Binding of the Coronavirus Mouse Hepatitis Virus A59 to Its Receptor Expressed from a Recombinant Vaccinia Virus Depends on Posttranslational Processing of the Receptor Glycoprotein

MICHAEL N. PENSIERO,[†] GABRIELA S. DVEKSLER, CHRISTINE B. CARDELLICHIO, GUI-SEN JIANG, PATRICK E. ELIA, CARL W. DIEFFENBACH, AND KATHRYN V. HOLMES*

Department of Pathology, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814-4799

Received 2 December 1991/Accepted 6 April 1992

Recently, we showed that a murine member of the carcinoembryonic antigen family of glycoproteins serves as a cellular receptor (MHVR) for the coronavirus mouse hepatitis virus A59 (MHV-A59) (G. S. Dveksler, M. N. Pensiero, C. B. Cardellicchio, R. K. Williams, G.-S. Jiang, K. V. Holmes, and C. W. Dieffenbach, *J. Virol.* 65:6881–6891, 1991; R. K. Williams, G.-S. Jiang, and K. V. Holmes, *Proc. Natl. Acad. Sci. USA* 88:5533–5536, 1991). To examine the role of posttranscriptional modification of MHVR on virus-receptor interactions, a vaccinia virus-based expression system was employed. Expression from the vaccinia virus recombinant (Vac-MHVR) in BHK-21 cells resulted in high levels of MHVR glycoprotein on the cell surface and made these cells susceptible to MHV-A59 infection. Nonglycosylated core MHVR proteins were made in Vac-MHVR-infected BHK-21 cells in the presence of tunicamycin by *in vitro* translation of MHVR mRNA in a rabbit reticulocyte cell-free system in the absence of microsomal membranes and by expression of an N-terminal deletion clone of MHVR lacking its signal peptide. These three nonglycosylated MHVR proteins were recognized by polyclonal antibody against affinity-purified receptor but did not bind antireceptor monoclonal antibody (MAb) CC1 or MHV-A59 virions. Partial glycosylation of MHVR, either expressed in Vac-MHVR-infected cells treated with monensin or synthesized by *in vitro* translation with microsomal membranes, restored both the MAb CC1- and the virus-binding activities of the MHVR glycoprotein. Deletion of 26 amino acids at the carboxyl terminus of MHVR resulted in a secreted protein which was able to bind MAb CC1 and MHV-A59. These results suggest that either a carbohydrate moiety is an element of the MHVR-binding site(s) for virus and MAb CC1 or a posttranslational membrane-associated process is required for functional conformation of the receptor glycoprotein.

Mouse hepatitis virus (MHV) is a large group of murine coronavirus strains which cause respiratory, enteric, and/or neurological infections or hepatitis in mice (2, 30). The susceptibilities of inbred strains of mice to MHV vary considerably and are controlled by several host genes (10, 23, 25). One host gene affecting MHV susceptibility encodes a glycoprotein (MHVR) that serves as a virus receptor on host cell membranes (4, 31). In hepatocyte membranes or intestinal brush border membranes (BBM) from MHV-susceptible BALB/c mice, the receptor for the A59 strain of MHV (MHV-A59) is a cellular glycoprotein with a mass of approximately 110 kDa (4, 7, 31). Virus binds specifically to this glycoprotein in virus overlay protein blot assays (VOPBAs) of BBM and hepatocyte membrane preparations (4). The binding of virus to BALB/c membranes and to murine fibroblasts *in vitro* is blocked by antireceptor monoclonal antibody (MAb) CC1, which also recognizes the receptor glycoprotein on immunoblots (31). MAb CC1 was used for affinity purification of the receptor glycoprotein, and the amino-terminal amino acid sequence of the purified receptor showed it to be a member of the carcinoembryonic antigen (CEA) family of glycoproteins in the immunoglobulin superfamily (31, 32).

A full-length cDNA clone, MVHR₁, encoding a murine CEA-related glycoprotein was obtained from a lambda gt11 library of BALB/c livers (7). When hamster or human cell

lines, which are normally resistant to infection with MHV-A59, were transfected with MHVR₁, they became susceptible to MHV-A59 infection (7). Preincubation of these transfected cells with MAb CC1 protected them from infection after challenge with MHV-A59 (7). Although the levels of MHVR produced in mouse fibroblast cell lines and in the BHK-21 cell line stably transfected with MHVR₁ were sufficient to permit MHV-A59 infection, the levels of expression were too low for detailed biochemical characterization of the receptor protein. One goal of our research was to develop a higher-level expression system for the MHVR glycoprotein to permit evaluation of the role of posttranscriptional processing on its biological functions. Numerous studies have shown that glycoproteins expressed by vaccinia virus recombinants are efficiently processed and targeted to their proper cellular compartments (16, 17). We therefore constructed a recombinant vaccinia virus (Vac-MHVR) containing the MHVR₁ cDNA under the control of the early-late vaccinia virus P7.5 promoter and analyzed the biological activities of the resulting MHVR glycoprotein.

This paper shows that expression in BHK-21 cells of MHVR from a vaccinia virus expression system resulted in the synthesis of high levels of MHVR which was properly processed and targeted to the plasma membrane. The glycoprotein retained the ability to bind MHV-A59 virions, leading to productive MHV infection, and it also bound antireceptor MAb CC1. In contrast, the nonglycosylated core protein of MHVR bound neither MAb CC1 nor MHV-A59. Thus, posttranslational processing of MHVR is required for its activity as a virus receptor.

* Corresponding author.

[†] Present address: Virology Division, Genetic Therapy, Inc., Gaithersburg, MD 20878.

MATERIALS AND METHODS

Cells and viruses. BHK-21 cells obtained from J. Hay (Uniformed Services University of the Health Sciences) were grown in Dulbecco's modified Eagle's minimal essential medium (high glucose) supplemented with 10% heat-inactivated fetal calf serum (GIBCO Laboratories, Grand Island, N.Y.). CV-1 cells and human TK⁻ cells were propagated as previously described (18). L2p cells from D. Percy (University of Guelph, Guelph, Ontario, Canada) were grown in Dulbecco's modified Eagle's minimal essential medium and 10% fetal bovine serum. MHV-A59 (from L. Sturman, New York State Department of Health, Albany), rat coronavirus (RCV) strain Parker (from D. Percy) and human respiratory coronavirus strain HCV-229E (from M. Johnson-Lussenburg, University of Ottawa, Ottawa, Ontario, Canada) were propagated as previously described (19, 26). Vaccinia virus strain WR, kindly provided by B. Moss (National Institutes of Health, Bethesda, Md.), was propagated in CV-1 cells and used as the parent virus for all of the recombinants. vSC11 is a vaccinia virus recombinant containing no insert that has been previously characterized (18).

