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Human Carcinoembryonic Antigen and Biliary Glycoprotein Can Serve as Mouse Hepatitis Virus Receptors

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Receptors for murine coronavirus mouse hepatitis virus (MHV) are members of the murine carcinoembryonic antigen (CEA) gene family. Since MHV can also infect primates and cause central nervous system lesions (G. F. Cabirac et al., Microb. Pathog. 16:349–357, 1994; R. S. Murray et al., Virology 188:274–284, 1992), we examined whether human CEA-related molecules can be used by MHV as potential receptors. Transfection of plasmids expressing human carcinoembryonic antigen (hCEA) and human biliary glycoprotein into COS-7 cells, which lack a functional MHV receptor, conferred susceptibility to two MHV strains, A59 and MHV-2. Domain exchange experiments between human and murine CEA-related molecules identified the immunoglobulin-like loop I of hCEA as the region conferring the virus-binding specificity. This finding expands the potential MHV receptors to primate species.

The host range of a virus is often determined by the patterns of expression of viral receptors. Although the expression of a viral receptor may not always be sufficient to confer susceptibility to a given virus, the lack of its expression would certainly restrict infection. The receptors for many viruses have been identified. While most viruses utilize a single type of receptor, increasing evidence suggests that some viruses can use more than one receptor. For example, human immunodeficiency virus may utilize either CD4 or variable-region heavy-chain 3 immunoglobulin (Ig) molecules (VH3) as receptors in different cell types (3). Murine coronavirus mouse hepatitis virus (MHV) also has been shown to utilize multiple receptors, including several different members of the murine carcinoembryonic antigen (CEA) gene family (5, 7, 8, 17, 25, 26). Thus, the requirement for virus-receptor interaction may be flexible. The ability of a virus to utilize several different molecules, which are differentially expressed in various tissues, may allow the virus to expand its tissue and host range.

MHV has long served as a murine model for human demyelinating diseases such as multiple sclerosis (23). The natural hosts for MHV are mice, which can develop hepatitis, enteritis, encephalitis, and/or demyelination upon MHV infection (18). Experimental intracerebral inoculation of MHV into rats also causes systemic infection and central nervous system lesions (22). Recent studies have shown that intracerebral or peripheral inoculation of MHV into primates, such as owl or African green monkeys, also led to infection, causing central nervous system demyelination similar to that seen in mice (4, 16). How MHV causes cross-species infection is not known. Conceivably, MHV may be able to use a receptor molecule of primate origin.

Several naturally occurring CEA molecules of murine origin have been shown to function as MHV receptors. These include $MHVR_1$, $MHVR_2$, and bgp2 (7, 8, 17, 25), which are members of the biliary glycoprotein (BGP) subgroup of the murine CEA gene family. Recently, a novel member of the pregnancy-specific glycoprotein (PSG) subgroup of the murine CEA family, which is expressed predominantly in the brain and is named bCEA, has also been shown to serve as an MHV receptor (5). All of these receptors contain several Ig-like loop domains (Fig. 1); the N-terminal loop (referred to as loop I here) resembles the Ig variable loop and has been shown to be the virus-binding site of an MHV receptor (9). Interestingly, despite their sequence variability, CEA-like molecules from different animal species, including humans, contain a consensus motif in loop I (Fig. 2). Although the precise amino acid residues required for virus binding have not been defined, the significant sequence homology between human and murine CEA-like molecules in the virus-binding domain suggests the interesting possibility that human CEA-related molecules may serve as MHV receptors. If this is found to be the case, it will provide a potential mechanism for cross-species infection by MHV. In this article, we report such a possibility.

