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Antigenic Differentiation of Mouse Hepatitis Viruses by Neutralization Test

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Mouse hepatitis viruses (MHV) are members of the coronavirus group, which contain a single-stranded polyadenylated RNA genome (13, 18, 20). The discovery of cell lines suitable for MHV replication and the development of assay systems have made it possible to investigate the mode of replication and other important characteristics of MHV (6, 21, 24). As a result, some information has become available on the molecular aspects of MHV multiplication in cultured cells (13, 17, 18, 24). MHV is also known to cause hepatitis and demyelinating disease in mice. Variation in pathogenicity and organ tropism among many strains has been shown to occur (8, 16). In addition, studies of recent isolates of MHV from athymic nude mice dying with wasting disease (9) or from suckling mice with severe diarrhea (5, 10, 12) revealed some new aspects of the pathogenicity of MHV in mice (10, 11). However, despite extensive studies of MHV multiplication and many pathological examinations, little is known about the serological differences among MHV strains (5, 24), though cross-reactivity of some strains of MHV with other coronaviruses has been demonstrated (16).

In this communication, we report serological differences among six established and six freshly isolated strains of MHV, obtained by the neutralization test (NT) which is the only currently available method for the differentiation of closely related MHV strains.

The MHV strains MHV-1 (4), MHV-2 (15), MHV-3 (2), JHM (1), MHV-S (19), and MHV-A59 (14) were kindly supplied by Dr. J.C. Parker, Microbiological Associates, Inc., Bethesda, Md. MHV-A, MHV-U, MHV-Nu66 (9), MHV-K (11), and MHV-Nu67 were isolated from the livers of nude mice with hepatitis, and MHV-D was isolated from the intestines of suckling mice with severe diarrhea (10). These viruses were propagated in DBT cells (6) which were grown in Eagle's minimal essential medium (EMEM, Nissui, Tokyo) supplemented with 10% tryptose phosphate broth (TPB, Difco, Detroit, Mich.) and 8% calf serum. The virus stock used in the NT was prepared as described previously (22). Antisera to MHV-3, JHM, and MHV-S were produced in rabbits by injection of purified viruses emulsified with Freund's complete adjuvant, as previously reported (23). For NT, a modification of the method of Dulbecco (3) was employed, which was more convenient than the original method and more sensitive than the serum

dilution method (22) for the the differentiation of MHV strains.

A preliminary test with the serum dilution method indicated that JHM was distinct from MHV-S. Therefore, NTs were performed with antisera to JHM and MHV-S and, as antigens, JHM, MHV-S, and MHV-2 which is clearly different from the other two viruses in terms of cytopathic effect (6, 7). Antiserum was diluted in 10-fold steps with EMEM containing 10% TPB (EMEM-TPB) and 0.4 ml of the dilution was mixed with an equal volume of EMEM-TPB containing 2×10^5 PFU/0.2 ml of virus. The mixture was incubated at 37 C for 45 min in a humid CO_2 incubator and titrated for remaining infectious virus. The results, shown in Fig. 1, indicate that anti-JHM serum neutralized JHM very efficiently and MHV-2 moderately, but did not neutralize MHV-S (Fig. 1A), and that the anti-MHV-S serum gave a reverse pattern of neutralization (Fig. 1B). These

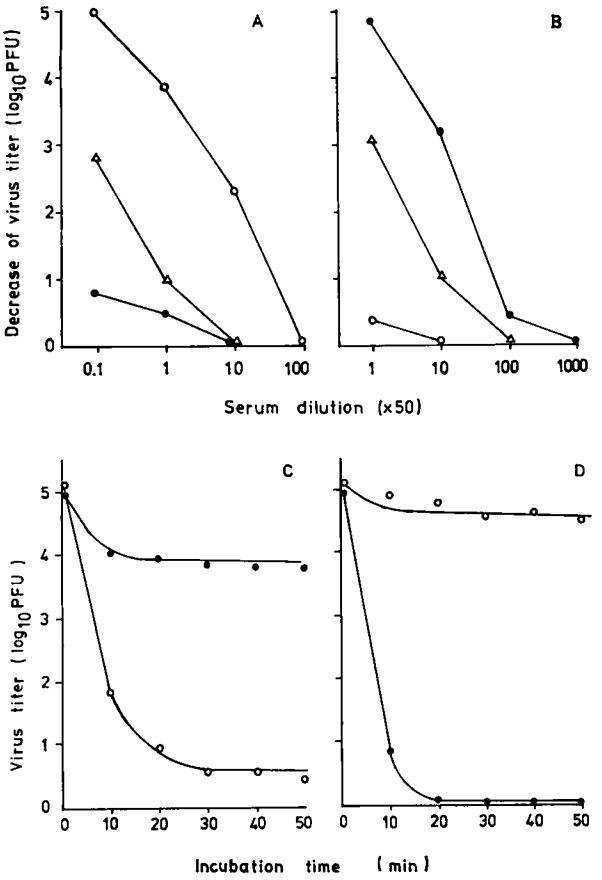

Fig. 1. Neutralization of JHM (○), MHV-S (●), and MHV-2 (△) by 10-fold serially diluted anti-JHM (1A) and anti-MHV-S (1B) sera, and kinetics of neutralization of JHM (○) and MHV-S (●) by anti-JHM (1C) and anti-MHV-S (1D) sera. For details, see the text.

