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Monoclonal Antibodies to Murine Hepatitis Virus-4 (Strain JHM) Define the Viral Glycoprotein Responsible for Attachment and Cell-Cell Fusion

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Received November 23, 1981; accepted February 14, 1982

Hybridoma cell lines producing monoclonal antibodies to the JHM strain of mouse hepatitis virus-4 (MHV-4) were established. By indirect immunofluorescence and immune precipitation, monoclonal antibodies of three viral polypeptide specificities were characterized. Monoclonal antibodies to nucleocapsid reacted in the cytoplasm of infected cells and precipitated the 60,000d nucleocapsid polypeptide (VP-4) of MHV-4. Other monoclonal antibodies reacted both in the cytoplasm and on the surface of infected cells and were found to precipitate the 170,000d viral glycoprotein (GP-1). A third set of monoclonal antibodies reacted both in the cytoplasm and on the surface of infected cells and precipitated the 25,000d viral glycoprotein (GP-5) and its precursor VP-6 (23,000d). Anti-GP-1 alone had direct neutralizing activity for MHV-4 virus, while in the presence of complement both anti-GP-1 and anti-GP-5 neutralized virus. Only anti-GP-1 had the ability to inhibit the spread of infection due to fusion in L241 cells. Thus, the viral glycoprotein GP-1 likely contains both the attachment and fusion activities of MHV-4.

INTRODUCTION

At least four murine coronavirus are associated with hepatitis and/or encephalitis in mice (Gledhill and Andrewes, 1951; Nelson, 1952; Dick et al., 1956; Manaker et al., 1961; Bailey et al., 1949), and these viruses are collectively referred to as murine hepatitis viruses (MHV). Strains of MHV exhibit distinct organ tropisms, being predominantly either hepatotropic or encephalitogenic, and may cause persistent infections in mouse colonies. MHV-4 (JHM strain) is an encephalitogenic strain which causes fatal acute encephalomyelitis following intracerebral inoculation in its natural host (Lampert et al., 1973; Weiner, 1973). Mutants of MHV-4 have been isolated which produce a high incidence of demyelination in mice with destruction of oligodendrocytes (Haspel et al., 1978). In vivo studies with JHMV and its mutants may provide valuable insights into the pathogenesis of human demyelinating diseases; however, a careful analysis of the pathogenesis of primary demyelination has been hampered by the lack of sensitive and specific probes for viral products as well as a firm understanding of coronavirus structure, replication, and mechanism(s) of persistence. Serologic evidence of infection and detection of virus antigens in target organs has been difficult to demonstrate due to the poor quality of serum antibodies elicited in mice. Antigens responsible for cross-reactivity among murine coronaviruses have not been well defined. The importance of making accurate comparisons is underscored by a recent report of serologic cross-reactivity between murine coronaviruses and two human coronavirus isolates from brains of multiple sclerosis patients (Burks et al., 1980). The replication of MHV-4 and other
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Murine coronavirus has not been well defined. The virion contains positive-stranded RNA and infected cells reportedly contain seven major and two minor mRNA species, the largest of which is the same size as the genome (Wege et al., 1981). The mode of synthesis and processing of these mRNAs has not been elaborated. Six polypeptides, four of which are glycosylated, have been reported in infected SAC cells by Siddell et al. (1981). Of these six polypeptides, the two largest are the glycopeptides GP-1 (170,000d) and GP-2 (98,000d) which appear to represent dimeric and monomeric forms, respectively, of the same glycopeptide as shown by tryptic peptide mapping. A 60,000d nonglycosylated polypeptide, VP-4, is associated with the viral nucleocapsid. The two smallest polypeptides, GP-5 (25,000d) and VP-6 (23,000d), found in the infected cells, are identical to those found in the virion envelope (Wege et al., 1979). Pulse-chase experiments have shown that VP-6 is processed to GP-5 by the addition of polysaccharide. A minor glycopeptide GP-3 (65,000d) appeared in gels when dithiothreitol was used as reducing agent, and its dimeric form (125,000d) was found in gels when β-mercaptoethanol was used (Wege et al., 1979).

