Fc RECEPTOR-LIKE ACTIVITY OF MOUSE HEPATITIS VIRUS E2 GLYCOPROTEIN

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INTRODUCTION

The JHM strain of mouse hepatitis virus (MHV-JHM) is a member of the coronavirus family, which experimentally induces encephalomyelitis in susceptible mice and rats. In surviving animals a chronic white matter disease ensues.[1] The development of demyelinating lesions is thought to be a primary effect of infection of oligodendrocytes.[2,3] MHV-JHM virus has been shown to persist in the infected brain as long as 1 year after infection. Persistent infections of mice with other strains of MHV such as MHV-A59 and MHV-3 have also been reported.[4,5,6] Murine coronaviruses have four structural proteins: a matrix like transmembrane glycoprotein (E1), a nucleocapsid protein (N), a peplomer protein (E2), and for some laboratory strains of MHV-JHM a 65,000 Da glycoprotein.[7,8] The E2 glycoprotein (180,000 Da) is responsible for the attachment of MHV to the host cell plasma membrane, induction of cell-to-cell fusion and eliciting of the production of neutralizing antibody.[9,10] In the course of immunological staining of MHV-infected cells with rabbit antisera we observed at moderate dilution (1:50 - 1:100) normal and preimmune serum stained MHV-JHM infected, but not uninfected, cells. This staining could not be removed by absorption of rabbit antiserum with uninfected cells. This observation suggested that infection of cells with MHV may result in the expression of receptors for the Fc region of immunoglobulin G (IgG). It has been reported, that herpesviruses induce FcR on the surface of infected cells. The IgG Fc-binding receptors induced by HSV-1 are composed of a complex of two virally encoded glyproteins, gE and gI, and both of them are required for Fc receptor activity.[11,12] We report here that MHV-JHM infected cells express Fc binding ability. We have demonstrated that the E2 protein is responsible for Fc binding on infected cells.

MATERIALS AND METHODS

Viruses and Cells: The L-2 and WEHI-3 cell lines have been described previously.[13,14] The origin and growth of MHV-JHM, MHV-A59 and MHV-3 have been described.[8,15]

Antibodies: The 1.38.1 mab specific for the MHV E2 glycoprotein[16] and a hyperimmune rabbit serum which recognizes N and E2 were develop in this laboratory. Goat antiserum against purified MHV-A59 glycoprotein

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E2 was a generous gift from Dr. K. Holmes. Rat anti-surfactant monoclonal antibodies were generously supplied by Dr. D. Strayer. The rat anti-mouse FcγR monoclonal antibody 2.4G2 was originally described by Unkeless.[17] Purified whole rabbit IgG specific for Micrococcus lysodeikticus as well as $F(ab')_2$ fragments were a generous gift of Dr. D. Rodkey. 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).

Metabolic labeling of cells and immunoprecipitation. Cytoplasmic lysates of MHV infected cultures which had been labeled with ^{35}S-methionine were prepared as described previously.[8] Secondary antibody coated Staphylococcus aureus Cowan strain (SAC) were washed with PBS and incubated with the desired primary antibody for one hour on ice. Unbound antibodies were washed away from the SAC cells with PBS and the SAC-antibody complexes were resuspended in 10 mM phosphate, pH 7.4, 500 mM NaCl, 0.25% NP-40, 0.2 TIU/ml of aprotinin, 1 mM PMSF. ^{35}S-labeled cell lysate was added to antibody coated SAC and incubated on ice for 1 hour. The precipitated material was extensively washed with the buffer described above. Bound antigens were dissociated by heating at 70°C in SDS-PAGE sample buffer and subsequently analyzed by SDS-polyacrylamide gel electrophoresis.[18]

Indirect immunofluorescence microscopy was carried out by standard techniques.

Partial proteolysis mapping: V-8 protease mapping was carried out by the technique described by Cleveland.[19]

RESULTS

In the course of immunofluorescence staining MHV-infected cells with rabbit antisera raised against MHV nonstructural proteins we observed that normal rabbit serum (NRS) at dilutions of 1:50 to 1:100 stained MHV-JHM infected L-2 cells (Fig. 1, Panel B) but not uninfected cells (Fig. 1, Panel E). Similar staining was observed with purified rabbit IgG specific for M. lysodeikticus (Panel C), but not with $F(ab')_2$ fragments of rabbit IgG (Panel F). Neither rabbit IgG specific for M. lysodeikticus nor its $F(ab')_2$ fragments stained uninfected cells (not shown). These data suggested, that the staining of MHV-JHM infected cells requires the Fc portion of immunoglobulin. NRS, rabbit IgG, and rabbit anti-MHV serum (Panel A) stained syncytia but not uninfected cells. The immune reaction was diffuse and restricted to the cytoplasm and the cell membrane.

