

Antigenic Relationships of Murine Coronaviruses: Analysis Using Monoclonal Antibodies to JHM (MHV-4) Virus

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Monoclonal antibodies were produced to JHMV-DL, a neurotropic member of the mouse hepatitis virus (MHV) or murine coronavirus group. Of 23 antibodies isolated, 10 were specific for the major envelope glycoprotein, gp180/90, 10 for the nucleocapsid protein, pp60, and 3 for the minor envelope glycoprotein, gp25. Eleven different MHV isolates were used in antibody binding assays to study antigenic relationships among the viruses. Each MHV isolate tested had a unique pattern of antibody binding, indicating that each is a distinct strain. Conservation of JHMV-DL antigenic determinants varied among the three proteins, with pp60 showing intermediate conservation, gp180/90 little conservation, and gp25 marked conservation in the different MHV strains. Monoclonal antibodies to pp60 proved most useful in delineating antigenic relationships among MHV strains. These antigenic groups correlated with pathogenic types, indicating that pp60 may be one of the gene products which mediates the distinct disease patterns manifested by different murine coronaviruses.

INTRODUCTION

Coronaviruses are enveloped viruses containing a single-stranded RNA genome of positive polarity (ter Meulen *et al.*, 1982; Sturman and Holmes, 1983). The murine coronavirus or mouse hepatitis virus (MHV) group is of particular interest because of the wide range of diseases produced by its members. Although the majority of MHV strains usually cause inapparent or latent gastrointestinal infections in nature (Gledhill and Niven, 1955; Rowe *et al.*, 1963), different MHVs have been reported to spontaneously or experimentally produce hepatitis, enteritis, peritonitis, lower respiratory infections, panencephalitis, demyelination, choro-

doependymitis, meningitis, myeloproliferation, and wasting disease in susceptible hosts (Virelizier *et al.*, 1975; Ishida *et al.*, 1978b; Hierholzer *et al.*, 1979; Wege *et al.*, 1982).

Despite marked variations in pathogenicity, the MHVs have relatively simple structural features, usually consisting of a major envelope glycoprotein, a minor envelope glycoprotein, and a nucleocapsid protein. The major glycoprotein, gp180/90, forms the projecting spikes or peplomers. These are important for cell attachment (Sturman and Holmes, 1983) and may influence tropism for specific tissues. The minor glycoprotein, gp25, is largely an internal protein, although a small segment projects through the virion envelope (Sturman, 1982). This protein is probably analogous to the matrix protein of other enveloped viruses and is thought to play a role in the control of viral maturation and establishment of virion stability (Holmes *et al.*, 1982). The nucleocapsid protein, pp60, is intimately associated with the viral RNA. The simplicity of the struc-

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ture of the virion makes the MHV group an ideal subject for comparative studies of pathogenesis, as, in principle, variations in disease patterns can be correlated with changes in protein structure.

The relatedness of the MHV strains has been examined by a number of methods, including cross-neutralization with hyperimmune serum (Hierholzer, 1979; Wege, *et al.*, 1981) and, more recently, kinetic neutralization (Childs *et al.*, 1983). Most MHV strains are antigenically distinct by these methods, although the MHV3 and A59 strains are very similar. Analysis of the viral polypeptides has shown that some MHVs have proteins with unique migration patterns by sodium dodecyl sulfate polyacrylamide gel electrophoresis (Bond *et al.*, 1979; Cheley *et al.*, 1981; Lai and Stohman, 1981). More recently, several studies have compared the oligonucleotide maps of closely related MHVs that cause different diseases in order to identify the genes responsible for different disease patterns (Lai *et al.*, 1981; Wege *et al.*, 1981; Stohman *et al.*, 1982).

JHM virus (JHMOV) is a neurotropic MHV which may cause encephalitis and demyelination (Bailey *et al.*, 1949; Weiner, 1973). Its structural features (Massalski *et al.*, 1982), proteins and genome (Lai and Stohman, 1981), are those of a typical MHV. In animal models of chronic demyelination, JHMOV persistence has been demonstrated for extended periods (Stohman and Weiner, 1981; Knobler *et al.*, 1982). Recently two plaque-size variants of JHMOV have been isolated and characterized: a large plaque isolate, JHMOV-DL, with a high propensity to acute encephalitis, and a small-plaque variant, JHMOV-DS, which predominantly causes chronic demyelination (Stohman *et al.*, 1982). In this manuscript, we report the use of a panel of JHMOV-DL-specific monoclonal antibodies to study the antigenic relationships of the structural proteins of 11 separate MHV isolates.

