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Characterization of a Variant Virus Isolated from Neural Cell Culture after Infection of Mouse Coronavirus JHMV

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Our previous experiments showed that a variant virus with a larger envelope glycoprotein encoded by a larger mRNA3 (cl-2) multiplied predominantly in the brain of rats after wild type (wt) JHMV infection (F. Taguchi, S. Siddell, H. Wege, and V. ter Meulen, 1985, *J. Virol.* 54, 429–435). We could isolate similar but not identical variant virus after infection of cultured neural cells from rat brain with wt JHMV (designated CNS virus), which also had a larger mRNA3 and produced larger envelope E2 glycoprotein in infected cells. CNS virus multiplied to a higher degree in cultured astrocytes from rat than wt JHMV and cl-2. During infection with these variant viruses in neural cells, virus populations generated did not change, in contrast to consistent selection of viruses with larger mRNA3 after wt JHMV infections. CNS virus produced abundant mRNA2a as well as 65K glycoprotein while the productions of both were trace in cl-2 infected cells. The present experiments, together with our previous observation, suggest that the larger E2 glycoprotein may be of importance for the replication in rat brain cells.

Mouse hepatitis virus (MHV) belongs to the coronavirus family, which is an enveloped single stranded RNA virus (*1, 2*). Among many strains of MHV, JHMV is of particular interest, since this virus produces a chronic central nervous system (CNS) disease both in rats (*3, 4*) and mice (*5*). It was recently shown that lymphocytes sensitized by basic protein were largely involved in the establishment of subacute demyelinating changes in rats and that the sensitization of lymphocytes was triggered by JHMV infection (*6*). These findings indicate that JHMV can be used as a model for immunologically mediated demyelinating diseases of humans in which a viral etiology is suspected.

Recently, we discovered that a variant of JHMV multiplied preferentially in the brain of rats infected with wild type (wt) JHMV and that the original wt JHMV was hardly recovered from the tissue (*7*). The variant virus selected in the brain, cl-2, contained larger mRNA3 and larger E2 glycoprotein as compared with those of the original wt JHMV (*7*). In primary culture of neural cells from rats, another variant virus with larger mRNA3 as well as larger E2 glycoprotein was selectively propagated. This report describes the comparison of wt JHMV, cl-2, and a new isolate, tentatively named CNS virus.

Primary glial cell cultures prepared from newborn Lewis rat brain were infected with JHMV. Virus grew slowly and maintained a relatively low titer as compared with those in highly sensitive DBT or Sac (−) cells. Virus titers in the culture medium were 10^2 to 10^3 PFU/ml throughout the experimental period until 1 week postinfection (p.i.), and syncytia formation was not detectable until 5 to 6 days p.i. (manuscript in preparation). DBT cells (*8*) were infected with the viruses derived from infected primary glial cultures at various times p.i. and the RNA was isolated from the cells as reported previously (*7, 9*). Viral specific mRNAs were examined by Northern blot hybridization as described elsewhere (*7*)

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with ^{32}P-labeled cDNA made from mRNA7 of JHMV (*10*). As shown in Fig. 1, 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 of 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 (*7*). With time of glial cell infection, viruses with larger mRNA3 became prominent, and on Day 6, in one case, only larger mRNA3 was detected (Fig. 1, lane 6). This shows that the virus with larger mRNA3 possesses the growth advantage in cultured neural cells as compared with one with wt mRNA3. This is compatible to the growth advantage of cl-2 with larger mRNA3 in the brain as reported recently (*7*). We inoculated plaque-purified wt JHMV on astrocyte cultures and in every case we could isolate the virus with larger mRNA3. However, it is not clear at present whether the virus with larger mRNA3 was only selected or arose and was selected in astrocyte culture. 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 shown in Fig. 1 (lane 6), and this virus was tentatively designated as CNS virus. CNS virus was proven to be slightly, but clearly different from cl-2 virus in the patterns of mRNA and intracellular protein as described below.

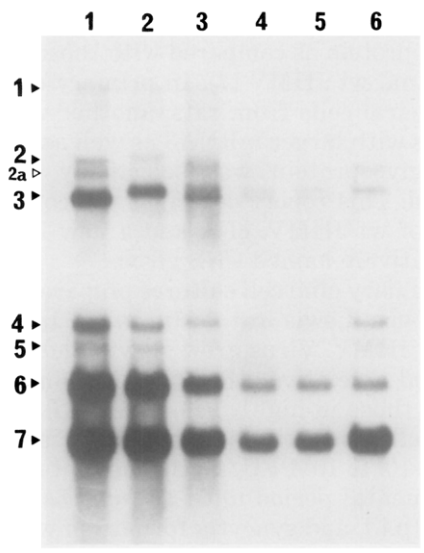

FIG. 1. Northern blot analysis of mRNA patterns of viruses isolated from neural cells after infection with wt JHMV. Primary culture of neural cells was infected with wt JHMV and virus produced in the cells at various time after infection were analyzed for mRNA pattern. 1, wt JHMV; 2, cl-2; 3 to 6, viruses isolated from infected neural cells on Days 2 (3), 4 (4), or 6 (5 and 6).

The mechanisms for generation of larger mRNAs are at present unknown. We could detect only one difference in oligonucleotide fingerprinting spots of genomic RNA between cl-2 and wt JHMV (*7*), which might be involved in the generation of larger mRNAs. Comparison of the sequences of two different mRNA3 could explain the mechanisms, which is now in progress.

