

## Comparison of Six Different Murine Coronavirus JHM Variants by Monoclonal Antibodies against the E2 Glycoprotein

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We have examined six different JHMV variants, sp-4 (recloned wt JHMV), cl-2, CNSV, DL, DS, and JHM-X, in terms of the sizes of the mRNA<sub>3</sub> and E2 glycoprotein as well as their reactivity to a panel of monoclonal antibodies to the E2 glycoprotein. Two of these variants, sp-4 and JHM-X, were found to have smaller mRNA<sub>3</sub> and E2 glycoprotein species compared with those of the other four variants. In addition, sp-4 and JHM-X were distinguished from the other four variants by their inability to bind to monoclonal antibodies recognizing two antigenic domains of the E2 molecule. Thus, six JHMV variants could clearly be divided into two groups with respect to the size and antigenicity of their E2 glycoproteins. © 1989 Academic Press, Inc.

Mouse hepatitis virus (MHV) belongs to the Coronaviridae whose name is based on the characteristic morphology of petal-shaped spikes of these viruses (1). They are enveloped viruses with a large nonsegmented, positive-stranded RNA genome (2, 3). MHV produces seven to eight discrete mRNAs. They form a 3'-coterminal nested set extending different lengths in 5' direction and each mRNA has a common 5'-leader sequence of about 70 bases (4, 5). In MHV-infected cells, three major viral proteins are detected. A nucleoprotein (N) of ca. 60,000 Da is the most abundant and is translated from mRNA<sub>7</sub> (6-8). Two other glycoproteins, E1 and E2, of ca. 23,000 and 120,000 to 160,000 Da, are translated from mRNAs 6 and 3, respectively (6-8). Among these three viral structural proteins, the E2 or peplomer glycoprotein has been shown to have particularly interesting biological activities (9-11). It is believed that the fusion of cells infected with MHV is caused by the E2 glycoprotein, which is fusogenic only after it has been cleaved proteolytically (12, 13). Another important biological function of the E2 glycoprotein is thought to be its binding to receptors on the cell surface as an initial step in virus replication in cells (10). The E2 glycoprotein is also believed to be involved in the pathogenesis of MHV on the basis of studies on monoclonal antibodies (14-17) and variant viruses isolated from persistent infections (18).

Recently, we have shown that the highly virulent variant viruses with larger E2 glycoproteins were preferentially isolated from rat brain (19) and cultured astrocytes (20) after infection by wild-type (wt) JHMV which contains a small mRNA<sub>3</sub> and E2 glycoprotein. These re-

sults suggest that there are at least two different types of JHMV with respect to the sizes of their E2 glycoproteins. It is of interest, therefore, to compare the E2 glycoproteins of various JHMV variants. In this communication, we report the comparison of six different JHMVs obtained from different laboratories with respect to the sizes of their mRNA<sub>3</sub> and E2 glycoproteins as well as their reactivities to a panel of monoclonal antibodies against the E2 glycoproteins.

The variant viruses used for the present experiment were cl-2 and CNSV isolated by passaging wt JHMV in rat brain and primary glial cell cultures, respectively, as previously reported (19, 20), and kindly provided by Dr. ter Meulen, University of Wurzburg; sp-4, which is a wt JHMV originally maintained in Germany and which had been subjected to four cycles of plaque-purification on DBT cells; DL and DS, which are plaque morphology variants of wt JHMV (21), kindly provided by Dr. Stohman, University of Southern California; and JHM-X (22), which is a derivative of wt JHMV maintained in Japan and was kindly provided by Dr. Makino at the same University. First, a comparison of the mRNA patterns of the six different JHMV variants was made, focusing on the size differences of mRNA<sub>3</sub>. RNA was extracted from DBT cells infected with each of the six JHMV variants, electrophoresed on agarose gels, and transferred onto nitrocellulose filters. Virus-specific mRNAs were detected by hybridization with <sup>32</sup>P-labeled cDNA prepared from mRNA<sub>7</sub> of JHMV as previously reported (23). As shown in Fig. 1, there were at least eight distinct mRNAs with different mobilities on agarose gels. There were no substantial differences in the mRNA patterns among the six different JHMV variants except for the sizes of mRNAs 2, 2a, and 3. The variants sp-4 and

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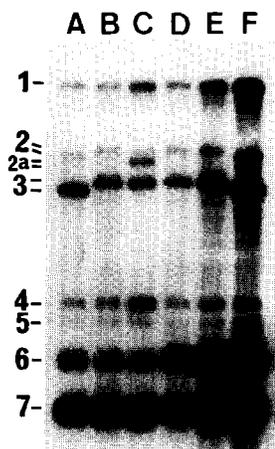


Fig. 1. Northern blot analysis of mRNA patterns of six different variants. RNA was extracted from DBT cells infected with each virus and virus-specific mRNAs were detected by hybridization with  $^{32}\text{P}$ -labeled cDNA. (A) sp-4; (B) cl-2; (C) CNSV; (D) DL; (E) DS; (F) JHM-X.

JHM-X produced mRNA 2, 2a, and 3 of smaller sizes (ca. 150,000 Da) compared with those produced by other variant viruses. In addition to these differences in the sizes of particular mRNAs, a striking difference in the amount of mRNA2 produced by CNSV was observed. In all other variants, mRNA2 was shown to be more abundant than mRNA2a; however, CNSV produced an excess of mRNA2a.