MATERIALS AND METHODS

Viruses and cells. Viruses were propagated in DBT cells as described previously (Stohman and Weiner, 1978), except when

virus in serum-free media was desired for use in radioimmunoassay (RIA). In this case, infected cells were first cultured in Dulbecco's Modified Eagle's Medium (DMEM, Gibco Laboratories, Grand Island, N. Y.) containing 1% fetal calf serum; when cytopathic effect (CPE) was first visible, the cells were washed twice with serum-free DMEM, and DMEM supplemented with 20 mM HEPES buffer, pH 7.2, was then added. At maximal CPE, the supernatant was collected, clarified by centrifugation at 375 *g* for 10 min at 4°, and stored at -70°.

The derivation and characterization of the two plaque morphology variants of JHMOV (MHV-4), DL (large plaque) and DS (small plaque), have been described recently (Stohman *et al.*, 1982). MHV-1, MHV-2, MHV-3, and MHV-S viruses were plaque-purified from virus stocks obtained from Dr. M. Collins, Microbiological Associates, Bethesda, Maryland and K. Fujiwara, University of Tokyo, Tokyo, Japan. The MHV-K, MHV-D, and MHV-Nuu viruses were obtained as cloned stocks from Dr. K. Fujiwara. The MHV-M virus, an isolate from nude mice with wasting disease, was obtained from Dr. M. Collins. Vesicular stomatitis virus (VSV) and herpes simplex virus, type 1 (HSV-1), were obtained from Dr. P. Brayton and Dr. D. Willey, respectively, both of the University of Southern California, School of Medicine.

For the production of the monoclonal antibodies, M5 cells, a horse serum-adapted line of SP2/0-Ag14, obtained from Dr. J. Davie, Washington University, St. Louis, Missouri, were grown in DMEM supplemented with 10% horse serum, 20 mM HEPES, 100 IU/ml penicillin, 100 µg/ml streptomycin, 2 mM L-glutamine, 10 mM MEM nonessential amino acids (Gibco Laboratories, Grand Island, N. Y.), and 5×10^{-5} M 2-mercaptoethanol. During selection, HAT was added to the medium (final concentrations: 1×10^{-4} M hypoxanthine, 4×10^{-7} M aminopterin, and 1.6×10^{-5} M thymidine) (Littlefield, 1964).

Virus neutralization. The ability of the monoclonal antibodies to neutralize virus was assayed as previously described (Stohman and Weiner, 1981). Briefly, 25-

μ l serial dilutions of antibody (heat inactivated at 56° for 30 min) were added to wells of a 96-well microtiter plate; subsequently, 25 μ l of a virus suspension containing 10-TCID₅₀ doses (50% tissue culture infective doses) was added to each well. The plates were incubated at 37° for 1 hr, and then 2×10^4 DBT cells were added to each well. The neutralizing titer was expressed as the highest dilution of antibody which prevented CPE in 50% of the wells.

Antibody binding assay. The solid phase radioimmunoassay (RIA) for antiviral antibodies has been described previously (Fleming *et al.*, 1983). Viral antigens were used in excess; preliminary assays showed that each virus stock contained sufficient antigen to produce maximal binding with a hyperimmune anti-MHV standard serum. After cloning, 25 μ l of supernatant from established hybridomas was assayed at 10^{-1} dilution. Serial dilutions of antibody showed that this concentration fell within the linear region of the assay. In cross reactive RIA, the binding of antibodies at 10^{-1} dilution was tested against homologous virus (JHMV-DL) and compared to the binding to heterologous viruses.

Relative binding was expressed by a modification of the convention of Gerhard *et al.* (1981). The counts per minute (cpm) bound to homologous (JHMV-DL) antigen were normalized to 100%. Binding of greater than 50% of this value was considered strongly positive; 25-50%, moderately positive; less than 25% but more than twice background, weakly positive; and less than twice background, negative. Results shown in the block diagrams are the composite of at least three assays, each of which consisted of quadruplicate samples.

Antisera. Hyperimmune antisera to JHMV-DL and A59 were made by serial immunizations of C57BL/6J (B6) mice obtained from Jackson Laboratories, Bar Harbor, Maine. Normal mouse serum free of anti-MHV antibody was obtained from B6 mice immediately after being received. Control antibodies used included monoclonal anti-mouse Ia (Harmon *et al.*, 1982), and rabbit anti-HSV-1, kindly supplied by Dr. D. Willey of the University of Southern California, School of Medicine.