Molecular mimicry of MHV E2 glycoprotein and mouse FcγR.

To examine the hypothesis that FcR-like structures are responsible for the immunostaining of MHV-JHM infected cells we carried out immunoprecipitation experiments using purified rabbit IgG specific for M. lysodeikticus and its purified $F(ab')_2$ fragments. This IgG immunoprecipitated a polypeptide of 180,000 Da from extracts of ^{35}S-labeled MHV-JHM infected cells (Figure 2, lane j) but not from uninfected cells. However $F(ab')_2$ fragments did not immunoprecipitate any polypeptides from MHV-JHM infected cells (lane i). This 180,000 Da polypeptide corresponded to 180,000 Da polypeptide precipitated by the neutralizing anti-E2 monoclonal antibody 1.38.1 (Figure 2, lane c and k). Furthermore, rat mab 2.4G2 specific for FcγR also

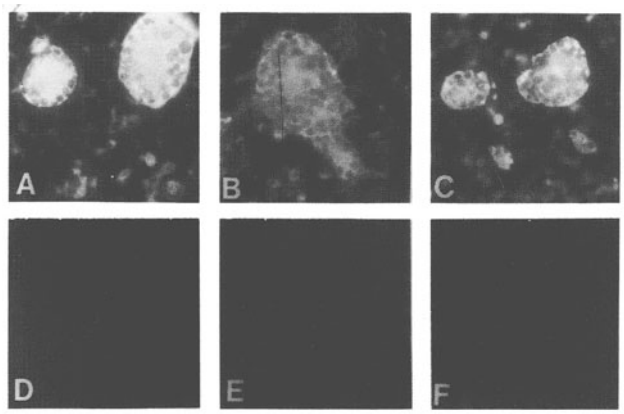

Fig. 1. Indirect immunofluorescence staining of MHV-JHM infected cells. MHV-JHM infected L-2 cells were stained with rabbit anti-MHV serum (A) (1:100); NRS (B) (1:100); purified rabbit IgG 50μg, (C); rabbit F(ab')2 fragments 50μg, (F). Rabbit IgG (E) and rabbit anti-MHV serum (D) were used to stain mock infected cells.

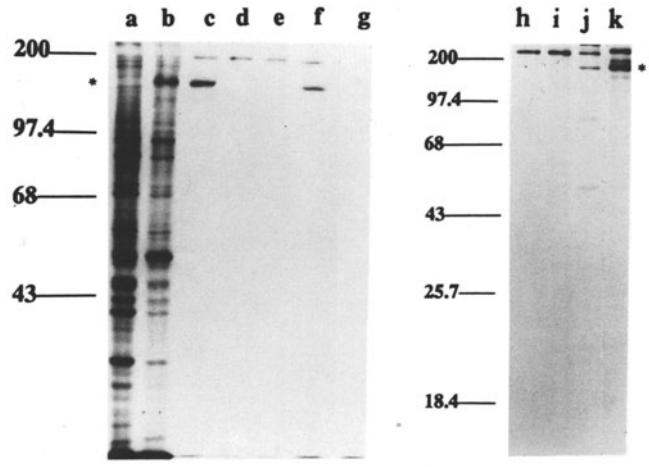

Fig. 2. Immunoprecipitation of MHV-E2 glycoprotein by rabbit anti-M. lysodeikticus IgG. [^{35}S]-methionine labeled MHV-JHM infected and mock-infected cytoplasmic extracts were prepared as described in Materials and Methods. Lanes a and b display 0.5μl of mock-infected and MHV-JHM infected lysate respectively. MHV-JHM infected cell lysates were immunoprecipitated with the anti-E2 mab 1.38.1 (lane c and k), with goat anti-mouse IgG (lane d), goat anti-rat IgG (lane e), rat anti-FcγR mab 2.4G2 (lane f), goat anti-rabbit IgG (lane h), 50μg rabbit anti-M.lysodeikticus $F(ab')_2$ fragments (lane i), 50μg of purified rabbit anti-M.lysodeikticus IgG (lane j). Mock-infected cell lysate was immunoprecipitated with the 2.4G2 mab (lane g). Immunoprecipitates were resolved by SDS-PAGE on a 8% gel (lanes a-g) or 10% gel (lanes h-k). The position of the 180,000 Da polypeptide is indicated (asterisk).

immunoprecipitated a 180,000 Da polypeptide from MHV-JHM-infected (Figure 2, lane f), but not from uninfected cells (Figure 2, lane g). Secondary goat anti-mouse IgG, goat anti-rat IgG and goat anti-rabbit IgG did not immunoprecipitate this 180,000 Da band.