Antisera. Polyclonal rabbit antiserum directed against affinity-purified MHV receptor (no. 655; anti-MHVR) was prepared as previously described (7) and adsorbed with vSC11-infected BHK-21 cells. MAb CC1 directed against MHV-A59 receptor glycoprotein (MHVR) was prepared from a hybridoma obtained by the fusion of SP2/0 cells with spleen cells from SJL/J mice immunized with a deoxycholate extract of BALB/c BBM as previously described (22, 32). Antiserum directed against MHV-A59 was collected from adult BALB/c mice 4 weeks after oronasal inoculation with infectious virus.

Isolation of MHVR cDNAs. A 710-bp partial cDNA clone of MHVR was generated by RNA polymerase chain reaction from BALB/c mouse colon mRNA and subcloned as previously described (7). Clones p10.4 and MHVR₁ were obtained by screening a lambda gt11 library of BALB/c mouse cDNA libraries with the p710 insert (7). The partial cDNA clone p10.4 started at base 197 (corresponding to amino acid 10 of the mature MHVR protein) and was identical in sequence to MHVR₁ up to base 1586.

Recombinant plasmids. The generation of recombinant plasmids is illustrated in Fig. 1 and summarized below.

(i) **p10_{ATG}.** A single-base substitution of an A for a G at amino acid 11 of p710 was effected by site-directed mutagenesis according to the manufacturer's instructions (Bio-Rad, Richmond, Calif.); this resulted in the generation of p710_{ATG}, which contains a methionine at codon 11. Clone p10_{ATG} was obtained by ligation of the *SmaI*-*Bam*HI fragment of p710_{ATG} with clone p10.4 at its unique *Bam*HI site. This resulted in an open reading frame which extended from amino acid 11 to the stop codon (base 1444 of MHVR₁).

(ii) **pVSV-10_{ATG}.** Two complementary oligonucleotides containing the 16 amino acids of the vesicular stomatitis virus (VSV) G protein signal sequence plus the first 2 amino acids of the mature G protein were synthesized. Hybridization of these oligos into the unique *Eco*RI site of p10_{ATG} resulted in an in-frame VSV signal sequence preceding the MHVR open reading frame.

(iii) **psMHVR.** MHVR₁ was digested with *Eco*RI and *Stu*I, and the resultant fragment was cloned into pSC11 as described below. All clones were confirmed by Sanger dideoxy sequencing with Sequenase (U.S. Biochemical, Cleveland, Ohio).

(iv) **Vac-MHVR, Vac-sMHVR, and Vac-VSV-10_{ATG}.** The

*Eco*RI-*Not*I and *Eco*RI-*Stu*I fragments from MHVR₁ and the *Sal*I-*Not*I fragment from pVSV-10_{ATG} were rendered blunt by incubation with 25 mM deoxynucleoside triphosphates in the presence of T4 DNA polymerase. The resultant fragments were then cloned into the unique *Sma*I site of the vaccinia virus shuttle vector pSC11 (5) to generate the clones pSC11-Vac-MHVR, pSC11-sMHVR, and pSC11-VSV-10_{ATG}. The correct orientation was confirmed by using asymmetric restriction digestion.

Generation of vaccinia virus recombinants. CsCl-purified DNA from pSC11-Vac-MHVR, pSC11-sMHVR, and pSC11-VSV-10_{ATG} was used to transfect CV-1 cells already infected with vaccinia virus. TK⁻ vaccinia virus recombinants were isolated as blue plaques, as previously described (5). All recombinants were subjected to three rounds of plaque purification on human TK⁻ 143 cells in the presence of 25 µg of bromodeoxyuridine (BUdR) per ml. The resultant vaccinia virus recombinants were named Vac-MHVR, Vac-sMHVR, and Vac-VSV-10_{ATG}.

Analysis of recombinant proteins. BHK-21 cells were grown on 60-mm-diameter plates and infected with each recombinant vaccinia virus at a multiplicity of infection (MOI) of 10 PFU per cell. For analysis of the amount, size, and virus- or antibody-binding activities of the recombinant proteins, at various intervals after inoculation, cells were lysed in 0.5 ml of disruption buffer (2.3% sodium dodecyl sulfate [SDS], 10% glycerol, 5% β-mercaptoethanol, 62.5 mM Tris-Cl, pH 6.8). For some experiments, 3 h after the inoculation of BHK-21 cells with vaccinia virus recombinants (MOI, 10 PFU per cell), we added inhibitors of glycosylation (monensin, 10⁻⁷ to 10⁻⁵ M, or tunicamycin, 1 to 20 µg/ml) and then incubated the cultures for an additional 17 h. Cell extracts were prepared as described above. Samples were subjected to SDS-8% polyacrylamide gel electrophoresis (PAGE) (11) and transferred to nitrocellulose. Typically, each lane contained 40 µl of sample equivalent to approximately 1.6 × 10⁵ cells. Immunoblots and VOPBAs were performed as previously described (4, 28).

Superinfection with MHV. BHK-21 cells grown on glass coverslips were inoculated with Vac-MHVR or vSC11 at an MOI of 0.1, 1.0, or 10 PFU per cell and incubated at 37°C for 3 h, and then they were challenged with MHV-A59 (MOI, 10 PFU per cell). For some experiments, cells were infected with Vac-MHVR or vSC11 and then incubated with MAb CC1 (50 µg/ml) or a control monoclonal antibody of the same isotype for 2 h prior to infection with MHV. After an additional 8 or 17 h, coverslips were processed for the detection of viral antigens by immunofluorescence. Briefly, cells were washed once with phosphate-buffered saline and then fixed in -20°C acetone for 10 min. Coverslips were air dried and frozen at -20°C until they were labeled with anti-MHV-A59 antiserum, polyclonal anti-MHVR antiserum, or MAb CC1, as previously described (7).