Preparation of monoclonal antibodies. The technique of Kohler and Milstein (1975) as modified by Harmon *et al.*, (1982), was followed. B6 mice, 6 weeks of age, were inoculated intraperitoneally with approximately 10^5 plaque-forming units of JHMV-DL grown in serum-free medium. Secondary immunization, usually 6 weeks later by the intravenous route, also consisted of 10^5 plaque-forming units of virus in serum-free medium. Suspensions of immune spleen cells were fused to M5 cells at a ratio of 2.5:1 using 34% polyethylene glycol MW 1500 (Aldrich Chemical Co., Milwaukee, Wis.) at 37°. The suspension was then washed, resuspended in HAT medium, and cultured in 24-well plates at 1×10^6 cells per well, supplemented by 1×10^6 feeder spleen cells/well prepared from nonimmune B6 mice. Following incubation for 4 days at 37°, half of the medium was removed from each well and replaced with fresh HAT medium containing 1×10^6 feeder cells. On Day 8 this step was repeated with HT media (aminopterin-free) and feeder cells. On Day 11, wells were screened by RIA for antiviral antibody. Cells in positive wells were cloned by visual inspection and limiting dilution in 96-well plates. Clones were expanded, and supernatants were subsequently harvested and reassayed by RIA. Monoclonal antibodies were assayed for immunoglobulin class and subclass by Ouchterlony immunodiffusion, using antiserum to murine immunoglobulin isotypes (Litton Bionetics, Kensington, Md.).

Radioimmunoprecipitation. The specificity of the monoclonal antibodies for viral proteins was determined by radioimmunoprecipitation (RIP). DBT cells were inoculated with virus at a multiplicity of infection of 1-5 for 1 hr at 37°. After removal of the inoculum, the cultures were incubated in the presence of serum-free DMEM containing 1 μ g/ml actinomycin D. The medium was removed and replaced with prewarmed methionine-free DMEM (MFDMEM) for 15 min. The medium was again removed and replaced with prewarmed MFDMEM containing 20 μ Ci/ml ³⁵S-methionine (New England Nuclear, Boston, Mass.). Following incubation for

30-45 min, the cultures were placed on crushed ice, washed 2 times with ice-cold PBS, and solubilized with a buffer composed of 10 mM Tris-HCL pH 7.4, 150 mM NaCl, 0.5% NP-40, 1 mg/ml *N-p*-tosyl-L-lysine-chlormethyl ketone HCL (TLCK), 200 µg/ml phenylmethylsulphonyl fluoride (PMSF), and 500 u/ml aprotinin. The lysate was clarified by centrifugation at 500 *g* for 5 min and stored at -70°. For immunoprecipitation, the lysates were adjusted to 0.2% sodium dodecyl sulfate (SDS) and incubated with an equal volume of polyvalent antiserum or monoclonal antibody as described by McMillan *et al.* (1981). The immune complexes were adsorbed to a suspension of protein-A bearing *Staphylococcus aureus* as described by Kessler (1981). The final pellet was resuspended in a buffer containing 67 mM Tris-HCL, pH 6.8, 2.66% SDS, 5.0% 2-mercaptoethanol, heated at 56° for 2 min, and then centrifuged for 1.5 min at 10,000 *g*. The supernatants were analyzed by electrophoresis on 6-15% discontinuous slab polyacrylamide gels.

RESULTS

Production and characterization of monoclonal antibodies to JHM-DL virus. Twenty-three cloned hybridomas produced antiviral antibodies as determined by RIA. The specificity of each antibody was determined by precipitation of JHMV-DL proteins from a lysate of infected cells. Figure 1 shows examples of the immunoprecipitates analyzed on six 15% gradient polyacrylamide gels. Lysates harvested late in infection contained various quantities of the degradation products of the nucleocapsid protein (lanes 7, 8, 9, 10, and 11 in Fig. 1 and lanes 6 and 8 in Fig. 5). In addition, a few monoclonal antibodies immunoprecipitated other weak protein bands (see lane 13 in Fig. 1 and lane 11 in Fig. 5) which could be eliminated by increasing the SDS to 0.5% in the reaction mixture. Based on the data obtained by immunoprecipitation, the 23 monoclonal antibodies were placed in three groups. Table 1 shows that 10 antibodies reacted with the major glycoprotein, gp180/90, 10 reacted with the nucleocapsid protein,

pp60, and 3 reacted with the minor envelope glycoprotein gp25. All the antibodies were tested for their ability to neutralize JHMV-DL. Six of the ten that reacted with gp180/90 neutralized JHMV-DL. None of the antibodies reactive with either pp60 or gp25 were able to neutralize JHMV-DL (Table 1). The heavy-chain immunoglobulin isotype of each antibody was tested by immunodiffusion. All the antibodies reacted with only a single anti-isotype antiserum. The isotypes for each monoclonal antibody are shown in Table 1.

