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Immunoglobulin Fc Binding Activity Is Associated with the Mouse Hepatitis Virus E2 Peplomer Protein

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Antigenic variation among murine coronaviruses is associated primarily with the surface peplomer protein E2 (180,000 Da). E2 is responsible for attachment of the virus to the host cell, MHV-induced cell fusion, and eliciting neutralizing antibody. We report here the molecular mimicry between E2 and Fc γ receptor (FcγR). Molecular mimicry between E2 and FcγR may allow the escape of virus-infected cells from destruction by immunological mechanisms. Rabbit IgG, monoclonal rat IgG_1 and IgG_{2b}, monoclonal mouse IgG_{2a} and IgG_{2b}, and the rat anti-mouse FcγR monoclonal antibody 2.4G2 immunoprecipitated from MHV-JHM-infected cells a polypeptide with a molecular mass identical to that immunoprecipitated by anti-E2 antibodies. $F(ab')_2$ fragments of rabbit IgG did not immunoprecipitate any proteins from MHV-infected cells. All of these antibodies did not immunoprecipitate any proteins from uninfected cells. The anti-mouse FcγR monoclonal antibody 2.4G2 immunoprecipitated from MHV-JHM-, MHV-3-, or MHV-A59-infected L-2 cells and 17CL-1 cells, or MHV-JHM-infected cultures of neonatal BALB/c brain cells, a protein with a molecular weight identical to that of MHV-JHM E2. The anti-FcγR monoclonal antibody did not immunoprecipitate any proteins from uninfected cells. Furthermore, the 2.4G2 monoclonal antibody (mab), unrelated rat and mouse monoclonal antibodies, and a goat antiserum against E2, but not normal goat serum, immunoprecipitated a 75,000- to 77,000-Da molecule from uninfected WEHI-3 cells, a FcγR bearing cell line. Several lines of evidence demonstrated that the protein immunoprecipitated by the anti-FcγR mab from MHV-JHM-infected cells is the E2 glycoprotein: (1) Partial proteolytic maps obtained by *Staphylococcus aureus* V-8 protease treatment of the 180,000-Da proteins immunoprecipitated by the anti FcγR mab and the anti-E2 mab were identical. (2) Sequential immunoprecipitation experiments from MHV-JHM-infected cells revealed that the same polypeptide chain was recognized by the anti-E2 mab and by the anti-FcγR mab 2.4G2. (3) Actinomycin D did not influence the induction and expression of the 180,000-Da polypeptide chain that was immunoprecipitated by the anti-FcγR mab, demonstrating that this protein is of viral origin.

INTRODUCTION

The JHM strain of mouse hepatitis virus (MHV), a member of the Coronavirus family, experimentally produces several diseases in mice and rats, depending on the dose and route of inoculation and the age and strain of the infected animals (LePrevost *et al.*, 1975; Levy-LeBlond *et al.*, 1979; Sorensen *et al.*, 1982, 1984). MHV-JHM is highly neurotropic (Lampert *et al.*, 1973; Weiner, 1973; Robb *et al.*, 1979; Knobler *et al.*, 1981). Infection of adult mice by this virus results in encephalomyelitis featuring prominent demyelination. Ten percent of the animals survive the acute infection and subsequently develop a persistent, demyelinating disease, reminiscent of multiple sclerosis. The development of demyelinating lesions is thought to be a primary effect of infection of oligodendrocytes, rather than being immunologically mediated (Lampert *et al.*, 1976; Wege *et al.*, 1982). The MHV genome encodes a nucleocapsid protein (N), a matrix-like transmembrane glycoprotein (E1), and a peplomer protein (E2) as well as several nonstructural proteins (Siddell *et al.*, 1981; Sturman and Holmes, 1983; Bond *et al.*, 1984). The E2 glycoprotein is responsible for the attachment MHV to the host cell plasma membrane and elicits the production of neutralizing antibody (Collins *et al.*, 1982). Monoclonal antibodies reacting with E2 have been used to identify several antigenic sites on MHV-JHM E2, some of which have been shown to be important determinants of pathogenicity (Talbot *et al.*, 1984; Talbot and Buchmeier, 1985). The E2 glycoprotein is synthesized on membrane-bound polyribosomes (Holmes *et al.*, 1981; Siddell *et al.*, 1981). The primary translation product, a 120-kDa polypeptide, is rapidly glycosylated to a 150-kDa E2 precursor which is subsequently further glycosylated to a 180-kDa species. The predominant intracellular form of E2 in MHV-infected cells has an apparent molecular weight of 180,000 Da. Shortly before the release of virions from cells, some of the cell-associated 180,000-Da E2 protein is cleaved to yield two 90,000-Da glycoproteins, 90A and 90B (Ricard and Sturman, 1985; Sturman *et al.*, 1985). The cleavage of E2 is host cell-dependent, with the ratio of uncleaved E2 (180,000 Da) to cleaved E2 (90A and

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90B) varying from cell line to cell line and depending on the physiologic state of the host cells (Frana *et al.*, 1985). Cleavage of E2 is necessary to activate its membrane fusing activity (Sturman *et al.*, 1985).

In the course of immunologic staining of MHV-infected cells with polyclonal rabbit antisera we observed that normal and preimmune sera gave unusually high background staining. This background staining was only observed in infected cells, never in uninfected cells. This observation led us to investigate the hypothesis that MHV, like the herpesviruses, induce infected cells to display Fc receptor (FcR) activity (Watkins, 1964; Yasuda and Milgram, 1968). The IgG Fc-binding receptors induced by herpes simplex virus type I (HSV-1) have been characterized in detail (Longnecker *et al.*, 1987; Johnson *et al.*, 1988). They are composed of a complex containing the virally encoded glycoproteins gE and gI (g70), with both of these polypeptides being required for Fc receptor activity. The functional significance of the HSV-induced FcR is not known.

Fc receptors (FcR) for all immunoglobulin classes are found on a variety of B lymphocytes, some T cells, macrophages, NK cells, and polymorphonuclear leukocytes. They provide a link between humoral and cellular immune responses by targeting immune complexes to effector cells (Mellman *et al.*, 1983; Adams *et al.*, 1984; Leslie, 1985).