In vitro transcription and translation. MHVR₁ linearized with *Not*I was used as a template for mRNA synthesis by using T7 RNA polymerase according to the manufacturer's instructions (Promega, Madison, Wis.). The transcription reaction was terminated with phenol-chloroform and was followed by multiple ethanol precipitations. A rabbit reticulocyte lysate containing [³⁵S]methionine was programmed with 1 µg of MHVR₁ mRNA in the presence or absence of canine pancreatic microsomal membranes (Promega). Translation efficiency was monitored by spotting 2 µl of the translation reaction mixture on Whatman 540 filters and measuring the incorporation of radiolabel into hot trichloroacetic acid-precipitable counts. Translated proteins from

FIG. 1. Clones used in this study. Refer to Materials and Methods for the specifics of how each clone was generated. (A) p710 is a partial cDNA clone which encodes a protein beginning at amino acid 10 of MHVR. p710_{ATG} is derived from p710 by site-directed mutagenesis resulting in the substitution of a methionine for a valine. Ligation of the *Sma*I-*Bam*HI fragment of p710_{ATG} into p10.4 cut with *Eco*RV-*Bam*HI resulted in p10_{ATG}. Two complementary oligonucleotides encoding the VSV signal sequence were hybridized and then ligated into p10_{ATG} cut with *Kpn*I-*Eco*RI to generate the chimera pVSV-10_{ATG}. Fragments from pVSV-10_{ATG} and MHVR₁ (below) were used to clone into the unique *Sma*I site of the vaccinia virus shuttle vector pSC11. (B) Schematic representation of the full-length MHVR cDNA clone, MHVR₁ (7). The *Stu*I restriction site at base 1322 was used to generate psMHVR. The termination codon, TGA, is located at base 1444.

optimized reaction mixtures were immunoprecipitated with either MAb CC1 or rabbit polyclonal anti-MHVR or, as controls, a monoclonal antibody of the same immunoglobulin G1 isotype directed against an unrelated antigen or with preimmunization rabbit serum. The immunoprecipitated proteins were analyzed on 7.5% SDS-polyacrylamide gels, and the products were detected by autoradiography.

Nucleotide sequence accession numbers. The sequences of the cDNA clones described here have been deposited with

GenBank, and accession numbers M64035 and M77196 have been assigned to clones p10.4 and MHVR₁, respectively.

RESULTS

Expression of the MHV receptor glycoprotein from a vaccinia virus recombinant. To obtain a level of expression of MHVR higher than that from cell lines transiently or stably transfected with MHVR₁ in a Rous sarcoma virus vector, we

FIG. 2. Time course of MHVR expression from Vac-MHVR-infected BHK-21 cells. Cell lysates were prepared from BHK-21 cells at the indicated times after inoculation with Vac-MHVR (R) or vSC11 (V) (MOI, 10 PFU per cell). Total cell proteins were analyzed by SDS-PAGE and immunoblotted with rabbit polyclonal anti-MHVR to identify the MHVR glycoprotein and related proteins. Molecular masses (in kilodaltons) are on the right.

used a vaccinia virus expression system. Using the vaccinia virus shuttle vector pSC11, we placed MHVR₁ into vaccinia virus under the control of the vaccinia virus early-late promoter P7.5. The resultant vaccinia virus recombinant was designated Vac-MHVR. BHK-21 cells were infected with Vac-MHVR or vSC11 (vaccinia virus without an insert), and, at various times after infection, cellular proteins in immunoblots were analyzed with antireceptor antibody. Figure 2 shows that Vac-MHVR expressed a 110-kDa glycoprotein which comigrated with MHVR from BALB/c intestinal BBM. This protein was not observed with samples from vSC11-infected (V) or mock-infected (M) BHK-21 cells. Recombinant protein was detected as early as 3 h postinfection, and its expression increased for up to 30 h. Samples in the immunoblots represented crude protein from approximately 1.6×10^5 cells, equivalent to 1/10 of a confluent 60-mm-diameter tissue culture dish. We calculated that the level of MHVR expression from Vac-MHVR was at least 2,500-fold higher than that in BHK-21 cells stably transformed with MHVR₁ under the Rous sarcoma virus promoter (7). Additionally, the vaccinia virus expression system gave a much higher level of expression of MHVR than was found with the L2p or 17 Cl1 line of mouse fibroblasts, both of which express sufficient MHVR to permit virus attachment but do not make enough to be detected in immunoblots under these conditions (data not shown).

Although immunoblotting with rabbit polyclonal anti-MHVR or MAb CC1 showed both the 110-kDa MHVR and a related 55- to 58-kDa glycoprotein species in BALB/c intestinal BBM and hepatocytes, (31, 32), we did not detect this species in Vac-MHVR-infected BHK-21 cells (Fig. 2). Thus, the 55- to 58-kDa glycoprotein was not a proteolytic cleavage product of the 110-kDa glycoprotein. Immunoblots with polyclonal anti-MHVR antibody showed that BHK-21 cells infected with Vac-MHVR expressed an MHVR-related protein with a mass of approximately 88 kDa, and a protein of this size and antigenic reactivity was occasionally detected in BALB/c BBM. This glycoprotein may result from incomplete processing or glycosylation of MHVR.

Large amounts of plasma membrane-associated MHVR were detected by indirect immunofluorescence of BHK-21 cells infected with Vac-MHVR by using rabbit polyclonal anti-MHVR (Fig. 3a). No MHVR antigen was observed on BHK-21 cells which were mock infected or infected with vSC11 alone (data not shown).

Superinfection of Vac-MHVR-infected BHK-21 cells with MHV-A59. To determine whether MHVR₁ expressed from Vac-MHVR could make BHK-21 cells susceptible to MHV-A59 infection, we first either infected cells with Vac-MHVR or vSC11 (MOI, 0.1 PFU per cell) or mock infected them and then superinfected cells with MHV-A59 (MOI, 10 PFU per cell) at 3 h after vaccinia virus inoculation. We chose to superinfect with MHV at 3 h after vaccinia virus inoculation for two reasons: first, this was the earliest time at which we could detect MHVR protein by immunoblotting, and second, at this time the shutoff of host protein synthesis by vaccinia virus was not complete. MHV-induced fusion was detected as early as 8 h after MHV inoculation. Only those BHK-21 cells that had previously been infected with Vac-MHVR formed multinucleate syncytia typical of MHV infection of permissive murine cell lines (Fig. 4d). Cell fusion was not observed with mock-infected cells (Fig. 4a), Vac-MHVR-infected cells alone (Fig. 4c), or cells infected with vSC11 and superinfected with MHV (Fig. 4b). Although some cell rounding due to vaccinia virus infection was observed (Fig. 4b through d), Vac-MHVR-infected cells remained attached to the substrate and continued to produce MHVR glycoprotein for up to 48 h. Figure 3b shows MHVR on the surface of an MHV-induced multinucleated cell infected with Vac-MHVR and superinfected with MHV-A59.