Since MHV infects both mice and rats we investigated the ability of several irrelevant rat and mouse monoclonal antibodies to immunoprecipitate the E2 protein. Rat mabs specific for lung surfactant (IgG1 and IgG2b) immunoprecipitated a 180,000 Da polypeptide which coelectrophoresed with the protein immunoprecipitated with the 2.4G2 mab (Fig. 3). These rat mabs immunoprecipitated the 180,000 Da polypeptide from cells infected with additional strains of MHV, namely MHV-A59 and MHV-3 (Fig. 3).

Irrelevant mouse mab of the IgG2a subclass (OKT3, OKT8, MKD6) and IgG2b subclass (OKT4) but not IgG1 (anti-Leu 4 and mab specific for the T cell receptor gamma chain) also immunoprecipitated a 180,000 Da polypeptide from MHV-JHM infected cells but not from uninfected cells (data not shown). Therefore, we concluded that rat, mouse and rabbit IgG immunoprecipitated from MHV-JHM infected L-2 cells 180,000 Da polypeptide, which has identical molecular size to the MHV-E2 protein.

MHV-E2 glycoprotein and mouse FcγR are structurally identical

To determine if the 180,000 Da polypetide immunoprecipitated by the anti-E2 mab 1.38.1 and the anti-Fc γR mab 2.4G2 are structurally related we employed V-8 protease peptide map analysis.[19] V-8 protease digestion of the polypeptides immunoprecipitated by anti-E2 mab yielded an identical peptide map to that obtained by V-8 digestion of the polypeptide immunoprecipitated by the anti-FcγR mab (Fig. 4).

Since rabbit, rat and mouse IgG immunoprecipitated E2 glycoprotein from MHV-JHM infected L-2 cells we tested the ability of goat anti-E2 antibodies to bind to FcγR on the representative FcR bearing cell line, WEHI-3. The 2.4G2 mab, a rat anti-surfactant mab (IgG1), mouse mab MKD6 specific for I A^d and goat anti-E2 serum immunoprecipitated a 75,000 - 77,000 Da polypeptide typical of FcγR from ^{35}S-methionine labeled WEHI-3 cells (Fig. 5). Nonimmune goat serum did not precipitate any labeled proteins from WEHI-3 cells, suggesting, that the goat anti-E2 antibody immunoprecipitated the FcγR via its Fab region.

DISCUSSION

Molecular mimicry is defined as the presence of common antigenic sites, either linear or conformational, between microorganisms and normal host cell components.[20] The immune response initiated against foreign viral material, which is homologous to "self" host protein may lead to autoimmune disease.[21] In this work we report the antigenic mimicry between FcγR and the MHV-JHM E2 glycoprotein. Purified rabbit IgG, but not $F(ab')_2$ fragments, irrelevant rat mabs (IgG1 and IgG2b); irrelevant mouse mabs (IgG2a and IgG2b) immunoprecipitated a 180,000 Da polypeptide from MHV-JHM infected L-2 cells. Furthermore a rat 2.4G2 mab specific for FcγRII also immunoprecipitated also a 180,000 Da polypeptide from MHV-JHM infected cells which coelectrophoresed with the 180,000 Da molecule immunoprecipitated by a mab specific for MHV-E2 glycoprotein. The 180,000 Da polypeptide recognized by the 2.4G2 anti-FcγR mab and the 1.38.1 anti-E2 mab yield identical V-8 protease peptide maps (Fig. 4) and therefore, are almost assuredly the same molecular species. Furthermore, actinomycin D did not inhibit the expression of the 180,000 Da polypeptide that was immunoprecipitated by the anti-FcγR