Reactivities of antibodies with nucleocapsid protein pp60. Ten monoclonal antibodies reactive with the nucleocapsid protein of JHMV-DL, pp60, were used to examine the antigenic conservation of this protein in MHV strains that induce diverse types of disease. Antigenic preservation was determined in an RIA which tested the 10 antibodies with different MHV strains serving as antigens. Table 2 shows an example of this type of experiment. Anti-pp60 antibodies were tested for their ability to bind to the homologous antigen (JHMV-DL), the small-plaque variant of this virus (JHMV-DS), and a related murine coronavirus (MHV-A59), as well as control antigens (HSV-1, VSV). Table 2 shows binding of at least 25% of the values for homologous virus in 6 of 10 reactions of anti-pp60 antibodies with MHV-A59 and none of 10 with JHMV-DS. Since viral antigens were used in excess and binding of antibodies to the glycoproteins of JHMV-DS was equal to the binding to the glycoproteins of homologous virus (see below), we do not attribute the very low binding of antibodies to JHMV-DS nucleocapsid protein, pp60, to limited antigenic density of the viral preparation. Anti-pp60 monoclonal antibodies did not react with control viral antigens, and control monoclonal antibody did not react with MHV antigens.

The relative binding of antibodies to different MHV strains in a larger series of experiments was used to construct the block diagram presented in Fig. 2. The pattern of reactivities indicates a moderate degree of antigenic conservation of pp60 among MHV strains. By inspection, the viruses tested could be divided into two broad

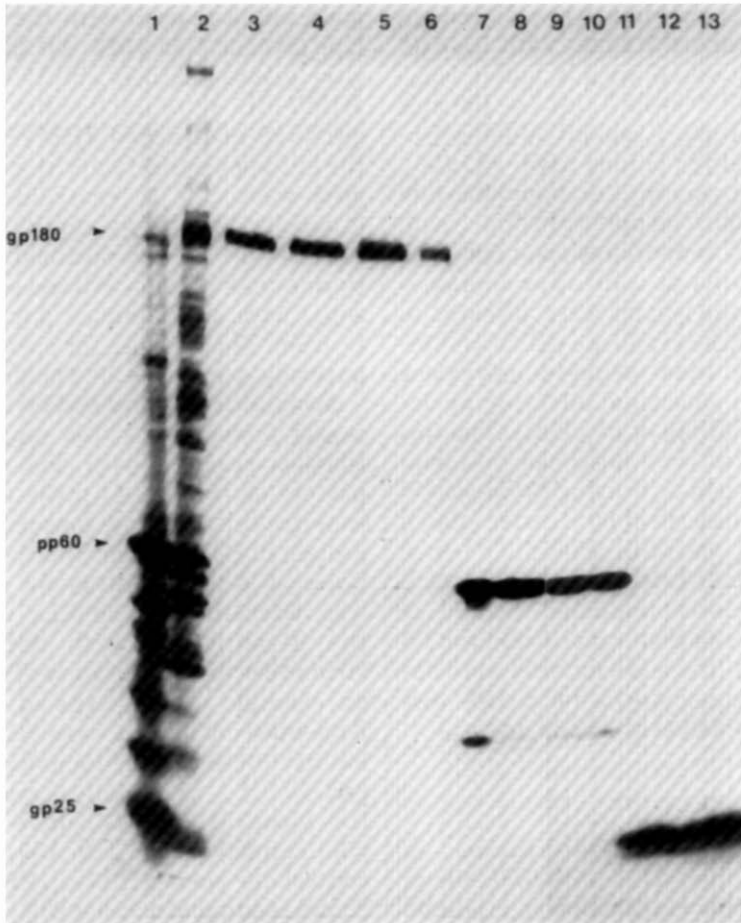


FIG. 1. Immunoprecipitation of JHMV proteins by monoclonal antibodies from [35 S]methionine-labeled infected cell lysate. Lanes 1 and 2 are whole lysates prepared at 5.5 hr post-infection (lane 1) and 7.0 hr postinfection (lane 2). Lane 3 through 6 are monoclonal antibodies reactive with gp180/90 (lane 3, J.1.2; lane 4, J.2.5; lane 5, J.7.5; lane 6, J.7.6). Lanes 7 through 10 are monoclonal antibodies reactive with pp60 (lane 7, J.2.1; lane 8, J.3.7; lane 9, J.3.3; lane 10, J.1.1). Lane 11 (J.1.3), lane 12 (J.2.7), and lane 13 (J.3.11) are monoclonal antibodies reactive with gp23. Samples were analyzed on 6–15% gradient polyacrylamide slab gels.

groups based on their reactivities. The first group consisted of A59, MHV-3, MHV-D, MHV-K, and MHV-Nuu viruses, all of which did not react with monoclonal antibodies J.1.1, J.3.14, J.3.7, and J.3.15. The antigenic sites recognized by these antibodies are apparently conserved in the second group, composed of MHV-2, MHV-S, and MHV-1 viruses. In addition to these two groups, two individual viruses, JHMV-DS and MHV-M, were notable for their lack of marked binding (less than 25% cpm versus homologous virus) with any of the 10 anti-pp60 monoclonal antibodies tested.

