CORONAVIRUS RECEPTOR SPECIFICITY

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INTRODUCTION

Bang and his colleagues first showed the importance of the host in determining susceptibility to coronavirus infection, using mouse hepatitis virus MHV-2 as a model system which could cause death of Pri mice but not of C3H mice or macrophages isolated from them (1). Subsequently, as additional coronaviruses were recognized, it became clear that these viruses generally have narrow host ranges and show marked tissue specificity for replication (2). We have studied the importance of virus-receptor interactions in the host range and tissue tropism of coronavirus infections.

IDENTIFICATION OF A RECEPTOR FOR MOUSE HEPATITIS VIRUS MHV-A59

Our first approach to identifying a receptor for MHV-A59 utilized a solid phase virus binding assay in which virus bound to undenatured intestinal brush border membranes (BBM) from MHV-A59-susceptible BALB/c mice (3). In contrast, the virus did not bind

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to BBM from adult SJL/J mice, which are much more resistant to MHV-A59 infection than BALB/c mice (3). This suggests that differences in virus binding to membranes of these mouse strains might play a role in the observed difference in susceptibility to MHV infection. Incubation of viruses with BBM proteins separated by SDS-PAGE in a virus-overlay protein blot assay (VOPBA) showed that MHV-A59 bound to a 110-120 kDa glycoprotein and, less stongly, to a 58 kDa glycoprotein in BALB/c BBM, but not to BBM proteins from SJL/J mice. This 110 kDa glycoprotein was a candidate for a virus receptor.

Antibody that recognized the 110 kDa glycoprotein was raised by immunizing SJL/J mice with BBM from BALB/c mice (4). Both the polyclonal mouse antibody and a monoclonal antibody, MAb-CC1, recognized the 110 kDa and 58 kDa BALB/c glycoproteins in immunoblots of BBM proteins and blocked virus infection of several mouse fibroblast and macrophage cell lines (5). Anti-receptor MAb-CC1 also partially protected infant mice from infection with MHV-A59 (6). Williams et al. isolated the glycoprotein from Swiss Webster mouse liver by immunoaffinity chromatography with MAb-CC1, and showed by N-terminal amino acid sequencing that the 110 kDa glycoprotein was a murine member of the carcinoembryonic antigen (CEA) family of glycoproteins in the immunoglobulin superfamily (5).

CEA-related glycoproteins are expressed on many epithelial cell membranes where they are believed to play a role in intercellular adhesion (7). A radioimmunoassay (RIA) that measured binding of radiolabeled MAb-CC1 to crude membrane preparations from many tissues showed that the MHV receptor glycoproteins were present in highest amounts on BALB/c colon, small intestine and liver which are the principal targets for virus replication in vivo (5). This RIA was not sensitive enough to detect the receptor on membranes of other tissues or of cultured mouse cells that are susceptible to MHV-A59 virus infection. The presence of this receptor antigen on membranes of mouse cell lines was demonstrated by the finding that MAb-CC1 could block MHV-A59 infection of the cells. These experiments suggest a correlation between the tissue tropism of MHV-A59 in vivo with the degree of expression of a receptor moiety.

The cDNA encoding the 110 kDa BALB/c protein (MHVR) that binds MHV-A59 was cloned and expressed in BHK hamster kidney cells and human RD cells (8). These cell lines are normally resistant to MHV-A59 infection, but they became susceptible to infection following transfection with MHVR cDNA. This indicates that MHV-A59 cannot infect hamster cells because they do not express a suitable receptor for the virus. The importance of receptor specificity in determining the host range of mouse coronavirus MHV was further supported by the observation that MHV-A59 did not bind detectably to intestinal BBM of any species except mice (9).

A series of recombinant MHVR proteins with deletions of specific domains was made and transiently expressed in BHK cells which were then tested for susceptibility to MHV-A59 infection (10). These experiments showed that both MHV-A59 virus and MAb-CC1 bound to the first 133 amino acids of MHVR corresponding to the N-terminal immunoglobulin-like domain.

As shown for many other CEA-related glycoproteins, we found that many different isoforms of MHVR glycoproteins can be co-expressed in murine cells in various combinations and ratios. As described in the accompanying paper (11), we examined the ability of each of 5 of these murine glycoproteins to serve as functional receptors for MHV-A59 when expressed in hamster cells. We found that MHVR and several splice variants were functional receptors as was a glycoprotein from SJL/J mice that is homologous to the 58 kDa glycoprotein of MHVR (10). This is the first report of multiple alternative receptors for an enveloped virus. It raises the important questions of whether all of these glycoprotein isoforms are equally effective as receptors for MHV, or whether some strains of virus may preferentially utilize different receptor isoforms. If there were differences in receptor utilization among various MHV strains, then differences in tissue

tropism and virulence of these virus strains might be related to the specificity of virus-receptor interactions. Quantitative evaluation of the affinity of MHV S glycoproteins with the alternative MHV receptors will be required to address this novel and important aspect of the role of virus receptors in virus strain differences.

STUDIES ON THE HOST RANGE AND TISSUE TROPISM OF RAT CORONAVIRUS

Rat coronaviruses are closely related to MHV serologically, but they cause quite different diseases. Sialodacryadenitis virus (RCV-SDAV) and Parker's rat coronavirus (RCV-P) infect the respiratory epithelium, salivary and lacrimal glands of rats, but not intestine or liver (12,13). RCV-SDAV was found to bind to membrane proteins of rat respiratory epithelium, salivary and lacrimal glands but not to rat or mouse intestine or liver. Thus, the specificity of binding of the rat coronavirus to membranes reflects both the host range and tissue tropism of the virus in vivo (14).