The human CEA gene family consists of three subgroups, the PSGs, the BGPs, and the CEAs (20). Only the PSG and the BGP subgroups have murine homologs (19), both of which have been shown to serve as MHV receptors (5, 7, 17, 25). In this study, we tested human CEA and BGP (hCEA and hBGP) for their ability to serve as MHV receptors. hCEA clone F (2), containing nucleotides (nt) 1 through 2485 of the hCEA cDNA (the coding region is nt 115 through 2223), was cloned into the PECE vector (10) at the *Sal*I site in the multiple cloning site, placing the expression of hCEA under the control of the simian virus 40 T-antigen promoter. Also, nt 1 through 2197 of hBGP I cDNA (13) (the coding region is nt 82 through 1662) were cloned into the expression vector pHbAPr-1-neo under the control of the human beta-actin promoter (12). These two plasmids were transfected separately into COS-7 cells (11), which lack a functional MHV receptor but can otherwise support MHV infection once provided with a functional MHV receptor (1, 24). The transfected COS-7 cells were then infected with various MHV strains at 42 h posttransfection, and the supernatant was collected at 24 h postinfection (48 h postinfection for MHV-2 and MHV-3) and assayed for virus

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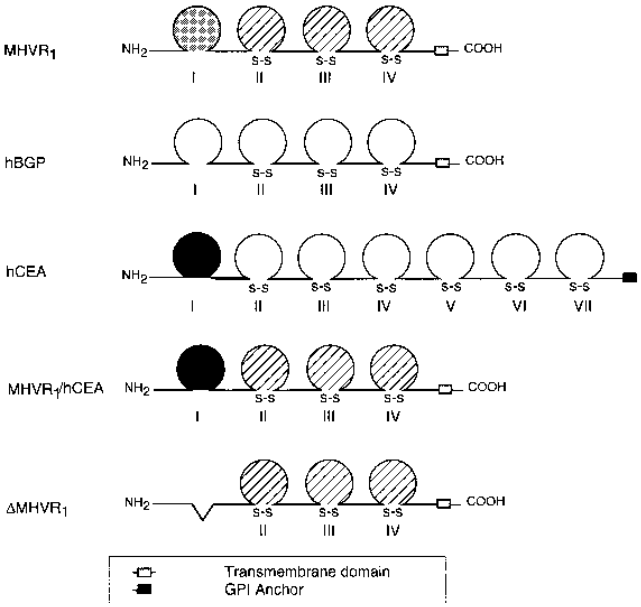

FIG. 1. Schematic diagram of the structure of CEA molecules used in this study. Ig-like loops are shown. The V-shaped line in $\Delta MHVR_1$ represents the deletion of loop I. GPI, glycosyl phosphatidylinositol.

titer (Table 1). The results showed that COS-7 cells transfected with the control vector were completely resistant to infection by the A59 strain of MHV. However, after the cells were transfected with either hCEA or hBGP, they became susceptible to A59 infection, yielding a virus titer of approximately 2×10^3 PFU/ml. Although the virus yield was slightly lower (by approximately 1 log) than that produced from cells transfected with $MHVR_1$, which is the prototype MHV receptor (8), this result indicated that both hCEA and hBGP can serve as receptors for the A59 strain of MHV. The virus infection of the transfected cells was confirmed by immunofluorescence staining of the transfected COS-7 cells 24 h after the transfected cells were infected with MHV A59 by using a monoclonal antibody against MHV N protein (Fig. 3). The results showed that, in hBGP- and hCEA-transfected cells, there was cytoplasmic staining of viral proteins (Fig. 3B and C). Some of the virus-infected cells showed syncytium formation. In contrast, in the cells transfected with the vector plasmid, no such staining was observed (Fig. 3A).

We next examined whether other MHV strains also can utilize hBGP and hCEA as receptors. The results showed that MHV-2 infection of COS-7 cells which had been transfected with hBGP or hCEA produced a virus titer approximately 1.5 log higher than that from cells transfected with the vector plasmid (Table 1). The virus yields from the hBGP- and

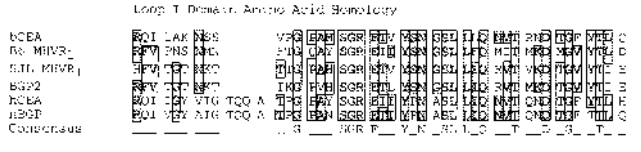

FIG. 2. Comparison of the deduced amino acid sequences of the loop I domains of the known MHV receptors with those of two hCEA family members. Boxed residues represent amino acids conserved in at least three of the molecules. The bottom line shows the consensus residues that are conserved among all of the molecules. The sequences were derived from bCEA (5), B6 $MHVR_1$ and SJL $MHVR_1$ (24), BALB/c bgp2 (16), hCEA (2), and hBGP (13).