In this work we report that MHV-JHM-infected cells also contain an Fc binding activity. This activity is mediated by the E2 protein, which has an affinity for the Fc domain of immunoglobulin G. Furthermore a rat monoclonal antibody (mab) directed against the mouse FcR immunoprecipitated a 180,000-Da molecule from MHV-JHM-infected cells, which was identical to the E2 peplomer protein.

MATERIALS AND METHODS

Virus and cells

The L-2, 17CL-1, and WEHI-3 cell lines have been previously described (Rothels *et al.*, 1959; Sturman and Takemoto, 1972; Hogarth *et al.*, 1987). The cells were grown at 37° as monolayer cultures in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) with standard concentrations of penicillin, streptomycin, and glutamine. WEHI-3 cells were grown in the presence of heat-inactivated FBS. Primary dissociated mixed brain cell cultures were prepared from BALB/c mice 10–13 days of age as described (McCarthy and DeVellis, 1980). The origin and growth of MHV-JHM, MHV-A59, and MHV-3 have been described (Robb and Bond, 1979; Levy *et al.*, 1981).

Antibodies

The 1.38.1 mab specific for the MHV E2 glycoprotein (Leibowitz *et al.*, 1986) and a hyperimmune rabbit serum which recognizes N and E2 were developed in this laboratory. Rat anti-surfactant monoclonal antibodies (tissue culture supernants) were generously supplied by Dr. David Strayer (University of Texas Medical School, Houston). All other monoclonal antibodies used in this work were kindly provided by Dr. C. D. Platsoucas of the M. D. Anderson Cancer Center. The OKT3 (IgG_{2a}), OKT4 (IgG_{2b}), and OKT8 (IgG_{2a}) mab have been described (Kung *et al.*, 1979; Reinherz *et al.*, 1979; Thomas *et al.*, 1980). Purified anti-Leu4 (IgG_1) monoclonal antibody (Platsoucas, 1984) was purchased from Beckton–Dickinson (Mountainview, CA). The 3D5 anti-γ chain T cell receptor mab (IgG_1) has been described (Nanno *et al.*, 1989). MKD6 mouse mab specific for I-A^d (IgG_{2a}) and 15-5-5^s mouse mab specific for D^k, K^d, H-2^f (IgG_{2a}) originated from the ATCC (Rockville, MD). The rat anti-mouse FcγR antibody 2.4G2 was originally described by Unkeless (Unkeless, 1979). Goat antiserum against purified MHV-A59 glycoprotein E2 was a generous gift from Dr. K. Holmes (Uniformed Services University for Health Sciences). The 1.38.1 anti-E2 mab, the 3D5 anti-T cell receptor mab, the anti-I-A^d, and the 15-5-5^s mab were used as ammonium sulfate concentrates of tissue culture supernates. The 2.4G2 anti-FcR mab was used as tissue culture supernate without concentration. OKT3, OKT4, and OKT8 mab were purchased from Orthodiagnostic Corp. (Raritan, NJ) and used as diluted ascitic fluids. Purified whole rabbit IgG specific for *Micrococcus lysodeikticus* as well as $F(ab')_2$ fragments were a generous gift of Dr. S. Rodkey (University of Texas, Health Science Center at Houston). The $F(ab')_2$ fragments were prepared as described (Nisonoff *et al.*, 1961). Residual intact IgG molecules were removed from our $F(ab')_2$ preparation by affinity chromatography over a protein A–Sepharose column. The purified $F(ab')_2$ fragments were determined to be free of undigested IgG by Ouchterlony immunodiffusion assays. Affinity-purified rabbit anti-goat IgG, goat anti-rabbit IgG, rabbit anti-mouse IgG, and goat anti-rat IgG, and their FITC-conjugates, were purchased from Jackson Immunoresearch Laboratories (West Grove, PA) and were heavy and light chain specific.

Metabolic labeling of cells and immunoprecipitation

Monolayers of L-2 cells in 6-well Costar plates were infected with MHV-JHM using 1–3 PFU/cell. Virus was removed after adsorption at 37° for 1 hr. Cells were radiolabeled, usually from 8 to 9 hr after infection, with 400 μCi/ml [^{35}S]methionine (ICN Radiochemicals, Ir-

vine, CA) in methionine-free DMEM supplemented with 2% FBS. Just before labeling cells were washed twice with methionine-free DMEM. After radiolabeling, cells were chilled on ice and washed with cold phosphate-buffered saline (PBS), and a cytoplasmic extract was prepared in 250 μl of lysing buffer (10 m*M* Tris–HCl, pH 7.4, 10 m*M* NaCl, 1.5 m*M* $MgCl_2$, 0.5% NP40, 0.2 TIU/ml of aprotinin) at 0°. Cytoplasmic extracts were stored at −70°. Antibody-coated *Staphylococcus aureus* Cowan strain (SAC) cells were prepared as follows: 200 μl of 10% (wt/vol) formalin-treated SAC cells (Calbiochem, San Diego, CA) were pelleted in a microfuge, washed once with PBS, and incubated for 1 hr on ice with the appropriate affinity purified secondary antibody (20–40 μg per 200 μl SAC). Pellets were washed twice with PBS and incubated on ice with the desired primary antibody for an additional hour. Unbound antibodies were washed away from the SAC cells with PBS and the SAC–antibody complexes were resuspended in MRIP buffer (10 m*M* phosphate, pH 7.4, 500 m*M* NaCl, 0.25% NP40, 0.2 TIU/ml aprotinin, 1 m*M* PMSF). Cell lysate (50 μl) was added to antibody-coated SAC and the mixture incubated on ice for 1 hr. The SAC-immunocomplexes were collected by centrifugation and washed five to six times with MRIP buffer. Bound antigens were eluted by heating at 70° for 5 min in SDS–PAGE sample buffer. Samples corresponding to approximately 1×10^5 cells were resolved by SDS–PAGE at 10 mA for about 10 hr as described by Laemmli and Favre (1973). The gels were fixed and processed for fluorography using EN^{3}HANCE (New England Nuclear) and exposed to preflashed Kodak XAR-2 X-ray film.

