been shown to induce an experimental allergic encephalitis-like disease (7). In contrast, most animals develop acute encephalitis, which leads to death within 14 days. During acute encephalitis, it was found that a virus with a larger mRNA3 as well as E2 glycoprotein compared with wild-type (wt) JHMV was selectively propagated in the brain of rats infected with wt JHMV. It was also observed that a similar virus with a larger mRNA3 and E2 glycoprotein was selected in primary rat neural cell culture. These facts suggest that the virus with a larger E2 glycoprotein has a growth advantage in rat brain cells as compared with wt JHMV. In this paper, we describe these observations together with a detailed analysis of variants isolated from rat brain and cultured rat neural cells.

RESULTS AND DISCUSSION

Four to 5-week-old Lewis rats were inoculated intracerebrally with cloned wt virus, and virus growth in the brains was examined by plaque assay of tissue homogenates on DBT cells (8). Infectious viruses could first be detected in brains 3 days p.i. Within 5 to 6 days p.i., the virus titer reached a peak of 10^4 to 10^5 PFU per brain. The titer in the brains decreased gradually

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thereafter. Sixty to 70 % of infected rats showed clinical central nervous system symptoms 6 to 9 days p.i., and almost all of these animals died within 2 weeks. Brain material containing high levels of infectious viruses obtained 5 to 7 days p.i. were analyzed directly for viral RNAs by Northern blot hybridization. RNA from the brain tissues was collected by the method described by Chirgwin et al (9) and ^{32}P labeled cDNA made from mRNA7 of JHMV (10) was used for Northern blot analysis. In all cases in which MHV-specific mRNAs were detected, the mRNA profile differed from that produced during a lytic infection of DBT cells with the wt JHMV. Whereas no differences in the electrophoretic mobilities of genome-sized RNA and subgenomic mRNAs4, 5, 6 and 7 could be detected, mRNAs 2 and 3 were reproducibly and significantly larger in the brain-derived material. We have never detected the mRNA profile characteristic of wt JHMV in material derived from brain tissue, nor have we been able to reisolate from animal brains a virus that produced the wt virus mRNA profile in the tissue culture. We conclude, therefore, that acute encephalitis is associated with the propagation of a variant virus population and not of wt virus. The development of acute encephalitis is apparently dependent upon mutation within the wt JHMV population and selection within the animal of a specific population of variant viruses.

To facilitate biochemical analysis of the viruses that replicated in the brains of infectd animals, we plaque purified a virus, cl-2 virus. As shown in Fig. 1, both mRNAs 2 and 3 of cl-2 were larger, by ca. 150,000 daltons, than the corresponding mRNAs produced by wt virus. There was no detectable difference in the size of any other viral mRNAs. It was also revealed that during passages in DBT cells, the mRNA profile characteristic of cl-2 virus remained constant. Also, the virus population that preferentially replicated in the brains of animals inoculated with cl-2 virus, as well as viruses subsequently reisolated from such animals, all showed mRNA profiles identical to that of cl-2 virus. As for the growth and cytopathic effects of wt and cl-2 viruses in cultured DBT cells, no big difference was evident.

Genomic RNA labeled in vivo with ^{32}P phosphate was extracted

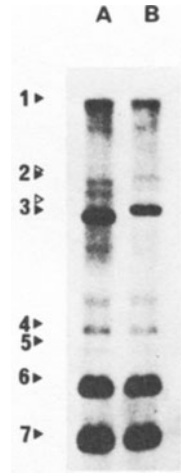

Fig.1. Northern blot analysis of RNA from wild-type (A) and cl-2 (B) infected DBT cells.

from wt and cl-2 virions, purified by urea agarose gel electrophoresis and analyzed by T_1-resistant oligonucleotide fingerprinting as previously reported (11). The fingerprint of the wt JHMV used in these studies is very similar to that obtained in other laboratories, and we therefore numbered the oligonucleotides according to previous publications (11,12). When the wt and cl-2 virus genomic RNA are compared, there are only two differences in the fingerprinting pattern. Oligonucleotide 7 is missing in the genome RNA of cl-2 and, instead, a new oligonucleotide designated 7a was found. The similar electrophoretic mobilities of oligonucleotides 7 and 7a suggests that they are closely related in base composition. In contrast, there is no detectable difference in the oligonucleotide fingerprints of mRNA3, although mRNA3 of cl-2 virus was ca. 500 nucleotides larger than that of wt virus.