results are compatible with those obtained by the serum dilution method (data not shown). Figure 1 also shows that it is sufficient for the differentiation of MHV strains to perform the NT with one antiserum dilution; the antiserum neutralized almost completely the infectivity (1×10^5 PFU/0.2 ml) of the homologous virus. As a next step, the mixture of virus and antiserum was tested at various times during the incubation period to determine the equilibrium of neutralization. An aliquot of 0.4 ml of 5-fold-diluted anti-JHM or 50-fold-diluted anti-MHV-S serum was mixed with 0.4 ml of JHM or MHV-S containing 2×10^5 PFU/0.2 ml in EMEM-TPB and the mixture was incubated at 37 C for 10 to 50 min. Figures 1C and 1D show that neutralization reached an equilibrium 20 to 30 min after incubation in all cases under these conditions. On the basis of these results, NT was performed with 12 strains as follows. Anti-MHV-3, anti-JHM, and anti-MHV-S sera were diluted with EMEM-TPB to the point at which the homologous virus at a titer of 1×10^5 PFU/0.2 ml was neutralized. To each antiserum, an equal volume of the desired MHV strain titering 2×10^5 PFU/0.2 ml was added, and the mixture was incubated at 37 C for 45 min in a humid CO_2 incubator and assayed for remaining infectious virus. As shown in Fig. 2, the 12 strains could be divided into three groups: 1) strains neutralized by anti-MHV-3 serum (MHV-1, MHV-2, MHV-3, MHV-S, MHV-A59, MHV-U, MHV-Nu66, and MHV-Nu67), 2) those neutralized by anti-JHM serum (JHM, MHV-A, and MHV-K), and 3) MHV-D which was not neutralized by any of the sera. Most strains group 1 were also neutralized by anti-MHV-S serum, and MHV-2 was partially neutralized by anti-JHM serum, but MHV-3 was not neutralized by either of them.

Of the seven strains neutralized by both anti-MHV-3 and anti-MHV-S sera (in group 1), all except MHV-2 and MHV-A59 are of low-virulence for weanling

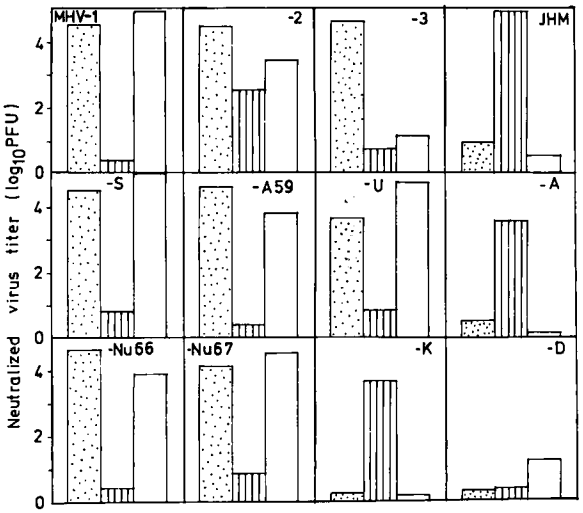

Fig. 2. Neutralization of 12 MHV strains by anti-MHV-3 (▒), anti-JHM (▥), and anti-MHV-S (□) sera. For details, see the text.

mice (8, 9, 16). High-virulence strains MHV-2, MHV-3, and MHV-A59 were neutralized by anti-MHV-3 serum, but the high-virulence of these strains did not coincide with serological relatedness, because many other low-virulence strains were also neutralized by anti-MHV-3 serum. This indicates that there is no correlation between virulence and serological type in MHV strains. Demyelinating disease can be produced in mice by infection with JHM (16) and MHV-S (16, 22), but these viruses are serologically different. This also suggests that there is no correlation between the disease picture and serological type of MHV.

MHV-D, which is different from all the other strains tested, produces in suckling mice primary enteritis due to infection of intestinal epithelial cells by the orally administered virus (10). Such enteritis was not produced by the other strains tested in the present study, though enteritis as a result of severe hepatitis has been reported for MHV-S (22) and MHV-3 (16). In preliminary experiments with anti-MHV-D mouse serum, MHV-S and MHV-2 were neutralized to the same extent as MHV-D, while MHV-3 and JHM were not neutralized (data not shown). On the other hand, anti-MHV-S rabbit serum reacted with MHV-S but not with MHV-D. Similar data were obtained with MHV-S/CDC isolated from infant mice with diarrhea (5). Anti-MHV-S/CDC serum neutralized MHV-S as well as MHV-S/CDC, but antibodies to MHV-S neutralized MHV-S/CDC less efficiently. It was shown in the same study, by complement fixation, that antiserum to MHV-S/CDC unilaterally reacted with another agent of infant diarrhea, lethal intestinal virus of infant mice (LIVIM) (12). These observations suggest the possibility that MHV-D, MHV-S/CDC and LIVIM comprise a group distinct from other MHV strains with regard to serological cross-reactivity and pathogenicity for suckling mice.

Recently, Wege et al determined the antigenic relationships among several MHV strains by NT (24); their results generally agree with those obtained by Hierholzer et al (5). They showed that individual strains of MHV were neutralized most effectively by the antiserum to the homologous virus, though cross-reactivity was observed among some strains. The essential difference between our experimental system and those of the other authors (5, 24) are the animal species used for the production of antisera and the procedures used for immunization, *i.e.*, we injected purified virus with complete Freund's adjuvant into the footpad of rabbits (23), while the other authors injected infectious virus into mice by the peritoneal route (24). These differences might be responsible for the inconsistency in the results.

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