Sturman et al. (1980) have recently proposed a structural model for the membrane proteins of the mouse hepatitis virion based on experimental data obtained with the A-59 strain. In this proposed model, the largest virion glycopeptides GP-1 (180,000d) and GP-2 (98,000d) are associated with the virion peplomers and have been designated E-2 protein. GP-5 (25,000d) and VP-6 (23,000d) are deeply embedded in and may transsect the viral envelope, interacting internally with the nucleocapsid protein. In the proposed model, these polypeptides form a structure designated the E-1 protein which plays an essential role in virus maturation intracellularly (Holmes et al., 1981). The viral glycoproteins bearing receptor sites for virus adsorption and cell–cell fusion during MHV infection have not been defined. In this report we describe the generation and characterization of monoclonal antibodies to MHV-4 and their use in initial studies to define biological activities associated with the virion glycopeptides.

Materials and Methods

Virus and cell culture. The JHM strain of MHV-4 was provided by L. Weiner (University of Southern California). It was plaque purified in NCTC-1469 cells and then propagated and plaque assayed in L-241 cells obtained from L. Sturman (New York State Department of Health, Albany). To label intracellular MHV polypeptides, cells were infected at an m.o.i. of 0.1 in 60- or 100-mm tissue culture dishes, then 50 μCi/ml [35S]methionine (specific activity > 600 Ci/mmol, Amersham/Searle, Chicago, Ill.) in methionine-free medium was added for 2 hr at 18–20 hr after infection.

Production of monoclonal antibodies to JHMV. Balb/c mice 4–6 weeks of age were primed by ip inoculation of 10^3 to 10^4 plaque-forming units (PFU) of MHV-4 grown in L-241 cells. After 3 weeks, boosting doses consisting of 0.1 ml of a 10% (v/v) extract of virus-infected cells were inoculated ip for 3 consecutive days prior to fusion. Fusion with P3 × 63 Ag8 clone 6531 nonsecretor plasmacytoma cells was done essentially by the method of Kohler and Milstein (1975) as we have described elsewhere (Buchmeier et al., 1981). Briefly, 10^8 spleen cells were fused with 10^7 plasmacytoma cells using PEG 1000 at 50% concentration. Cells were diluted in HAT medium and plated out in eight 96-well plates. Wells were observed for 3–4 weeks for hybrid colonies and these colonies were screened for production of antibody to MHV-4. Antibody producing cultures were immediately subcloned by limiting dilution and rechecked.

Screening of culture fluids for antiviral antibody was usually done by indirect immunofluorescence on both acetone-fixed and paraformaldehyde-fixed cells. As controls, uninfected L-241 cells and culture supernatant from parental myeloma cells were included in each assay. Fluorescein isothiocyanate-conjugated goat anti-mouse IgG was prepared in this laboratory
Clones of each culture producing the highest titers of antibody in vitro were selected for ascites production in pristane (2,6,10,14-tetramethylpentadecane)-primed Balb/c mice.

**Specificities of monoclonal immunoglobulins.** Virus-specific antibody concentrations in cell culture and ascites fluids were estimated by indirect immunofluorescence endpoint titration on acetone-fixed target cells. IgG concentrations were estimated by radial immunodiffusion using sheep anti-mouse IgG. Determination of immunoglobulin isotype was determined by agar gel diffusion using subclass-specific antisera (Meloy Laboratories, Springfield, Va.).

**Immune precipitation.** MHV-4 polypeptide specificities of monoclonal antibodies were determined by immune precipitation of $^{35}$S methionine-labeled viral proteins from infected L-241 cells. Previously described methods (Schauffhausen et al., 1978; Buchmeier et al., 1981) were adapted as follows. Radiolabeled cytosol extracts of infected and control L-241 cells were prepared by solubilizing 0.5 to $1.0 \times 10^7$ infected cells in $2.5$ to 5 ml of lysis buffer ($20 \text{ mM Tris-Cl}, pH 9.0; 137 \text{ mM NaCl}; 1 \text{ mM CaCl}_2; 0.5 \text{ mM MgCl}_2; 1\% \text{ Aprotinin}; 1\% \text{ Nonidet P 40}; \text{ and } 10\% (v/v) \text{ glycerol}), then centrifuging at $10,000 g$ for 30 min to remove nuclear debris. A volume of 200 $\mu l$ of cytosol was mixed with 100 $\mu l$ of hybridoma culture fluid or 5 $\mu l$ of ascites fluid and incubated at 37°C for 1 hr. Heat-killed, formalin-fixed *Staphylococcus aureus* bacteria (SA) in SABC buffer (0.1 $M$ phosphate-buffered saline, pH 7.2; 0.5% Nonidet P-40; 2 $mM$ methionine; 0.22% sodium azide; 1 mg/ml ovalbumin) were added to each mixture in volumes of 100 $\mu l$ of a 10% (v/v) suspension, and incubation was continued for 30 min at 22°C. Pellets were collected by centrifugation at 2500 $g$ for 10 min and washed three times in Tris-Cl (0.1 $M$, pH 9) containing 0.5 $M \text{ LiCl}$. The final pellet was resuspended in 100 $\mu l$ of 2% SDS, 1% mercaptoethanol sample preparation buffer and heated at 100°C for 2 min. Bacteria were removed by centrifugation and samples were analyzed by SDS-PAGE on 10.5% gels. Controls included precipitation of polypeptides from uninfected cytosol and inability to precipitate MHV-4 proteins by monoclonal antibodies directed against an unrelated virus (LCMV).