MHV-A59 antigens in the cytoplasm were detected by immunofluorescence only in cultures infected with Vac-MHVR and superinfected with MHV-A59 (Fig. 5a and c). When BHK-21 cells were inoculated with Vac-MHVR at MOIs ranging from 0.1 to 10 PFU per cell and then superinfected with MHV, cell fusion was much more extensive in cultures infected with lower amounts of vaccinia virus than in those with more recombinant vaccinia virus (data not shown). This suggests that the higher amounts of vaccinia virus at least partially inhibited coronavirus protein synthesis as well as cellular protein synthesis. Interestingly, it appears that MHV at least partially escaped the vaccinia virus-induced inhibition of host protein synthesis, since the production of the MHV nucleocapsid N protein in Vac-MHVR-infected cells at 17 h postinfection with MHV-A59 was comparable to that seen with MHV-infected murine cells (data not shown).

We previously showed that pretreatment of MHV-susceptible murine fibroblasts with antireceptor MAb CC1 blocks infection with MHV-A59 (32). To show the viral specificity of the receptor expressed by Vac-MHVR, we incubated Vac-MHVR-infected BHK-21 cells with antireceptor MAb CC1 for 2 h prior to challenge with MHV-A59. No cell fusion was observed with cultures treated with MAb CC1 (data not shown), and no MHV-specific antigens were detected in the MAb CC1-treated, Vac-MHVR-infected cells at 8 or 24 h after MHV inoculation (Fig. 5b and d, respectively). A monoclonal antibody of the same isotype as MAb CC1, directed against an unrelated antigen, failed to protect Vac-MHVR-infected cells from infection with MHV-A59 (data not shown). Thus, MAb CC1 selectively blocked MHV infection of Vac-MHVR-infected BHK cells, even though the MHVR was vastly overexpressed relative to L2p cells. This indicates that the affinity of MAb CC1 for the receptor

FIG. 3. Expression of MHVR in BHK-21 cells infected with Vac-MHVR. (a) MHVR expression in BHK-21 cells 15 h after inoculation with Vac-MHVR (MOI, 0.1 PFU per cell); (b) MHVR expression of Vac-MHVR-infected BHK-21 cells after inoculation with MHV-A59 (MOI, 10 PFU per cell). MHVR was detected both on single Vac-MHVR-infected cells and on MHV-induced syncytia (panel b, upper left corner).

is at least as great as or greater than the affinity of the MHV spike glycoprotein for MHVR.

Virus specificity of MHVR. Most coronaviruses infect only one species or several closely related species. For murine coronavirus MHV-A59, this block in virus replication appears to be at the level of virus-receptor interaction (6). To determine whether coronaviruses of other species could use the same receptor glycoprotein as MHV-A59, we used RCV which is antigenically related to MHV-A59 and a human coronavirus (HCV-229E) which is serologically unrelated to MHV to challenge BHK-21 cells infected with Vac-MHVR for 3 h and examined the development of coronavirus antigens by immunofluorescence. The L2p line of murine fibroblasts which naturally expresses MHVR is permissive for both MHV-A59 and RCV, as shown by the development of viral antigens in the cytoplasm and cell fusion for MHV-A59 (Fig. 6a) and the development of viral antigen and the rounding of cells infected with RCV (Fig. 6b) (19). However, only MHV-A59 was able to infect Vac-MHVR-infected BHK-21 cells (Fig. 6c), and no development of RCV-induced antigens or cytopathology was detected (Fig. 6d). These data suggest that RCV cannot utilize MHVR as a receptor to initiate infection, even when this receptor is expressed at very high levels. Similarly, HCV-229E did not infect BHK-21 cells infected with Vac-MHVR (data not shown).

Effects of posttranslational processing of MHVR on virus and anti-MHVR antibody binding. To examine the effects of glycosylation upon the virus- and antibody-binding activities of MHVR, we used three complementary approaches to obtain MHVR proteins with different levels of glycosylation. The first approach was to translate the MHVR₁ clone in a cell-free rabbit reticulocyte lysate in the presence or absence of canine microsomal membranes. Our second approach was to use the glycosylation inhibitors tunicamycin and monensin in BHK-21 cells infected with Vac-MHVR. The resulting nonglycosylated and partially glycosylated forms of MHVR were then analyzed for their abilities to bind rabbit polyclonal anti-MHVR and MAb CC1 in immunoblots and MHV-A59 virions in a VOPBA (4). The third approach was to study MHVR protein expressed from a vaccinia virus construct which contains a short N-terminal deletion in MHVR (clone 10.4). To allow proper translocation of this protein, we engineered a VSV signal sequence onto the N terminus of this deletion clone (Vac-VSV-10_{ATG}).

In vitro-transcribed MHVR₁ mRNA was translated in a rabbit reticulocyte cell-free lysate in the presence and absence of canine microsomal membranes. The [³⁵S]methionine-labeled proteins were immunoprecipitated with rabbit polyclonal anti-MHVR or MAb CC1 or, as controls, preimmunization rabbit serum or an unrelated monoclonal anti-

FIG. 4. BHK-21 cells infected with recombinant vaccinia virus and/or MHV-A59. BHK-21 cells were infected at an MOI of 0.1 PFU per cell with Vac-MHVR (c and d) or vSC11 (b) or were mock infected (a). At 3 h postinfection, the cells were superinfected with MHV-A59 at an MOI of 10 (a, b, and d). At 8 h after inoculation with MHV-A59, the cells were fixed in 2% paraformaldehyde and photographed. Although all cultures infected with vaccinia virus exhibited some cell rounding (b through d), only BHK-21 cells expressing MHVR from Vac-MHVR and challenged with MHV-A59 fused to form multinucleate syncytia (arrow in panel d).