Reactivities of antibodies with major glycoprotein 180/90. The major virion envelope glycoprotein, gp180/90, is capable of a number of functions, including recognition of the host cell receptor (Sturman and Holmes, 1983). It was therefore of interest to investigate the antigenic relationships of gp180/90 in a number of different MHV strains which produce a variety of diseases. All monoclonal antibodies recognized antigenic determinants of the small plaque variant JHMV-DS equally well as the immunogen, JHMV-DL, indicating the close antigenic relationship of

TABLE 1
CHARACTERIZATION OF MONOCLONAL
ANTIBODIES TO JHMV-DL^a

Antigen specificity	Monoclonal antibody	Isotype	Neutralizing titer ^b
gp180/90	J.1.2	IgG 2b	—
	J.7.18	IgG 3	1:256
	J.2.2	IgG 2b	1:512
	J.7.2	IgG 2b	1:1024
	J.2.5	IgG 2a	—
	J.2.6	IgG 2b	1:128
	J.7.1	IgG 2a	—
	J.7.5	IgG 2b	1:512
	J.7.6	IgG 2b	1:256
	J.1.16	IgG 2b	—
pp60	J.2.1	IgG 2b	—
	J.3.13	IgG 2b	—
	J.3.5	IgG 2a	—
	J.3.1	IgG 2a	—
	J.3.3	IgG 2a	—
	J.3.4	IgG 2a	—
	J.1.1	IgG 2b	—
	J.3.14	IgG 2b	—
	J.3.7	IgG 2b	—
	J.3.15	IgG 2a	—
gp25	J.1.3	IgG 2b	—
	J.2.7	IgG 2a	—
	J.3.11	IgG 2b	—

^a Monoclonal antibodies were obtained from hybridoma tissue culture supernatants. Antigen specificity was determined by RIP, neutralizing titer by microassay, and immunoglobulin isotype by immunodiffusion as described under Materials and Methods.

^b Symbol (—) indicates no neutralization at 1:4 dilution.

these two viruses (Fig. 3). By contrast, very few of the antigenic determinants on the other MHVs were closely related to those of JHMV-DL, indicating the lack of conservation of JHMV major glycoprotein antigens among the rest of the MHV strains.

Several antibodies, such as J.1.2, J.7.18, J.2.2, and J.7.2, recognize only JHMV variants DL and DS and thus may appear to have identical specificities. Nevertheless, differences in fine specificity were demonstrated in further experiments. We first isolated variant JHM viruses which escaped neutralization by monoclonal antibodies. When these variant viruses were

used as antigens, each of the 10 anti-gp180/90 monoclonal antibodies in our panel showed a distinctive pattern of reactivity to them, indicating each antibody in this set recognized a unique antigenic determinant (Fleming, unpublished observations).

Reactivity of antibodies with minor glycoprotein gp25. The smaller viral glycoprotein, gp25, is embedded within and extends through the viral envelope. It has been suggested that it may function as a matrix protein and is essential in viral budding and in the formation of the viral envelope (Holmes *et al.*, 1982). Three monoclonal antibodies were obtained which are specific for gp25. This protein appears to be highly conserved among the MHV strains (Fig. 4). Nevertheless, this finding must be regarded as tentative, in view of the small numbers of available antibodies with specificity for gp25.

Cross immunoprecipitations. To confirm the pattern of antibody binding determined by RIA, anti-JHMV-DL monoclonal antibodies were tested for their ability to immunoprecipitate the appropriate viral protein from a lysate of cells infected with the related MHV, A59. Figure 5 shows that those anti-JHMV-DL antibodies (J.7.5, J.2.1, J.1.3, and J.2.7) which react with A59 by RIA were also able to immunoprecipitate the appropriate A59-specified protein, indicating that the shared antigens detected by RIA were in fact on the analogous protein. By contrast, those antibodies that were unreactive with A59 virus by RIA (J.2.5, J.1.2, J.3.7, and J.1.1) did not immunoprecipitate detectable radiolabeled A59 virus proteins. Taken together, the immunoprecipitation data confirm the specificities of the anti-JHMV-DL antibodies as determined by RIA.

DISCUSSION

Although murine coronaviruses have a relatively simple structure, they cause a wide variety of diseases. In an attempt to understand the basis for their diverse pathogenicities and to obtain information on the antigenic characteristics of these viruses, we have used 23 monoclonal an-