**Virus neutralization.** Virus neutralizing capacity of monoclonal antibodies was quantitated by a semimicro plaque reduction assay. Ascites fluids were first centrifuged at 10,000 $g$ heat inactivated at 56°C for 30 min, then preadsorbed with L-241 cells ($1 \times 10^6$ cells per 200 $\mu l$ of ascites) for 30 min at 4°C. Virus was diluted to contain 50–100 PFU in 100 $\mu l$ and mixed with 100 $\mu l$ of diluted antibody. Virus-antibody mixtures were incubated for 1 hr at 4°C, then plated onto L-241 cell monolayers in 24-well plates (Flow Laboratories, McLean, Va.). The mixtures were adsorbed for 1 hr and then removed and replaced with culture medium. Agar overlay was not necessary due to the short (<24 hr) incubation period and cell-associated nature of JHMV. Neutralization endpoint titers were expressed as the reciprocal of the highest antibody dilution which gave a 50% reduction in plaque number (PRD 50%). Controls included ascites fluids containing neutralizing monoclonal antibodies to an unrelated virus.

**Inhibition of spread of virus in cell culture.** The growth of MHV-4 in L-241 cells is characterized by rapid formation of large syncytia and resultant intercellular virus spread. Effect of virus-specific hybridoma antibodies on spread of virus infection in cell cultures was determined by incubation of infected monolayers with monoclonal antibodies in the culture medium. L-241 cell monolayers in 24-well Linbro plates were infected at 50–100 PFU per well and incubated at 37°C for 1 hr. Incubation was removed and culture medium containing serially diluted monoclonal antibody prepared as described above was added at the concentrations indicated and incubation continued for 22–24 hr at which time plates were fixed and stained with 0.1% crystal violet. The inhibition titer of monoclonal antibody was expressed as the highest dilution which inhibited the number of visible plaques and syncytia by 50%. Inhibitory effect of virus-specific monoclonal antibody on syncytium formation
was also visualized microscopically by indirect immunofluorescence staining. After 6, 9, and 12 hr of incubation in the presence of monoclonal antibody, coverslip cultures were washed three times in PBS and fixed in acetone. Fluorescent staining for detection of syncytia was performed using monoclonal antibody to MHV-4 nucleocapsid protein (4B-6.2).

Surface labeling of monoclonal antibody bound to infected cells. Cell surface binding of monoclonal antibodies was assessed both by indirect immunofluorescence and by electron microscopic examination of immunoferritin-labeled infected cells. L-241 cells were infected for 1 hr at 37° with MHV-4 in suspension at an m.o.i of 1.0, washed, then incubated at 37° for 6 hr. Cells were then transferred to an ice bath, washed in cold medium, and viable cells enumerated by trypan blue exclusion. Al-iquot of 1 × 10⁶ viable cells were mixed with 100 µl of monoclonal ascites fluid at 1:100 dilution for 30 min at 4°. Cells were then washed three times with cold medium and reacted for 1 hr on ice with 50 µl of rabbit anti-mouse IgG conjugated to ferritin (Cappel Laboratory, Dorrington, Pa.). After incubation, cells for electron microscopy were washed four times with cold medium, once with PBS, and fixed with 2.5% glutaraldehyde in 0.125 M cacodylate buffer and examined as described elsewhere (Knobler et al., 1981). For surface immunofluorescence cells were reacted with monoclonal antibody as described above, then with sheep anti-mouse IgG coupled to biotin. After 30 min at 4° cells were washed then reacted with FITC coupled Avidin (E-Y Laboratories, San Mateo, Calif.) for 5 min. Cells were then washed and examined using a Zeiss fluorescence microscope equipped for incident illumination.