body (Fig. 7). Translation in the absence of membranes (Fig. 7, lanes 5 to 8) yielded a 53-kDa core glycoprotein which was recognized by polyclonal antibody but not by MAb CC1. Inclusion of microsomal membranes in the *in vitro* translation reaction mixture (Fig. 7, lanes 1 to 4) resulted in the generation of a 75-kDa glycoprotein which was immunoprecipitated by polyclonal anti-MHVR antiserum and by MAb CC1 but not by the control antisera. Translation reaction mixtures (Fig. 7, lanes 9 to 12) without MHVR₁ mRNA failed to generate labeled products which were recognized by any of the antisera. Although polyclonal anti-MHVR antiserum precipitated both glycosylated and nonglycosylated species from translation reaction mixtures containing microsomal membranes (Fig. 7, lane 1), MAb CC1 only precipitated the higher-molecular-weight glycosylated form (Fig. 7, lane 2). The p10_{ATG} clone which contained a functional ATG initiation codon at amino acid 11 of the mature MHVR but lacked a signal sequence was also used to generate mRNA in an *in vitro* transcription system. Translation of this mRNA resulted in the production of a 50-kDa core protein which was immunoprecipitated by polyclonal anti-MHVR antiserum but not by MAb CC1 (data not shown). These experiments suggest that a posttranslational processing step requiring membranes, such as the addition of high-mannose oligosaccharides, was required for recognition of the MHVR protein by MAb CC1.

Tunicamycin or monensin was added to Vac-MHVR-infected BHK-21 cells 4 h postinoculation, and the infection

was allowed to proceed for an additional 20 h. When either inhibitor was added at the time of Vac-MHVR infection, expression of MHVR was completely blocked. The deleterious effects of the inhibitors were minimized by allowing Vac-MHVR to grow for 4 h prior to adding the tunicamycin or monensin. Additionally, both inhibitors reduced total cellular protein synthesis; therefore, 20 h was needed to detect the nonglycosylated MHVR species in immunoblot assays. These cultures also contained some MHVR which had been synthesized in the 4 h before the drugs were added and had undergone the normal posttranslational processing steps. MHVR translated after the addition of tunicamycin did not have N-linked oligosaccharides added. MHVR translated after the addition of monensin would have core N-linked oligosaccharides added, but the arrest of glycoprotein transport at the *cis*-Golgi would result in a failure to modify the N-linked oligosaccharides by glycosyl transferases in the medial or *trans*-Golgi or to add any O-linked glycans to the MHVR. These different glycosylated forms of MHVR were immunoblotted with polyclonal anti-MHVR or MAb CC1 or were analyzed by VOPBA (Fig. 8A, B, and C, respectively). Fully processed MHVR from BALB/c intestinal BBM (120 kDa) (Fig. 8, lanes 2) and affinity-purified MHVR from BALB/c liver membranes (110 kDa) (Fig. 8, lanes 1) were detected by polyclonal anti-MHVR antiserum, MAb CC1, and MHV-A59. The 55- and 58-kDa proteins in membrane preparations from mouse livers and intestines (Fig. 8, lanes 1 and 2, respectively) were also well recog-

FIG. 5. Effects of MAb CC1 on MHV-A59 superinfection of BHK-21 cells expressing MHVR from Vac-MHVR. One hour after the infection of BHK-21 cells with Vac-MHVR (MOI, 0.1 PFU per cell), cells were incubated with antireceptor MAb CC1 (b and d) or without any antiserum (a and c). Two hours after the addition of antiserum, cells were inoculated with MHV-A59 and then incubated for 8 h (a and b) or 24 h (c and d). Pretreatment of cells expressing MHVR from Vac-MHVR with MAb CC1 prevented MHV infection of cells.

nized by polyclonal anti-MHVR and MAb CC1, but they bound MHV-A59 virus much less than did the 110-kDa MHVR. The fully glycosylated MHVR from cells infected with Vac-MHVR without inhibitors of glycosylation (Fig. 8, lanes 4) and the MHVR synthesized prior to the addition of the inhibitors were also recognized by polyclonal anti-MHVR, MAb CC1, and MHV-A59 (Fig. 8, lanes 3 and 5). However, the nonglycosylated 50-kDa MHVR made in the presence of tunicamycin (Fig. 8, lanes 5) was recognized only by polyclonal anti-MHVR and not by MAb CC1 or MHV-A59. The additional bands in Fig. 7, lanes 5, were unrelated to MHVR expression because they were also seen in immunoblots from vSC11 (empty vaccinia virus)-infected BHK cells (data not shown). Longer exposure of these autoradiograms failed to show any MAb CC1 or MHV-A59 binding to the 50-kDa core MHVR protein. The size of the core protein correlated well with the molecular weight of the protein predicted from the open reading frame of the MHVR₁ nucleotide sequence (7) and with the *in vitro* translation product translated in the absence of microsomal membranes (Fig. 7, lane 5). Monensin treatment of Vac-MHVR-infected cells resulted in the production of a broad glycoprotein band with a mass of approximately 78 kDa (Fig. 8, lanes 3) which was recognized by polyclonal anti-MHVR,

MAb CC1, and MHV-A59. The Vac-VSV-10_{ATG} recombinant expressed a protein slightly smaller (48 kDa) than the core MHVR protein seen in the presence of tunicamycin (Fig. 8, lanes 6). The smaller molecular mass probably resulted from the deletion of nine amino acids from the N terminus of this clone. The Vac-VSV-10_{ATG} protein appeared as a single band and was recognized only by polyclonal anti-MHVR and not by MAb CC1 or MHV-A59. Possibly, the VSV signal sequence added to this construct was unable to initiate translocation into membranes resulting in a nonglycosylated product. Thus, nonglycosylated core forms of MHVR were not recognized by MAb CC1 or MHV-A59, and posttranslational processing, such as the addition of mannose-rich core oligosaccharides, and transport to the *cis*-Golgi were required to achieve the conformation and composition of MHVR which were recognized by MAb CC1 and MHV-A59.

BHK-21 cells infected with Vac-sMHVR produced a secreted MHVR protein that was recognized by polyclonal anti-MHVR, MAb CC1, and MHV-A59 (Fig. 8, lanes 7). sMHVR samples represent 50 μ l of culture medium from infected cells; the amount of MHVR expressed was comparable to that of MHVR obtained from 1.6×10^5 cells infected with Vac-MHVR (compare lanes 4 versus 7 in Fig. 8). Thus,

FIG. 6. Virus specificity of MHVR. L2p cells were infected with MHV-A59 (a) or Parker RCV (b) for 15 h. BHK-21 cells were inoculated with Vac-MHVR at an MOI of 0.1 PFU per cell (c and d) and 3 h later were superinfected with MHV-A59 (c) or RCV (d) for an additional 21 h. Panels a and c were stained with polyclonal rabbit anti-MHV-A59; panels b and d were stained with rabbit anti-RCV. BHK-21 cells expressing MHVR are not susceptible to infection with RCV.