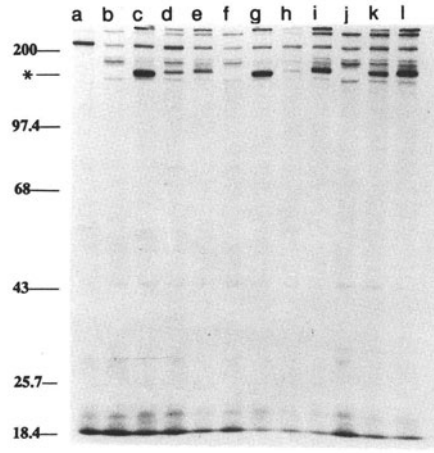

Fig. 3. Immunoprecipitation of MHV-E_2 by 2.4G2 anti-FcγR mab and anti-surfactant antibodies (IgG1 and IgG2b). MHV-JHM infected cells were immunoprecipitated with 2.4G2 mab (lane c), rat anti-surfactant mab (IgG1) (lane g) and rat anti-surfactant mab (IgG2b) (lane k). MHV-A-59 infected cells reacted with 2.4G2 mab (lane d); rat anti-surfactant mab (IgG1) (lane h) and rat anti-surfactant mab (IgG2b) (lane l). MHV-3 infected cells reacted with 2.4G2 mab (lane e); rat anti-surfactant mab (IgG1) (lane i). Goat anti-rat IgG were reacted with MHV-JHM infected cells (lane a). Mock-infected cells were reacted with 2.4G2 mab (lane b); rat anti-surfactant (IgG1) (lane f) and rat anti-surfactant (IgG2b) (lane j).

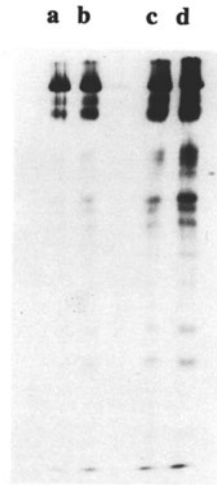

Fig. 4. Comparison of the Cleveland partial proteolytic fragments derived from 180,000 Da polypeptide immunoprecipitated by 2.4G2 anti-FcγR mab (lanes a,b) and by 1.38.1 anti-E_2 mab (lanes c,d). Lanes b and d contained approximately twice as much material as lanes a and c.

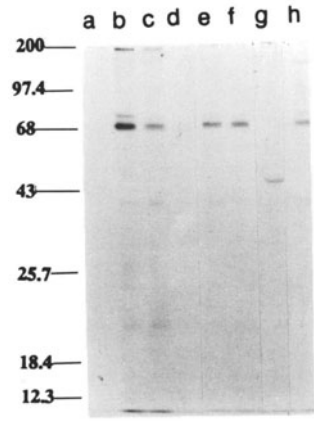

Fig. 5. Radioimmunoprecipitation of FcγR by goat anti-E2 antibodies. ^{35}S-methionine labeled WEHI-3 cell lysates were immunoprecipitated with goat anti-rat IgG (lane a); mab 2.4G2 (lane b); rat anti-surfactant mab (lane c); goat anti-mouse IgG (lane d); mab MKD6 (lane e); anti E2 mab (lane f); normal goat serum (lane g); goat anti-E2 (lane h).

mab, indicating that this protein is of viral origin (data not shown). Rabbit IgG, the 2.4G2 anti-FcγR mab and polyclonal goat anti-E2 specific serum immunoprecipitated a typical FcγR molecule of 75,000-77,000 Da from the myelomonocytic WEHI-3 cell line (Fig. 5). Nonimmune 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. The binding of goat anti-E2 to the FcγR suggests that this molecule shares an antigenic determinant with the MHVE2 protein as well as an Fc binding activity.

Fc receptors for all immunoglobulin classes are found on B lymphocytes, some T cells, macrophages, NK cells and polymorphonuclear leukocytes.[22] The function of these Fc receptors is to bind Ig via the Fc region of the Ig molecule. They provide a link between humoral and cellular immune response by permitting antibody-dependent cell mediated cytotoxicity (ADCC) to take place.[23,24,25] FcγRII recognized by the 2.4G2 mab is present on macrophages and lymphocytes[17]. The receptor is a transmembrane glycoprotein with two extracellular domains that are homologous to members of the Ig gene superfamily.[26]

Expression of receptors for the Fc regions of IgG are induced also by members of Herpesviridae: HSV-1, HSV-2, cytomegalovirus and varicella zoster[11,27,28,29,30] The FcγR- like molecules induced by HSV-1 are encoded in the virus genome and are comprised of a complex containing gE and gI (g70) glycoproteins.[12,31] Both are required for Fc receptor activity. No significant 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.[32]

The antigenic mimicry observed here between E2 viral antigen and FcγR may have important biological implications. It has been postulated that coating of infected cells with IgG molecules attached by their Fc fragment may mask viral antigens from neutralizing anti-viral antibody.[33] Such binding may allow the infected cells to escape destruction by immunological mechanism, namely ADCC and complement mediated lysis, and may contribute in maintenance of persistent infection.

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