In order to compare the growth capability of these viruses in neural cells, passaged secondary neural cells consisting of more than 95% astrocytes were infected with these JHMVs at a m.o.i. 0.1 and virus titers in the culture medium were plaque assayed (*11*). As shown in Fig. 2, CNS virus multiplied to reach highest titer, 10^3 to 10^4 PFU/0.1 ml, among three viruses. To analyze the virus population growing in neural cells, the mRNA patterns of viruses released from infected neural cells (shown in Fig. 2) were examined after infection on DBT cells. As shown in Fig. 3, 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 facts 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 in Fig. 3 as well as in Fig. 1 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

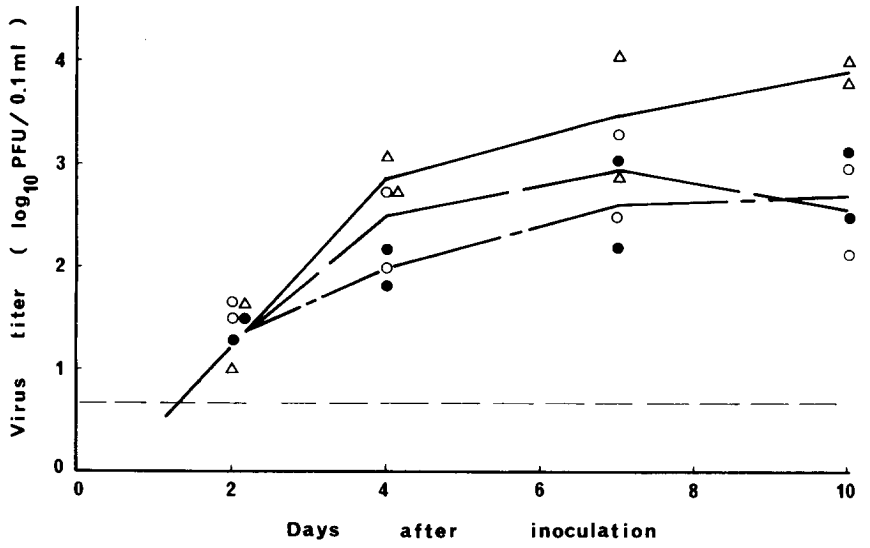

FIG. 2. Growth kinetics of wt JHMV (● - - ●), cl-2 (○ - - - ○), and CNS virus (△ — △). Secondary cultures of neural cells were infected at a m.o.i. 0.1 and virus titers in the culture medium sampled at intervals after infection were plaque assayed. For each virus, two petri dishes of culture were used. Dotted line indicates the lowest level for detection.

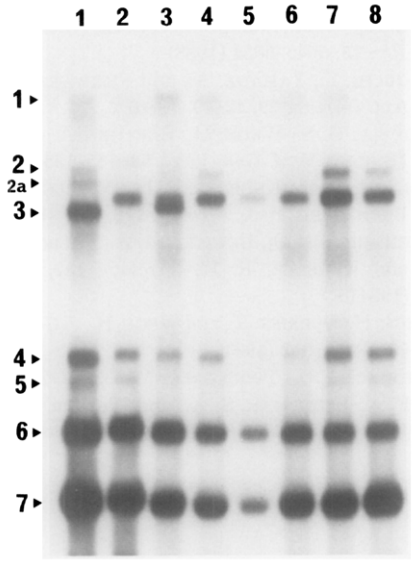

FIG. 3. Northern blot analysis of mRNA of viruses derived from secondary neural cells after infection with wt JHMV, cl-2, or CNS virus. DBT cells were infected with viruses collected from neural cells on Day 4 or 10 of infection and virus specific mRNAs produced in DBT cells were analyzed by Northern blot hybridization. 1 and 2, wt JHMV (1) and cl-2 (2) mRNA patterns as control; 3 and 4, wt JHMV collected on Day 4 (3) and Day 10 (4); 5 and 6, cl-2 collected on Day 4 (5) and Day 10 (6); 7 and 8, CNS virus collected on Day 4 (7) and Day 10 (8).

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.

For the comparison of intracellular proteins produced by these three JHMVs, DBT cells were infected at a m.o.i. 1 with viruses and infected cells were labeled with [^{35}S]methionine for 30 min when CPE covered 80 to 90% of monolayer, 8 to 10 hr p.i. Viral specific intracellular protein was examined by immune precipitation and SDS-polyacrylamide gel electrophoresis (SDS-PAGE) as mentioned elsewhere (*9*, *12*). As shown in Fig. 4, 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 65K protein was much more abundant in

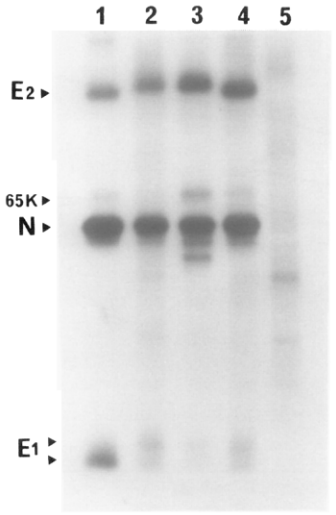

FIG. 4. Electrophoresis of intracellular virus-specific proteins. DBT cells infected with wt JHMV (1) and (4), cl-2 (2), CNS virus (3) or mock infected (5) were labeled with [^{35}S]methionine and cytoplasmic lysates were immunoprecipitated with anti-JHMV rabbit serum.

CNS virus-infected cells than in those infected with cl-2. The mRNA encoding 65K protein and the function of the protein is not yet known.

It is reported that E2 glycoprotein is important for virus adsorption onto susceptible cells and cell fusion (*13*) as well as for the virulence to animals (*14–16*). The selective growth of variant viruses with larger E2 glycoprotein in neural cell culture as shown in this paper as well as in rat brain also suggests that the larger E2 glycoprotein plays an important role for the virus to replicate in rat brain cells.

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