Vac-sMHVR expressed a high level of sMHVR. This confirms the prediction from the nucleotide sequence that MHVR is a class I glycoprotein, anchored by a single membrane-spanning domain near its carboxyl terminus. The data also suggest that the carboxy terminus of MHVR is not needed for the posttranslational processing of MHVR required for MAb CC1 or MHV-A59 binding.

DISCUSSION

We have shown that a recombinant vaccinia virus, Vac-MHVR, containing the cDNA encoding MHVR can direct high-level expression of a functional receptor for MHV-A59 which binds MHV-A59 and the antireceptor monoclonal antibody MAb CC1. MHV-A59 superinfection of BHK-21 cells infected with Vac-MHVR resulted in MHV infection, the development of MHV antigens, and the formation of MHV-induced multinucleated syncytia. The high levels of

MHVR expression achieved with this recombinant allowed us to elucidate some of the biochemical properties of MHVR as well as the biological properties of virus-receptor interactions which we were unable to determine with the stably transfected BHK_{MHVR1} cell lines (7).

This report shows that posttranslational processing of MHVR is required for MHV-A59 and MAb CC1 binding. Nonglycosylated core MHVR protein generated from either tunicamycin-treated, Vac-MHVR-infected BHK-21 cells or from the translation of MHVR₁ mRNA in reticulocyte lysates failed to react with MAb CC1 or MHV-A59. However, MHVR protein made in the presence of monensin containing high-mannose oligosaccharides bound MHV and MAb CC1. Several alternative explanations could account for the requirement of posttranslational processing of MHVR for virus or antibody binding. The simplest hypothesis is that N-linked core oligosaccharides are required to generate the proper tertiary structure of MHVR which is recognized by

FIG. 7. In vitro translation of MHVR mRNA. A rabbit reticulocyte cell-free lysate containing [^{35} S]methionine was incubated with MHVR mRNA in the presence of canine pancreatic microsomal membranes (lanes 1 to 4), with MHVR mRNA without microsomes (lanes 5 to 8), with microsomal membranes alone (lanes 9 and 10), or without microsomes or MHVR mRNA (lanes 11 and 12). Reaction mixtures were then incubated with either polyclonal rabbit anti-MHVR antiserum (lanes 1, 5, 9, and 11), MAb CC1 (lanes 2, 6, 10, and 12), preimmune rabbit antiserum (lanes 3 and 7), or an unrelated immunoglobulin G1 monoclonal antibody (lanes 4 and 8). After 2 h of incubation with the indicated antiserum, immune complexes were collected with Staph A-Sepharose beads. The nonglycosylated MHVR precursor band appeared as a doublet, possibly because of premature chain termination. MAb CC1 was able to immunoprecipitate only glycosylated forms of MHVR (compare lane 2 with lane 6). Molecular masses (in kilodaltons) are on the left.

virus or antiviral antibodies. Alternatively, MHV may interact with a complex binding site, such as one containing both carbohydrate and peptide moieties. A third possibility is that MHV recognizes a unique sugar moiety on MHVR. Perhaps such a unique processing event would occur only in certain strains of mice, and others would therefore be resistant. We have shown that an N-terminal peptide antiserum directed against MHVR (anti-NTR) recognizes a glycoprotein with a similar molecular mass in livers from MHV-A59-resistant SJL mice and MHV-A59-susceptible BALB/c mice (31). Vac-MHVR infection of cells from different strains of mice may reveal whether the observed differences in the binding of virus to MHVR and its homologs made in different mouse strains depend upon different posttranslational processing of the same core protein in different mouse strains. However, the posttranslational processing step required for MHV and MAb CC1 recognition of MHVR is not necessarily a glycosylation step. Translocation of MHVR across a membrane or

the addition of a noncarbohydrate moiety such as a lipid might be the step required to achieve the proper conformation of MHVR for virus binding.

Three well-characterized virus receptors are PVR, the poliovirus receptor, CD4, the receptor for human immunodeficiency virus, and intercellular adhesion molecule-1 (ICAM-1), the major-group rhinovirus receptor. The role of glycosylation in the function of glycoprotein receptors for these viruses has not been resolved. Antibody directed against β -galactosidase-poliovirus receptor fusion proteins failed to block poliovirus infection of HeLa cells (33). Zibert et al. suggested that the fusion proteins either lacked the proper conformation or needed additional modifications in order to induce an antibody that blocked virus binding. Thus, poliovirus binding to its receptor depends on proper conformation and/or modification of the receptor. In contrast, Fenouillet et al. (8) found that deglycosylation of soluble ^{125} I-labeled CD4 (sCD4) with endoglycosidase F or

FIG. 8. Effects of glycosylation inhibitors on the binding of anti-MHVR antibodies or MHV-A59 virions to MHVR. Vac-MHVR-infected BHK-21 cells were incubated with 1 μ g of tunicamycin per ml (lanes 5) or 10^{-5} M monensin (lanes 3) or without any inhibitor (lanes 4), as described in Materials and Methods. Positive controls were affinity-purified receptor from the livers of Swiss Webster mice (lanes 1) (28) and intestinal BBM preparations from BALB/c mice (lanes 2). Lanes 6 represent lysates from BHK-21 cells infected with Vac-VSV-10_{ATG}. Lanes 7 represent (undiluted) culture medium from Vac-sMHVR-infected BHK-21 cells. All samples were run on 8% acrylamide gels, blotted to nitrocellulose, and incubated with either polyclonal anti-MHVR (655), MAb CC1, or MHV-A59 followed by anti-S antiserum. Bound antibodies were detected by the incubation of immunoblots with ^{125} I-Staph A. MAb CC1 and MHV-A59 do not recognize core, nonglycosylated forms of MHVR.

N-glycosidase did not prevent it from binding to HIV gp120. Lineberger and coworkers (15) showed that a membrane-associated event, possibly glycosylation, was required for the specific recognition of ICAM-1 by anti-ICAM-1 monoclonal antibodies or rhinovirus, even though both virus- and monoclonal antibody-binding sites have been mapped to the nonglycosylated first loop of ICAM-1.