In some experiments MHV-infected or uninfected cells were labeled with [^{35}S]methionine in the presence of actinomycin D. 17CL-1 cells were infected with MHV-JHM using 1–3 PFU/cell essentially as described (Bond *et al.*, 1984). Actinomycin D, 10 μg/ml (Sigma Chemical Co., St. Louis, MO), was added to the medium at Time 0. Control MHV-JHM-infected cells were cultured in medium without actinomycin D. The cells were labeled with [^{35}S]methionine, lysed, and immunoprecipitated with 1.38.1 anti-E2 mab and 2.4G2 anti-FcR mab as described above. Under these conditions actinomycin D inhibited RNA synthesis of control cells by 94% as determined by [^{3}H]uridine incorporation (Leibowitz and DeVries, 1988).

Immunofluorescent microscopy

L-2 cells were trypsinized and plated on 8 chamber slides (Tissue culture chamber slides, Miles Scientific, Naperville, IL) at $2–3 \times 10^5$ cells per chamber. After the formation of monolayers (18 hr) the cells were infected with MHV-JHM at a m.o.i. of 1–3 PFU/cell in DMEM supplemented with 2% FBS. One hour later the virus was aspirated and the medium replaced. When syncytia involved about 80–90% of the cell monolayer, about 8 hr postinfection, the cells were washed once with PBS and fixed for 5 min with buffered 2% paraformaldehyde. Slides were treated with 0.15 *M* glycine for 15 min, washed twice with PBS, and stained by indirect immunofluorescence. Staining with the 2.4G2 mab was performed on live cells, omitting paraformaldehyde fixation and exposure to glycine. All incubations of cells with primary and appropriate FITC-conjugated secondary antibodies were performed on ice for 20 min.

Partial proteolysis mapping

V-8 protease mapping was carried out by the technique described by Cleveland with minor modifications (Cleveland *et al.*, 1977). Polypeptides to be compared were located by direct autoradiography of SDS polyacrylamide gels. The initial gel was dried directly after washing in water for 10 min without prior fixation or staining. After autoradiography proteins of interest were excised from the dried gel. The gel slices were then placed in wells of a 15% polyacrylamide gel and rehydrated for 30 min in 20 μl of a buffer containing 0.1% SDS, 10 m*M* Tris–HCl, pH 6.8, 10% glycerol, 5% 2-ME, and a trace of bromphenol blue. An additional 60 μl of the same buffer containing 100 ng of *S. aureus* V-8 protease (Boehringer-Mannheim, Indianapolis, IN) was added to each well and the gel slices were incubated at room temperature for 30 min. Electrophoresis was at a constant current of 7 mA.

RESULTS

Immunofluorescence staining of MHV-JHM-infected cells by rabbit IgG

During the development of polyclonal rabbit antibodies specific for MHV-JHM nonstructural proteins we observed that at moderate (1:50 to 1:100) dilutions normal rabbit serum (NRS) stained MHV-JHM-infected L-2 cells (Fig. 1B) but not uninfected cells (Fig. 1E). This effect was also observed with purified rabbit IgG specific for *M. lysodeikticus* (Fig. 1C). In contrast to this finding F(ab')$_2$ fragments of rabbit IgG specific for *M. lysodeikticus* did not stain either infected (Fig. 1F) or uninfected cells (not shown), suggesting that the Fc portion of the antibody molecule is required for staining of MHV-JHM-infected cells. Both the rabbit sera and purified rabbit IgG stained the plasma membrane and the cytoplasm of infected cells. There was no difference in the pattern of staining between the NRS (Fig.