Percy and his colleagues showed that rat coronaviruses could replicate in mouse L2 cells which also support the replication of MHV-A59 (15). To determine whether RCV-SDAV could utilize the MHV receptor to enter L2 cells, we pre-treated the cells with MAb-CC1 and then challenged them with the virus (14). We found that MAb-CC1

Table 1. Susceptibility of Mouse Strains to Rodent Coronavirus Infection and Protection by Anti-receptor Antibody MAb-CC1

Cell Line	Virus	Susceptibility to Infection	Protection by MAb-CC1
L2(Percy)	MHV-A59	Yes	Yes
	RCV-SDAV	Yes	No

prevented MHV-A59 infection of these cells, but failed to inhibit replication of RCV-SDAV (Table 1). These observations suggest that rat coronavirus may utilize not MHVR or its splice variants but a different receptor on L2(Percy) cells.

Unlike MHV-A59, rat coronaviruses did not bind to only one major membrane protein from its target tissues in VOPBAs. Rat tissues do, however, express CEA-related glycoproteins homologous to MHVR (16). To determine whether a rat CEA-related glycoprotein could serve as a receptor for RCV-SDAV, we used this virus to challenge COS cells transfected with the rat glycoprotein, called Ecto-ATPase (17), that is a homolog of mouse MHVR and human biliary glycoprotein. No infection was detected by immunofluorescence of cells with anti-viral antibody at 8 hours after virus challenge.

Additional studies on the virus receptor activities of other CEA-related glycoproteins of the rat are in progress. These studies raise the interesting possibility that closely related coronaviruses of the mouse and rat utilize two different types of receptors on the membranes of their host cells, and that availability of these receptors is an important determinant of virus susceptibility.

IDENTIFICATION OF A RECEPTOR FOR HUMAN CORONAVIRUS HCV-229E

Human coronaviruses (HCVs) are found in two different antigenic groups of

coronaviruses. Although HCV-OC43 is serologically related to MHV and RCV, its receptor has not yet been identified. HCV-229E is in a serogroup with feline infectious peritonitis virus, canine coronavirus, and porcine enteric coronavirus TGEV (18,19). We prepared monoclonal antibodies directed against human cell lines that are susceptible to infection with HCV-229E, and tested them for their ability to protect these cells from HCV-229E infection. MAb-RBS was found to block infection, and this antibody immunoprecipitated a 150 kDa glycoprotein from membranes of the human cell lines (20). As found for TGEV (21), a receptor for HCV-229E is aminopeptidase N (APN), a zinc-

Table 2. Correlation of Expression of Coronavirus Receptors Tissues in their Natural Hosts with Sites of Virus Infection.

Virus	Receptor	Properties	Sites of	
			Receptor Expression	Infection
MHV	MHVR	110 kDa	Liver	Liver
	MHVR(2d), mmCGM2	58 kDa	Intestinal epithelium	Intestinal epithelium
			Respiratory epithelium	Respiratory epithelium
			Brain	Brain
			Spleen	Spleen
RCV	Unknown	?	Salivary glands	Salivary glands
			Lacrimal glands	Lacrimal glands
			Respiratory epithelium	Respiratory epithelium
HCV-229E	Human amino-peptidase N	150 kDa	Respiratory epithelium	Respiratory epithelium
			Enteric epithelium	
			Monocytes	
			Granulocytes	
			Synapses	

binding metalloprotease found on monocytes, granulocytes, intestinal brush border and nerve synapses (22,23) and respiratory epithelium (24). Hamster or mouse cells are resistant to infection with HCV-229E unless they are tranfected with the cDNA encoding hAPN. Both the activity of the enzyme and virus infection can be blocked by treatment of the cells with MAb-RBS or by chelating the zinc ions from the medium (20,22). These experiments show that the receptor for HCV-229E is hAPN, a cell membrane glycoprotein unrelated to MHVR.

CONCLUSIONS

The recognition of different types of cellular receptors by the spike glycoproteins of MHV-A59 and HCV-229E appears to be an important determinant of tissue tropism and host range of these coronaviruses. Accordingly, mutations in the gene encoding the S glycoprotein might be expected to result in alterations in receptor specificity and altered host range and/or tissue tropism of the virus. Naturally occurring strains of MHV and IBV and mutants selected with monoclonal antibodies have been shown to have major differences in the size, amino acid sequence and antigenicity of their S glycoproteins. In some cases these mutations have been correlated with alterations in biological properties (25-27). Development of methods to introduce recombinant mutant glycoproteins into the coronavirus genome will permit more detailed studies on the biological significance of receptor specificity as a determinant of tissue tropism and host range.

The levels of receptor expression in different tissues and selective use of alternative receptors by different virus strains are probably important factors of virus-receptor interactions that affect the biological activity of receptors. Although the principal target tissues for MHV-A59 are the tissues in which the receptors are expressed in the highest amounts (5), other tissues in which MHVR-related glycoproteins are also expressed are not common sites of virus infection or symptomatology. Table 2 shows the murine and human coronavirus receptors and some of the cell types in which they are known to be expressed (28-30). It is likely that in some of these tissues, host factors that affect steps in virus replication subsequent to virus attachment to its receptor will be found to determine susceptibility to coronavirus infection.

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