The Vac-VSV-10_{ATG} recombinant generated a 50-kDa core protein which was not properly glycosylated and failed to bind virus. Challenge of Vac-VSV-10_{ATG}-infected BHK-21 cells with MHV-A59 did not result in MHV-A59 infection (data not shown). Thus, the VSV signal sequence could not substitute for the highly conserved 34-amino-acid signal peptide found in all members of the CEA family. Studies with rotavirus glycoprotein VP7 show that the VP7 signal peptide (H2) is necessary for the direct targeting of this protein to the endoplasmic reticulum (24). The substitution of the influenza HA signal sequence for the H2 signal peptide resulted in the secretion of VP7 (24). Similarly, it is possible that the cleaved 34-amino-acid signal peptide of MHVR interacts with nascent MHVR and stabilizes it. The absence of this signal peptide could result in aberrant folding and/or improper transport of MHVR.

The requirement for posttranslational processing of MHVR for MAb CC1 or virus binding explains why screening lambda gt11 libraries of BALB/c mouse liver cDNA with these reagents did not yield a cDNA clone of MHVR. Recombinant MHVR expressed in bacteria would not be posttranslationally processed in the same way as it is in eukaryotic cells.

Because secreted MHVR bound to MHV-A59 virus and was recognized by antireceptor MAb CC1, it is apparent that the native configurations of the virus- and antibody-binding domains are conserved in sMHVR. Therefore, we will be able to use the vaccinia virus system to express deletions of MHVR and to determine which domains of the MHV S glycoprotein are required for virus binding. The vaccinia virus expression system will yield sufficient amounts of

secreted MHVR protein for crystallization and the analysis of structure by X-ray crystallography.

The CEA family of glycoproteins is highly conserved among mammals. Multiple isoforms of CEA-related glycoproteins are formed by complex alternative splicing in different tissues (1), and the level of expression of murine CEA-related glycoproteins is developmentally regulated in various tissues (9). The highest levels of human CEA are expressed in gastrointestinal tumors and in the fetal digestive tract (21). Immunoblots of BALB/c liver or intestinal membrane proteins with polyclonal anti-MHVR or anti-NTR or those with MHV-A59 detected two glycoproteins, one with a molecular mass of 110 to 120 kDa and the other with a molecular mass of 56 to 58 kDa. We previously postulated that this smaller glycoprotein could either result from proteolytic cleavage of the 110- to 120-kDa MHVR glycoprotein or be generated by alternative splicing (4). Since cells infected with Vac-MHVR did not produce the 56- to 58-kDa species (Fig. 8), it is clear that this glycoprotein was not generated by proteolysis of the 110-kDa MHVR. Probably, the 56- to 58-kDa proteins result from alternative splicing of the primary mRNA transcript that encodes MHVR₁ (1). It is important to determine whether these small MHVR-related proteins can also function as receptors for MHV-A59. This can be accomplished only by cloning and expressing each of the murine CEA-related glycoproteins in BHK-21 cells and then challenging them with infectious virus.

The normal cellular functions of the CEA-related proteins of humans and rodents are being studied in many laboratories. Several CEA-related proteins, including cell CAM 105, a rat liver homolog of MHVR, and a mouse isoform of MHVR, suggest that they mediate intercellular adhesion (3, 14, 27, 29), and one report also suggests that a rat homolog of MHVR has ecto-ATPase activity (13). The highest levels of mouse CEA-related glycoproteins, however, are found not at intercellular junctions but on the apical membranes of epithelial cells in the colon, small intestine, and kidney tubule and in the bile canaliculi of hepatocytes (9). Although

the cellular functions of these molecules on epithelial surfaces are not known, the molecules in intestinal epithelial cells are in an ideal location to serve as receptors for intestinal bacteria (12) or for viruses such as MHV, which can be spread by oral-fecal contamination. Recently, it was shown that pathogenic strains of *Escherichia coli* isolated from humans can bind to CEA from human colons and to the antigenically related nonspecific cross-reacting antigen isolated from human granulocytes (12, 20). Because the binding of *E. coli* to deglycosylated CEA was significantly reduced (12), Leusch et al. suggested that mannosyl residues of CEA and nonspecific cross-reacting antigen may be involved in the recognition of the bacteria by human colonic epithelial cells and human granulocytes. The vaccinia virus expression system for cloned CEA-related proteins provides a high level of expression in cultured cells, comparable to that observed in apical BBM of the intestine. This should greatly facilitate studies of the cellular functions of the CEA-related glycoproteins. The broad host range of vaccinia virus also offers another special advantage to this expression system in that it can express MHVR or related glycoproteins in a wide variety of cell lines and tissues. This will permit the evaluation of differential host cell processing of MHVR in different cell types, providing additional information about the specific processing steps required for virus or MAb CCl binding.

ACKNOWLEDGMENTS

We are grateful to Hanh Nguyen and Marc Wolfgang for their excellent technical assistance and Richard K. Williams and John Hay for fruitful discussions and helpful suggestions with the manuscript.

This research was supported by NIH grants AI25231 and AI25376.