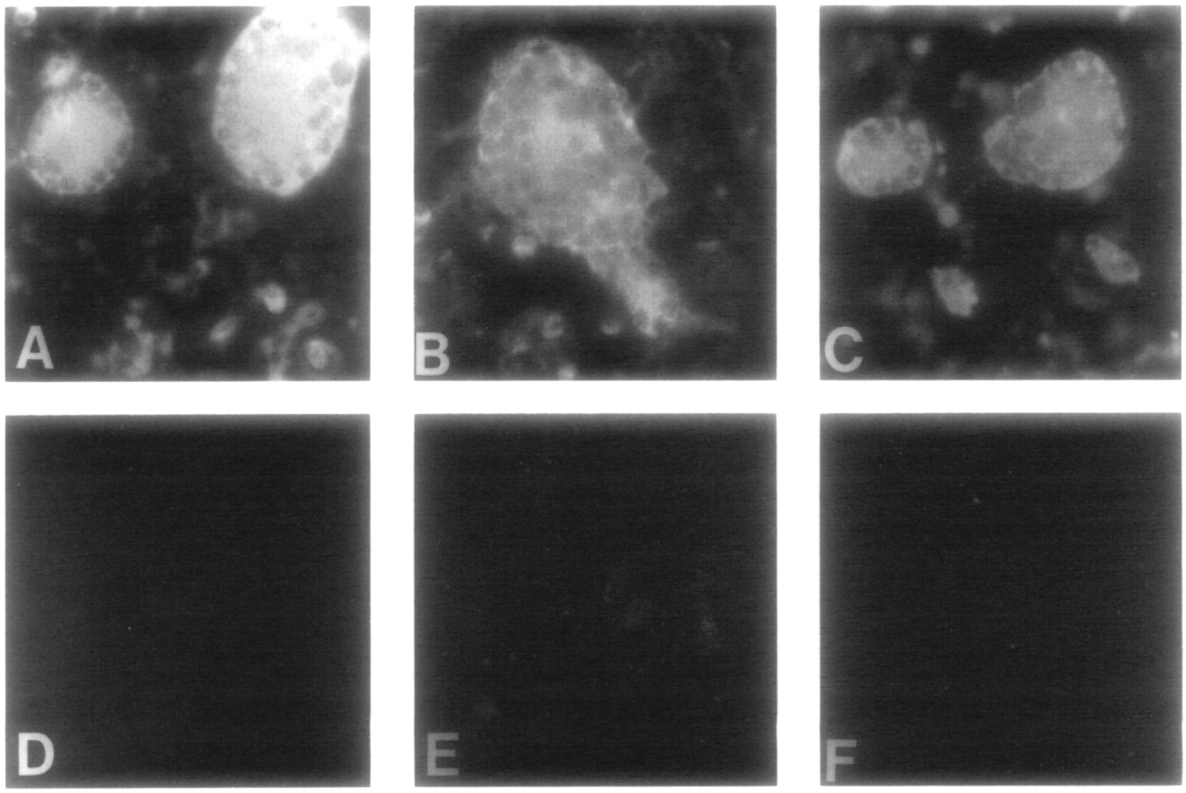

FIG. 1. Indirect immunofluorescence staining of MHV-JHM-infected cells. L-2 cells were infected with MHV-JHM or mock infected and stained for immunofluorescent microscopy as described under Materials and Methods. (A) Infected cells stained with polyclonal rabbit anti-MHV (1:100). (B) Infected cells stained with normal rabbit serum (1:100). (C) Infected cells stained with purified rabbit IgG (50 μg). (D) Mock-infected cells stained with polyclonal rabbit anti-MHV (1:100). (E) Mock-infected cells stained with normal rabbit serum (1:100). (F) Infected cells stained with rabbit F(ab')$_2$ fragment (50 μg).

1B) and the rabbit anti-MHV serum (Fig. 1A), although the latter was able to stain cells at higher dilutions (1:500) than the former (1:100). The rabbit anti-MHV serum (Fig. 1A) and the purified anti-*M. lysodeikticus* IgG (Fig. 1C) stained syncytia and single infected cells but not uninfected cells (Figs. 1D and 1E). FITC-conjugated goat anti-rabbit antibodies did not stain either infected or uninfected cells (not shown).

Molecular mimicry of mouse FcγR by MHV glycoprotein E2

The results of the immunofluorescence experiments suggested that the Fc portion of rabbit IgG is required for the observed staining of MHV-JHM-infected cells by irrelevant rabbit sera. To examine the hypothesis that this staining is mediated by an FcR-like molecule expressed in virus-infected cells we employed a purified rabbit IgG specific for *M. lysodeikticus*. This IgG preparation precipitated a polypeptide chain of 180 kDa from cytoplasmic extracts prepared from [^{35}S]methionine-labeled MHV-JHM-infected cells (Fig. 2, lane g). In contrast a F(ab')$_2$ fragment prepared from the same *M. lysodeikticus*-specific purified rabbit IgG did not precipitate any polypeptide chains from MHV-JHM-infected cells (Fig. 2, lane h). A 180-kDa polypeptide was also immunoprecipitated by a rat mab (2.4G2; IgG$_{2b}$) specific for FcγR (Fig. 2, lane d). All of these 180,000-Da proteins coelectrophoresed with MHV-JHM E2 protein precipitated by the neutralizing anti-E2 monoclonal antibody 1.38.1 (Fig. 2, lane a). The 150,000-Da E2 precursor was recognized by the 1.38.1 antibody but not by the other immunoglobulins. A 180,000-Da molecular weight protein was not observed in samples immunoprecipitated with secondary goat anti-rat IgG (lane b) or goat anti-rabbit IgG (lane e) in which the primary antibodies were omitted. Similar results were obtained in performing immunoprecipitations from several different cell lines, including 17Cl-1, L-2, and mouse neonatal brain cells (data not shown).

To further investigate this phenomenon we tested several irrelevant rat monoclonal antibodies for their ability to bind to the E2 protein. As shown in Fig. 3, rat mab (IgG$_1$) specific for lung surfactant and a second rat anti-surfactant mab (IgG$_{2b}$) both precipitated a

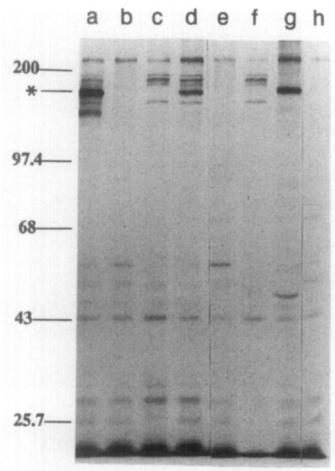

FIG. 2. Immunoprecipitation of E2 by rabbit anti-*Micrococcus lysodeikticus* IgG. MHV-JHM-infected or mock-infected cells were labeled for 1 hr with [^{35}S]methionine at 8 hr after infection and cytoplasmic extracts prepared. Aliquots (10 μl) of infected cell extract were immunoprecipitated with mouse anti-E2 mab 1.38.1 (lane a), goat anti-rat IgG (lane b), monoclonal antibody (rat) 2.4G2 (lane d), goat anti-rabbit IgG (lane e), 100 μg of purified rabbit anti-*M. lysodeikticus* IgG (lane g), and 100 μg rabbit anti-*M. lysodeikticus* F(ab')$_2$ fragments (lane h). Similarly, 10-μl aliquots of mock-infected cell extract were immunoprecipitated with monoclonal antibody (rat) 2.4G2 (lane c) and 100 μg of purified rabbit anti-*M. lysodeikticus* IgG (lane f). Immunoprecipitates were resolved by SDS–PAGE on a 8% gel. The positions of molecular weight standards (kDa) are shown. The position of the 180-kDa E2 is indicated with an asterisk.

180,000-Da polypeptide which coelectrophoreses with the protein precipitated by 2.4G2. Furthermore, a 180,000-Da polypeptide was also precipitated from cells infected with two other strains of MHV, namely MHV-A59 and MHV-3, by the 2.4G2 mab and rat monoclonal antibodies specific for lung surfactant (Fig. 3).