RESULTS

Polypeptide Specificity of Monoclonal Antibodies to MHV-4

Fifty-two cell lines making monoclonal antibodies to MHV-4 were established from 243 cultures. Initial screening by immunofluorescence segregated these into those which reacted with antigens at the surface of infected cells, and those which reacted with antigens expressed only in the cytoplasm. To determine the polypeptide specificity of these antibodies, we immunoprecipitated radiolabeled viral polypeptides from cytosol extracts of infected cells. Under the conditions which we employed, the monoclonal antibodies fell into three groups: Those which precipitated GP-1, those reacting with VP-4, and those reacting with GP-5 and VP-6. Figure 1 illustrates these results for selected antibodies. Correlating these data with immunofluorescence observations, we found that antibodies which stained cell surface viral antigens at 6, 12, or 24 hr reacted with either GP-1 or GP-5, VP-6 whereas antibodies which stained antigens only in the cytoplasm immunoprecipitated VP-4. Under the conditions employed with [³⁵S]methionine label, we observed little GP-2 (98,000d) surface glycopeptide; however, preliminary experiments using glucosamine label (data not shown) suggest that anti-GP-1 antibodies also react with GP-2. Forty-four of the fifty-two monoclonal antibodies were analyzed for their immunoglobulin isotype using monoclonal typing reagents. Of these, eight were IgG-1, 12 were IgG-2A, two were IgG-2B, six were IgG-3, three were IgA, four were IgM and nine were undetermined.

Detection of Viral Antigens on the Surface of Infected Cells

The replication of murine coronaviruses is characterized by synthesis of viral RNA and proteins in the cytoplasm, envelopment of the nucleocapsid, and maturation by budding into intracellular vesicles. Cell fusion and resultant syncytium formation are not essential for maturation (Robb and Bond, 1979); however, cell–cell fusion between infected cells and uninfected cells may contribute to intercellular spread of infection. Viral antigens at the cell surface can also serve as targets for immune attack. Presence of viral glycoproteins GP-
1 and GP-5 on the surfaces of infected cells was examined using two monoclonal antibodies, 5B-19.2 (anti-GP-1) and 5A-5.2 (anti-GP-5). L-241 cells were examined 6 hr after infection with MHV-4 by indirect immunofluorescence and by immunoferritin labeling for surface antigen. Six hours was selected for detailed study because this preceded both release of mature virus into the supernatant medium and cell death. Both anti-GP-1 (Fig. 2B) and anti-GP-5 (Fig. 2C) stained infected cells at 6 hr; however, anti-VP-4 did not (Fig. 2A). The binding of anti-GP-1 was visualized by immune electron microscopy using ferritin-conjugated rabbit anti-mouse IgG (Fig. 3). Antigen at the cell surface was observed in the absence of virions.

**Neutralization with Monoclonal Antibodies**

The MHV-4 antigen responsible for adsorption to target cells has not been identified. In order to address this point, we selected four monoclonal antibodies demonstrated by immunoprecipitation to react with the GP-1 polypeptide, three which precipitated GP-5, and as controls, two which precipitated VP-4. Monoclonal ascites fluids were absorbed with L-241 cells and heat inactivated prior to screening for virus neutralizing activity. The immuno-