Since the mRNA3 produced by cl-2 virus was significantly larger than that of wt virus, we examined whether the protein encoded by mRNA3, E2 glycoprotein, was also larger in cl-2 infected cells. DBT cells were infected and labeled with ^{35}S methionine (13). Virus specific proteins were examined by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) after immunoprecipitation with an antiserum raised against purified wt JHMV as reported previously (13). As shown in Fig. 2, the intracellular precursor to the E2 protein produced in cl-2 virus infected cells is ca. 15,000 daltons larger than wt virus intracellular E2 precursor. As this apparent size difference could be due to differences in glycosylation, we compared the sizes of the E2 polypeptides synthesized in the presence of tunicamycin. The E2 polypeptide in cl-2 virus infected cells was ca. 15,000 daltons larger than that found in wt JHMV infected cells.

These data suggest that the virus with a larger E2 glycoprotein preferentially replicated in the brain of rat, which may be due to the selective advantage of such viruses in the brain tissue. To see whether such selection occurs in cultured neural

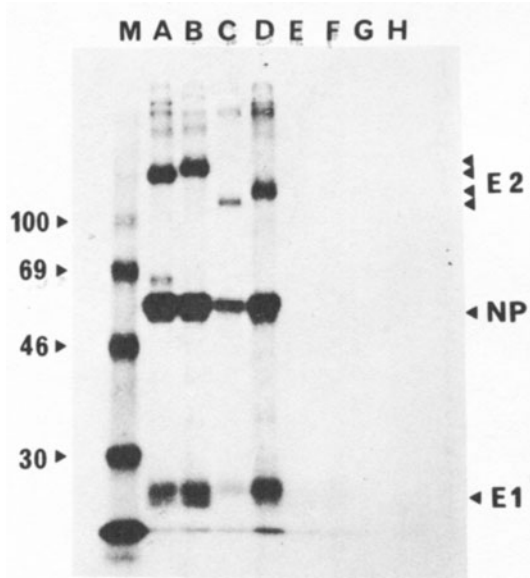

Fig.2. Electrophoresis of intracellular virus specific protein after immunoprecipitation. A, C, E and G: lysates from wt infected cells, B, D, F and H: lysates from cl-2 infected cells, C, D, G and H: from tunicamycin treated cells, A, B, C and D: precipitated with anti-JHMV serum, E, F, G and H: precipitated with normal rabbiat serum. M: molecular weight marker.

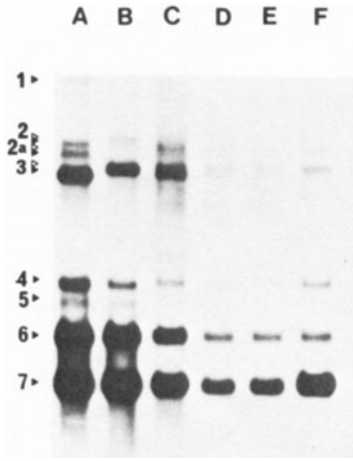

Fig.3. Northern blot analysis of mRNA patterns of viruses isolated from neural cells after infection of wt JHMV. A: wt JHMV, B: cl-2, C to F: viruses isolated on day 2 (C), 4 (D), or 6 (E and F).

cells, primary glial cell cultures prepared from newborn Lewis rat brain were infected with wt JHMV. The virus grew slowly and maintained a relatively low titer as compared with sensitive DBT cells, and syncytia formation was not detectable until 5 to 6 days p.i. DBT cells were infected with the viruses derived from infected primary glial cultures at various times p.i. The RNA was isolated from the cells and virus specific mRNAs were examined by Northern blot hybridization. As shown in Fig. 3, the major band of mRNA3 detected in DBT cells infected with virus derived from an early phase of glial cell infection was shown to be similar to wt JHMV. However, another band of mRNA3 with a larger molecular weight was detected, which corresponded in electrophoretic mobility to the mRNA3 of cl-2 virus. With increasing time of glial cell infection, viruses with larger mRNA3s became prominent, and on day 6, only larger the mRNA3 was detected. This shows that the virus with a larger mRNA3 possesses a growth advantage in cultured neural cells as compared with one with wt mRNA3. This is compatible to the growth advantage of cl-2 virus in the brain.