TABLE 2
REACTIONS OF ANTI-pp60 MONOCLONAL ANTIBODIES TO HOMOLOGOUS AND HETEROLOGOUS VIRUSES^a

Antibodies ^b	JHMV-DL	Viruses JHMV-DS	A59	VSV	HSV-1
J.2.1	14,570 ± 750	910 ± 14 (6.2%)	12,777 ± 180 (87.6%)	—	—
J.3.13	18,145 ± 169	2150 ± 91 (11.8%)	12,420 ± 367 (68.4%)	—	—
J.3.5	5,831 ± 510	43 ± 40 (.7%)	5,176 ± 245 (88.8%)	—	—
J.3.1	18,227 ± 95	2535 ± 77 (13.9%)	12,267 ± 1092 (67.3%)	—	—
J.3.3	23,125 ± 1011	2715 ± 558 (11.7%)	21,122 ± 1665 (91.3%)	—	—
J.3.4	11,886 ± 1731	1863 ± 286 (15.7%)	4,896 ± 470 (41.2%)	—	—
J.1.1	3,675 ± 90	—	—	—	—
J.3.14	4,051 ± 258	—	—	—	—
J.3.7	347 ± 31	—	—	—	—
J.3.15	1,721 ± 27	—	—	—	—
anti-Ia	—	—	—	NT	NT
anti-HSV-1	NT	NT	NT	NT	34,902 ± 1672

^a Sample RIA: Data are given as counts per minute with mean value and one standard deviation of four replicate samples. Cpm for JHMV-DS and A59 are also expressed as a percentage of cpm for JHMV-DL (parenthesis). Symbol (—) indicates background cpm and NT indicates that the interaction was not tested.

^b All antibodies were tested at 10⁻¹ dilution. Control antibodies are anti-mouse Ia (monoclonal antibody produced by an identical protocol) and anti-HSV-1 (hyperimmune rabbit serum).

tibodies derived from the immunization of mice with the neurotropic strain JHMV-DL to examine the relationships of the structural proteins of the principal MHV strains.

Assessment by RIA and RIP showed that the degree of conservation of JHMV-DL antigens, as judged by this panel of antibodies, varied among the three principal MHV structural proteins. The nucleocapsid protein pp60 showed intermediate conservation, the major glycoprotein gp180/90 little conservation, and the minor glycoprotein gp25 strong conservation. Each MHV strain had a unique pattern of reactivities to the set of monoclonal antibodies used, confirming previous studies by Childs *et al.* (1983) which showed that these MHV isolates are in fact separate and distinct strains.

Relative binding to anti-pp60 antibodies allowed grouping of the MHV strains into several antigenic families. In general, this group of antibodies showed substantial binding to all the murine coronaviruses tested, with the exception of JHMV-DS and MHV-M, indicating that the nucleocapsid protein antigens are substantially conserved in most MHV strains. Analysis of the relatedness of the pp60 proteins divided

the viruses into two groupings. The first consisted of A59, MHV-3, MHV-D, MHV-K, and MHV-Nuu viruses, which were unreactive with monoclonal antibodies J.1.1, J.3.14, J.3.7, and J.3.15. The second group, consisting of MHV-2, MHV-S, and MHV-1 viruses, conserved these antigenic specificities, as evidenced by strong reactions with the same set of monoclonal antibodies. Although these strains have not all been previously compared in a single study, previous investigations do support this general division. For example, the oligonucleotide maps of MHV-1 and MHV-S are distinct from the other MHVs examined (Lai and Stohman, 1981), as are the peptide maps of the pp60 from these two strains (Cheley *et al.*, 1981). In addition, the oligonucleotide maps of A59, MHV-3, MHV-D, and MHV-K are very similar (Lai and Stohman, 1981; Lai, unpublished data). MHV-M has an oligonucleotide map which is very different from any other MHV examined (Lai, unpublished data), and it is therefore not surprising that there is little recognition of its antigens.

The finding that none of the 10 anti-pp60 antibodies showed moderate or strong binding to JHMV-DS was very unexpected, since JHMV-DL and JHMV-DS have a

MONOCLONAL ANTIBODIES TO pp 60	VIRUSES												
	JHM DL	JHM DS	A59	MHV 3	MHV D	MHV K	MHV Nuu	MHV 2	MHV S	MHV 1	MHV M	VSV	HSV
J 2 1	■	▨	■	■	■	■	■			▨			
J 3 13	■	▨	■	■	■	■	■	■	■	▨	▨		
J 3 5	■	▨	■	■	■	■	■		▨	▨			
J 3 1	■	▨	■	■	■	■	■	■	■	▨	▨		
J 3 3	■	▨	■	■	■	■	■	■	■	▨	▨		
J 3 4	■	▨	▨	■	▨	▨	▨	■	▨	▨			
J 1 1	■							■	■	▨			
J 3 14	■							■	■	■			
J 3 7	■							■	■	■			
J 3 15	■							■	■	■			

FIG. 2. Reactivities of monoclonal antibodies with the MHV nucleocapsid protein, pp60. The binding of anti-pp60 monoclonal antibodies in RIA with different MHV antigens is expressed as a percentage of mean cpm relative to homologous virus (JHNV-DL). The blocks represent >50% (■), 25-50% (▨), twice assay background-25% (▩) and less than twice background cpm (□). All determinations represent an average of three assays.