![Immune precipitation of viral polypeptides from cytosol of MHV-4-infected L-241 cells by monoclonal antibodies. Cells were infected with MHV-4, then pulse labeled with [35S]methionine, and a cytosol extract prepared (shown in lane 1) and immunoprecipitated as described in the text. Monoclonal antibodies and their polypeptide specificity were 4B-6.2 (anti-VP-4, lane 2); 4B-11.6 (anti-GP-1, lane 3); 5A-13.5 (anti-GP-1, lane 4); 5A-5.2 (anti-GP-5, VP-6, lane 5); and 5B-128 (anti-GP-5, VP-6, lane 6). Background precipitation of GP-1 and VP-6 is evident in lane 2 and of VP-6 in lanes 3 and 4. Specificity controls included precipitation of control cell cytosol by monoclonal antibody to MHV (5A-5.2) (lane 7) and precipitation of MHV-infected cytosol by monoclonal antibody to lymphocytic choriomeningitis virus (LCMV IIWE-6) (lane 8).
Fig. 2. Surface immunofluorescence staining of MHV-4 antigens by monoclonal antibodies. L-241 cells were infected for 1 hr in suspension, washed, and incubated 6 h further prior to staining with monoclonal antibodies. Panel A, infected cells stained with monoclonal anti-VP-4 (4B-6.2). No specific staining was observed indicating that VP-4 is not on the cell surface. Panel B, infected cells stained with monoclonal anti-GP-1 (5B-19.2) and panel C, stained with anti-GP-5 (5B-11.5). Results indicate that both GP-1 and GP-5 are expressed on the cell surface.
globulin class, IgG concentration, and immunofluorescence endpoint titer were also determined. The ability of these monoclonal antibodies to neutralize MHV infectivity is illustrated in Fig. 4. Neutralization was mediated only by those monoclonal antibodies which precipitated GP-1. One anti-GP-1 (5B-93.9) which did not neutralize was of the IgA class. Monoclonal antibodies to the GP-5 glycoprotein and to NP showed no significant neutralizing activity in the absence of complement; however, we found that the addition of guinea pig complement to the reaction mixture substantially increased the neutralizing activity of the GP-5 antibodies. In one such experiment with antibody 5A-5.2 (anti-G5) the titer of 10,000 PFU of JHMV was reduced by only 14% without complement, but by 98% with the addition of 1/40 fresh guinea pig complement. In contrast, antibody 4B-11.6 (anti-GP-1) neutralized 99 and 97% of the infectivity without and with complement, respectively. Neutralizing antibody titers did not always correlate with IgG concentration, and this may reflect differences in avidity. Other controls consisting of monoclonal antibodies to JHMV VP-4 and neutralizing antibodies to an unrelated virus (LCMV) showed no significant neutralizing activity against MHV-4.

Inhibition of Spread of Infection by Monoclonal Antibodies

An interesting biological property of MHV-4 is its ability to spread infection to adjacent uninfected cells by fusion. This property is especially evident in certain cells such as L-241, primary macrophage, primary brain, and embryonic fibroblast cultures from susceptible mouse strains such as Balb/c. Other cell lines such as the 17-Cl-1 line of mouse fibroblasts do not demonstrate extensive fusion after infection with MHV-4 (Knobler, Collins, unpublished observation). The viral glycopeptide associated with fusion activity has not been identified. Monoclonal antibodies with specificity for GP-1 and GP-5 were tested for ability to inhibit spread of infection in L-241 cells. Inhibition of spread was quantitated by incubating previously infected cells in medium containing monoclonal antibodies and scoring the number of visible plaques and foci of syncytia 22–24 hr after infection. Only incubation with monoclonal antibodies to GP-1 resulted in inhibition in plaque and syncytium number (Fig. 5), indicating involvement of that glycopeptide in cell to cell spread of infection by fusion. Titers observed were unrelated to the neutralizing antibody titer but did correlate with indirect immunofluorescence antibody titers. For example, considering the data summarized in Table 1, the relative titers of three different anti-GP-1 antibodies (5B-170.3, 5B-19.2, and 4B-11.6) in neutralization assays were less than, equal to, and greater than their respective titers in inhibition of spread of infection. Measurement of antibody activity by inhibition of spread of infection was approximately 10-fold less sensitive than indirect immunofluorescence.

The inhibitory effects of monoclonal antibody to GP-1 upon development of syncytia was also observed morphologically by indirect immunofluorescence on MHV-4-infected L-241 cells. Monoclonal antibodies at a final dilution of 1/40 were added to the medium of L-241 cultures on coverslips one hour after infection with MHV. As early as 9 hr after infection, a marked inhibition both in the numbers and size of syncytia was observed in cultures incubated with monoclonal antibody to GP-1. Inhibition of development of syncytia was even more evident in such cultures 12 hr after infection (Fig. 6). Monoclonal antibodies to GP-5, VP-4, and an unrelated virus (LCMV, not shown) had no effect on the development of syncytia.