Irrelevant mouse mab of the IgG$_{2a}$ (OKT3, OKT8, MKD6, and 15-5-5^S) and IgG$_{2b}$ subclasses (OKT4), but not IgG$_1$ mouse mab (anti-Leu4 and 3D5), also immunoprecipitated a 180-kDa polypeptide from MHV-JHM-infected cells (Fig. 4). These results suggested that the precipitation of a 180,000-Da polypeptide by rat, mouse, and rabbit IgG was mediated through binding of this polypeptide to the Fc portion of the immunoglobulin molecules. The 180,000-Da polypeptide was specifically expressed by virus-infected cells. Mock-infected cells did not express the 180-kDa polypeptide (Fig. 4A, lane b), nor was the 180-kDa protein precipitated from mock-infected cells by monoclonal antibodies (Fig. 4B), even upon prolonged exposure of the autoradiogram. Precipitation of this 180,000-Da polypeptide was not via the secondary goat antibodies (goat anti-rat, goat anti-rabbit, or goat anti-mouse) used in these experiments since goat IgG alone did not precipitate a detectable protein of this size from MHV-infected cells. The polypeptide precipitated via the Fc portion of rat, mouse, and rabbit IgG had a molecular size, as judged by SDS–PAGE, identical to the MHV E2 protein precipitated by a neutralizing anti-MHV-JHM E2 mab.

Structural identity of the 180-kDa polypeptides recognized by anti-E2 and anti-FcγR monoclonal antibodies

To determine if mab against FcγR recognized the same molecule as that of mab specific for E2 on MHV-JHM-infected cells we first employed sequential immunoprecipitation of viral lysates with both antibodies. After five rounds of immunoprecipitation of MHV-JHM-infected cell lysate with anti-FcγR mab we could no longer detect the 180,000-Da band by SDS–PAGE (Fig. 5, lane g). The anti-E2 mab did not immunoprecipitate any polypeptides from lysate depleted by anti-FcγR antibody, demonstrating that the epitopes recognized by the 1.38.1 anti-E2 mab and the anti-FcγR mab are carried on the same molecule (Fig. 5). Actinomycin D did not inhibit the induction of the 180,000-Da molecule precipitated by mab 1.38.1 or 2.4G2 (data not shown), supporting the concept that the 180,000-Da

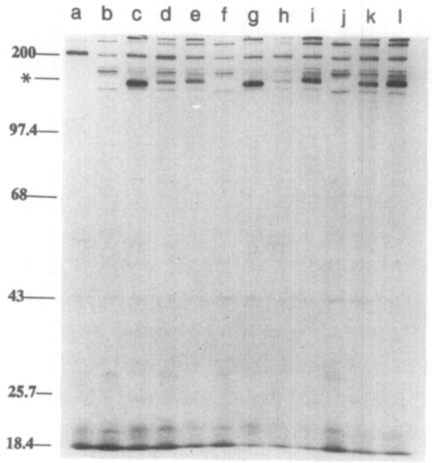

FIG. 3. Rat anti-FcγR and anti-surfactant antibodies recognize MHV E2. L-2 cells were infected with either MHV-JHM, MHV-A59, MHV-3, or mock infected. At 8 hr postinfection the cells were labeled with [^{35}S]methionine for 60 min and cytoplasmic extracts prepared. Goat anti-rat IgG was reacted with 10 μl of mock-infected cell extract (lane a). Rat anti-FcγR monoclonal antibody 2.4G2 was reacted with mock-infected cell extract (lane b), MHV-JHM-infected cell extract (lane c), MHV-A59-infected extract (lane d), and MHV-3-infected extract (lane e). A rat anti-surfactant monoclonal antibody (IgG$_1$) was reacted with mock-infected extract (lane f), MHV-JHM-infected extract (lane g), MHV-A59-infected extract (lane h), and MHV-3-infected extract (lane i). A rat anti-surfactant monoclonal antibody (IgG$_{2b}$) was reacted with mock-infected extract (lane j), MHV-JHM-infected extract (lane k), and MHV-A59-infected extract (lane l). Immunoprecipitates were resolved as described in the legend to Fig. 2. The position of the 180-kDa E2 is indicated with an asterisk.

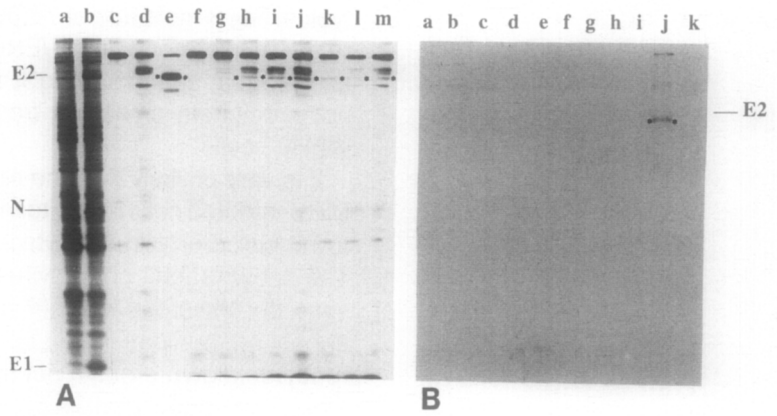

FIG. 4. Immunoprecipitation of a MHV-JHM E2 by murine IgG. [^{35}S]Methionine-labeled MHV-JHM-infected and mock-infected cytoplasmic extracts were prepared as described under Materials and Methods. (A) Lanes a and b display 1 μl of mock-infected and MHV-JHM-infected lysate, respectively. Mock-infected cell extract was immunoprecipitated with the anti-E2 mab 1.38.1 (lane d). MHV-JHM-infected cell extracts were immunoprecipitated with goat anti-mouse IgG (lane c), normal mouse serum (lane k), and the mouse monoclonal antibodies 1.38.1 (IgG_1) anti-E2 (lane e), anti-Leu4 (IgG_1) (lane f), anti-TCR γ chain (IgG_1) 3D5 (lane g), OKT3 (IgG_{2a}) (lane h), OKT4 (IgG_{2b}) (lane i), OKT8 (IgG_{2a}), (lane j), MKD6 (IgG_{2a}) (lane l), 15-5-5^S (IgG_{2a}) (lane m). The samples were resolved by SDS–PAGE on an 8% gel. Lanes a, b, and e are from an autoradiogram exposed 50% as long as the autoradiogram used for the other lanes. Positions of MHV-JHM viral proteins are indicated, on the left side of the autoradiogram. The positions of the 180-kDa E2 protein precipitated in lane e and lanes h–m are further indicated with asterisks. (B) Mock-infected extracts were immunoprecipitated with the mouse monoclonal antibodies 3D5 (lane b), anti-Leu4 (lane c), OKT3 (lane d), OKT4 (lane e), OKT8 (lane f), MKD6 (lane g), 15-5-5^S (lane h), and with normal mouse serum (lane i). MHV-JHM-infected lysates were immunoprecipitated with goat anti-mouse IgG (lane a) and the anti-E2 monoclonal antibody 1.38.1 (lane j). The immunoprecipitates were resolved by SDS–PAGE on an 8% polyacrylamide gel.