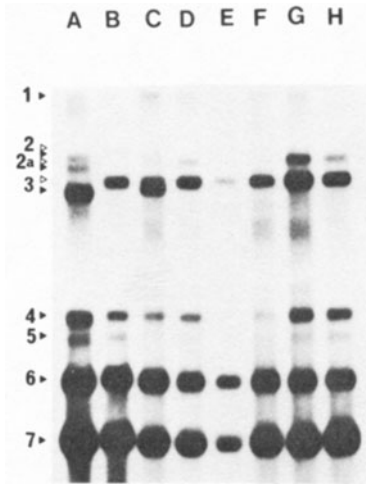

Fig.4. Northern blot analysis of mRNA of viruses derived from secondary neural cells after infection with wt JHMV, cl-2 or CNS virus. A: wt JHMV, B: cl-2, C and D: wt JHMV collected on day 4 (C) and day 10 (D), E and F: cl-2 collected on day 4 (E) and 10 (F), G and H: CNS virus collected on day 4 (G) and 10 (H).

We obtained a virus clone by plaque purification from the supernatant of cultured glial cells infected with wt JHMV, which has identical mRNA profile as the virus replicated in neural cells, and this virus was tentatively named CNS virus. The growth capability of wt JHMV, cl-2 and CNS virus in neural cells, passaged secondary neural cells consisting of more than 95 % astrocytes were examined. CNS virus multiplied to reach highest titer, 10^3 to 10^4 PFU/ 0.1 ml, among these viruses. To analyze the virus population growing in neural cells, the mRNA patterns of viruses released from infected neural cells were examined after infection on DBT cells. As shown in Fig. 4, in the case of wt JHMV, two different bands corresponding to wt JHMV mRNA3 and also the larger cl-2 type mRNA3 were present 4 days p.i. The lower band was more abundant compared with the upper one. However, virus samples collected on day 10 p.i. showed only the larger cl-2 virus type mRNAs. These results indicate that wt JHMV is a major population among the viruses being produced in astrocytes as early as 4 days p.i., but they are mostly replaced by CNS-like virus by day 10 p.i. As for the mRNA patterns of cl-2 and CNS virus from neural cells, no such change was observed for 10 days after infection. It is also shown that mRNAs 2 and 3 as well as one band found between them, designated tentatively 2a, were shown to be larger in cl-2 and CNS virus as compared with those from wt JHMV. However, these two variant viruses were different from one another in that mRNA2 was much more abundant than 2a in cl-2 infected cells whereas CNS virus had more mRNA2a than 2. The virus obtained 10 days after infection of astrocytes with wt JHMV did not differ from CNS virus in terms of mRNA pattern. This suggests that CNS virus was selected for its growth capacity in astrocytes.

Comparison of intracellular proteins produced by these three JHMVs in DBT cells were made by immunoprecipitation and SDS-PAGE (Fig. 5). The difference of intracellular viral protein pattern observed among these viruses was that the E2 glycoproteins of cl-2 and CNS virus were shown to have the same electrophoretic mobility in SDS-PAGE and they were larger than that of wt JHMV. The prominent difference observed between cl-2 and CNS virus was that the 65K protein was much more abundant in CNS virus-infected cells than in those infected with cl-2. The mRNA encoding 65K protein and the function of the protein is not

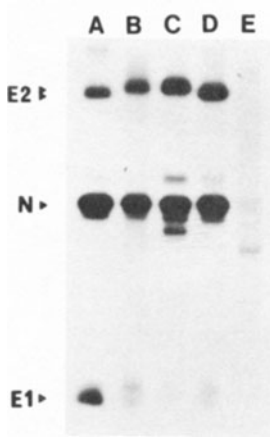

Fig.5. Electrophoresis of intracellular virus-specific proteins after immunoprecipitation with anti-JHMV serum. A and D: wt JHMV, B: cl-2, C: CNS virus, E: mock infection.

yet known. However, it should be noted that mRNA2a is appaarently more abundant in the cells infected with CNS virus than those infected with cl-2 virus.

All of above data suggest that viruses with larger E2 glycoprotein encoded by a larger mRNA3 multiply preferentially in the brain cells of rats, resulting in fatal acute encephalitis. E2 glycoproteins are believed to play a very important role for the virulence to animals. Recently, variant viruses in terms of virulence or pathogenicity were obtained by selection with monoclonal antibodies (14,15). Such findings indicate that particular regions of the E2 molecule are deeply involved in the high-virulence for rats. Detailed molecular biological analysis of JHMV variants with different virulence for animals will help to delineate the critical regions of E2 glycoprotein which may influence the high virulence for rats.