REFERENCES

- Barnett, T. R., A. Kretschmer, D. A. Austen, S. J. Goebel, J. T. Hart, J. J. Elting, and M. E. Kamarck. 1989. Carcinoembryonic antigens: alternative splicing accounts for the multiple mRNAs that code for novel members of the carcinoembryonic antigen family. *J. Cell Biol.* **108**:267-276.
- Barthold, S. W., and A. L. Smith. 1989. Virus strain specificity of challenge immunity to coronavirus. *Arch. Virol.* **104**:187-196.
- Beauchemin, N., C. Turbide, J. Q. Huang, S. Benchimol, S. Jothy, K. Shiota, A. Fuks, and C. P. Stanners. 1989. Studies on the function of carcinoembryonic antigen, p. 49-64. In A. Yachi and J. E. Shively (ed.), *The carcinoembryonic antigen gene family*. Elsevier Science Publishers BV (Biomedical Division), New York.
- Boyle, J. F., D. G. Weismiller, and K. V. Holmes. 1987. Genetic resistance to mouse hepatitis virus correlates with absence of virus-binding activity on target tissues. *J. Virol.* **61**:185-189.
- Chakrabarti, S., K. Brechling, and B. Moss. 1985. Vaccinia virus expression vector: coexpression of β -galactosidase provides visual screening of recombinant virus plaques. *Mol. Cell. Biol.* **5**:3403-3409.
- Compton, S. R. 1988. Coronavirus attachment and replication. Ph.D. Dissertation. The Uniformed Services University of the Health Sciences, Bethesda, Md.
- Dveksler, G. S., M. N. Pensiero, C. B. Cardellicchio, R. K. Williams, G.-S. Jiang, K. V. Holmes, and C. W. Dieffenbach. 1991. Cloning of the mouse hepatitis virus (MHV) receptor: expression in human and hamster cell lines confers susceptibility to MHV. *J. Virol.* **65**:6881-6891.
- Fenouillet, E., B. Clerget-Raslain, J. C. Gluckman, D. Guétard, L. Montagnier, and E. Bahraoui. 1989. Role of N-linked glycans in the interaction between the envelope glycoprotein of human immunodeficiency virus and its CD4 cellular receptor. Structural enzymatic analysis. *J. Exp. Med.* **169**:807-822.
- Huang, J. Q., C. Turbide, E. Daniels, S. Jothy, and N. Beauchemin. 1990. Spatiotemporal expression of murine carcinoembryonic antigen (CEA) gene family members during mouse embryogenesis. *Development* **110**:573-588.
- Knobler, R. L., L. A. Tunison, and M. B. Oldstone. 1984. Host genetic control of mouse hepatitis virus type 4 (JHM strain) replication. I. Restriction of virus amplification and spread in macrophages from resistant mice. *J. Gen. Virol.* **65**:1543-1548.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* **227**:680-685.
- Leusch, H. G., S. A. Hefta, Z. Drzeniek, K. Hummel, Z. Markos-Pusztai, and C. Wagener. 1990. *Escherichia coli* of human origin binds to carcinoembryonic antigen (CEA) and non-specific crossreacting antigen (NCA). *FEBS Lett.* **261**:405-409.
- Lin, S. H. 1989. Localization of the ecto-ATPase (ecto-nucleotidase) in the rat hepatocyte plasma membrane. Implications for the functions of the ecto-ATPase. *J. Biol. Chem.* **264**:14403-14407.
- Lin, S. H., and G. Guidotti. 1989. Cloning and expression of a cDNA coding for a rat liver plasma membrane ecto-ATPase. The primary structure of the ecto-ATPase is similar to that of the human biliary glycoprotein I. *J. Biol. Chem.* **264**:14408-14414.
- Lineberger, D. W., D. J. Graham, J. E. Tomassini, and R. J. Colonno. 1990. Antibodies that block rhinovirus attachment map to domain 1 of the major group receptor. *J. Virol.* **64**:2582-2587.
- Mackett, M., and G. L. Smith. 1986. Vaccinia virus expression vectors. *J. Gen. Virol.* **67**:2067-2082.
- Mackett, M., T. Yilma, J. K. Rose, and B. Moss. 1985. Vaccinia virus recombinants: expression of VSV genes and protective immunization of mice and cattle. *Science* **227**:433-435.
- Pensiero, M. N., G. B. Jennings, C. S. Schmaljohn, and J. Hay. 1988. Expression of the Hantaan virus M genome segment by using a vaccinia virus recombinant. *J. Virol.* **62**:696-702.
- Percy, D., S. Bond, and J. MacInnes. 1989. Replication of sialodacryoadenitis virus in mouse L-2 cells. *Arch. Virol.* **104**:323-333.
- Sauter, S. L., S. M. Rutherford, C. Wagener, J. E. Shively, and S. A. Hefta. 1991. Binding of nonspecific cross-reacting antigen, a granulocyte membrane, to *Escherichia coli* expressing type 1 fimbriae. *Infect. Immun.* **59**:2485-2493.
- Shively, J. E., and J. Beatty. 1985. CEA-related antigens: molecular biology and clinical significance. *Crit. Rev. Oncol. Hematol.* **2**:355-399.
- Smith, A. L., C. B. Cardellicchio, D. F. Winograd, M. S. deSouza, S. W. Barthold, and K. V. Holmes. 1991. Monoclonal antibody to the receptor for murine coronavirus MHV-A59 inhibits virus replication in vivo. *J. Infect. Dis.* **163**:879-882.
- Smith, M. S., R. E. Click, and P. G. Plagemann. 1984. Control of mouse hepatitis virus replication in macrophages by a recessive gene on chromosome 7. *J. Immunol.* **133**:428-432.
- Stirzaker, S. C., and G. W. Both. 1989. The signal peptide of the rotavirus glycoprotein VP7 is essential for its retention in the ER as an integral membrane protein. *Cell* **56**:741-747.
- Stohlman, S. A., and J. A. Frelinger. 1978. Resistance to fatal central nervous system disease by mouse hepatitis virus strain JHM. I. Genetic analysis. *Immunogenetics* **6**:277-281.
- Sturman, L. S., and K. V. Holmes. 1977. Characterization of coronavirus. II. Glycoproteins of the viral envelope: tryptic peptide analysis. *Virology* **77**:650-660.
- Svalander, P. C., P. Odin, B. O. Nilsson, and B. Obrink. 1987. Trophoblast surface expression of the cell adhesion molecule cell-CAM 105 on rat blastocysts. *Development* **100**:653-660.
- Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA* **76**:4350-4354.
- Turbide, C., M. Rojas, C. P. Stanners, and N. Beauchemin.

1991. A mouse carcinoembryonic antigen gene family member is a calcium-dependent cell adhesion molecule. *J. Biol. Chem.* **266**:309–315.
30. **Wege, H., S. Siddell, and V. ter Meulen.** 1982. The biology and pathogenesis of coronaviruses. *Curr. Top. Microbiol. Immunol.* **99**:165–200.
31. **Williams, R. K., G.-S. Jiang, and K. V. Holmes.** 1991. Receptor for mouse hepatitis virus is a member of the carcinoembryonic antigen family of glycoproteins. *Proc. Natl. Acad. Sci. USA* **88**:5533–5536.
32. **Williams, R. K., G.-S. Jiang, S. W. Snyder, M. F. Frana, and K. V. Holmes.** 1990. Purification of the 110-kilodalton glycoprotein receptor for mouse hepatitis virus (MHV)-A59 from mouse liver and identification of a nonfunctional, homologous protein in MHV-resistant SJL/J mice. *J. Virol.* **64**:3817–3823.
33. **Zibert, A., H. C. Selinka, O. Elroy-Stein, B. Moss, and E. Wimmer.** 1991. Vaccinia virus-mediated expression and identification of the human poliovirus receptor. *Virology* **182**:250–259.