polypeptide recognized by the anti-FcγR mab 2.4G2 was of viral origin. To determine if the 180,000-Da polypeptides immunoprecipitated by the anti-E2 monoclonal antibody 1.38.1 and the anti-FcγR antibody 2.4G2 are structurally related, we employed V-8 protease peptide map analysis (Cleveland *et al.*, 1977). This method allows the unambiguous identification of structural similarities between specific proteins. Lysates of MHV-JHM-infected cells were immunoprecipitated with anti-E2 and anti-FcγR mabs, the precipitates were electrophoresed on SDS polyacrylamide gels, and the 180,000-Da bands were cut out, partially digested with V-8 protease, and subjected to electrophoresis as described under Materials and Methods. The partial V-8 digestion products of the 180,000-Da protein immunoprecipitated by the anti-E2 mab were identical to those obtained by V-8 digestion of the polypeptides precipitated by the anti-FcγR mab (Fig. 6).

To further elucidate the relationship between E2 and FcγR we investigated whether mab specific for the MHV E2 protein recognize FcγR expressed by a representative FcγR-bearing cell line, WEHI-3. As shown in Fig. 7, a 75,000- to 77,000-Da polypeptide typical of FcR was immunoprecipitated by (1) a 2.4G2 rat anti-FcγR mab; (2) a rat anti-surfactant mab; (3) a mouse mab specific for E2; (4) mouse mab MKD6, specific for I-A^d; (5) a goat anti-E2 serum. Nonimmune goat serum, goat anti-mouse IgG, and goat anti-rat IgG did not precipitate any labeled proteins from [^{35}S]methionine-labeled WEHI-3 cells.

Computer analysis

Computer analysis of the MHV-JHM E2 peplomer and the Fcγ receptor protein sequences using the Dayhoff Align program (Dayhoff *et al.*, 1983) identified three short domains (6–13 residues) of sequence similarity. These domains were from residues 108 to 118 (Fc receptor) with amino acids 457 to 467 (MHV-JHM E2), residues 129 to 134 (Fc receptor) with amino acids 499 to 506 (MHV-JHM E2), and residues 112 to 124 (Fc receptor) with amino acids 259 to 271 (MHV-JHM E2). Monte Carlo analysis of these regions of sequence similarity indicated that they were only of possible significance with Monte Carlo scores between 4.0 and 6.0. An optimal alignment was obtained by combining two nearly adjacent domains into a single unit spanning residues 457 to 506 for E2 and residues 108 to 134 for the Fc receptor (Fig. 8, domain 1). Monte Carlo analysis of the optimally aligned sequences indicated that this sequence similarity is probably significant for MHV-JHM E2 and the Fc receptor (Monte Carlo score = 7.35). Similar results were obtained using the MHV-A59 E2 protein sequence.

DISCUSSION

We describe here antigenic mimicry between the mouse FcγR and the MHV-JHM E2 glycoprotein. Anti-

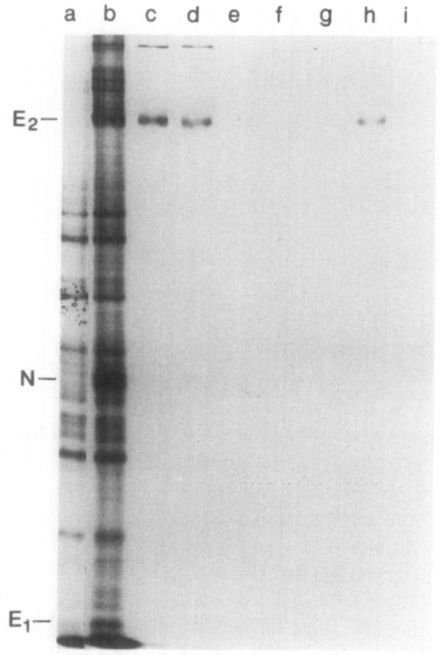

FIG. 5. Sequential precipitation of MHV-JHM-infected cells with anti-FcγR and anti-E2. An immunosorbant was prepared by reacting sequentially SAC cells with goat anti-rat IgG and anti-FcγR. MHV-JHM-infected cell lysate (50 μl) was reacted with 200 μl of this immunosorbant. The bound antigen was recovered by centrifugation (lane c) and any unbound antigen reacted in four subsequent rounds of immunoprecipitation with the anti-FcγR immunosorbant (lanes d–g). The supernate containing unbound antigens was then reacted with a second immunosorbant prepared using SAC cells, goat anti-mouse IgG, and anti-E2 antibody 1.38.1. Antigens bound to 1.38.1 were recovered by centrifugation (lane i). The 1.38.1 immunosorbant was also reacted with undepleted MHV-JHM-infected lysate (lane h). Lanes a and b show the original lysates from mock-infected and MHV-JHM-infected cells, respectively. Electrophoresis was on a 10% polyacrylamide gel.