TABLE 1. Virus titers from COS-7 cells after transfection with various cDNAs[a]

DNA transfected	Titer (PFU/ml) of virus strain:			
	A59	MHV-2	MHV-3	JHM
Control (PECE vector alone)	nd[b]	1.8×10^4	nd	nd
$MHVR_1$	3.3×10^4	6.1×10^5	6.3 ± 10^2	3.6×10^2
hCEA	2.0×10^3	6.2×10^5	nd	nd
hBGP	2.6×10^3	4.8×10^5	nd	nd
$MHVR_1$/hCEA	3.6×10^3	5.0×10^5	nd	nd
$\Delta MHVR_1$	nd	1.2×10^4	nd	nd

[a] COS-7 cells were transfected with plasmid DNA by using DOTAP or Lipofectin in accordance with the published method (26) and infected with various viruses (multiplicity of infection, 5 to 50) at 48 h postinfection. Media were harvested 24 or 48 h postinfection, and 0.5 ml of media was used for the plaque assay on DBT cells (14).

[b] nd, not detectable.

hCEA-transfected cells were similar to that produced from the cells transfected with the prototype MHV receptor, $MHVR_1$. As previously observed (5), MHV-2 yielded a high background level of virus in COS-7 cells transfected with the vector plasmid. We could not distinguish whether this was due to nonspecific adsorption of the virus to the COS-7 cells or to virus utilization of specific endogenous surface molecules on COS-7 cells as receptors. Nevertheless, the hBGP- and hCEA-transfected cells reproducibly yielded significantly higher virus titers than did cells transfected with the vector alone. In contrast, when MHV-3 and JHM strains were used for infection, only $MHVR_1$-transfected cells yielded a detectable virus titer; neither hCEA- nor hBGP-transfected cells produced any virus (Table 1). These combined results demonstrate that both hCEA and hBGP can serve as receptors for at least two MHV strains, A59 and MHV-2, but not for JHM or MHV-3. Thus, hCEA and hBGP are potential virus-strain-specific MHV receptors.

To further demonstrate that the virus infection of hCEA- and hBGP-transfected cells was due to the surface expression of hCEA or hBGP, we selected transfected cells that expressed hCEA or hBGP on the cell surface by fluorescence-activated cell sorting (FACS) with antibodies against either hCEA or hBGP. As shown in Fig. 4, in the samples transfected with hCEA or hBGP, a significant percentage of the cells expressed various amounts of hCEA or hBGP on their surfaces (panels 2 and 4). These hCEA- or hBGP-expressing cells were calculated to account for approximately 8 to 16% of the total cell numbers (panels 2 and 4 of Fig. 4a), depending on the level of surface expression. This percentage was roughly equivalent to the transfection efficiency, as determined by the expression of β-galactosidase, quantitated by using the same transfection protocol (data not shown). In contrast, less than 0.2% of the untransfected COS-7 cells (panels 1 and 3) were positive by the FACS analysis. The cells with the higher levels of surface expression of hCEA (panel 2 of Fig. 4a) were collected, plated on the culture dish, and used for infection by MHV A59. At 24 h postinfection, cells were subjected to immunofluorescence staining by using an antibody against the MHV N protein. The result showed that approximately 15 to 25% of the cells showed cytoplasmic staining of viral proteins, indicating that many of the cells were infected (right panel of Fig. 4b). In contrast, the cells that did not express hCEA on their cell surfaces (collected from the peak fraction [panel 2 of Fig. 4a]) did not show any staining for viral proteins (left panel of Fig. 4b). These results suggest strongly that viral infection of these cells was the result