SUMMARY

After intracerebral inoculation of wild type (wt) JHMV into 4 to 5 week-old Lewis rats, only variants with larger mRNA3 were selectively propagated and no wt JHMV was reisolated from the brain. Detailed analysis of a cloned virus from infected rat brain, cl-2, showed that the virus had larger mRNAs 2, 2a and 3 as compared with those of wt JHMV, while there was no such difference for other mRNAs. The E2 glycoprotein of a variant virus was also shown to be larger as compared with that of wt JHMV. Such selective replication of variants were also observed in neural cell culture after infection with wt JHMV. However, these variants isolated from the brain of infected rat (cl-2) and from infected neural cells (CNS virus) differed from each other in the amounts of mRNAs 2 and 2a as well as 65 K protein. All of these data suggest that the viruses with larger E2 glycoprotein have the growth advantage in rat brain cells, which could be responsible for acute encephalitis of rats after infection with wt JHMV.

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REFERENCES

1. K. Nagashima, H. Wege, R.Meyermann, and V. ter Meulen. Coronavirus induced subacute demyelinating encephalomyelitis in rats. A morphological analysis. Acta Neuropathol. 44:63 (1987)
2. M. Koga, H. Wege, and V. ter Meulen. Sequence of murine coronavirus JHM induced neuropathological change in rat. Neuropathol. Appl. Neurobiol. 10:173 (1984)
3. H. Wege, R. Watanabe, and V. ter Meulen. Relapsing subacute demyelomyelinating encephalomyelitis in rats during the course of coronavirus JHM infection. J. Neuroimmunol. 6:325 (1984)
4. M. V. Haspel, P. W. Lampert, and M. B. A. Oldstone. Temperature-sensitive mutants of mouse hepatitis virus produce a high incidence of demyelination. Proc. Natl. Acad. Sci. 75:4033 (1978)
5. L. R. Knobler, P. W. Lampert, and M. B. A. Oldstone. Virus

persistence and recurring demyelination produced by a temperature-sensitive mutant of MHV-4. Nature 298:279 (1982).
6. P. W. Lampert, J. K. Sims, and A. Kniazett. Mechanism of of demyelination in JHM virus encephalomyelitis. Electronmicroscopic studies. Acta Neuropathol. 24:76 (1973)
7. R. Watanabe, H. Wege, and V. ter Meulen. Adoptive transfer of EAE-like lesions from rats with coronavirus-induced demyelinating encephalomyelitis. Nature 305:150 (1983)
8. F. Taguchi, A. Yamada, and K. Fujiwara. Resistance to highly virulent mouse hepatitis virus acquired by mice after low-virulence infection: Enhanced antiviral activity of macrophages. Infect. Immun. 29:42 (1980)
9. J. M. Chirgwin, A. E. Przybyla, R. J. MacDonald, and W. J. Rutter. Isolation of biologically active ribonucleic acid from sources enriched in ribonulease. Biochemistry, 18:5294 (1979)
10. M. A. Skinner, and S. Siddell. Coronavirus JHM: nucleotide sequence of the mRNA that encodes nucleocapsid protein. Nucleic Acids Res. 15:5045 (1983)
11. S. Makino, F. Taguchi, N. Hirano, and K. Fujiwara. Analysis of genomic and intracellular viral RNAs of small plaque mutants of mouse hepatitis virus, JHM strain. Virology, 139:138 (1984)
12. S. A. Stohlman, P. R. Bryton, J. O. Fleming, L. P. Weiner and M. C. Lai. Murine coronaviruses: Isolation and characterization of two morphology variants of JHM neurotropic strain. J. Gen. Virol. 63:265 (1982)
13. S. Siddell, H. Wege, A. Barthel, and V. ter Meulen. Coronavirus JHM: Intracellular protein synthesis. J. Gen. Virol. 53:145 (1981)
14. J. O. Fleming, M. D. Trousdale, F. A. K. 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 (1986)
15. R. G. Dalziel, P. W. Lampert, P. J. Talbot, and M. J. Buchmeier. Site-specific alteration of murine hepatitis virus type 4 peplomer glycoprotein E2 results in reduced neurovirulence. J. Virol. 59:453 (1986)