FcγR mab as well as purified rabbit IgG (but not $F(ab')_2$ fragments) immunoprecipitated from MHV-JHM-infected L-2 cells a polypeptide with a molecular weight (180,000 Da) identical to that of the MHV-JHM E2 glycoprotein. We demonstrated that the molecules recognized by these two antibodies yield identical V-8 maps, and that the E2 protein has an FcγR-like affinity for the Fc portion of IgG. Additional supporting evidence includes (1) sequential immunoprecipitation experiments from MHV-JHM-infected cells, first by the anti-FcγR mab and then by the anti-E2 mab followed by SDS–PAGE, that revealed that the same polypeptide chain was recognized by the two mabs. (2) Actinomycin D did not inhibit the expression of the 180,000-Da polypeptide chain that was immunoprecipitated by the anti-FcγR mab, further indicating that this protein is of viral origin. (3) A mouse mab specific for E2, a polyclonal goat anti-E2-specific serum, the 2.4G2 anti-FcγR mab, and a rat anti-surfactant mab all immunoprecipitated a typical FcγR molecule of 75,000–77,000 Da from cells of the myelo-monocytic WEHI-3 cell line. Normal goat serum, goat anti-mouse IgG, and goat anti-rat IgG did not precipitate any band from ^{35}S-labeled lysates of WEHI-3 cells.

It is very unlikely that the reaction of rabbit IgG with MHV-infected cells is due to the presence of antibodies to rabbit coronaviruses which cross-react with MHV. The known rabbit coronaviruses and mouse hepatitis virus do not cross-react (R. Baric and D. Small, personal communications). It is highly unlikely that a currently unknown cross-reacting rabbit anti-rabbit coronavirus antibody to MHV E2 would not be detected by our immunoprecipitation experiments with $F(ab')_2$. The goat anti-rabbit IgG that we have used as secondary antibody in our immunoprecipitations is heavy and light chain specific and recognizes light chains of rabbit IgG in immunodiffusion experiments (data not shown). Thus if the reactivity of rabbit IgG with E2 were at the antigen combining site of the IgG molecule, the $F(ab')_2$ fragments of the rabbit IgG would have precipitated a 180-kDa protein as did the intact IgG molecules. We

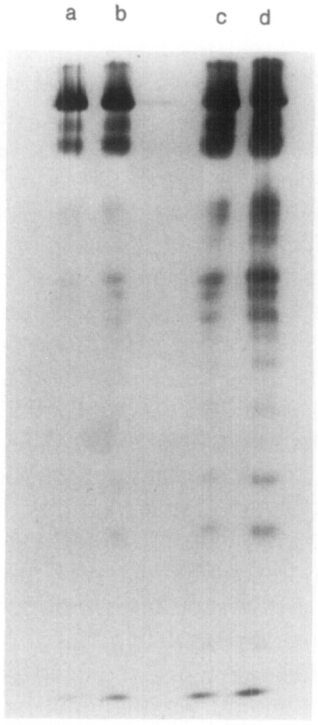

FIG. 6. Partial proteolysis mapping of 180-kDa proteins recognized by anti-E2 and anti-FcγR antibodies. The 180-kDa proteins immunoprecipitated from MHV-JHM-infected cells by the mouse anti-E2 monoclonal antibody 1.38.1 or the rat anti-FcγR antibodies were purified by SDS–PAGE and digested with *Staphylococcus aureus* V8 protease. The partial digestion products of the protein recognized by anti-FcγR (lanes a and b) and anti-E2 (lanes c and d) were resolved on a 15% SDS–polyacrylamide gel. Lanes a and c contained approximately 50% as much material as lanes b and d.

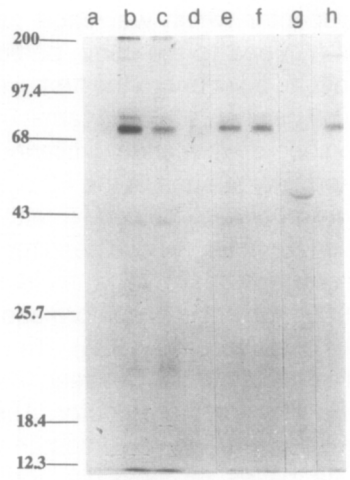

FIG. 7. Radioimmunoprecipitation of FcγR by anti-E2 antibodies. WEHI-3 cell monolayers were labeled for 16 hr with [^{35}S]methionine and cytoplasmic extracts prepared. The radiolabeled extracts were reacted with lane a, goat anti-rat IgG; lane b, monoclonal antibody 2.4G2; lane c, rat anti-surfactant monoclonal antibody (IgG_1); lane d, goat anti-mouse IgG; lane e, mouse monoclonal antibody MKD6; lane f, mouse anti-E2 monoclonal antibody 1.38.1; lane g, normal goat serum; lane h, goat anti-E2. Immunoprecipitates were resolved by SDS-PAGE on a 10% acrylamide gel.

have shown that these $F(ab')_2$ fragments did not immunoprecipitate any detectable MHV proteins.

Another possible explanation for the observed precipitation of E2 with IgG would be the association of E2 with a host-encoded molecule with Fc binding activity. Although we cannot completely exclude this possibility we view this as being unlikely since it is necessary to postulate that this molecule is associated with E2 in L-2 cells, 17Cl-1 cells, and cultured mouse neonatal brain cells. Furthermore, this hypothesis fails to explain the precipitation of the 75,000-Da Fc receptor from WEHI cells with goat anti-E2 antibodies but not normal goat serum.

Fc receptors for all immunoglobulin classes are found on a variety of B lymphocytes, some T cells, macrophages, NK cells, and polymorphonuclear leukocytes (Hubscher and Eisen, 1971; Dickler and Sachs, 1974; Tsay *et al.*, 1980; Teilland, 1985). They provide a link between humoral and cellular immune responses by permitting antibody-dependent cell-mediated toxicity (ADCC) to take place (Nathan *et al.*, 1980; Mellman *et al.*, 1983; Leslie, 1985). To date three distinct murine FcγR have been identified: a low-affinity, trypsin-resistant receptor for IgG_1 or IgG_{2b} (Unkeless, 1979; Hibbs *et al.*, 1986; Lewis *et al.*, 1986), a high-affinity, trypsin-sensitive receptor for monomeric IgG_{2a} (Unkeless and Eisen, 1975), and a receptor for IgG_3 (Diamond and Yelton, 1981). One of the best characterized is the mouse macrophage–lymphocyte receptor for IgG_1 or IgG_{2b} (FcγRII). The study of the latter receptor has been facilitated by the development of the rat mab 2.4G2 directed against FcγR present on macrophages and lymphocytes (Unkeless, 1979; Weinshank *et al.*, 1988). The receptor is a transmembrane glycoprotein with two extracellular domains that are homologous to members of the Ig superfamily (Williams and Barclay, 1988).