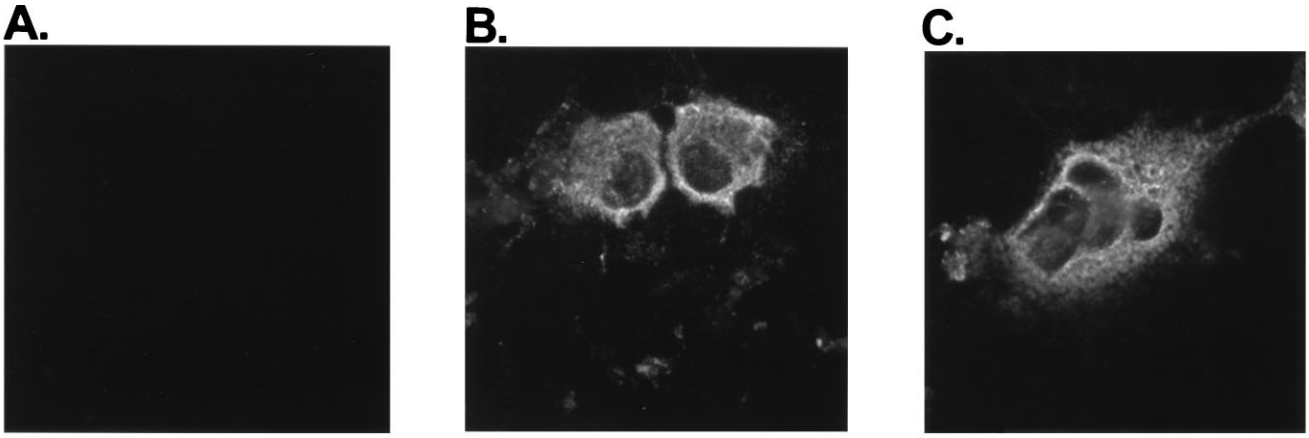

FIG. 3. Immunofluorescence staining of MHV antigens in COS-7 cells transfected with PECE vector (A), hBGP (B), or hCEA (C) and infected with MHV strain A59. The primary antibody used was a monoclonal antibody against the N protein of MHV.

of the surface expression of hCEA. Similar results were obtained for hBGP (data not shown). It is not clear why all of the cells were not positively stained with the anti-MHV antibody. It is possible that cell infectivity is related to the level of receptor expression or that some COS-7 cells are intrinsically resistant to viral infections.