MONOCLONAL ANTIBODIES TO gp 180/90	VIRUSES												
	JHM DL	JHM DS	A59	MHV 3	MHV D	MHV K	MHV Nuu	MHV 2	MHV S	MHV 1	MHV M	VSV	HSV
J.1.2	■	■											
J.7.18	■	■											
J.2.2	■	■											
J.7.2	■	■											
J.2.5	■	■									▨		
J.2.6	■	■					▨	■			▨		
J.7.1	■	■	▨				▨	■	▨	■			
J.7.5	■	■	■		▨	▨							
J.7.6	■	■	■		▨		■						
J.1.16	■	■	■	■		▨							

FIG. 3. Reactivities of monoclonal antibodies with the MHV major glycoprotein, gp180/90. The binding of anti-gp180/90 monoclonal antibodies in RIA to different MHV antigens is expressed as described for Fig. 2.

MONOCLONAL ANTIBODIES TO gp 25	VIRUSES													
	JHM DL	JHM DS	A59	MHV 3	MHV D	MHV K	MHV Nuu	MHV 2	MHV S	MHV 1	MHV M	VSV	HSV	
J.1.3	■	■	■	■	▨	▨				▩				
J.2.7	■	■	■	■	▨	▨	■	■	■	■				
J.3.11	■	▩	▩	■	▨	▨	■	■	▩	■	▩			

FIG. 4. Reactivities of monoclonal antibodies with the MHV minor glycoprotein, gp25. The binding of anti-gp25 monoclonal antibodies in RIA to different MHV antigens is expressed as described for Fig. 2.

common passage history, and their genomes share all but two oligonucleotide spots (Stohlman *et al.*, 1982). Recently, oligonucleotide fingerprints of mRNA No. 7, which encodes pp60, were studied in JHMV-DL and JHMV-DS. The fingerprints of the two variants were indistinguishable (Lai, unpublished observations). We presently have no firm explanation for the difference between the data obtained with monoclonal antibodies and oligonucleotide fingerprinting. Very likely, the monoclonal antibodies detected antigenic sites which are represented by genetic sequences not detectable by T₁-oligonucleotide fingerprinting. This striking result implies that there is a significant antigenic change in the nucleocapsid protein of the small plaque variant, JHMV-DS. The lack of antigenic differences among JHMV-DL and JHMV-DS in the other two virion proteins suggests that the change in pp60 may be correlated with differences in pathogenicity between the two viruses (Stohlman, *et al.*, 1982). However, this conclusion is provisional and should be supported by further studies, such as the analysis of nonstructural proteins and the complete sequencing of the genomes of the viruses.

Gp180/90 is an external protein which serves as the target for neutralizing antibody, induces cell fusion, and may play a key role in determining host cell range (Holmes *et al.*, 1982; Collins *et al.*, 1982). None of the anti-gp180/90 monoclonals indicated any major antigenic conservation of this protein among the wide range of murine coronaviruses, except for JHMV-DS. The fact that none of the gp180/90

antigens of the other MHVs shared many determinants with JHMV is not surprising, as marked variability of external proteins, usually in the context of conservation of internal proteins, has been well-documented in viruses such as vesicular stomatitis virus (Doel and Brown, 1978), poliovirus (Nottay *et al.*, 1981), and the murine retroviruses (Niman and Elder, 1982). This phenomenon may relate to the high mutation frequency of RNA viral genomes in general and the selective pressure that host immune systems may preferentially exert on neutralization-reactive surface components of viruses (Holland *et al.*, 1982).

Antigenic determinants of the minor glycoprotein gp25 were highly conserved among the MHV strains tested. This result was not unexpected, since gp25 is thought to serve as a matrix protein (Holmes *et al.*, 1982; Sturman, 1982), and therefore it is possible that many mutations involving gp25 might be lethal to the virus. This finding is consistent with previous studies showing that the matrix proteins of vesicular stomatitis virus (Doel and Brown, 1978) and influenza virus (Laver and Downie, 1976) are highly conserved among different strains.