DISCUSSION

We have prepared a library of monoclonal antibodies to MHV-4 and have identified their polypeptide specificity toward the viral proteins GP-1, VP-4, GP-5, and VP-6. These antibodies have been used in
FIG. 3. Immunoferritin labeling of cells infected by MHV-4 as described in the legend to Fig. 2. Panel A, cells labeled with monoclonal antibody to GP-1 (5B-19.2) and panel B, infected cells labeled with antibody to MHV VP-4 (4B-6.2). Cell surface deposits of ferritin (arrows) were seen only on cells reacted with antibody to viral glycoprotein GP-1 (panel A). ×40,000.
Coronaviruses mature by budding into intracytoplasmic vesicles (reviewed in Robb and Bond, 1979). We have demonstrated that despite this intracellular mode of maturation both viral GP-1 and GP-5 are expressed at the surfaces of infected cells. Surface expression occurred by 6 hr, prior to release of progeny virus, and was

Fig. 4. Neutralization of JHMV infectivity by monoclonal antibodies. Diluted monoclonal ascites fluid and virus were mixed and incubated for 1 hr as described under Materials and Methods then titered on monolayers of L-241 cells in 24-well plates. The first well in each row contained no antibody (No Ab) and all others contained monoclonal antibody diluted as indicated. Significant neutralization was observed only with antibodies 4B-11.6 and 5B-19.2 both directed against the viral GP-1. Endpoint titers of 1/12,000 and 1/1,200, respectively, were achieved with these antibodies.
shown by immunoelectron microscopy to occur in areas free of morphologically distinguishable virions. This observation has several implications with respect to host-virus interactions. First, expression of virus-specific surface components during eclipse may render the infected cell susceptible to specific antibody-dependent or cellular host defense mechanisms. Thus the infected cell can potentially be eliminated prior to release of new virus. Conversely, spread of MHV infection in culture and presumably in vivo is largely cell to cell, and is facilitated by the ability of viral polypeptides to mediate cell fusion resulting in syncytia formation. Presence of these viral polypeptides on the cell surface suggests that cell fusion may begin prior to virus release. We have observed cell fusion in culture as early as 6 hr after infection, coincident with the appearance of GP-1 and GP-5 at the cell surface. Sturman and co-workers (1980) have proposed that GP-5 and VP-6 are structurally associated with a transmembrane protein complex which they have termed E-1. This protein is thought to be involved in virus maturation along the endoplasmic reticulum. The ability of anti-GP-5 to bind to the infected cell surface and to the intact virion indicates that this putative transmembrane polypeptide of the coronaviruses is expressed at the cell surface independent of virus maturation and differs

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**Fig. 5.** Inhibition of spread of virus infection. Monolayers of L-241 cells in 24-well plates were infected with approximately 50 PFU of MHV and incubated for 1 hr at 37° as described in the text. Supernatant fluid containing monoclonal antibodies at the final dilutions indicated were added to the monolayers and incubation was continued for 22 hr before cells were fixed and stained. In this assay, both clear plaques indicative of detached syncytia and large syncytia, which appeared as darkly stained foci, were observed and enumerated. Antibodies to GP-1 (4B-11.6 and 5B-170.3) inhibited development of syncytia at PRD 50 dilutions of 1/1020 and 1/300, respectively, while antibody to GP-5 (5B-119.4) had no effect at a 1/20 or 1/80 dilution.
TABLE 1

INHIBITION OF BIOLOGICAL ACTIVITIES BY MONOCLONAL ANTIBODIES TO MHV-4 GLYCOPROTEINS

| Monoclonal antibody | Isotype | Polypeptide specificity | Neutralization titer (PRD 50) | Inhibition of spread of infection (PRD 50) |
|---------------------|---------|------------------------|------------------------------|------------------------------------------|
| 5B-170.3            | IgG-1   | GP-1                   | 180                          | 300                                      |
| 5B-19.2             | IgG-1   | GP-1                   | 1200                         | 1300                                     |
| 5B-93.9             | IgA     | GP-1                   | <100                         | <20                                      |
| 4B-11.6             | IgG-2A  | GP-1                   | 12,000                       | 1020                                     |
| 5B-119.4            | IgG-2A  | GP-5                   | <100                         | <20                                      |
| 5A-5.2              | IgG-3   | GP-5                   | <100                         | <20                                      |
| 5B-11.5             | IgG-2A  | GP-5                   | <100                         | <20                                      |
| 4B-4.5              | ND      | VP-4                   | <100                         | <20                                      |
| 4B-6.2              | IgG-1   | VP-4                   | <100                         | <20                                      |
| LCM 2-11.15         | IgG-2A  | —                      | <100                         | <20                                      |

Note. Summary of antibody specificity, virus neutralization, and inhibition of spread of infection obtained in experiments illustrated by Figs. 4 and 5. Antibodies to GP-1 both neutralized viral infectivity and inhibited spread of MHV infection in culture while antibodies to GP-5 and VP-4 did not.