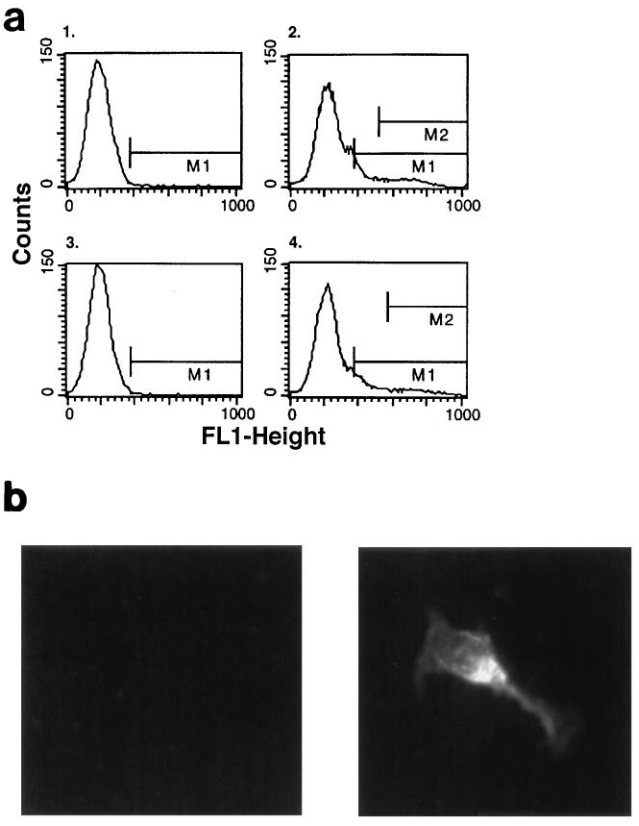

FIG. 4. (a) FACS analysis of hCEA- and hBGP-transfected cells using antibodies against hCEA (panels 1 and 2) or hBGP (panels 3 and 4). Panels 1 and 3, untransfected cells; panel 2, cells transfected with hCEA; panel 4, cells transfected with hBGP. M1 and M2 indicate the fractions of cells collected for counting the numbers of hCEA- and hBGP-expressing cells. (b) Viral infection of FACS-separated cells. The peak fraction and M2 fraction shown in panel 2 of Fig. 4a were collected separately, plated on culture plates, and infected with MHV A59. Immunofluorescence staining, with an N protein-specific antibody, of the infected cells was performed at 24 h postinfection. (Left panel) peak fraction (nonexpressing cells); (right panel) M2 fraction (hCEA-expressing cells).

Previous studies have shown that loop I of $MHVR_1$ is the domain responsible for MHV virus binding and that the sequences of the remaining loops are not important for viral receptor function (9). Since hCEA and hBGP can be used as receptors by some, but not all, MHV strains, in contrast to $MHVR_1$, which confers the receptor function for all of the virus strains tested (Table 1), we attempted to determine whether the loop I domain of hCEA is responsible for its virus strain specificity. For this purpose, we constructed $MHVR_1$/hCEA, an $MHVR_1$ whose loop I domain has been replaced with the loop I domain of hCEA. We also constructed a control, $\Delta MHVR_1$, an $MHVR_1$ sequence without a loop I. Transfection of $MHVR_1$/hCEA into COS-7 cells conferred susceptibility to A59 and MHV-2, yielding virus titers comparable to those from hCEA- and hBGP-transfected cells, but not to JHM or MHV-3 (Table 1). This virus susceptibility pattern was similar to those of hCEA and hBGP. In contrast, cells transfected with $\Delta MHVR_1$ were not susceptible to infection by any MHV strains (Table 1). These results indicate that the hCEA loop I domain determines the virus strain specificity of an MHV receptor.

The results presented here suggest that at least two MHV strains can utilize hCEA gene products as virus receptors. This is the first report that MHV can utilize molecules of different species as receptors. This property may allow MHV to cause cross-species infection. It is most likely that the consensus sequences among the loop I domains of these molecules (Fig. 2) are involved in the virus-receptor interaction. The virus strain specificity in the utilization of the various MHV receptors may be caused by the sequence divergence among these molecules. It is interesting to note that there is a gradation of virus strain specificity in receptor utilization. $MHVR_1$, a murine BGP-like molecule, is utilized by all MHV strains and thus may be considered the prototype MHV receptor. Murine bCEA, a PSG-like molecule, is utilized by all MHV strains but JHM (5), whereas hCEA and hBGP can be utilized only by A59 and MHV-2. Such a selectivity may be solely determined by the loop I sequence, as demonstrated by the domain swap experiments presented here. This result is also consistent with the previous finding that the loop I domain of $MHVR_1$, when fused to the C-terminal portion of the poliovirus receptor, can also function as an MHV receptor (6).

It should be noted that the presence of a functional MHV

receptor in the human species does not necessarily indicate that MHV can infect humans in nature and cause the clinical pathology seen in rodents. In fact, no human or other primate cell lines have been shown to be infectable with MHV in vitro, suggesting either that the expression of the CEA-like molecules in these cultured cells is limited or that there is another restriction step in the virus entry process. The reports that MHV-related nucleic acid sequences were detected in some multiple sclerosis brain specimens (15, 21) remain unconfirmed. Nevertheless, our findings do suggest a potential mechanism for MHV to cause cross-species infection. Similar CEA-like molecules in other primate species may also serve as MHV receptors, and this may explain the finding that MHV can establish infection and cause demyelination in monkey brain after intracerebral or peripheral inoculation (4, 16).

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