Next, we compared the sizes of the E2 glycoproteins produced by the six different JHMV variants. In addition, we examined the sizes of N proteins as control, because mRNA7, encoding N protein, was not different in length among the six viruses. Cell lysates prepared from DBT cells infected with each of variants were im-

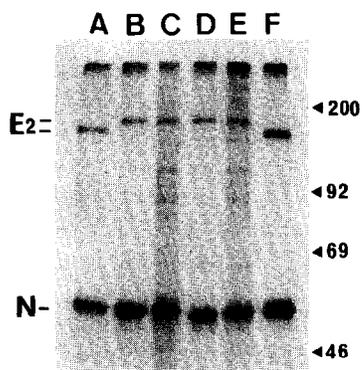


Fig. 2. Immunoprecipitation with monoclonal antibodies of E2 and N proteins of six different variants. DBT cells infected with each of six different variants were labeled with  $[^3\text{H}]$ leucine and cytoplasmic lysates were immunoprecipitated with monoclonal antibodies against E2 and N proteins. (A) sp-4; (B) cl-2; (C) CNSV; (D) DL; (E) DS; (F) JHM-X. Arrows indicate the molecular weights in kilodaltons.

MONOCLONAL ANTIBODIES	SPECIFICITY	sp-4	JHM-X	cl-2	CNSV	DL	DS
J. 7. 2	E 2			■	■	■	■
J. 2. 5	E 2			■	■	■	■
J. 1. 2	E 2			■	■	■	■
J. 7. 5	E 2			■	■	■	■
J. 7. 6	E 2			■	■	■	■
J. 2. 6	E 2			■	■	■	■
J. 2. 2	E 2			■	■	■	■
J. 7. 1	E 2	■	■	■	■	■	■
J. 2. 1	N	■	■	■	■	■	■
J. 3. 1	N	■	■	■	■	■	■
J. 3. 3	N	■	■	■	■	■	■
J. 1. 3	E 1	■	■	■	■	■	■
J. 2. 7	E 1		■	±		■	■

Fig. 3. Antigenic comparison of the structural proteins of the six different JHMV variants. Major antigenic domains "A," "B," and "not-A, not-B" on the E2 glycoprotein were determined by competitive binding studies, as previously described (15, 26). Antigenic relatedness of the different variants was determined by solid-phase ELISA using the panel of monoclonal assays, each employing triplicate samples. Binding is expressed as a percentage of the optical density relative to the DL strain, the virus used to produce the monoclonal antibodies. The blocks represent greater than 80% binding (■) or less than 15% binding (□); in ± an intermediate result was found. Of the anti-E2 monoclonal antibodies, J.2.2 is directed to site "B," J.7.1 to a "not-A, not-B," and all the rest are against site "A."

munoprecipitated with a monoclonal antibody against the E2 glycoprotein and N protein, and these proteins were analyzed by SDS-polyacrylamide gel electrophoresis as described elsewhere (24). As shown in Fig. 2, there was a clear difference in the mobility of the E2 glycoprotein among the six variants. E2 glycoproteins produced by sp-4 and JHM-X, both of which synthesized a small mRNA3, were shown to be approximately 15,000 Da smaller than those produced by the other variant viruses with larger mRNA3s. No significant differences were observed in the sizes of N proteins produced by the variants.

The antigenic properties of the E2 glycoproteins of the variants were determined by testing their binding to a panel of monoclonal antibodies produced by immunization with the DL strain as previously reported (25). As shown in Fig. 3, the monoclonal antibodies uniformly had excellent binding to all the viruses tested, with the exception of sp-4 and JHM-X, the two variants

with small mRNA3s and E2 proteins. These two variants did not bind to monoclonal antibodies recognizing two major antigenic regions on the peplomer, which have been designated E2(A) and E2(B). Monoclonal antibody J.7.1, recognizing a third site on E2, binds all viruses with essentially equal intensity, indicating that the E2 molecule is present in normal amounts in all variants tested. All monoclonal antibodies to N protein and one to E1 protein bound strongly to all six strains. However, the other monoclonal antibody to the E1 protein, J.2.7, did not bind variants cl-2, CNSV, and sp-4, showing that J.2.7 can distinguish variants maintained in West Germany from other JHMs. These results suggest that the determinants E2(A) and E2(B) are either antigenically altered or deleted in variants with small E2 glycoproteins. The second possibility seems more likely, in view of the uniformly high binding of the monoclonal antibodies to variants with large E2 proteins and the consistent lack of any binding of the antibodies to variants with small E2 glycoproteins. It seems very likely that these antigenic determinants reside in the domain consisting of a ca. 15,000-Da protein region in large E2 glycoproteins which is missing in small E2 glycoproteins. It was recently reported (26) that the recombinant viruses having approximately two-thirds of the JHMV mRNA3 coding region at the 5'-end and one-third of A59 mRNA3 at the 3'-end have lost their reactivity to monoclonal antibodies recognizing E2(A) and E2(B) of the JHMV E2 molecule, indicating that these two antigenic domains are likely encoded by one-third of the mRNA3 of JHMV at the 3'-end. From such observations, it may be speculated that ca. 500 bases found only in larger mRNA3s are located in one-third of the mRNA3 at the 3'-end. At present, we are trying to obtain cDNA which encode the larger E2 protein in order to localize the E2 domains specifically recognized by E2(A) and E2(B) monoclonal antibodies.

It has recently been reported that only the viruses with the larger E2 glycoprotein were isolated from the rat brain after infection with wt JHMV with a small E2 glycoprotein (19). The isolated virus, cl-2, was shown to replicate very well in the rat brain, causing severe encephalitis. This indicates that the viruses with the larger E2 glycoprotein may have a growth advantage in rat brain, resulting in acute and semiacute encephalomyelitis. Experiments are now in progress to determine the relationship between the size of E2 glycoproteins and neurovirulence of JHMV for rats.

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