Both FcγR and E2 bind rat IgG_1 and IgG_{2b}, and rabbit IgG, but not goat IgG. It has been shown that the 2.4G2 antibody recognizes a site on the FcγR molecule which is either identical or overlaps with the Fc binding do-

```
                    DOMAIN 1

A59     546 RCQIFANILLNGINSGTTCSTDLQLPNTEVVTGICVKYDLY 586
            ||*   | |||                          | |  |
FcR     108 RCHSWRNKLLN--------------RISFFHNEKSVRYHHY 134
            ||*   | |||                         |||  |
JHM     457 RCQIFANILLNGINSGTTCSTDLQLPNTEVATGVCVRYDLY 506


                    DOMAIN 2

A59     259 WVTPLVKRQYLFN 271
            |   |  |  *|*
FcR     112 WRNKLLNRISFFH 124
            |   || |  *|*
JHM     259 WVTPLLKRQYLFN 271
```

FIG. 8. Amino acid sequence comparison of Fc receptor to MHV E2. Alignments were performed using the Dayhoff Align program. The positions of the sequence similarity domains within the published amino acid sequences are indicated (Schmidt *et al.*, 1987; Luytjes *et al.*, 1987; Lewis *et al.*, 1986).

main (Mellman and Unkeless, 1980). The binding of goat anti-E2 to the FcγR is an indication that the FcγR shares an antigenic determinant with the MHV E2 protein as well as an Fc binding activity. We have not as yet unequivocally determined if the antigenic site recognized by our goat anti-E2 antibody and shared by E2 and the FcγR and the Fc binding site on E2 and FcγR are equivalent. The FcγR which is recognized by antibody 2.4G2 binds mouse IgG_1, IgG_{2a}, and IgG_{2b}. MHV E2 binds mouse IgG_{2a} and IgG_{2b}, but not IgG_1. The difference in the ability of MHV E2 and FcγR to bind mouse IgG_1 could be an indication that their Fc binding sites have somewhat different structures. It is interesting in this regard that 2.4G2 did not neutralize MHV infectivity in a plaque reduction assay (data not shown).

Expression of receptors for the Fc regions of IgG are induced also by herpesviruses, mainly HSV-1, HSV-2, cytomegalovirus, and varicella zoster virus (Watkins, 1964; Ogata and Shigeta, 1979; Para *et al.*, 1982; Murayama *et al.*, 1986; Eizuru and Minamishima, 1988). For HSV-1, the best studied of these viruses, Fc receptors are composed of a complex containing the virally encoded gE and gI (g70) glycoproteins (Johnson and Feenstra, 1987; Johnson *et al.*, 1988). Both are required for Fc receptor activity. The FcγR-like molecules on MHV-JHM-infected cells are also encoded in the virus genome. The Fc binding activity of E2 was conserved among the three strains of MHV we tested. Furthermore, MHV-A59 E2 reacted with the anti-FcγR monoclonal antibody 2.4G2 as did MHV-JHM E2 (data not shown). No obvious common structural basis for the Fc binding activities of the MHV E2, HSV-1 gE and gI, and FcγR molecules could be demonstrated on the basis of their primary sequences using the Dayhoff Align program (Dayhoff *et al.*, 1983). Comparisons of secondary structure using the Garnier and Robson method (Garnier *et al.*, 1978) were also unrewarding. The demonstration of an Fc binding activity which has been conserved among the MHV strains tested, and the presence of a similar activity among the herpesviruses, suggests that binding of IgG via the Fc portion of the molecule must provide some selective advantage for these viruses.

As expected, a computer search of the PIR data base using the Dayhoff Align program (Dayhoff *et al.*, 1983) revealed homologies between FcγR and a number of members of the immunoglobulin gene superfamily (Williams and Barclay, 1988). In addition, two regions of sequence similarity between the MHV E2 protein and the FcγR were detected (Fig. 8). The data do not currently allow us to determine if either of the two E2 regions we have identified with sequence similarity with the Fc receptor are responsible for either the Fc binding activity of E2 or its antigenic cross-reactivity with the Fc receptor which we report here. Both of these regions mapped to the same portion of the Fc receptor molecule, adjacent to the first cysteine of the second loop (Lewis *et al.*, 1986). In terms of the E2 protein, these regions of sequence similarity are located within the N-terminal subunit of the E2 peplomer. Molecular mimicry between molecules with similar degrees of sequence similarity have been reported (Dyrberg and Oldstone, 1986). However, molecular mimicry can also occur on the basis of conformational determinants (Dyrberg and Oldstone, 1986). The current data do not permit us to distinguish between these two possibilities for E2 and the Fc receptor. The molecular mimicry we report here led us to search the PIR and Genebank data bases for possible homologies between E2 and other polypeptides which belong to the immunoglobulin gene superfamily. Small degrees of homology of uncertain significance were detected between E2 and some members of this family.

The antigenic mimicry observed here between E2 viral antigen and FcγR may have important biological implications. It has been postulated that binding of polyclonal IgG lacking antiviral specificity to viral antigens exposed on the cell surface could mask these antigens from specific anti-viral antibody by sterically hindering the attachment of specific antibody (Adler *et al.*, 1978). Such binding could reduce complement and cell-mediated lysis of infected cells. These effects could possibly be important in allowing virus-infected cells to avoid the host immune response, especially ADCC-mediated destruction, and potentially play a role in MHV persistent infections. This may be especially important in experimentally produced persistent infection of rats, since the binding of rat IgG to E2 appears to be of a higher affinity than the binding of mouse IgG.

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