We investigated the biological roles of the GP-1 and GP-5 polypeptides in initiation and spread of infection using monoclonal antibodies. Neutralization tests employing several anti-GP-1, anti-GP-5, and anti-VP-4 monoclonals demonstrated that only anti-GP-1 possessed strong virus-neutralizing activity. Antibodies to GP-5 did not neutralize virus infectivity unless complement was added to the reaction. Thus the virion polypeptide GP-1 probably contains the site of attachment of virion to host cell. One anti-GP-1 (5B-93.9) did not neutralize infectivity despite a binding titer of 1/16,000. It is likely that this antibody recognizes an epitope on GP-1 that is unrelated to the attachment site. Blocking experiments utilizing 5B-93.9 to attempt to competitively inhibit binding to known neutralizing antibodies are currently under way to clarify this question. Despite their failure to neutralize, antibodies to GP-5 clearly bind to virions. The addition of fresh guinea pig complement to anti-GP-5 antibody + virus reaction mixtures resulted in virus neutralization comparable to that seen with anti-GP-1 antibody and this may reflect a lytic effect of complement on the virus. Since both complement and antibody-dependent cell-mediated cytotoxicity (ADCC) mechanisms are operative in the whole animal, the ability of these antibodies to modify the course of disease in vivo must be considered.

Virus-specific membrane antigens clearly also play a role in the intercellular spread of infection through the mechanism of cell fusion. Fusion activity has been described for many enveloped viruses. Among the paramyxoviruses this activity is the function of a distinct fusion glycoprotein (F₁) (Hommen and Ohuchi, 1973; Scheid and Choppin, 1974). In the orthomyxoviruses a hydrophobic region of the hemagglutinin peplomer has fusion activity (Lazarowitz and Choppin, 1975; Klenk et al., 1975). When we examined our collection of monoclonal antibodies for the ability to inhibit spread of infection, only anti-GP-1 was effective, suggesting that the virion peplomer contains the active site for cell–cell
FIG. 6. Inhibition of syncytium formation by monoclonal antibodies. Cells were infected with MHV as described in the legend to Fig. 5 and incubation was continued for 12 hr with or without antibody; then the cells were stained with monoclonal antibody to VP-4 (4B-6.2) to mark internal viral polypeptides. Panel A, uninfected L-241 cells; panel B, 12 hr after infection in absence or antibody; panel C, 12 hr after infection incubated in the presence of anti-GP-1 (5B-19.2); panel D, incubated in the presence of anti-GP-5 (5B-11.5). Syncytia which developed in cultures incubated with anti-GP-1 antibody were decreased in number and involved fewer cells than those in control or anti-GP-5-incubated cultures.
fusion. The mechanism by which anti-GP-1 inhibits spread is not clear. Anti-GP-1 may directly inhibit the molecular events of membrane fusion or may bind to a site which sterically hinders membrane penetration. Although our experiments suggest that GP-5 is not directly involved in cell fusion the possibility exists that GP-5 participates in events occurring after initiation of fusion by GP-1. Alternatively, expression of GP-5 at the surface of the infected cell may be merely a fortuitous occurrence. Recent data (Holmes et al., 1981) suggest that the GP-5, VP-6 complex E-1 plays an essential role in the intracellular maturation of MHV. Thus surface expression of this polypeptide may reflect a breakdown in the normal pathways of glycoprotein expression in the infected cell. Clearly, monoclonal reagents will be valuable tools to probe the precise mechanisms of infection, cell-cell spread, and cell fusion by coronaviruses.

ACKNOWLEDGMENTS

This is publication No. 2603 from Scripps Clinic and Research Foundation, La Jolla, California. We thank Michael B. A. Oldstone for helpful discussion; Ricarda DeFries, Hannah Lewicki, and Linda Tunison for excellent technical assistance, and Susan Edwards for manuscript preparation.

The work described here was supported by NIH Grants NS 12428, NS14068, and AI 16102, and was performed during the tenure of an American Heart Association Established Investigatorship granted to M.J.B. R.L.K. is the recipient of a National Multiple Sclerosis Society Postdoctoral Fellowship Award.

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