Studies of viral pathogenicity are also consistent with the divisions proposed based on the reactivity with the anti-pp60 monoclonal antibodies. Most MHV strains cause hepatitis under natural or appropriate experimental conditions. Nevertheless, the members of the first group characteristically produce other diseases in addition to hepatitis: A59 can cause demyelination (Robb *et al.*, 1979), MHV-3

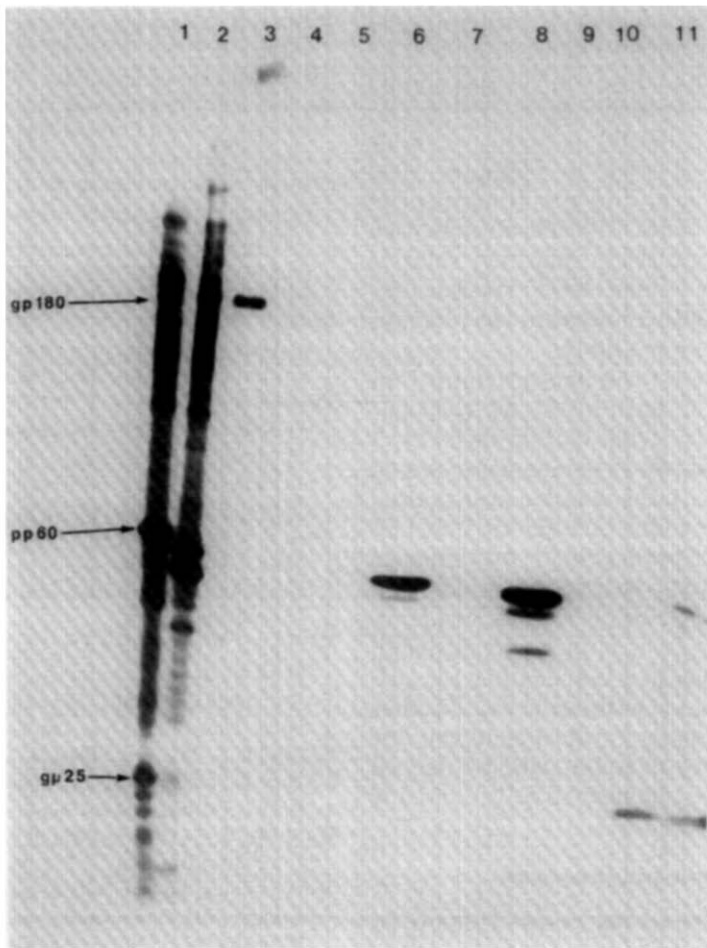


FIG. 5. Cross immunoprecipitations: Monoclonal antibodies specific for the three JHMV-DL-specified proteins were tested for their ability to react with A59 virus proteins by immunoprecipitation using a [35 S]methionine-labeled lysate of A59-infected cells as antigen. Lane 1 is a lysate of JHMV-DL-infected cells. Lane 2 is a lysate of A59 virus-infected cells showing the faster migration of pp60. Lane 3 (J.7.5), lane 4 (J.2.5), and lane 5 (J.1.2) are monoclonal antibodies reactive with the JHMV-DL gp180/90 protein. Lane 6 (J.2.1), lane 7 (J.3.7), lane 8 (J.3.3), and lane 9 (J.1.1) are monoclonal antibodies reactive with JHMV-DL pp60. Lane 10 (J.1.3) and lane 11 (J.2.7) are monoclonal antibodies reactive with JHMV-DL gp23. Samples were analyzed on 6-15% gradient polyacrylamide slab gels.

choroidoependymitis (Virelizier *et al.*, 1975) MHV-D enteritis (Ishida *et al.*, 1978a), and MHV-K myeloproliferation in nude mice (Ishida *et al.*, 1978b). By contrast, the members of the second group (MHV-2, MHV-S, and MHV-1) have been reported to produce primarily hepatitis; MHV-1 and MHV-S have also been considered to be of relatively low pathogenicity (Cheley *et al.*, 1981; Wege *et al.*, 1982).

Thus using the present panel of monoclonal antibodies to JHMV-DL, antibodies

to pp60, rather than gp180/90 or gp25, are most useful in establishing antigenic relationships both among MHV strains and between plaque variants of one MHV strain, JHMV. The fact that these antigenic divisions to some extent correlate with pathogenicity raises the possibility that pp60 may play an important role in disease potential. However, genetic regions coding for other gene products may contain alterations which would result in variations not detectable in this study, as shown pre-

viously by oligonucleotide fingerprinting of JHMV-DL and JHMV-DS (Stohlman *et al.*, 1982). Therefore, these conclusions should be considered tentative. Also, the major glycoprotein, gp180/90, is highly variable and may be expected to influence viral pathogenicity, e.g., by controlling tropism for specific cell types. This has already been suggested in one study in which relative hepatotropism among MHV strains may be correlated with changes in the gene for gp180/90 (Lai *et al.*, 1981).

Taken together, these findings imply that determinants on both gp180/90 and pp60 of MHV may play critical differential roles in pathogenesis. Such a conclusion would be consistent with the detailed information available concerning the genetic and molecular bases of pathogenicity of influenza (Rott, 1979) and reoviruses (Fields and Greene, 1982). For these viruses, virulence has been shown to be multigenic, although individual genes are responsible for different aspects of pathogenesis. Further studies, including an evaluation of the role of nonstructural proteins, will be needed to extend these conclusions to the murine coronaviruses.

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